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The Chemistry of SYNTHETIC DYES

VOLUME VII

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PREFACE

For a few years I considered writing an entirely new edition of "The Chemistry of Synthetic Dyes." As a result of discussions with many friends and colleagues who are familiar with the two volumes, I decided in 1967 that the urgent need was not for a revision, because very little of the contents of the 1952 publication had become obsolete, but for a review of the subsequent developments.

The progress made in the chemistry of synthetic dyes in the last twenty years has been amazing. The discovery of reactive dyes is one major advance. There has also been extensive research on intermediates, disperse dyes, cationic dyes, cyanine dyes, pigments, and metal complexes, all of which have led to much new chemical knowledge. Consequently I realized that it was no longer possible for a single author to give accurate and authoritative accounts of the progress made in each specialized area of synthetic dyes. I have been fortunate in the response I have had to my invitations to contribute to the additional volumes. All the chapters have been written by acknowledged authorities, whose names are associated with many patents and papers, and who have worked for many years on the topics they have covered.

In addition to the synthesis and application of dyes of all types, fluorescent brightening agents and such fundamental topics as color and electronic states of organic molecules, photochemistry of dyes, physical chemistry of dyeing, and the relation between structure and technical properties are covered.

These volumes are intended primarily for chemists and technologists who are concerned with the synthesis of dyes and their applications, but since most of the chapters constitute essays in synthetic organic chemistry, they should be of interest to organic chemists in general. An important feature is the very thorough coverage and critical assessment of patent literature as well as publications in scientific journals. The record of achievement presented in these volumes also indicates the directions of future research.

I am indebted to the authors for accepting my invitation and to the companies who made it possible for their leading scientists to spare the necessary time. The plan for this international multiauthor effort took concrete shape during ten days I spent in the Research Department of Farbenfabriken Bayer. I am grateful to Professor Petersen, Dr. Delfs, and their colleagues, and also to Dr. R. R. Davies (Imperial Chemical Industries, Dyestuff Division) for their help

in the organization of this series. My thanks are due to Mr. J. V. Rajan, Mr. G. V. Kulkarni, and Mr. S. A. Nair for preparing the Subject Indexes, checking literature references, and dealing with the heavy correspondence. Academic Press has handled production problems with its usual efficiency, and it is a pleasure to thank the staff for their cooperation. Finally, I wish to make grateful acknowledgment of the hospitality of the National Chemical Laboratory provided by the Director, Dr. B. D. Tilak, the Director-General of Scientific and Industrial Research, Dr. Y. Nayudamma, and his predecessor Dr. Atma Ram, without which I could not have undertaken this project.

K. VENKATARAMAN

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LIST OF ABBREVIATIONS

Manufacturing companies (CI abbreviations have generally been followed) :

AAP	Koppers Co. Inc., Pittsburgh, Pennsylvania (American Aniline Products, Inc.)
ACC	Augusta Chemical Co., Augusta, Georgia
Acna	Aziende Colori Nazionali Affini A.C.N.A., Milan, Italy
B & BASF	Badische Anilin- und Soda-Fabrik A.G., Ludwigshafen a. Rhein, Germany
BrC	British Celanese, Ltd., Spondon, England
CCC	American Cyanamid Co., Bound Brook, New Jersey
CFM	Cassella Farbwerke Mainkur A.G., Frankfurt a. Main, Germany
Chinoin	Chinoin Gyogyszer-es Vegyeszeti Termelek Gyara, RT, Budapest, Hungary
CIBA	CIBA Ltd., Basle, Switzerland
CL	Celanese Corporation of America, New York, New York
CN	Compagnie Nationale de Matières Colorantes et de Produits Chimiques du Nord Réunies Etablissements Kuhlmann, Paris, France
DGS	Deutsche Gold- und Silber Scheideanstalt vormals Roessler, Frankfurt, Germany
DH	Durand & Huguenin S.A., Basle, Switzerland
Dow	Dow Chemical Co., Midland, Michigan
DuP	E. I. Du Pont de Nemours & Co., Wilmington, Delaware
EKCo	Eastman Kodak Co., Rochester, New York
Ethicon	Ethicon, Inc., Somerville, New Jersey
FBy	Farbenfabriken Bayer A.G., Leverkusen, Germany
FH	Farbwerke Hoechst A.G., Frankfurt/Main-Hoechst, Germany
Filature Provoust	Filature de Laine Provoust, Roubaix, France
Fran	Compagnie Française des Matières Colorantes, Paris, France
FW	Farbenfabrik Wolfen, Kr., Bitterfeld, Germany
G	General Aniline & Film Corporation, New York, New York
Gy	J. R. Geigy S. A., Basle, Switzerland
HCC	Hodogaya Chemical Co., Ltd., Tokyo, Japan
HH	Hardman and Holden, Ltd., Manchester, England
HWL	Hickson & Welch, Ltd., Castleford, England

IC	Interchemical Corporation, Hawthorne, New Jersey
ICI	Imperial Chemical Industries, Ltd., Manchester, England
IG	I. G. Farbenindustrie A.G., Frankfurt a. Main, Germany
K	Kalle & Co., A.G., Biebrich a. Rhein, Germany
Kewanee	Kewanee Oil Co., Bryn Mawr, Pennsylvania
KYK	Nippon Kayaku Co., Ltd., Tokyo, Japan
LBH	L. B. Holliday & Co., Huddersfield, England
MCI	Mitsubishi Chemical Industries, Ltd., Tokyo, Japan
MDW	Mitsui Chemical Industry Co., Ltd., Tokyo, Japan
MLB	Farbwerke vorm. Meister, Lucius & Brüning, Hoechst a. Main, Germany
NAC	Allied Chemical Corporation, New York, New York
Nepera	Nepera Chemical Co., Inc., Harriman, New York
NSK	Sumitomo Chemical Co., Ltd., Osaka, Japan
OBM	Otto B. May, Inc., Newark, New Jersey
PCC	Peerless Color Co., Passaic, New Jersey
PHO	Phoenix Color & Chemical Co., Paterson, New Jersey
Pitt	Pittsburgh Coke & Chemical Co., Pittsburgh, Pennsylvania
RL	Rohner Ltd., Pratteln, Switzerland
S	Sandoz Ltd., Basle, Switzerland
TE	Eastman Chemical Products (Eastman Kodak Co.), Kingsport, Tennessee
Ube-Ditto	Ube-Ditto Kasai, Ltd., Osaka, Japan
UCC	Union Carbide Corporation, New York, New York
VGF	Vereinigte Glanzstoff-Fabriken A.G., Wuppertal-Elberfeld, Germany
Vond	N.V. Fabriek van Chemische Producten, Vondelingenplaat, Holland
Whitten	H. A. Whitten Co., New York, New York
YDC	Yorkshire Dyeware & Chemical Co., Ltd., Leeds, England

Journals, Reports, and Books:

1961 *Chemical Abstracts List of Abbeviations* has been generally followed. The following special abbreviations have also been used.

BIOS	British Intelligence Objectives Sub-Committee Final Report
CA	Chemical Abstracts

CI	Colour Index, 2nd edition, 1956
CIOS	Combined Intelligence Objectives Sub-Committee Report
CIS	Colour Index, 2nd edition, Supplement 1963
CSD	The Chemistry of Synthetic Dyes, Academic Press
FIAT	Field Intelligence Agency Technical Report
PB	Technical Report of the Office of the Publication Board, Office of the Technical Services of the U.S. Department of Commerce
Ullmann	Ullmanns Encyclopädie der Technischen Chemie

Patents:

AustP	Austrian Patent
BeP	Belgian Patent
BP	British Patent
CP	Canadian Patent
CzechP	Czechoslovakian Patent
DAS	Deutsche Auslegeschrift
DBP	Deutsche Bundespatente
DP	Dutch Patent
DRP	Deutsche Reichspatente
EGP	East German Patent
FP	French Patent
IP	Indian Patent
JP	Japanese Patent
NethP	Netherlands Patent
PolP	Polish Patent
RP	Russian Patent
SAP	South African Patent
USP	United States Patent

CHAPTER I

SULFUR DYES

D. G. Orton

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CHARLOTTE, NORTH CAROLINA

I. Introduction

Historically, the name "sulfur dyes" has been applied to both a chemical class of dyes and a dyeing class. The first sulfur dyes were all made by thionation of various organic materials with either sulfur or

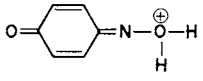
sodium polysulfide, and they were all applied to cotton from sodium sulfide solution. It was not long before other types of dye were made by thionation procedures, in particular the Hydron Blue type of vat dye. More recently, wholly synthetic dyes have appeared on the market which are applied from sulfide solution. While these are sulfur dyes from the point of view of the dyer, to the chemist they belong in the azo, phthalocyanine, anthraquinone, or oxazine class. It seems reasonable to define as sulfur dyes those dyes that are best applied from a sulfide dyebath, and this definition is adopted here.

Although cotton and other cellulosic fibers form a declining proportion of world fiber production, they still constitute a very large and important market and are likely to do so in the foreseeable future. Sulfur dyes are widely used for the production of fast dyeings on all kinds of cellulosic materials, especially in heavy shades where their cost advantage over vat dyes is greatest. During the last 20 years, there have been many changes in the sulfur dye market. Cotton rawstock dyeing is now much less common, but there has been a corresponding increase in continuous dyeing of piece goods and batch dyeing of yarn both in package and beam form. A number of dye manufacturers have ceased to offer sulfur dyes. In England there is now only one manufacturer, and in the United States two manufacturers have gone out of the business since 1965. Nevertheless, the sulfur dye market still continues to grow and is likely to do so while the manufacturers continue to improve their product line to meet changing circumstances.

This review covers progress in the chemistry, manufacture, and application of sulfur dyes since 1950, and is intended to supplement the chapter on sulfur dyes in *CSD II*. The reader is assumed to be familiar with that work.

II. Intermediates

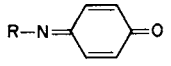
The condensation of *p*-nitrosophenol with amines in sulfuric acid, giving indophenols, has been studied.[1] It is concluded that in 75% sulfuric acid nitrosophenol reacts in the protonated quinonoid form (I).



(I)

[1] O. Manabe, T. Suzawa, and H. Hiyama, *J. Chem. Soc. Jap., Ind. Chem. Sect.* **57**, 326–328 (1954).

This seems reasonable in view of the fact that the products are the quinonoid indophenols (II), rather than the leucoindophenols (III).



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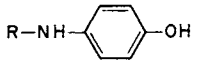


(III)

Kinetic studies of the formation of *o*-toluidine indophenol[2] show that the reaction is of second order with respect to both *o*-toluidine and *p*-nitrosophenol, and the reaction rate diminishes with sulfuric acid concentration. These results are interpreted as indicating reaction between the free base of *o*-toluidine and the conjugate acid of *p*-nitrosophenol. Similar results were obtained for the formation of dimethylaniline and diphenylamine indophenols.[3] The rate constant for the reaction with diphenylamine was shown to be several hundred times greater than for reaction with *o*-toluidine and dimethylaniline. This is in accordance with industrial practice, since diphenylamine indophenol may be made at $-10°$, while *o*-toluidine and dimethylaniline require temperatures between 6° and 10°. It has been shown that the troublesome deterioration of *o*-toluidine indophenol at higher temperatures is due to polymerization.[4]

III. The Chemistry of Thionation

Conventional sulfur dyes are still, for the main part, of unknown and variable constitution. Reproduction of shade from batch to batch is achieved partially by arbitrary control of reaction conditions and partially by checking the course of the thionation by means of laboratory dyeings. The lack of reliable structural formulas for these dyes hinders scientific control of industrial processes and discourages the academic research worker from any investigation of reaction mechanism.

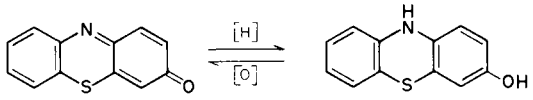
However, certain classes of dye have received much attention over the years, and despite conflicting evidence there is a measure of agreement on certain structures. In particular, the indophenol dyes are fairly well understood. This important class of dyes includes the blues and greens, the Indocarbon Blacks, and the red-browns derived from 4-

[2] O. Manabe, S. Moriwaki, and H. Hiyama, *J. Chem. Soc. Jap., Ind. Chem. Sect.* **57**, 520–523 (1954).

[3] O. Manabe, S. Moriwaki, and H. Hiyama, *J. Chem. Soc. Jap., Ind. Chem. Sect.* **57**, 523–525 (1954).

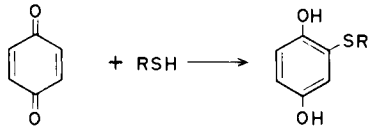
[4] H. Hiyama, A. Ito, and J. Matsumoto, *J. Chem. Soc. Jap., Ind. Chem. Sect.* **55**, 102–103 (1952).

hydroxydiphenylamine. It is agreed that the fundamental chromophore is the phenothiazinone group (IV), which accounts for the high color



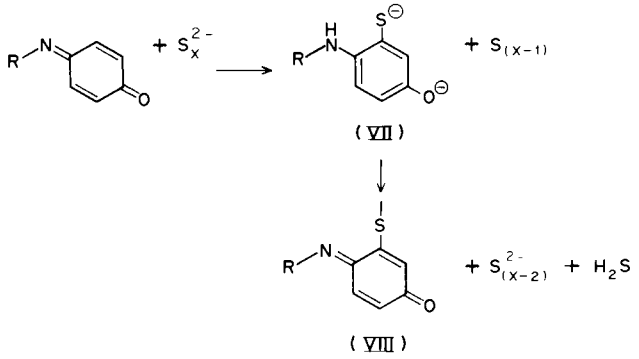
(IV) (V)

value of these dyes and for their reduction to pale yellow leuco compounds (V). It is further agreed that as many as three more sulfur atoms are attached to the quinoneimine ring. The formation of such structures from indophenol may be explained in terms of nucleophilic substitution by polysulfide ions. It is known that quinones react readily with mercaptans, forming substituted quinols (VI).[5] The analogous reaction of



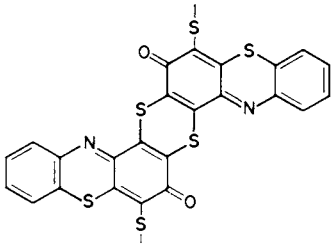
(VI)

polysulfide with quinoneimine is postulated as the first stage in a thionation, giving the substituted leucoindophenol (VII). This is then dehydrogenated by sulfur to the quinoneimine form (VIII), liberating hydrogen sulfide. This theory accounts for the inability of sodium sulfide to form sulfur dyes with indophenols, since sulfide is unable to oxidize the leuco form of the intermediates to the corresponding quinoneimine. This process of substitution followed by oxidation is repeated until thionation is complete and the dye is formed.



[5] J. M. Snell and A. Weissberger, *J. Amer. Chem. Soc.* **61**, 450 (1939).

The work of Zerweck, Ritter, and Schubert[6] indicates the presence of a thianthrene structure (IX) in indophenol dyes. This structure might



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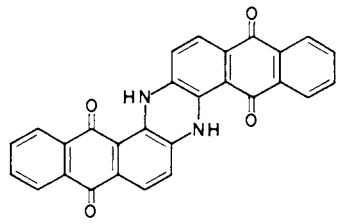
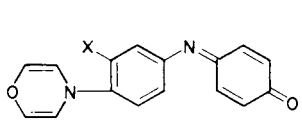
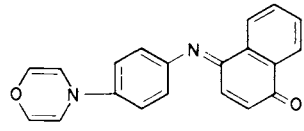


(X)

reasonably be expected to confer affinity for cotton, since it is isosteric with a number of vat dyes, e.g., indanthrone (X). Support for the thianthrene ring system is found in the properties of a group of sulfur dyes made from *N*-arylmorpholines.[7] The dyes derived from the indophenols (XI) were blue and had affinity for cotton, whereas the dye from



(XI) X = H, CH_3



(XII)

the naphthoquinoneimine indophenol (XII) was violet and had no affinity for cotton. The lack of affinity and violet color of this dye may be accounted for by the inability of the naphthoquinoneimine to form a thianthrene bridge with a second molecule.

Sulfur Black T has been the subject of various investigations. Hiyama[8] has studied the positions into which sulfur enters to form the dye. Analysis of sulfur black free from uncombined sulfur shows that a minimum of five sulfur atoms is needed for each 24 carbon atoms in order to form the dye. The effect of polysulfide composition on yield has been studied.[9] It is claimed that the maximum yield is obtained with Na_2S_4. Other authors[10] contend that the optimum polysulfide is between $Na_2S_{3.1}$ and $Na_2S_{3.9}$, depending on the molar ratio of polysulfide to dinitrophenol. These results are in agreement with industrial thionation procedures, which utilize $Na_2S_{3.6}$ to $Na_2S_{4.3}$.

[6] W. Zerweck, H. Ritter, and M. Schubert, *Angew. Chem., Ausg. A* **60**, 141–168 (1948).

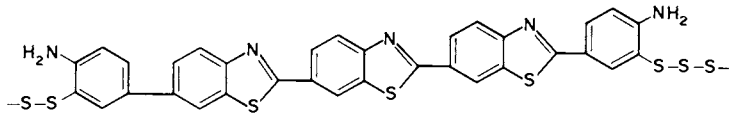
[7] N. Kuroki, A. Katayama, J. Nishino, and K. Konishi, *J. Chem. Soc. Jap., Ind. Chem. Sect.* **57**, 863–864 (1954).

[8] H. Hiyama, *J. Chem. Soc. Jap., Ind. Chem. Sect.* **51**, 92–96 (1948).

[9] K-C. Chang and K. K. Hua, *J. Chem. Eng.* (*Tientsin*) **16**, 18–24 (1949).

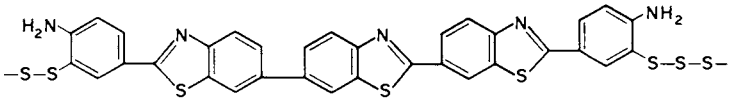
[10] C. C. Yin, W. H. Yuan, and H. H. Chang, *Chem. Ind. Eng.* (*Peiping*) **2**, No. 3, 80–85 (1951).

A degradative study of Immedial Supra Yellow GWL indicates that it consists mainly of (XIII).[11] The dye is therefore made by a sulfur bake



(XIII)

of benzidine with either Primuline Base, *p*-toluidine, or dehydrothio-*p*-toluidine and *p*-toluidine. Most probably Primuline Base is used, as the other alternatives would be expected to give rise to such structures as (XIV).

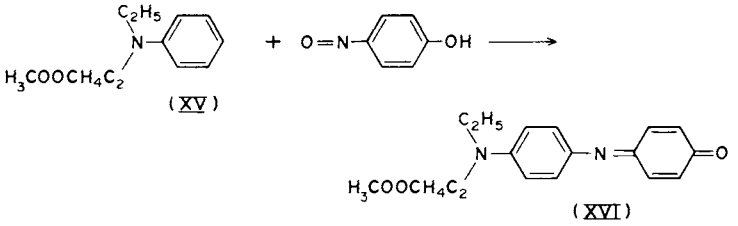


(XIV)

IV. New Sulfur Dyes Made by Thionation

A. Indophenols

There has been some interest in Japan in sulfur dyes for Vinylon. Thionation of indophenols carrying carbomethoxyalkyl groups gives blue dyes.[12] The intermediates are made by condensation of anilines with methyl acrylate. For instance, *N*-ethylaniline is heated with methyl acrylate and a catalytic quantity of hydroquinone in acetic acid. The resulting amine (XV) is condensed with *p*-nitrosophenol to form the



(XV) (XVI)

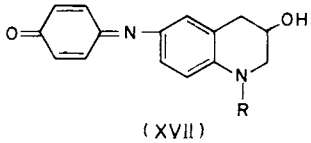
corresponding indophenol (XVI). Indophenols having hydroxyethyl groups are claimed to be applicable to synthetic fibers.[13] Some of these

[11] J. Marek and D. Markova, *Collect. Czech. Chem. Commun.* **27**, 1533–1548 (1962).

[12] N. Kuroki, A. Takaoka, E. Ota, and K. Konishi, *J. Chem. Soc. Jap., Ind. Chem. Sect.* **57**, 864–866 (1954).

[13] N. Kuroki, A. Katayama, and K. Konishi, *J. Chem. Soc. Jap., Ind. Chem. Sect.* **57**, 291–293 (1954).

indophenols are made by condensation of *p*-nitrosophenol with such amines as *N*,*N*-2-hydroxyethylallylaniline. Others are made by oxidative condensation of 1-naphthol with the appropriate *p*-amino-*N*,*N*-dialkylaniline. Similarly, *N*-arylmorpholines yield indophenols which are thionated to give violet and blue dyes for acrylic fibers.[7] Condensation of *p*-aminophenol with tetrahydro-1-alkyl-3-quinolinols in the presence of an oxidant gives indophenols (XVII) which are thionated in poly-



(XVII)

sulfide to yield blue dyes of good lightfastness for Vinylon.[14] Other blue and green dyes for Vinylon are made by thionating the indophenols obtained by oxidative condensation of *N*,*N*-dialkylphenylenediamines with cresols and xylenols.[15]

A number of blue indophenol sulfur dyes have been made from *N*-alkydiphenylamines.[16] Methyl, ethyl, and butyl diphenylamines yield bright greenish blue dyes, the methyl derivative being notably fast to chlorine. *N*-Alkyl-3-chlorodiphenylamines give significantly redder shades, but chlorine in the 4-position has little effect on shade.

The blue dyes made from 2,4-dinitro-4′-hydroxydiphenylamine are much improved in shade by a treatment with sodium cyanide in conjunction with an alkaline oxidation, the shade becoming significantly redder and brighter.[17] A similar cyanide treatment is claimed to improve the exhaust dyeing properties of Sulfur Black.[18]

B. Polycyclic Intermediates

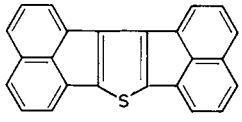
The commercial success of the lightfast orange dyes made by a sulfur bake of decacyclene (*CSD II*, 1072) has led to some activity in this and related areas.

Chlorination of decacyclene with sulfuryl chloride at 70° using an iron catalyst introduces 10 atoms of chlorine. This intermediate is baked with sulfur at 250°–270° for 6–8 hours and then desulfurized to yield a deep brown sulfur dye of good lightfastness.[19] The various nitrodecacyclenes are thionated with sulfur chloride in chlorosulfonic acid to yield

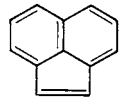
[14] N. Kuroki, A. Katayama, and K. Konishi, *Kogyo Kagaku Zasshi* **59**, 617–619 (1956).
[15] N. Kuroki, A. Katayama, and K. Konishi, *Kogyo Kagaku Zasshi* **59**, 615–617 (1956).
[16] Gy, *BP* 707,705.
[17] G. C. Strouse, *USP* 2,801,995.
[18] N. M. Bigelow, *USP* 2,418,816.
[19] CFM, *BP* 765,636.

brown sulfur dyes of outstanding fastness to light.[20] Trinitrodecacyclene and sulfur are stirred in chlorosulfonic acid at 20°. Chlorine is introduced, allowing the temperature to rise to 50°, and the mixture is stirred at 50° for 3 hours. It is then drowned in ice, and the precipitated dye is filtered off, washed, and desulfurized to give a yellow-brown dye of high lightfastness. Similarly, hexanitrodecacyclene yields a khaki dye, and enneanitrodecacyclene a black-brown dye. Decacyclene is brominated and then thionated in sulfur to give orange and yellow-brown sulfur dyes of excellent light- and wash-fastness.[21] For example, bromination of decacyclene at room temperature with an iodine catalyst in chlorobenzene gives a tribromodecacyclene. This is thionated by baking with sulfur at 195°–200° to give yellow-orange shades. Thionation at 250° gives red-brown shades. It is interesting that the brominated decacyclene can be thionated at such a relatively low temperature, whereas decacyclene itself requires temperatures in excess of 300°. The brominated intermediate evolves hydrogen bromide and hydrogen sulfide during thionation, so both bromine and hydrogen atoms are displaced by sulfur.

Decacyclene is itself made by a sulfur bake process in which acenaphthene is dehydrogenated with evolution of hydrogen sulfide. It would be economically very attractive if cheap acenaphthene could be directly converted to decacyclene orange by sulfur fusion. However, this has not been achieved. A considerable quantity of diacenaphtho[1,2-*b*:1′,2′-*d*]-thiophene (XVIII) is produced as a by-product, and further thionation of this yields brown dyes of inferior lightfastness.[22] Similar red-brown shades are obtained by sulfur bakes of acenaphthylene (XIX).[23] However, it is claimed that the dyes made by polysulfide bakes of nitro-



(XVIII)

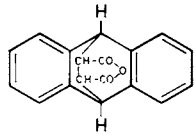


(XIX)

acenaphthenes have superior lightfastness.[24] 5-Nitroacenaphthene is baked with Na_2S_8 at 240°–260° to give a dye which gives red-brown shades from a violet-brown vat. Pure 5,6-dinitroacenaphthene gives black-brown shades, and the addition of other dinitroacenaphthene isomers gives this dye a redder tone. These products are claimed to be suitable for cotton, nylon, and Vinylon.

[20] CFM, *BP* 781,408.
[21] CFM, *BP* 862,218.
[22] O. Leminger and E. Marvel, *CzechP* 97,634.
[23] Nippon Kayaku Co., *JP* 112 (1967).
[24] Nippon Kayaku Co., *JP* 3291 (1963).

Sulfur bakes of pyrene, polychloropyrene, and acenaphthene in the presence of copper salts are claimed to give red-brown dyes of excellent light- and wash-fastness.[25] Pyrene is baked with sulfur at 280°–285° for 4 hours. The dye is isolated by dissolving the crude melt in caustic soda solution and acidifying with hydrochloric acid. Methylpyrenes have been shown to yield valuable sulfur dyes. 3-Methylpyrene is baked with sulfur at 240°–260° to give a brown sulfur dye. The 4-methyl isomer gives a redder shade of brown.[26] Addition of *p*-phenylenediamine to this melt gives yellow-olive to yellow-brown shades.[27] Similarly, 4-methylpyrene with *o*-phenylenediamine gives a brown dye, with *p*-toluidine a red-brown dye, and with 2-aminoanthraquinone a red-brown dye. Condensation of anthracene with maleic anhydride at 250°–260° gives 9,10-dihydroanthracene-9,10-*endo*-a,b-succinic anhydride (XX). When



(XX)

this intermediate is baked with sulfur the product is insoluble in sulfide, but dyes cotton dark olive from a hydrosulfite vat.[28] However, brown sulfur dyes are obtained if 2,4-diaminotoluene, benzidine, or dehydrothio-*p*-toluidine is added to the thionation charge.[28,29]

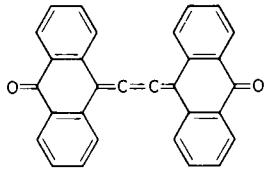
C. Anthraquinone Derivatives

Thionation of 2-aminoanthraquinone[30] and its halogen and sulfonic acid derivatives[31] with sodium polysulfide gives olive and brown vat dyes. Thus 2-aminoanthraquinone is baked with $Na_2S_{5.7}$ at 280° for 12 hours, giving a vat dye which dyes cotton in black-brown shades from a dark brown vat. Sodium 2-aminoanthraquinone-3-sulfonate baked with Na_2S_5 at 280°–300° for 12 hours gives an olive-brown vat dye. A similar dye is obtained from 2-amino-3-chloroanthraquinone, while 2-amino-1-chloroanthraquinone gives an olive-gray and sodium 2-amino-1-bromoanthraquinone-3-sulfonate, an olive-brown. These dyes have excellent fastness to washing and light, but not to chlorine.

[25] CIBA, *BP* 924,977.
[26] FW, *DBP* 1,102,939.
[27] FW, *DBP* 1,110,790.
[28] H. Hiyama and T. Sagara, *Kagaku To Kogyo* (*Osaka*) **32**, 308–311 (1958).
[29] H. Hiyama and T. Aihara, *JP* 2235 (1959).
[30] CFM, *BP* 791,498.
[31] CFM, *DBP* 1,057,266.

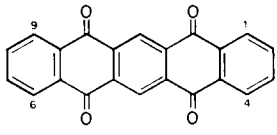
Sulfur dyes are obtained by polysulfide bakes of various 1-hydroxyanthraquinone derivatives.[32]

Reflux thionation of dianthronethylene (XXI) in ethanolic sodium



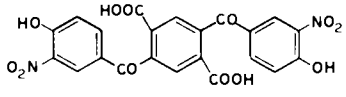
(XXI)

polysulfide gives a red-violet product which dyes cotton pink from a red hydrosulfite vat. Under similar conditions, 2,2′-dichlorodianthronethylene yields a violet vat dye.[33] Polysulfide thionations of pentacenediquinones (XXII) and their precursors give rise to fast brown and



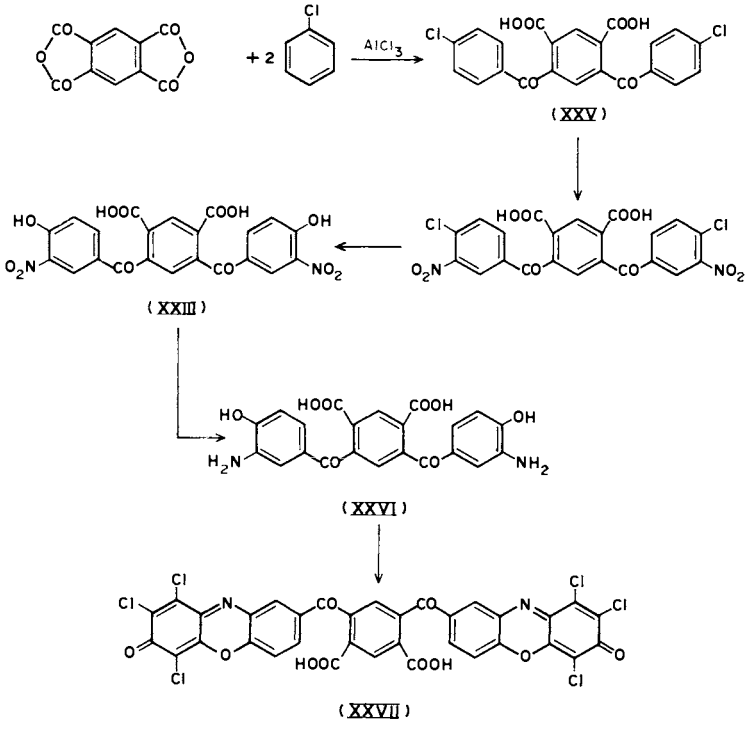
(XXII)

olive-green dyes.[34] Thus, baking 1,6-diacetylaminopentacenediquinone at 270° with sodium tetrasulfide forms an olive dye, while the 1,4,6,9-tetraphenoxy and the 1-amino derivatives give olive-green shades. The substituted dibenzoylbenzenedicarboxylic acids (XXIII) and (XXIV) yield an olive-brown dye. Condensation of pyromellitic dianhydride with chlorobenzene under Friedel-Crafts conditions gives 4,6-di-*p*-chlorobenzoylisophthalic acid (XXV). Nitration, alkaline hydrolysis, and sulfide reduction leads to the diamine (XXVI). Condensation with chloranil gives the dioxazine (XXVII), which may be thionated to yield an olive dye.



(XXIV)

[32] CFM, *DBP* 942,705.
[33] CFM, *DBP* 1,270,714.
[34] Vond, *NethP* 112,976.



(XXV)

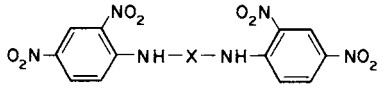
(XXIII)

(XXVI)

(XXVII)

D. Aromatic Amines

Brown sulfur dyes are made by baking compounds of general formula (XXVIII) with polysulfide.[35] Examples of such intermediates are



(XXVIII)

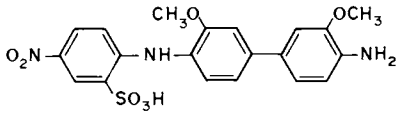
N,*N*′-bis(2,4-dinitrophenyl)ethylenediamine and *N*,*N*′-bis[2-(2,4-dinitrophenylamino)ethyl]amine, made by condensation of 2 moles of 2,4-dinitrochlorobenzene with the appropriate diamine.

Diphenylamine derivatives useful as sulfur dye intermediates are made by condensation of *o*-tolidine and *o*-dianisidine with 1 or 2 moles of a chloronitrobenzenesulfonic acid.[36] Thus *o*-dianisidine is condensed with 2-chloro-5-nitrobenzenesulfonic acid to give (XXIX). This is baked with polysulfide to give brown shades. The analogous intermediate from

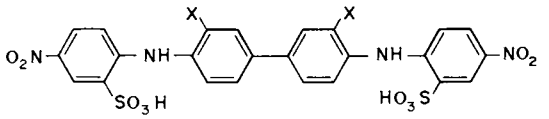
[35] CFM, *DBP* 818,541.

[36] Gh. Lupusor and S. Ifrim, *Bul. Inst. Politeh. Iasi;* [N.S.] **14**, 259–264 (1968).

o-toluidine gave a green-gray sulfur dye. The intermediates (XXX), made from 2 moles of the reactive chloro compound, give gray dyes. It is claimed[37] that ethylbenzene derivatives yield sulfur dyes superior to those derived from the homologous toluene derivatives. In particular, dinitro-, diamino-, and aminonitroethylbenzenes give useful olive-green and olive shades when baked with polysulfide.



(XXIX)



(XXX)

E. Copper Phthalocyanine

Conventional sulfur and polysulfide bakes of copper phthalocyanine do not yield any sulfur dye. This intermediate can be thionated with the aluminum chloride/sulfur chloride complex,[38] but the dark green sulfur dyes so made have rather poor solubility and have not been marketed. Brighter shades of green with better solubility are made by chlorosulfonation of copper phthalocyanine, followed by zinc or iron reduction of the sulfonyl chloride to mercaptan.[39] Thus copper phthalocyanine is heated with chlorosulfonic acid for 3 hours at 145°–150°, and the mixture is then poured onto ice. Zinc dust is added and the mixture is heated to 90° to complete the reduction. However, if the chlorosulfonation temperature is kept down to 100°–110°, iron reduction yields blue sulfur dyes.[40] Presumably fewer mercapto groups are introduced than in the green dyes. These products may be further thionated by baking with sulfur to give dark green sulfur dyes of higher sulfur content.[41] Thus copper phthalocyanine is chlorosulfonated at 145° and the product is reduced with iron and acid. The resulting green powder is baked with sulfur at temperatures between 220° and 245° to give strong dark green sulfur dyes of high light- and wash-fastness, the lower temperatures

[37] Nippon Kayaku Co., *JP* 22,031 (1961).
[38] A. L. Fox, *USP* 2,369,666.
[39] N. H. Haddock, *BP* 544,953.
[40] R. L. Mayhew, *USP* 2,484,300.
[41] CFM, *BP* 784,353.

giving a yellower shade. Similar results are obtained if the mercaptophthalocyanines are methylated before being thionated. Similarly tetra-(4)-thiocyanatocopper phthalocyanine is baked with sulfur at 240°–265° to give dark green sulfur dyes.

Addition of hydroxylamine salts and a catalytic quantity of molybdenum or vanadium oxide to the chlorosulfonation has a drastic effect on the shade of the dye. Iron reduction of the chlorosulfonation product gives green-gray sulfur dyes of good lightfastness.[42] Copper phthalocyanine with a trace of molybdic oxide is added to an excess of chlorosulfonic acid containing 5 moles of hydroxylamine sulfate. The mixture is heated at 150° for 2 hours, added to ice and an excess of iron powder, and then stirred at 50° for 24 hours. The product is filtered off and washed acid-free. Addition of sulfur chloride to the chlorosulfonation changes the shade of the final dye to violet-gray.[43] Thus, copper phthalocyanine is added to 3 moles of hydroxylamine sulfate and a trace of ammonium molybdate in excess chlorosulfonic acid. The mixture is heated to 125° and sulfur chloride (20 moles) is added dropwise. After 1–2 hours further heating at 125° the mixture is drowned in ice, iron powder, and sulfuric acid. The reduction is completed as above. A neutral gray shade is obtained if 50 to 90% of the chlorosulfonic acid is replaced by 100% sulfuric acid in the above procedure.[44] If the iron reduction stage is omitted the product is a greenish-gray sulfur dye.[45] The exhaust dyeing properties are improved by keeping the reaction temperature below 100°.

Copper phthalocyanine sulfonyl chlorides may be reduced with sodium sulfide or sodium hydrosulfide to give copper phthalocyanine sodium sulfinates, which may also have sulfonate or thiolsulfonate groups.[46] These are heated in an autoclave with sodium thiosulfate or sodium polysulfide to give green sulfur dyes.[47] Alternatively the sulfonyl chloride may be treated with thiourea to yield bright green sulfur dyes.[48]

F. Dioxazines

Dioxazine intermediates, like phthalocyanines, are not readily thionated by conventional procedures, but yield sulfur dyes by iron

[42] CFM, *BP* 761,287.
[43] CFM, *DBP* 1,059,133.
[44] CFM, *BP* 854,579.
[45] CFM, *DBP* 1,062,852.
[46] G. A. Gamlen and P. W. Hickmott, *BP* 960,643.
[47] W. J. Bryan and W. S. Griffith, *USP* 3,378,568.
[48] D. Razavi, *FP* 1,526,096.

TABLE I

Thionation of Dyes from Dioxazines

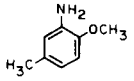
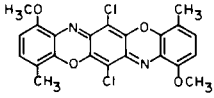
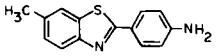
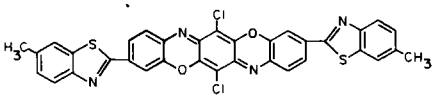
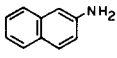
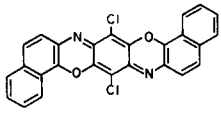
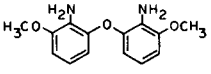
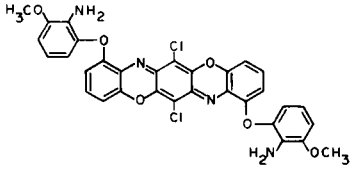
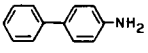
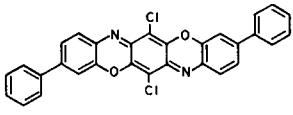
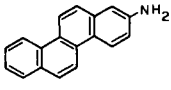
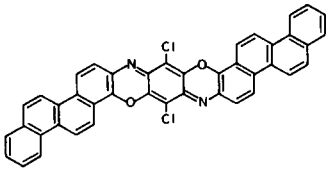
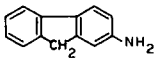
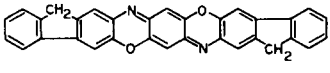
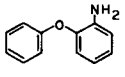
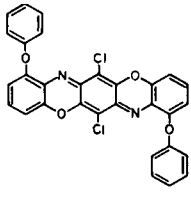
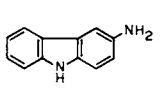
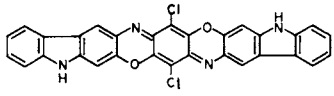
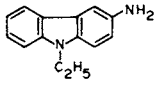
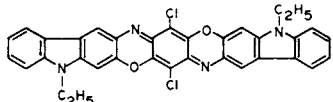
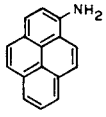
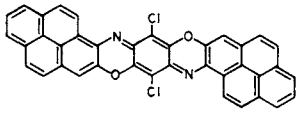
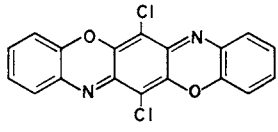
Amine	*Dioxazine*	*Color of vat*	*Shade on cotton*
		Green-blue	Red-violet
		Yellow	Blue-violet
		–	Violet
		–	Red-violet
		–	Purple
		–	Turquoise blue
		Yellow	Brilliant violet
		–	Brick red

TABLE I—*continued*

Amine	*Dioxazine*	*Color of vat*	*Shade on cotton*
		–	Violet-blue
	 (XXXII)	–	Blue
		–	Dark blue

reduction of their sulfonyl chloride derivatives to the thiols.[49] Condensation of chloranil with aniline gives 6,13-dichlorotriphenodioxazine (XXXI), which is chlorosulfonated at 130°–135°. The chlorosulfonation



(XXXI)

mass is drowned in ice and reduced with iron at 40°–55° to give a bronzy powder which dyes cotton from a green-blue sulfide vat in red to claret shades of good chlorine fastness. Other dioxazines may be treated similarly to yield sulfur dyes of varying shades. The starting amine, dioxazine intermediate, and the shade of dye obtained are listed in Table I.

The dioxazine (XXXII, see Table I) (*CSD II*, 786) yields navy blue sulfur dyes by treatment of its sulfonyl chloride or sulfinate with thiourea.[45]

G. Other Intermediates

Brown, khaki, and olive-green dyes are obtained by baking naphthols, nitrosonaphthols, and nitrosophenols with a copper salt and poly-

[49] CFM, *BP* 815,840.

sulfide.[50] The intermediates and shades of the resulting dyes are listed in Table II.

TABLE II

INTERMEDIATES FOR DYES MADE BY THIONATION

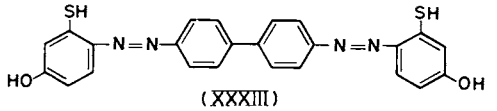
Intermediate	*Thionation temperature*	*Shade*
2-Naphthol	255°–260°	Khaki
1-Nitroso-2-naphthol	260°–265°	Brownish khaki
p-Nitrosophenol	260°–265°	Olive-green
1,5-Dihydroxynaphthalene	260°–265°	Brown

Under similar conditions phenolphthalein and fluorescein yield olive-green dyes.[51]

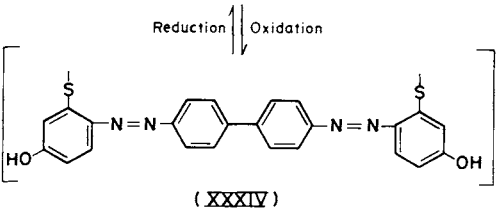
p-Dichlorobenzene recovered from DDT waste is nitrated and then hydrolyzed to give 4-chloro-2-nitrophenol. Reflux thionation of this intermediate in aqueous polysulfide gives a gray sulfur dye.[52]

V. Sulfur Dyes of Known Constitution

As early as 1922 there were attempts to synthesize dyes of definite structure, which would be applicable to cotton-like sulfur dyes.[53] Azo dyes were made having mercapto substituents, and these were soluble in sulfide and could be oxidized on the fiber to the disulfide. Benzidine was tetrazotized and coupled with 2 moles of *m*-mercaptophenol to give the dye (XXXIII). This dissolved in sodium sulfide to give a red solution which dyed cotton, and upon oxidation the polydisulfide form



(XXXIII)



(XXXIV)

[50] CIBA, *BP* 923,472.

[51] CIBA, *BP* 925,114.

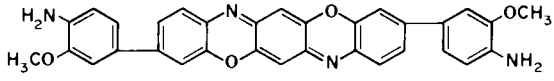
[52] M. Z. Shah, M. Sarwar, M. K. Bhatty, and Karimullah, *Pak. J. Sci. Ind. Res.* **9**, 274–276 (1966).

[53] E. R. Watson and S. Dutt, *J. Chem. Soc., London* **121**, 2414 (1922).

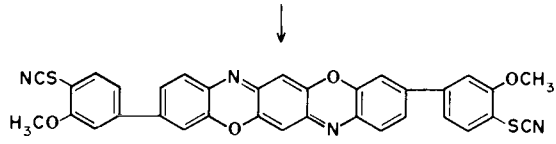
of the dye (XXXIV) was generated. This and other azodisulfide dyes had certain technical defects, and no useful synthetic sulfur dye was developed until the 1940s when red and green sulfur dyes were invented, based on perylene-3,4,9,10-carboxylic diphenylimide[54] and copper phthalocyanine,[55] respectively. The commercial success of the green dye has stimulated research in other areas, including azo and dioxazine dyes.

A. Dioxazines

Chloranil is condensed with 2 moles of dianisidine to give the diaminodioxazine (XXXV). This is tetrazotized and the tetrazonium salt is treated with sodium thiocyanate to yield the violet sulfur dye (XXXVI).[56]



(XXXV)



(XXXVI)

Condensation of dioxazine dicarboxylic acids with sulfur-substituted anilines yields orange to red sulfur dyes.[57] For example, 6,13-dichlorotriphenodioxazine-2,9-dicarboxylic acid (XXXVII) is heated under reflux in *o*-dichlorobenzene with thionyl chloride and a catalytic quantity of pyridine to form the acid chloride (XXXVIII). The excess thionyl chloride is distilled off and 4-thiocyanatoaniline is added. Heating for 1 hour at 125° completes the reaction, and the dye (XXXIX) is isolated by diluting the reaction mixture with alcohol and filtering. Other dioxazines which may be used are 6,13-dichlorotriphenodioxazine-3,10-dicarboxylic acid (XL), 3,6,10,13-tetrachlorotriphenodioxazine-2,9-dicarboxylic acid (XLI) and 6,13-dibromotriphenodioxazine-3,10-dicarboxylic acid (XLII). Table III gives the shades obtained from these intermediates with various sulfur-containing amines.

[54] N. H. Haddock, *USP* 2,409,851.
[55] N. H. Haddock, *USP* 2,395,117.
[56] G. B. Robbins, *USP* 2,600,690.
[57] G. B. Robbins, *USP* 2,564,381.

TABLE III

Dioxazine Dyes

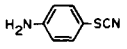
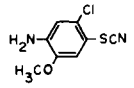
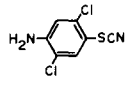
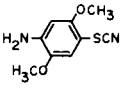
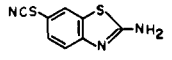
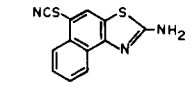
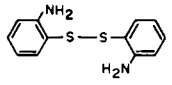
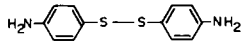
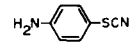
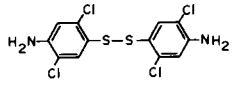
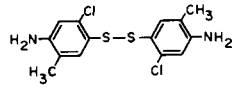
Dioxazine	*Amine*	*Shade*
(XXXVII)		Red
(XXXVII)		Red
(XXXVII)		Yellowish red
(XXXVII)		Bluish red
(XXXVII)		Bluish red
(XXXVII)		Bluish red
(XXXVII)		Red
(XXXVII)		Red
(XLII)		Orange
(XXXVII)		Yellowish red
(XXXVII)		Red

TABLE III—*continued*

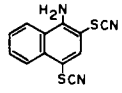
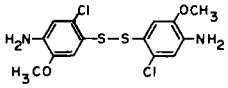
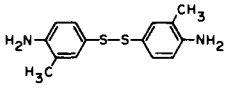
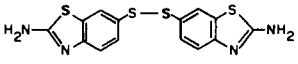
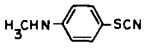
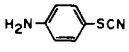
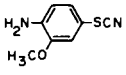
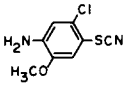
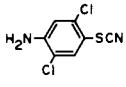
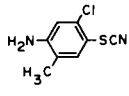
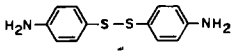
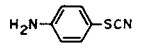
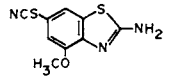
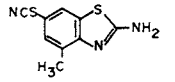
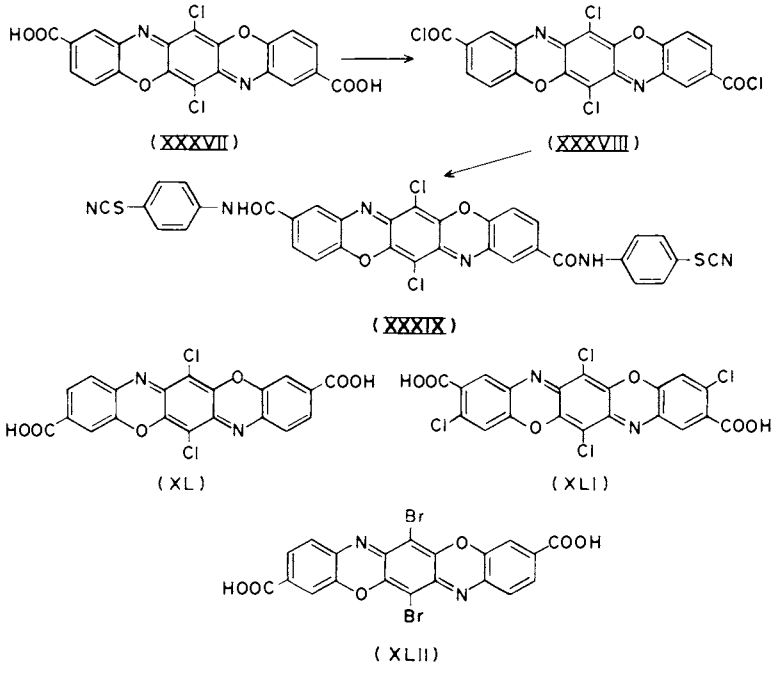
Dioxazine	*Amine*	*Shade*
(XXXVII)		Bluish red
(XXXVII)		Red
(XXXVII)		Yellowish red
(XXXVII)		Bluish red
(XXXVII)		Light red
(XL)		Bright orange
(XL)		Bright orange
(XL)		Bright orange
(XL)		Yellowish orange
(XL)		Yellowish orange
(XL)		Bright orange

TABLE III—*continued*

Dioxazine	*Amine*	*Shade*
(XLI)		Red
(XXXVII)		Bluish red
(XXXVII)		Bluish red



(XXXVII) (XXXVIII) (XXXIX) (XL) (XLI) (XLII)

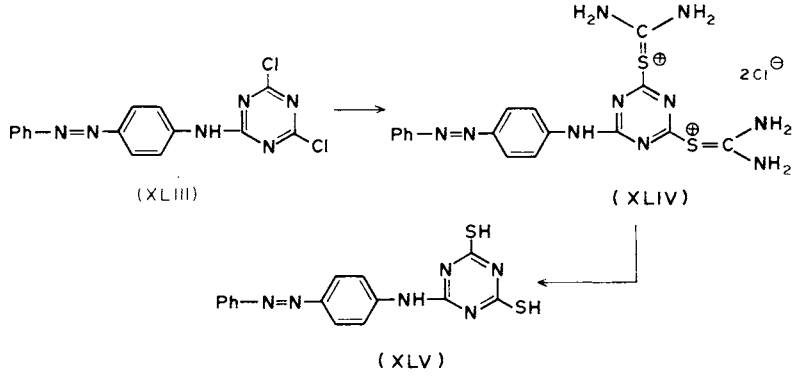
B. Hiyaman Dyes

In a 1959 monograph[58] Hiyama reviewed the chemistry of synthetic sulfur dyes and proposed a theory to account for the dullness of sulfur dyes. It is held that sulfur dyes are dull in shade not merely because they are mixtures, but because mercaptan groups added to a chromophore widen the absorption band. Supporting evidence is found in the ultraviolet absorption spectra of certain colorless aromatic mercaptans,

[58] H. Hiyama, "Synthetic Sulfur Dyes." Osaka Municipal Technical Research Institute, Osaka, 1959.

sulfides, and disulfides. The bright shades of certain sulfur yellows and blues are explained as being due to the dye's absorption band being only partially within the visible region. In order to synthesize bright sulfur dyes, it was considered necessary to separate the mercaptan groups from the chromophore by an insulating group which would prevent the electronic interaction that causes the absorption region to broaden out. Suitable insulating groups include *sym*-triazine and sulfonanilide groups.

Cyanuric chloride is condensed with water-insoluble dyes having one or more primary amino groups. The remaining chlorine atoms on the triazine ring are then replaced by reaction with thiourea, and subsequent reduction of the isothiourea group gives the dimercaptotriazinyl-substituted dye. Thus aminoazobenzene is condensed with cyanuric chloride in acetone below 5°, using sodium hydroxide as an acid-binding agent. The dichlorotriazinyl intermediate (XLIII) reacts with 2 moles of thiourea at 10°–20° in the same solvent. Sodium sulfide is added and the thiourea derivative (XLIV) is reduced at 40°–50° to give the dye

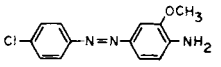
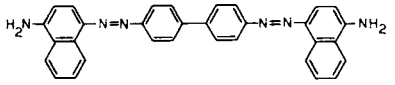
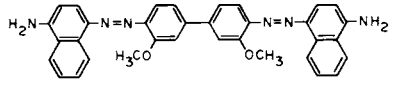
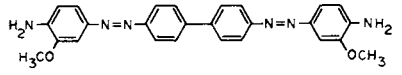
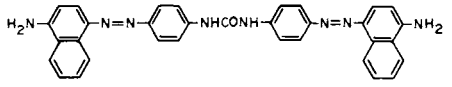
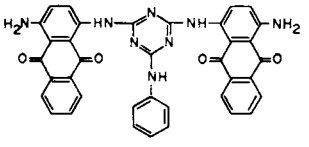


(XLV). The acetone is distilled off and the yellow dye is precipitated by acidifying with hydrochloric acid. The shades obtained from various other amino-substituted dyes are illustrated in Table IV.

Other dimercaptotriazinyl azo dyes are made by coupling diazonium salts with coupling components carrying the dimercaptotriazinyl group. Salicylic acid and *m*-nitroaniline are heated under reflux with phosphorus trichloride in toluene to form the *m*-nitroanilide (XLVI) in 85% crude yield. Iron reduction in dilute acetic acid gives (XLVII) in 80% yield. This is condensed with cyanuric chloride in acetone and subsequently treated with thiourea to give (XLVIII). The coupler (XLIX) is made from Naphtol AS-BS by sulfide reduction followed by condensation with cyanuric chloride, then thiourea, while (L) and (LI) are made analogously from the corresponding aminonaphthols. A wide variety of

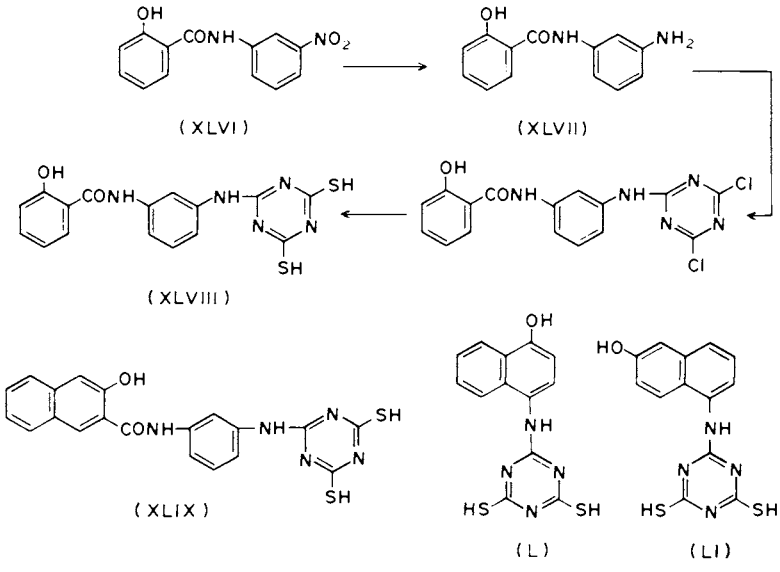
TABLE IV

DYES CONTAINING THE DIMERCAPTOTRIAZINYL GROUP

Amine	Shade of derived dye
	Yellow
	Brown
	Brown
	Orange yellow
	Yellow orange
	Violet

shades (from yellow to blue) has been produced by coupling these four intermediates with various diazonium salts. One of the technical disadvantages of these dyes is that mercaptan groups on the triazinyl ring are rather more difficult to oxidize than are aromatic thiols. Consequently air oxidation of the dyeings is incomplete, and the residual thiol groups give rise to inferior wash-fastness. This can be improved by treating the dyeing with a salt of a heavy metal, such as cadmium, or with an alkylating agent such as benzyldimethylphenylammonium chloride.

A number of dyes are described in which an azo or phthalocyanine pigment is chlorosulfonated and the sulfonyl chloride condensed with a diamine. The free amino group is diazotized and converted to thiocyanate. The products are dyes of general formula (LII). Their wash-fastness is not good, partially because of the acidic nature of the sulfonamide group, which makes the dyes somewhat soluble in alkali.



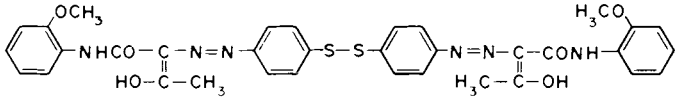
(XLVI) (XLVII) (XLVIII) (XLIX) (L) (LI)

$$Dye-SO_2NH-Ar-SCN$$

(LII)

C. Azo Disulfide Dyes

More recently azo disulfide dyes have been made in which the mercapto groups are not separated by insulating groups from the chromophore. Many of these dyes give bright shades and have technically satisfactory wash-fastness. Bright greenish yellow shades of good wash-fastness are obtained by coupling various diaminodiaryl disulfides with acetoacetanilides.[59] For example, 4,4′-diaminodiphenyl disulfide is tetrazotized in dilute hydrochloric acid at 0°, and coupled with a solution of acetoacet-*o*-anisidide in dilute caustic soda. The product (LIII) is filtered off, washed, and dried. Improved lightfastness is obtained by using 4,4′-diamino-2,2′-dichlorodiphenyl disulfide as the diazo component.[60] Coupling with pyrazolones gives yellow dyes, while derivatives of Naphtol AS give reds. These azo disulfide dyes all have rather low



(LIII)

[59] C. G. Jeremias and F. E. Barwick, *USP* 3,225,025.
[60] C. G. Jeremias and F. E. Barwick, *USP* 3,299,040.

affinity for cellulose and a special technique has been developed for their application.[61] The dyes are prepared in dispersion form and are padded on to the cloth, which is then dried. Reducing agent is then padded on and the cloth is steamed to fix the dye. After washing, the cloth passes through a sodium dichromate/acetic acid bath to oxidize the dye. The dyeing is then washed in water and soaped. Azo disulfide dyes may be applied by this method along with sulfur dyes, either as dispersed pastes, reduced liquids, or their thiosulfate derivatives. Instead of the above two-pad procedure the dye may be padded along with a compound such as thiourea or di(sodiocarboxymethyl)trithiocarbonate[62] which has no effect on the dye in the pad box, but reduces it in the steamer.

VI. Manufacture

Sulfur dye manufacturers are rather secretive about their processes, so there are few publications concerning process improvements.

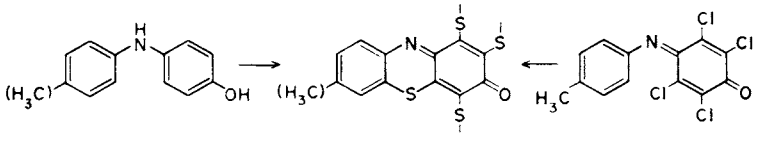
Continuous manufacture of sulfur dyes has been described. Sulfur Black can be made by feeding a stream of 2,4-dinitrophenol, sulfur, sodium hydroxide, and sodium sulfide through a reactor heated to 150°–160°. The dye is formed in 10 to 15 minutes.[63] Continuous polysulfide bakes have also been described.[64] Thus, 2,4-diaminotoluene, *p*-aminophenol, sulfur, and sodium sulfate are ground together and baked in a screw reactor for 10 minutes at 260° to give a yellow-brown dye. Likewise, a mixture of dinitronaphthalene, sodium sulfide, sodium hydroxide, sulfur, and copper sulfate is baked 10 minutes at 300° to give a dark brown dye. Continuous drying and oxidation of sulfur dyes in a ribbon dryer has been described.[65]

A notable improvement in batchwise thionation under reflux is brought about by the addition of hydrotropic agents, such as alkylbenzenesulfonates, to the thionation charge.[66] This results in a more fluid and homogeneous reaction mass throughout the duration of the reaction, a greater proportion of the intermediate being dissolved rather than suspended. Consequently the yield of dye is frequently increased, in some cases by as much as 75%. Furthermore, the reaction mass may be diluted to type strength by suitable additions of water, alkali, and sodium hydrosulfide, giving directly the reduced liquid sulfur dye.

[61] C. R. Holtzclaw and J. H. Benson, *USP* 3,264,053.
[62] D. F. Mason and T. E. Lesslie, *USP* 3,329,477.
[63] A. A. Kuznetsov, M. F. Silin, and M. M. Agal'tsov, *RP* 67,125 (1946).
[64] H. Bach and F. Schmidt, *EGP* 35,591 (1965).
[65] B. S. Sazhin, *Inzh.-Fiz. Zh.* 1, No. 9, 36–44 (1958).
[66] E. D. Robinson and D. F. Mason, *USP* 2,657,112.

Since this procedure incorporates the hydrotrope in the liquid dye the solubility of the dye is thereby improved, in some cases by as much as 100%, enabling stronger liquid dyes to be made. Converting the dye directly from a thionation mass to a reduced liquid has economic advantages. It is no longer necessary to air-oxidize the dye out of solution, filter it off, and dissolve the presscake, and the savings in equipment, time, and labor are significant. The air-oxidation stage is frequently a cause of color loss, due to over-oxidation, and further mechanical losses may occur during filtration. A further advantage of the use of hydrotropes is that the usage of solvents in the thionation may thereby frequently be reduced by 90%. Rather than use a relatively water-immiscible solvent like *n*-butanol, such water-soluble solvents as diethylene glycol monoethyl ether are used. The amount of solvent is then so small that it is not imperative to recover it, and it is incorporated in the liquid dye with good effect on the solubility of the dye. For example, CI Leuco Sulfur Black 11 is made by heating for 36 hours a charge containing 240 kg of *p*-hydroxyphenyl-2-naphthylamine, 150 liters of water, 100 kg of sodium *m*-xylenesulfonate, 120 kg of flake sodium sulfide, and 212 kg of sulfur. The dye is then diluted to standard strength by the addition of water, flake sodium sulfide, and sodium hydrosulfide solution.

Dyes that dye cotton bright red-brown shades, which are essentially identical with those of CI 53830, are now made by reflux thionation of 4-hydroxydiphenylamine and certain of their alkyl and chloro derivatives, rather than the relatively expensive phenothiazinone intermediate (LV).[66,67] Both of these intermediates are believed to give rise to phenothiazinone structures such as (LVI),[68,69] so it is not surprising



(LIV) (LVI) (LV)

that the shade, dyeing, and fastness properties are very similar. Thus 100 kg of 4-hydroxy-4′-methyldiphenylamine is heated under reflux for 36 hours in a polysulfide solution made from 90 kg of sulfur, 107 kg of flake sodium sulfide, 200 kg of ethyl Cellosolve, and 65 liters of water. The dye is isolated by adding sodium sulfide, steam-distilling the solvent,

[67] Gy, *BP* 753,764.

[68] W. N. Jones and E. E. Reid, *J. Amer. Chem. Soc.* **54**, 4397 (1932).

[69] A. J. Buchanan, *USP* 2,130,416.

air-oxidizing, and filtering. It is then air-oxidized again in dilute alkali, neutralized, filtered off, washed, and vacuum-dried. Alternatively the dye may be thionated in the presence of sodium xylenesulfonate and converted directly to a liquid product.

VII. Application

A. General

For many years sulfur dyes had limited utility for a variety of reasons. They were all rather dull in shade, and no red was available. They had no fastness to chlorine and the lightfastness of some dyes, particularly yellows, was poor. Being sold in the form of insoluble powders, they had to be pasted with water and dissolved by heating with sodium sulfide. The resulting solutions were highly alkaline and frequently contained insoluble particles which filtered out on packages and made circulating liquor dyeing impossible. Sulfur-dyed goods were harsh in handle and yarns were difficult to rewind. A particular problem was the tendering of sulfur blacks under certain storage conditions. Much progress has been made to remedy these defects, and sulfur dyes are now finding application in areas where they were at one time considered unsuitable.

B. Forms of Sulfur Dyes

A major technical advance was the marketing of prereduced liquid sulfur dyes, clarified to remove insolubles and ready to dye. This coincided with the development of continuous pad–steam dyeing of piece goods, for which the liquid sulfur dyes are particularly suitable. The economic advantages of bulk storage and liquid handling have essentially displaced powder brands from high-volume production. Furthermore, the alkalinity of the liquid sulfur dyes is lower than that of sulfur dye powders dissolved with sulfide,[70] and combined with their freedom from insoluble particles this renders them suitable for package and beam dyeing. In 1948, there appeared the water-soluble Thionol M brands of ICI, in which the dyes are presented as their thiosulfate derivatives, having water solubility but no affinity for cotton (see Chapter II). Addition of reducing agent converts the dye to its mercaptan form. This range was followed by the Sulphosol (James Robinson & Co.), Hydrosol (CFM), and other brands. The lack of affinity of these dyes in the unreduced state makes them particularly suitable for pene-

[70] L. Tigler, *Amer. Dyest. Rep.* **57**, 37 (1968).
[71] Japan Pigment Co., *JP* **19**, 837 (1961).

tration of difficult materials. Dispersed pastes are also available. These are made from sulfur dye presscakes by milling in the usual way with suitable dispersing agents.[70–72] These may be applied similarly to the water-soluble thiosulfate dyes and are also suitable for mass pigmentation of viscose rayon.[73]

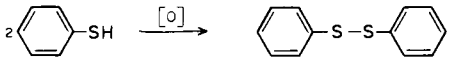
C. Shade and Lightfastness

The sulfur dye range has been extended by the addition of both bright synthetic dyes, including a red, and conventional sulfur dyes of superior lightfastness. Sodyesul Brilliant Orange 2Y Paste and Sodyesul Brilliant Yellow R Paste (Southern Dyestuff Co.) fill a need for bright shades at that end of the spectrum, and Sodyesul Liquid Red 2B[74] finds wide application, particularly in combination with conventional orange, red-brown, and blue sulfur dyes. Recently, new lightfast yellow, yellow-brown, olive, and gray shades have been introduced,[75] which are claimed to be suitable for work clothing, an area previously restricted to vat dyes. Bright green sulfur dyes of high lightfastness, based on copper phthalocyanine, are gaining wide acceptance.

D. Oxidation of Sulfur Dyes

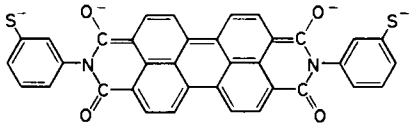
Most, if not all, sulfur dyes in their reduced state carry mercaptan groups. Oxidation of the dye produces disulfide bridges between dye molecules, resulting in high molecular weight, insolubility in water, and consequently good fastness to wet treatments. In addition, certain dyes, notably the indophenol colors, contain a reducible quinone or quinoneimine group, which in the reduced state forms a water-solubilizing sodium salt. The shade of any sulfur dye will depend on the extent to which it is oxidized, since the colors of the oxidized and reduced forms are commonly quite different. Until recently most sulfur-dyed materials were oxidized with either dichromate and acetic acid or else by water washing, the remainder being oxidized with hydrogen peroxide or other peroxy compounds. However, all of these methods have their disadvantages, and considerable work has been done by the dye manufacturers to find improved oxidants and dye fixing agents. Aspland has pointed out[76] that the oxidation of large, polyfunctional sulfur dyes immobilized on the fiber may not resemble very closely the oxidation of simple aromatic thiols to disulfides (LVII). Clearly there must be some

[72] FW, *BP* 1,001,004.
[73] J. Dvorak, E. Marval, and K. Bittner, *CzechP* **93**, 596 (1960).
[74] *J. Soc. Dyers Colour.* **85**, 424 (1969).
[75] *J. Soc. Dyers Colour.* **87**, 283 (1971).
[76] *Text. Chem. Color.* **2**, 229 (1970).

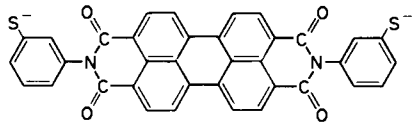


(LVII)

limit to the extent of such oxidation, and consequently there will be appreciable numbers of mercapto groups remaining, some of which may be then oxidized in different ways. Certain sulfur dyes are not even completely oxidizable in solution. Haddock's red dye (LIX)[54] dissolves in sodium sulfide to give a red-violet solution, presumably of the anion of the dihydro form (LVIII). Air oxidation yields a red water-soluble



(LVIII)



(LIX)

product whose color and lack of affinity for cellulose indicate it to be the dithiol (LIX).[77] The indophenol dyes vary considerably in the susceptibility of their quinoneimine groups to oxidation. CI Sulfur Red 5, made from *p*-hydroxydiphenylamine, is especially difficult to oxidize, whereas the related blues and greens which carry electron-donating groups *para* to the quinoneimine nitrogen, are readily oxidized.

Water washing has been widely used for the oxidation of sulfur dyeings. Removal of sulfide from the material leaves the dye accessible to reaction with dissolved oxygen. The method is simple, cheap, and raises no effluent-treatment problem, but its limitations restrict its use to certain dyes and methods of dyeing. CI Sulfur Red 5 cannot be oxidized by this method, and neither can the important bright green phthalocyanine dyes. Other dyes appear to be oxidized, but they change in shade considerably over a period of time. This method is unsuitable for continuous pad–steam work, since gradual accumulation of sulfide in the wash boxes causes the shade to shift during the run.

Sodium dichromate and acetic acid is the most widely used method of oxidation, giving stable shades with the majority of dyes and requir-

[77] D. W. Voigt, unpublished work (1972).

ing little control in use. Indophenol blues, greens, and blacks give their maximum color yield, and the treatment is cheap and easy to apply. However, there are several inherent disadvantages in the method. Many dyeings oxidized in this way have a harsh handle, which may be due to precipitation of chromium compounds in the goods from interaction of sodium sulfide and oxidant. This may be minimized by thorough washing off before oxidation, but certain dyes, notably sulfur black,[76] themselves form chromium complexes. Certain sulfur yellows, which exhibit a considerable divergence between the chrome- and peroxide-treated shades, complex with chromium, whereas those dyes which give the same shade with chrome as with other oxidants do not.[78] Chrome-oxidized sulfur black is quite water-repellent, and this is a disadvantage where resin finishes are to be applied subsequently. However, the major disadvantage of this oxidant concerns pollution of water by dyehouse effluent. Limitations on the permitted concentration of chrome in the effluent have obliged some dyers to look for alternative oxidants.

Hydrogen peroxide is coming into favor, especially since it raises no effluent problem of itself, being decomposed into water and oxygen. Sulfur dyes give clean bright shades with peroxide, and the material is soft and absorbent. Despite these advantages and its low cost, hydrogen peroxide is far from being universally accepted. The dyeings have significantly inferior wash-fastness, particularly if an excessive amount of peroxide is used. The cause of this impaired wash-fastness is unclear. One theory is that the mercapto groups become oxidized further than disulfide, forming sulfinate groups, for instance. This explanation supposes that peroxide is a more powerful oxidant than acid dichromate, but in fact peroxide will not oxidize Sulfur Red 2, whereas dichromate will. The addition of water-soluble salts of barium, calcium, magnesium, and strontium to the peroxide is claimed to be advantageous,[79] and continuous control of the peroxide concentration is necessary.[76]

Sodyelox 69, an oxidant of undisclosed composition, is capable of oxidizing all sulfur dyes and gives shades very similar to those obtained with chrome.[76] The wash-fastness is, however, significantly improved, and the goods are soft and absorbent. This product can be used at any temperature between 30° and 100°, and within the pH range 7 to 11. Like sodium dichromate, an excess of the oxidant will not affect the result, so close control is not required.

Sodium chlorite has been used for sulfur dye oxidation. It gives good absorbency and soft handle, but will not fully oxidize all sulfur colors.

[78] T. C. Crawford and D. G. Orton, unpublished work (1969).

[79] DuP, *BP* 1,020,146.

The oranges made from decacyclene and the red-browns, in particular, are not properly developed, and the blues do not give full color value.

Apart from these conventional oxidants, there has been some use of other fixing agents, including alkylating agents and the salts of heavy metals. Benzyldimethylphenylammonium chloride gives good fixation of the orange decacyclene dyes,[80] while chloroacetamide and the condensation products of amines with epichlorohydrin are also effective.[81] The use of 1,4-dichloro-2-butyne has been described.[82] None of these reagents is effective over the whole range of sulfur dyes, particularly the red-browns. The mercapto groups of sulfur dyes react with diazonium salts, forming sulfides (LX). This reaction forms the basis of a method

$$\text{Dye}-S^- + ArN_2^+ \longrightarrow [\text{Dye}-S-N=N-Ar] \longrightarrow \text{Dye}-S-Ar + N_2$$

(LX)

of fixing Indocarbon Blacks which is claimed to give improved fastness to chlorine.[83]

E. Resin Finishes

The development and widespread use of anticrease and durable press finishes has had marked effects on sulfur dye application. The first effect noticed was on the shade of the dyes. Certain sulfur dyes change shade considerably with resin treatments and are not recommended for such goods. Sodyesul Liquid Olive Yellow YCF is an example. This dye contains free primary amine groups, and the acid catalyst in the resin causes a distinct reddening in the shade, which is reversed by alkaline soaping treatments. Sodyesul Liquid Olive Yellow RFCF was developed to remedy this defect. The wash-fastness of sulfur dyes is materially improved by resin finishes, and lightfastness in some cases is marginally impaired. The chlorine-fastness of resin-treated sulfur dyes is improved remarkably, to the extent that many will withstand repeated severe launderings with the addition of bleach. Furthermore, chlorine treatments are detrimental to the resin finish, so the goods are frequently labeled "use no bleach."

F. Continuous Dyeing

Continuous dyeing of piece goods provides the greatest single use of sulfur dyes. The range comprises a padder, steamer, and open-width wash boxes, followed by a dryer. The cloth is scoured, but not necessarily

[80] W. Hagge and K. Haagen, *USP* 2,076,143.
[81] G. Schreiner, *Deut. Textiltech.* **19**, 91–96 (1969).
[82] H. Rath, *DBP* 1,265,701.
[83] G. F. Womble, *USP* 3,043,645.

bleached, and dried. Reduced dye is padded on and fixed by 30 to 60 seconds' steaming. The sulfide is then washed out, and the dye is oxidized in one or two boxes, washed, and dried. Padding requires careful control to give uniform results. Aside from such mechanical factors as even roller pressure, the affinity of reduced sulfur dyes raises problems. Dye exhausts out of the liquor even during the few seconds' immersion time, so that the expressed liquor returned to the trough is depleted. Consequently, the dye concentration in the feed is necessarily higher than in the original charge. Furthermore, individual dyes vary as to their degree of exhaustion, and all dyes exhaust more in pale shades than in heavy shades. As a result, the dyer requires considerable skill in maintaining uniformity throughout the run, particularly when combinations of dyes having different affinities are used. The degree of exhaustion will vary with the immersion time, which is kept constant by controlling the cloth speed and the level of dye liquor in the padder. Temperature is, of course, an important factor, which must be carefully controlled. To minimize exhaustion most dyes are padded at 40°. The cloth is steamed at 105° for 30 to 60 seconds. This operation can be omitted in the case of pale shades. Thorough washing is essential, and up to five wash boxes are used, increasing in temperature from 40° to 60°. Inadequate washing allows sulfide to be carried into the oxidation box, resulting in deposition of chromium salts on the fiber, and liberation of hydrogen sulfide gas. This makes the cloth harsh and water-repellent.

This dyeing procedure is the most widely used, but on certain cloth constructions may give an unsatisfactory surface appearance. In such cases the preferred procedure is to pad on the reduced dye, dry, and then pad through reducing agent before steaming. Alternatively, the cloth is padded with solubilized sulfur dyes or sulfur dye pastes which, having no affinity, penetrate the cloth uniformly. The cloth is then dried, padded through reducing agent, and steamed. This method gives exceedingly smooth and well-penetrated dyeings, but at somewhat higher cost.

Polyester/cotton blends are dyed continuously with combinations of sulfur and disperse dyes. The most common procedure is to pad on the disperse dye, dry, thermofix, pad on reduced sulfur dye, steam, wash, oxidize, wash, soap, and rinse. An alternative method is to pad simultaneously the disperse dyes and a sulfur dye paste or solubilized sulfur dye, dry, thermofix, pad with reducing agent, steam, and finish in the same way. Blends of nylon and cotton may be dyed with sulfur dyes alone, but the sulfur dyes on nylon are more difficult to oxidize, so the chrome bath is heated to 85°.

Cotton warp yarns are dyed continuously in rope form by the pad–

steam method, particularly in navy shades for the denim trade. The range speed is 20 to 30 yards per minute, in contrast to the 90 to 120 yards per minute used with piece goods. Cotton rawstock is dyed continuously, but no details of the machine are available.

G. Batch Dyeing

Package and beam dyeing have increased in recent years, as dyes and application procedures have improved. The development of clarified, reduced liquid dyes was a prerequisite for successful machine dyeing. Sulfur dyes are precipitated by calcium and magnesium, so a soft water supply is essential. The cotton itself is an increasing source of metallic contaminants, due to the use of ground water for irrigation and metal-containing defoliants in the cotton fields. A prescour with detergent and acetic acid removes these metals, and calcium-free salt is used in the dyeing. Tobin[84,85] has described detailed dyeing procedures for cotton and polyester/cotton blends.

Sulfur dyes, unlike vat dyes, can be applied successfully on the winch (beck) due to the greater stability to oxidation of sulfide compared to hydrosulfite. This method of dyeing is increasing, and all kinds of materials, from lightweight tubular knit goods to automobile carpeting, are dyed successfully. Jig dyeing is still in use for short lengths, and there is some batch dyeing of cotton rawstock.

H. Nontextile Applications

There has been considerable interest in recent years in the catalytic effect of sulfur dyes in oxidation reactions. Removal of mercaptans from petroleum fractions by sulfur dye-catalyzed atmospheric oxidation has been described,[86–89] and suitable equipment has been developed.[90] The indophenol greens and blues are particularly suited to this application. Sulfur Black is described as a catalyst for the atmospheric oxidation of hydrogen sulfide to sulfur in a weakly alkaline solution.[91] Various sulfur dyes catalyze the oxidation of sulfide to thiosulfate.[92,93]

[84] H. M. Tobin, *Amer. Dyest. Rep.* **55**, 451 (1966).
[85] H. M. Tobin, *Amer. Dyest. Rep.* **57**, 37 (1968).
[86] Compagnie Française de Raffinage, *BP* 788,559.
[87] Compagnie Française de Raffinage, *FP* 1,105,484.
[88] State Scientific Research Institute of the Petroleum Industry, *RP* 197,067 (1967).
[89] Japan Oil Co., *BP* 1,122,889.
[90] Compagnie Française de Raffinage, *FP* 1,230,502.
[91] Universal Oil Products, *USP* 3,104,951.
[92] F. Wolf, L. Eckert, and R. Heby, *Fortschr. Wasserchem. Ihrer Grenzgeb.* **10**, 81–92 (1968).
[93] Y. Maeda, Y. Nishiumi, and K. Ichikawa, *Kogyo Yosui* **106**, 60–67 (1967).

Among other applications, solubilized Sulfur Black is extensively used for dyeing paper, and Sulfur Black pigment has been described as a lubricant for wire-drawing.[94]

I. Sulfur Black Tendering

When Sulfur Black-dyed material is stored under conditions of high temperature and humidity, it loses tensile strength and may become unusable. This is no longer a serious practical problem since the cause of tendering is fairly well understood and effective measures can be taken to prevent it. Adverse storage conditions cause oxidation of the dye itself, not, as was once thought, of elemental sulfur, and the sulfuric acid formed catalyzes the hydrolytic degradation of the fiber. Dry storage conditions will not cause tendering, even if the dyed material is weakly acid to begin with.[95] Under adverse storage conditions tendering is minimized by oxidation of the dye with chrome, followed by an alkaline rinse immediately before drying.[70] Aromatic amines have long been known to afford protection against tendering.[96] More recently dicyandiamide, melamine,[97] and triethanolamine[98] have been proved effective. More important, all anticrease and durable press resins are effective inhibitors of Sulfur Black tendering. It is suggested that their mode of action is to reduce the water regain of the material,[70] but it is possible that the resins react with the labile sulfur in the dye and thereby prevent its oxidation on storage.

VIII. Analysis of Sulfur Dyes

Sulfur dyes are difficult to strip from dyed materials, and methods of determining the dye on the fiber based on stripping lead to considerable errors.[99] Accordingly, methods have been devised based on dissolving the fiber by acid hydrolysis and colorimetric determination of the residual dye.[100,101] Methods have been developed for the colorimetric

[94] Trefileries Leon Bekaert, *FP* 1,281,889.

[95] G. Nitschke, *Melliand Textilber.* **36**, 369–372 (1955).

[96] J. L. Crist, *USP* 2,193,328.

[97] Z. I. Sergeeva and I. L. Khmel'nitskaya, *Tekst. Prom.* (*Moscow*) **21**, No. 4, 42–47 (1961).

[98] S. S. Rakhlina and L. P. Kozlova, *Nauch.-Issled. Tr., Tsentr. Nauch.-Issled. Inst. Khlopchatobumazhn. Prom.* **2**, 33–42, 1961.

[99] E. Frieser, *Spinner, Weber, Textilveredl.* **78**, 620–621 (1960).

[100] N. A. Shubina, *Tekst. Prom.* (*Moscow*) **10**, No. 10, 41–42 (1950).

[101] C. Calin, V. Sandulescu, and E. Ionescu, *Text. Prax.* (*Moscow*) **21**, 822–826 (1966).

determination of sulfur dyes in substance.[102–104] These methods are liable to error because sulfur dyes frequently contain colored material which has no affinity for the fiber. The proportion of such nonsubstantive material in a dye may vary according to the conditions under which it was thionated.[105] Chromatographic separation of sulfur dyes is facilitated by the addition of alkylphenol/ethylene oxide condensates.[106] Free sulfur in sulfur dyes may be determined polarographically.[107]

[102] R. S. Chlenova, Ts. M. Gel'fer, and L. V. Basova, *Zh. Prikl. Khim.* (*Leningrad*) **33**, 475–478 (1960).

[103] C. Calin and V. Sandulescu, *Rev. Chim.* (*Bucharest*) **13**, 690–692 (1962).

[104] G. N. Kuritsyna, M. Ya. Daikhin, and L. P. Kladnitskaya, *Khim. Volokna* **5**, 71–72 (1966).

[105] D. W. Voigt, unpublished work (1972).

[106] N. Kolev and R. Kurteva, *Khim. Ind.* (*Sofia*) **34**, No. 3, 89–91 (1962).

[107] I. Takeshi, *Bunseki Kagaku* **11**, 1252–1254 (1962).

CHAPTER II

BUNTE SALT DYES

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I. Introduction

The dyes to be described can best be generically defined as "dyes substituted by the alkali metal or ammonium salts of *S*-alkyl or *S*-aryl thiosulfuric acids." Apart from conciseness, the title "Bunte Salt Dyes" is apt for several reasons; it recognizes at once the first discovery of organic thiosulfates by Hans Bunte at the Polytechnic Institute of Munich in 1874.[1] Klayman and Shine[2] note that "Bunte published only one, but truly excellent paper on organic thiosulfates and these compounds have traditionally been associated with his name." Although the work concerned alkyl thiosulfates, many authors now include aryl thiosulfates in the "Bunte salt" family.

Various chemical terminologies have been used in the literature for the compounds R—S—SO_3Na, e.g., thiolsulfates, thiosulfonates, sulfenyl sulfites, sulfenyl sulfonates, —*S*-sulfonic acids, thiosulfo acids; the terms *S*-alkyl thiosulfates and *S*-aryl thiosulfates are now most generally

[1] H. Bunte, *Ber. Deut. Chem. Ges.* **7**, 646 (1874).

[2] D. L. Klayman and R. J. Shine, *Quart. Rep. Sulfur Chem.* **3**, 191 (1968).

accepted and these will be used here in conjunction with the general terminology Bunte salts.

Dyestuffs substituted by thiosulfuric acid groups are used selectively in the dyeing and printing of cellulose fibers, wool, silk, polyamides, polyurethane, and in the coloring of leather and hair. In such applications, the thiosulfuric acid group may be functional chemically (a) in providing a route to thiols, disulfides, or polysulfides, and (b) as a fiber-reactive grouping for NH_2-containing fibers, or it may function as a relatively stable anionic grouping capable of ionic bonding to cationic substrates. Methods have been disclosed also for the use of such dyes in the coloring of secondary cellulose acetate, cellulose triacetate, and polyester fibers.

A convenient chemical classification differentiates *S*-alkyl thiosulfate and *S*-aryl thiosulfate dyes as dyes in which the divalent sulfur atom is bonded to alkyl carbon and aryl carbon, respectively.

II. Chemistry of Bunte Salts: $RSSO_3Na$, $ArSSO_3Na$

The chemistry of *S*-alkyl and *S*-aryl thiosulfate salts, dating from the first synthesis of sodium ethyl thiosulfate by Bunte[1] in 1874, has been well reviewed in recent years by Klayman and Shine,[2] Distler,[3] and Milligan and Swan.[4] Reactivity at the divalent sulfur atom has been widely studied both preparatively and mechanistically, and the concept of Bunte salts as sulfenyl sulfites derived from RS^+ has been proposed in recent years as an aid to an understanding of their susceptibility to nucleophilic attack.[5] The reactivity at divalent sulfur in relation to the scission of sulfur–sulfur bonds has been reviewed extensively by Parker and Kharasch.[6] A significant body of literature also relates to Bunte salts in protein chemistry. Bunte salt dyes have not previously been reviewed in breadth, and this chapter attempts to draw the literature and technology together into a cohesive whole. A survey of only those reactions most relevant to the synthesis and technology of Bunte salt dyes follows.

A. Preparative Reactions

1. S-*Alkyl Thiosulfates*

a. Reaction of Alkyl Halides (Usually Chlorides) with $Na_2S_2O_3$. This method[1] has been widely used and is standard for most known alkyl

[3] H. Distler, *Angew. Chem., Int. Ed. Engl.* **6**, 544 (1967).

[4] B. Milligan and J. M. Swan, *Rev. Pure Appl. Chem.* **12**, 72 (1962).

[5] N. Kharasch, Z. S. Ariyan, and A. J. Havlik, *Quart. Rep. Sulfur Chem.* **1**, 97 (1966).

[6] A. J. Parker and N. Kharasch, *Chem. Rev.* **59**, 583 (1959).

thiosulfates. According to Milligan and Swan,[4] substituents in the alkyl group do not usually interfere and aminoalkyl thiosulfates, such as the

$$RCl + Na_2S_2O_3 \longrightarrow RSSO_3Na + NaCl$$

following, have been prepared from the appropriate halide by Bretschneider,[7] Schlack,[8] and others.

$NH_2CH_2CH_2SSO_3H$ $\qquad$ $NH(CH_2CH_2SSO_3H)_2$

$CH_3NHCH_2CH_2SSO_3H$ $\qquad$ $N(CH_2CH_2SSO_3H)_3$

Lecher and Hardy[9] have proposed the use of thallous thiosulfate; since TlCl is only sparingly soluble in water it is separated without difficulty from the product.

In addition to the primary and secondary aminoalkyl halides, alkyl chlorides containing tertiary *N*-substituted benzyl halides, *N*-chloroacetyl derivatives, chlorotriazines, and chloroacetic esters may be converted readily into Bunte salts by this method. The reactions are usually conducted in water or aqueous alcohol under reflux for 1–2 hours.

b. Reaction of RSH with N*-Pyridinium Sulfonate.* The Baumgarten[10] synthesis of *S*-aryl thiosulfates has been applied successfully to the synthesis of *S*-aralkyl thiosulfates using substituted benzyl mercaptan. The Baumgarten method employs *N*-pyridinium sulfonate (from pyridine and SO_3) and yields the pyridine salt of the thiosulfuric acid

$$ArSH + C_5H_5N + SO_3 \longrightarrow ArSSO_3^- C_5H_5\overset{+}{N}H$$

esters. The yields are reported to be quantitative.

Chlorosulfonic acid may also be used in dye synthesis with an inert diluent.

c. Addition of Thiosulfate Ion to Activated Double Bonds. Kerber and Starnick[11] and Distler[3] have discussed new reactions in which thiosulfate ion adds nucleophilically to polarized double bonds in activated molecules such as acrylamide, vinylsulfonic acid, and divinyl sulfonate, to give β-thiosulfato-alkyl derivatives, e.g.,

$$RSO_2CH{=}CH_2 + Na_2S_2O_3 \underset{OH^-}{\overset{H^+}{\rightleftharpoons}} RSO_2CH_2CH_2SSO_3Na$$

The reaction is reversible according to pH so that the Bunte salt is formed under conditions of low alkalinity, but reverts to the unsaturated

[7] H. Bretschneider, *Monatsh. Chem.* **81**, 372 (1950).

[8] P. Schlack, *DBP* 869,066; 869,067.

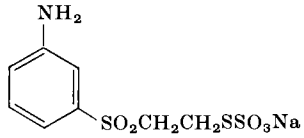
[9] H. Z. Lecher and E. M. Hardy, *J. Org. Chem.* **20**, 475 (1955).

[10] P. Baumgarten, *Ber. Deut. Chem. Ges.* B **63**, 1330 (1930).

[11] R. Kerber and J. Starnik, *Tetrahedron Lett.* p. 3007 (1966).

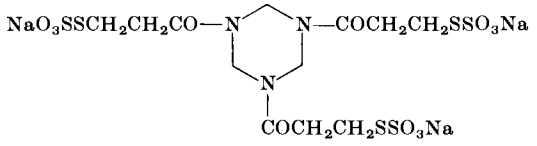
compound in alkali. The Bunte salts are prepared at pH 5–6 by gradual addition of acid.

Of particular interest are the derivatives of 1-amino-3-vinylsulfonylbenzene, which give dye intermediates of the type



These compounds were described by Meininger and Hoyer.[12]

Similar methods have been used to prepare[13] polyfunctional Bunte salts of the type



from tris(acryloyl)hexahydro-*s*-triazine.

2. S-*Aryl Thiosulfates*

a. The Scission of Disulfides by Sulfurous Acid, $NaHSO_3$, and Na_2SO_3. The most important route to *S*-aryl thiosulfates is based on the reaction

$$ArSSAr + SO_3^{2-} \rightleftharpoons ArS^- + ArSSO_3^-$$

Both the forward and reverse reactions are outstandingly important, the forward reaction as a synthetic route and the reverse reaction as a basis for the insolubilization of Bunte salt dyes in fibers. According to Milligan and Swan,[4] the position of the equilibrium is determined by many factors: the net charge carried by the disulfide molecule, the possible steric effects in the disulfide, the anionic stability of the thiolate ion, the pH, temperature, and electrolyte concentration.

The preparative forward reaction is promoted by simultaneous oxidation of ArS^- to ArSSAr, e.g., by air. The process is often referred to as "sulfitolysis."

The ionic scission of disulfide bonds by nucleophiles or bases including SO_3^{2-}, RS^-, ArS^-, etc., has been studied by Parker and Kharasch[14] for a series of nitro-substituted aromatic disulfides. It was concluded

[12] F. Meininger and E. Hoyer, *DBP* 1,204,666.

[13] R. Wegler and A. Ballauf, *Chem. Ber.* **81**, 527 (1948).

[14] A. J. Parker and N. Kharasch, *J. Amer. Chem. Soc.* **82**, 3071 (1960).

that cleavage is controlled thermodynamically by the relative S nucleophilicities of the entering group (e.g., SO_3^{2-}) and the leaving group (e.g., ArS^-) with the bond-breaking step rather than the bond-making step being the critical one. This approach has been discussed by Weston and Griffith[15] in relation to the technical behavior of Bunte salt dyes.

The sulfitolysis of aromatic disulfides using $NaHSO_3$ has been discussed by Lecher and Hardy.[9] Their studies were based primarily on 3-nitrophenyl disulfides and the conclusion was reached that, under certain conditions, $NaHSO_3$ or $KHSO_3$ converts the disulfide to the thiosulfate in excellent yield, even in the absence of air. Two independent reactions occurring simultaneously are proposed.

$$RSSR + KHSO_3 \longrightarrow RSH + RSSO_3K$$

$$4RSH + 2KHSO_3 \longrightarrow 2RSSR + K_2S_2O_3 + 3H_2O$$

Thus, thiol is oxidized by bisulfite to disulfide. The resultant reaction is formulated as

$$2RSSR + 6KHSO_3 \longrightarrow 4RSSO_3K + K_2S_2O_3 + 3H_2O$$

The success of this reaction scheme in relation to yield of Bunte salt is determined by the oxidation potential of RSH, which will in turn be determined by the electronegativity, and steric and charge characteristics of substituents in the aryl group. Good yields of Bunte salt were achieved with bis(3-nitrophenyl)-, bis(2-aminophenyl)-, and bis(2-benzamidophenyl) disulfides.

The sulfitolysis of disulfides has found wide application in the field of sulfur dyestuffs and in the preparation of aminoaryl thiosulfates as azo dye intermediates.

b. From Sulfenyl Halides and Sulfenamides. The synthesis of Bunte salts from sulfenyl halides and sulfenamides with sulfite and sulfurous acid does not appear to be widely used in dyestuff technology.

c. The Direct Substitution of $S_2O_3^{2-}$. The direct substitution of $S_2O_3^{2-}$ in an aromatic nucleus has been described in the case of 1,4-quinones and, under oxidation conditions, with *N*-substituted 1,4-phenylenediamines; the reactions have some significance as intermediate steps in dye synthesis, but have not been employed as a source of Bunte salt dyes.

B. Properties and Reactions Significant in the Technology

For technical purposes the water-soluble Bunte salt dyes are considered to have good aqueous stability under neutral conditions, although decomposition will usually result from prolonged refluxing.

[15] C. D. Weston and W. S. Griffith, *Text. Chem. Color.* **1**, 462 (1969).

1. *Acid Hydrolysis*

Bunte salts hydrolyze readily in hot mineral acid to yield the mercaptan or thiol

$$RSSO_3^- + H_2O \xrightarrow{H^+} RSH + HSO_4^-$$

The reactions are a useful technical source of dye thiols and proceed cleanly and quantitatively. The mechanism and kinetics have been studied by Kice *et al.*[16]

2. *Alkaline Hydrolysis*

Under alkaline conditions, the hydrolysis is complex, yielding mixtures of disulfides and sulfonic acids. In the special case of such polarized molecules as $RSO_2CH_2CH_2SSO_3Na$ alkaline reactions yield the unsaturated vinyl sulfones as previously discussed.

The decomposition of aryl thiosulfate under basic conditions is by no means understood completely, though many authors appear to favor the view that the complexities arise from the extreme instability of sulfenic acids R—SOH, which rearrange spontaneously. From a technical viewpoint, the use of highly caustic systems will rarely be compatible with efficient insolubilization processes for Bunte salts.

3. *Reaction with Inorganic Sulfides and Thiols*

The treatment of Bunte salts with inorganic sulfides leads to rapid displacement reactions, the end products of which may be thiols, aryl hydrodisulfides, di, tri-, and tetrasulfides, and equilibrium mixtures of these; *S*-alkyl thiosulfates invariably lead to di-[17] or polysulfides of the type RS_xR. *S*-Aryl thiosulfates yield soluble or insoluble end products, and the course of these reactions is determined by the reduction potential of the system and the pH in a complex way.[15] The preparation of trisulfides and tetrasulfides from Bunte salts has been studied by Milligan, Saville, and Swan.[18]

The reactions are displacement reactions of the type

$$S^{2-} + RSSO_3^- \rightleftharpoons RSS^- + SO_3^{2-}$$
$$RSS^- + RSSO_3^- \rightleftharpoons RSSSR + SO_3^{2-}$$

usually favored by the presence of formaldehyde, which removes sulfite

[16] J. L. Kice, J. M. Anderson, and N. E. Pawlowski, *J. Amer. Chem. Soc.* **88**, 5245 (1966).

[17] K. Schimmelschmidt, H. Hoffmann, and E. Baier, *Angew. Chem., Int. Ed. Engl.* **2**, 30 (1963).

[18] B. Milligan, B. Saville, and J. M. Swan, *J. Chem. Soc., London* p. 3608 (1963).

ion from the equilibrium; strontium salts are reported to behave similarly.

Thiolate ions are usually strongly reactive with Bunte salts, giving unsymmetrical disulfides which may rearrange to the symmetrical disulfides.[6] This reaction, which is very common in Bunte salt dye technology, was first studied by Footner and Smiles.[19]

4. *Reaction with Oxidizing Agents*

The oxidation of Bunte salts may lead to disulfides or sulfonic acids. Insolubilization to disulfide is best produced through the use of iodine, although it is reported that H_2O_2 may also be used. Neither process has achieved importance in the technology of Bunte salt dyes, but the writer has obtained highly efficient fixation of *S*-aryl thiosulfate dyes on cotton fabrics by treatment of the dyed fabric with iodine in brine at ambient temperatures.

In the technical fixation of *S*-alkyl thiosulfate dyes on cotton, Courtaulds North America, Inc.,[20] has used $NaClO_2$ at low pH. They propose the reaction sequence as

$$2RSSO_3^- + ClO_2^- \longrightarrow RSSR + 2SO_4^{2-} + \tfrac{1}{2}Cl_2$$

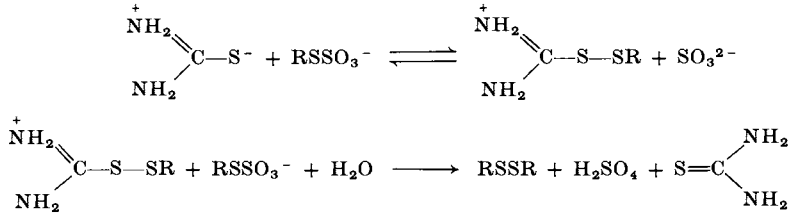
Iodine oxidations proceed as follows:

$$2RSSO_3^- + I_2 + 2H_2O \longrightarrow RSSR + 2HSO_4^- + 2HI$$

The iodine method was first reported by Price and Twiss.[21] Both alkyl and aryl thiosulfates are stable to nitrous acid under the conditions that prevail in diazotization processes provided $-SSO_3Na$ is not *ortho* to $-NH_2$.

5. *Reaction with Thiourea*

Thiourea is cited frequently as a reagent capable of insolubilizing Bunte salt dyes. It has been proposed that one mechanism may be as follows:



[19] H. B. Footner and S. Smiles, *J. Chem. Soc., London* p. 2887 (1925).

[20] Courtaulds North America, Inc., *BP* 1,081,019.

[21] T. S. Price and D. F. Twiss, *J. Chem. Soc., London* **95**, 1489 (1909).

Thiourea at temperatures in the neighborhood of its decomposition point is exceptionally reactive with Bunte salt dyes.

6. *Reaction with Amines*

Although there is very little in the Bunte salt dye technology relating specifically to reactions of this type, processes involving the use of Bunte salt dyes with polyamines and such groups as terminate the chains of polyamide fibers have been described. Bunte salts derived from chloroacetyl derivatives, e.g., $RNHCOCH_2SSO_3Na$, are reported[22] to give thiooxamides such as RNHCOCSNHR′ with amines. Yamase *et al.*[23] have reported that such groups are formed when polyamide fibers are dyed with Bunte salt dyes derived through chloroacetylation.

III. Dye Synthesis

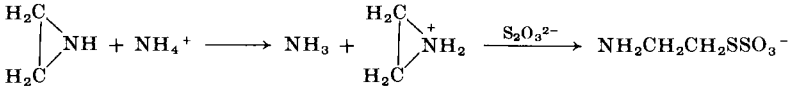
A. *S*-Alkyl Thiosulfates

The reactions employed in the synthesis of Bunte salt dyes and their intermediates of the *S*-alkyl thiosulfate type are summarized below; the letter D denotes a preformed dye.

1. *β-Aminoethylthiosulfates*

β-Aminoethylthiosulfuric acid is a key intermediate in the synthesis of many *S*-alkyl thiosulfate dyes. The product may be prepared[8] by the reaction of β-chloroethylamine with sodium thiosulfate or thallous thiosulfate; a method based on the ring opening of ethyleneimine by ammonium thiosulfate has also been described[24]:

$$ClCH_2CH_2NH_2 \cdot HCl + Na_2S_2O_3 \longrightarrow NH_2CH_2CH_2SSO_3Na$$



β-aminoethylthiosulfuric acid and the analogous products, β-(methylamino)ethylthiosulfuric acid, 2,2′-iminodi(ethylthiosulfuric acid), 3-aminopropylthiosulfuric acid, and 2-aminohexylthiosulfuric acid have been used extensively. Important turquoise blue dyestuffs are prepared by the chlorosulfonation of metallized phthalocyanines by condensation of the sulfonylchlorides with the above compounds.

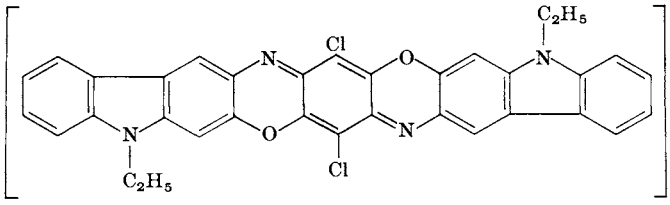
[22] B. Milligan and J. M. Swan, *J. Chem. Soc., London* p. 2969 (1959).

[23] I. Yamase, Y. Abe, N. Kuroki, and K. Konishi, *Kogyo Kagaku Konishi* **67**, 1411 (1964).

[24] D. L. Klayman, W. F. Gilmore, and T. R. Sweeney, *Chem. Ind. (London)* p. 1632 (1965).

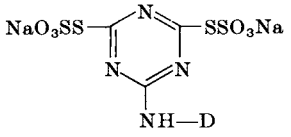
Schultheis *et al.* and Farbwerke Hoechst[25] describe the following preparation: 5.7 parts of copper phthalocyanine trisulfochloride is stirred 4 hours at 40°–45° with 7.1 parts of methylaminoethylthiosulfuric acid, 5.5 parts by volume of NaOH (33% w/w), and 100 parts of water. The blue dye is salted out. The product obtained is substantially $D \equiv (SO_2NMeCH_2CH_2SSO_3Na)_3$.

Other chromophores that may be used in like manner are dioxazine, and 1,4-diarylaminoanthraquinone,

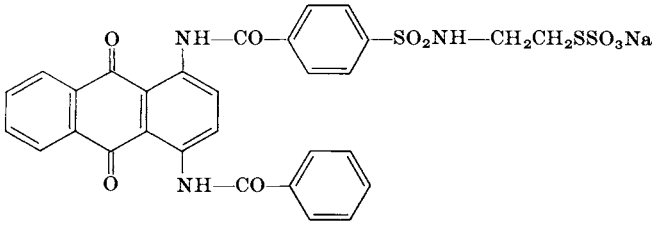


$\equiv (SO_2NHCH_2CH_2SSO_3Na)_4$

Interestingly, as well as the D—SO_2Cl and D—COCl derivatives, dyes partially condensed with cyanuric chloride may be further condensed with aminoalkane thiosulfates; Yamase *et al.*[26] have reacted dichlorotriazinyl dyes directly with sodium thiosulfate to give dyes fixable to fibers in the presence of acid-binding agents.



The introduction of Bunte salt groups into vat dyes through the use of aminoalkane thiosulfates has been achieved by CIBA[27]; again, the sulfochlorides of vat dyes are employed and a typical example is the following red dye:



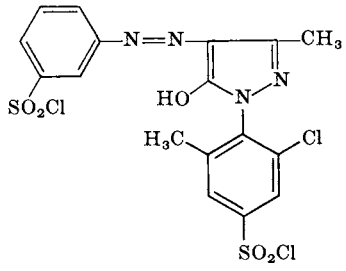
[25] W. Schultheis, K. Schimmelschmidt, H. Hoffman, and E. Baier, and FH, *USP* 3,236,860.

[26] I. Yamase, Y. Abe, M. Shimojoh, N. Kuroki, and K. Konishi, *Kogyo Kagaku Zasshi* **69**, 1310 (1966).

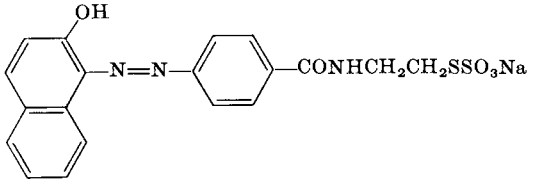
[27] M. Staeuble, K. Weber, and I. Hari, CIBA, *USP* 3,249,394.

Unlike the parent vat dye, the Bunte salts are water-soluble and are therefore much more readily reduced to the leuco form during dyeing processes. CIBA[28] also produced a vat dye from 2-chloro-4,6-bis[5-(sulfophenoxy)-1-anthraquinonylamino]-*s*-triazine condensed with 2-aminoethylthiosulfuric acid.

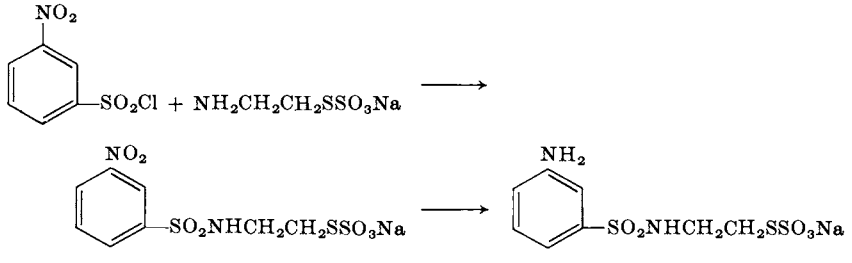
Dyes[29] for wool and polyamide fibers are obtained by the condensation of $CH_3NHCH_2CH_2SSO_3H$ with, for example,



In a study of the reactions of Bunte salt dyes with wool, Milligan and Swan[30] synthesized the following orange dye through the carbonyl chloride intermediate.



The condensation of nitroaryl sulfochlorides with aminoalkane thiosulfates and subsequent reduction to aminoaryl sulfonylamino β-ethylthiosulfuric acids has provided a source of diazotizable alkyl thiosulfates.



[28] CIBA, *BeP* 621,543.

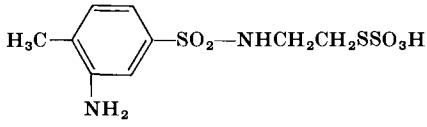
[29] W. Schultheis, K. Schimmelschmidt, H. Hoffmann, and E. Baier, and FH, *DBP* 1,275,232.

[30] B. Milligan and J. M. Swan, *Text. Res. J.* **31**, 18 (1961).

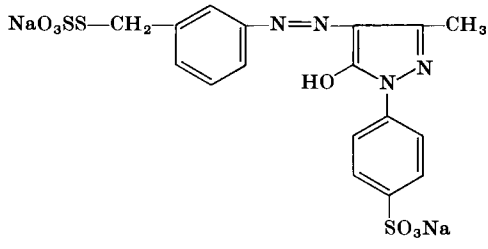
This broad approach to azo Bunte salt dyes forms the basis of the largest group of alkyl thiosulfates through the inventions of Farbwerke Hoechst (FH).[31,32] They successfully reduce the nitroarylsulfonamido-β-ethylthiosulfuric acids to the amines without degrading the thiosulfuric acid group through the use of iron and ferrous ions in a neutral or almost neutral aqueous medium (pH 5–7), e.g., excess iron powder in aqueous suspension in the presence of ferrous sulfate (0.02–0.2 mole per mole of nitro compound). The stability of the thiosulfuric acid group is surprising in view of the known sensitivity of Bunte salts to both reduction and hydrolysis reactions.

The nitroaryl compound may be substituted by alkyl, alkoxy, and halogen groups. In addition to the nitroarylsulfonamido-β-ethylthiosulfuric acid series, the method works well for the nitrobenzyl thiosulfates, *S*-nitroaryl thiosulfates, nitrophenoxyalkyl thiosulfates, and nitrobenzamidoalkyl thiosulfates.

Further examples of useful azo dye intermediates are as follows:



These intermediates provide the basis for a long series of water-soluble azo dyes containing one or more thiosulfuric acid groups.[33] The available intermediates are further extended by the use of *S*-alkyl thiosulfates of the type 3-(4′-aminobenzamido)benzenesulfonamido-β-ethylthiosulfuric acid and 3-(3′-aminobenzenesulfonamido)benzylthiosulfuric acid. Examples from the patent include

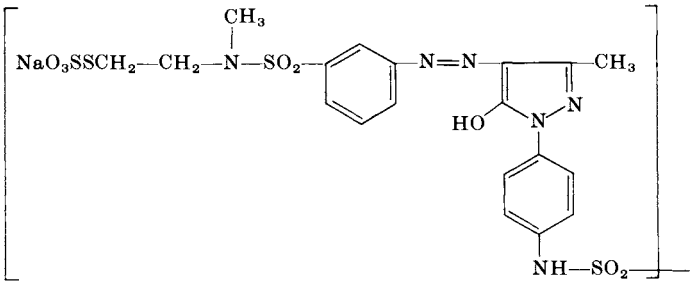


[31] K. Schimmelschmidt, H. Hoffmann, and FH, *USP* 3,151,144.

[32] H. Hoffmann and FH, *DBP* 1,173,485.

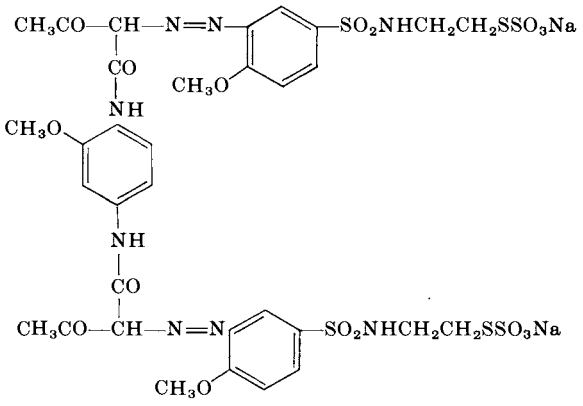
[33] W. Schultheis, K. Schimmelschmidt, H. Hoffmann, and E. Baier, and FH, *CP* 677,934.

and nickel phthalocyanine tetrasubstituted by



Dyes in the color range yellow, orange, red, brown, blue, and green are exemplified and are useful for the dyeing and printing of wool, silk, leather, polyamides and polyurethanes under acid conditions.

Yellow disazo dyes having similar dyeing properties have been prepared[34] in like manner, e.g.,



2. *β-Thiosulfatoethyl Sulfones*

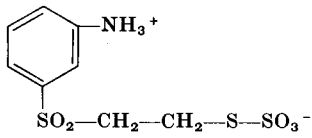
β-Thiosulfatoethyl sulfones as functional groups in dyes have been reported. Diazotizable amines are prepared by the addition of thiosulfate ion to aminoaryl vinyl sulfones, e.g.,

$$ArSO_2CH{=}CH_2 + S_2O_3^{2-} + H^+ \longrightarrow R{-}SO_2{-}CH_2CH_2SSO_3^-$$

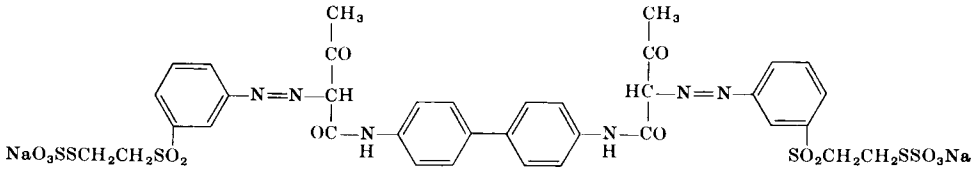
Thus, in aqueous solution sodium thiosulfate is added to *m*-aminophenyl vinyl sulfone at 50° and the pH maintained at 5.2–5.7 by the addition of

[34] W. Schultheis, K. Schimmelschmidt, H. Hoffmann, and E. Baier, and FH, *USP* 3,098,064.

acetic acid over 1 hour. The zwitterion separates from strongly acid solution.[12,35]



The product is neutralized, diazotized, and coupled to give the following yellow dye suitable for wool[36]:



The diazotizable bases may be substituted by hydroxy, halogen, lower alkyl, and lower alkoxy groups.

Blue anthraquinonoid dyes suitable for the dyeing and printing of cellulosic, wool, silk, polyamide, and polyurethane fibers are prepared[37] by the addition of thiosulfate ion to 1-amino-4-(3′-vinylsulfonylanilino)-anthraquinone-2-sulfonic acid. In like manner, azo dyes bearing vinyl sulfone groups are converted to *S*-alkyl thiosulfates. In a variation of the process the vinyl sulfone may be prepared *in situ* using a β-sulfatoethyl sulfone as starting material; the sulfate ester is heated in aqueous sodium carbonate, pH 9–9.5, for 50 minutes, neutralized with mineral acid, and reacted with sodium thiosulfate in the presence of sodium acetate over 4 hours at 70°–75° while maintaining a pH of 5.7–6.2 with mineral acid.

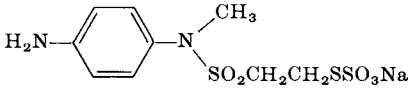
Caldwell and Milligan[38] prepared a series of β-thiosulfatoethyl sulfone dyes (designated Buntazol dyes) by treating β-sulfatoethyl sulfone dyes (Remazol Dyes—Farbwerke Hoechst) with sodium thiosulfate in similar fashion.

The β-thiosulfatoethyl sulfones share with the well-known β-sulfatoethyl sulfones the characteristic of reversibility to vinyl sulfones in the presence of base and such dyes may thus be covalently bonded to cellulosic and protein fibers. The reaction is discussed by Distler.[3]

Further *S*-alkyl thiosulfate dyes suitable for the dyeing of cotton in

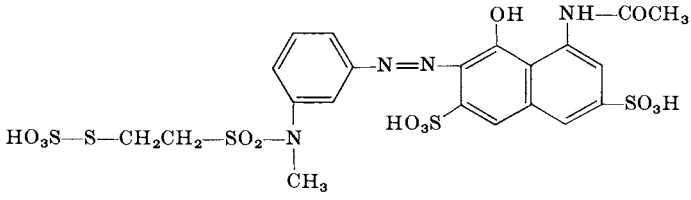
[35] F. Meininger, E. Hoyer, and FH, *USP* 3,301,884.
[36] FH, *BeP* 666,192.
[37] F. Meininger, E. Hoyer, and FH, *USP* 3,359,286.
[38] J. B. Caldwell and B. Milligan, *Text. Res. J.* **33**, 481 (1963).

the presence of acid-binding agents are derived[39] from diazo components of the general structure



obtained by thiosulfate ion addition to *p*-amino-*N*-methyl-*N*-vinylsulfonylaniline.

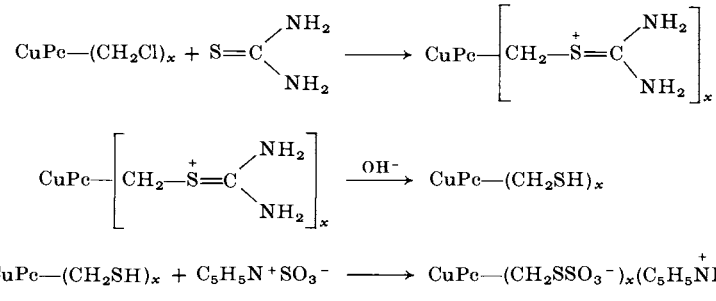
Such dyes are notable for purity and intensity of shade on cellulosic fibers, and when dyed with a base, for good stability to light and washing processes. Alternatively they may be applied substantively to wool and polyamides from weakly acid dyebaths. The invention is typified by the red dye:



Applied to cotton in the presence of Na_2CO_3, red shades are obtained fast to both light and washing.

3. *Dyes Bearing ω-Halo Alkyl Groups*

a. Polyhalomethyl Metal Phthalocyanines. Dyes particularly suitable for the coloring of cellulosic materials are produced, typically, by the chloromethylation of copper phthalocyanine and subsequent reaction with sodium thiosulfate.[40] Thus, chlormethylated copper phthalocyanine, having for example 2.9 chloromethyl groups per mole, is stirred in dimethyl sulfoxide containing $Na_2S_2O_3 \cdot 5H_2O$ for 3 hours at 80°–90°



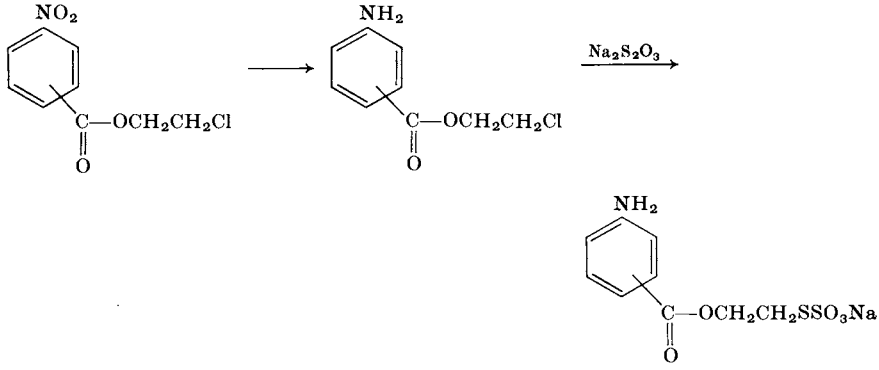
[39] FH, *BeP* 668,012.

[40] V. D. Poole and ICI, *BP* 955,004.

to give CuPc–$(CH_2SSO_3Na)_x$. The blue dye shows excellent fastness to light and wet treatments when printed on cellulosic fabrics in the presence of thiourea and $NaHCO_3$. The invention also provides an alternative route to CuPc–$(CH_2SSO_3Na)_x$ dyes.

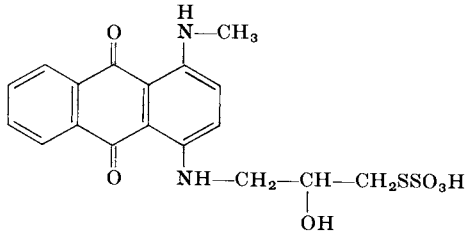
The inventors report that the two methods give identical products. The final reaction with SO_3 or chlorosulfonic acid in pyridine is an interesting extension of Baumgarten's synthesis[10] of aryl thiosulfates.

b. From Aryl Carboxylic Ester Derivatives. *S*-Alkyl thiosulfuric acid groups are attached to diazotizable bases through aryl carboxylic ester groups,[41] e.g.,



The amine is diazotized and coupled to a pyrazolone to give a yellow dye for acetate rayon.

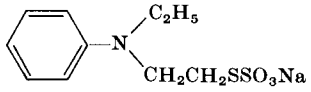
c. From Aminoanthraquinones. Red, violet, blue, and green dyes, for wool from acid baths and for printing acetate rayon, are obtained[42] through the addition of epichlorhydrin to aminoanthraquinones, followed by reaction with sodium thiosulfate. From 1-methylamino 4-aminoanthraquinone the following blue dye for acetate rayon is obtained:



[41] F. Felix, W. Muller and CIBA; *USP* 2,283,326.

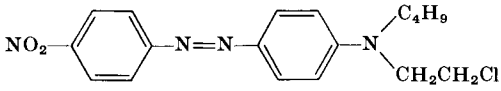
[42] F. Felix, P. Grossmann, M. Bommer, and CIBA, *USP* 2,180,805.

d. From Classical Disperse Dyes. Azo dyes from classical disperse dye structures have been produced[43] through the introduction of thiosulfate groups into the coupling component by the use of compounds such as

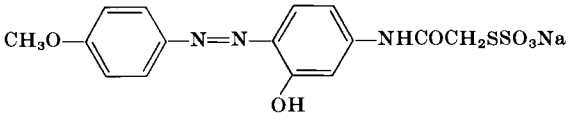


prepared, for example, from *N*-ethyl-(*N*-β-bromoethyl)aniline with sodium thiosulfate.

Alternatively the following dye may be treated with sodium thiosulfate in aqueous alcohol to give a red Bunte salt dye for acetate rayon:

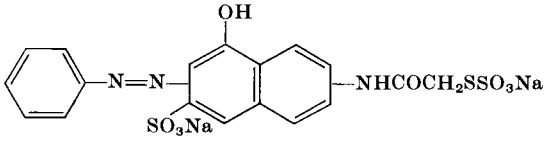


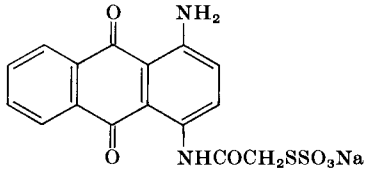
e. By Chloroacetylation. Through chloroacetylation of azo dyes having free amino groups, chloroacetamido groups are made available[44] for reaction with $Na_2S_2O_3$ to give dyes, for example, of structure



Chloroacetylation was used also by Milligan and Swan[30] as a route to alkyl thiosulfates.

A study[23] of the dyeing characteristics of *S*-alkyl thiosulfate dyes of the type D—$NHCOCH_2SSO_3Na$ on fibers containing amino groups, but free from disulfide bonds, e.g., polyamides, led to the preparation of eight dyes exemplified by





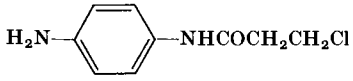
[43] IG, *BP* 490,945.

[44] F. Felix, W. Huber, and CIBA, *USP* 2,245,971.

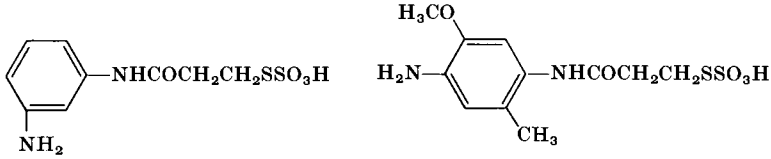
[45] FH, *BeP* 672,011 (1966).

The work proved of particular interest through evidence that a covalent bond between the thiosulfuric acid group and the amino group of the fiber is formed, e.g., D—NH—CO—CS—NH—Fiber.

Mono and disazo dyes have been prepared[45] through the use of Bunte salts derived, for example, from



The azo dyes have good solubility and good fastness to light and washing on cellulose, wool, silk, polyamides, polyurethanes, and leather. Other such diazo components bearing thiosulfate groups are, for example,



f. From Chlorotriazines. The use of reactive halogen groups as a route to thiosulfate dyes is further exemplified by the reaction products of chlorotriazine dyes; see Yamase *et al.*[26]

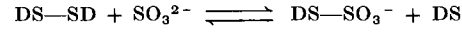
B. *S*-Aryl Thiosulfates

1. *The Solubilized Sulfur Dyes*

Solubilized sulfur dyes[46] constitute a large group of commercial products available under the proprietary names shown below:

Asathiosol	Nissen
Doxul	Solacva
Dykosol	Sulfanosol
Eclipsol	Sulfatin
Hydrosol	Sulfur Aquasol
Hydrosol Supra	Sulphosol
Kayasol	Thionol M

Generally speaking, they are prepared by reacting a sulfur dye in aqueous suspension with sodium bisulfite or sodium sulfite, usually under conditions of aeration:



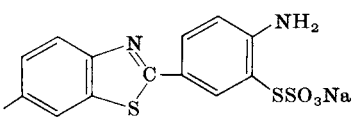
[46] *Colour Index* 3rd ed. S.D.C.

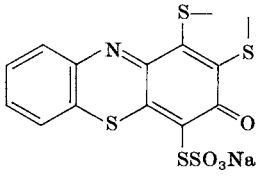
driven to completion by

$$2DS^- \xrightarrow{O_2} DS{-}SD$$

The solubilization of sulfur dyes through the use of $NaHSO_3$, Na_2SO_3, and their mixtures was established first in 1895 by simply treating a washed slurry of the sulfur dyes with sulfite for 3 days at room temperature.[47] In production procedures the slurry is heated until the dyestuff passes into complete solution. A more recent modification, suitable for sulfur vats, CI Vat Blues 42 and 43 and CI Sulfur Black II, simplifies the process.[48] The dye presscake is water-washed to reduce the electrolyte content to 1–4%, and kneaded as a paste containing 40–50% water with Na_2SO_3 at 60°–80° until the desired solubility is achieved.

The molecular constitutions of solubilized sulfur dyes have not been defined due to the uncertain structure of the parent chromophores. There is, however, a concurrence of opinion that they belong to the general class of *S*-aryl thiosulfate dyes.[49] Accordingly, it is suggested that molecular fragments of the following types may occur in solubilized sulfur dyes.





Nothing has been published concerning the degree of polyfunctionality with respect to Bunte salt groups, nor, so far as has been discovered, data on the effect of different thionation procedures on the technical properties of derived Bunte salts.

At the turn of the century, Green and Meyenberg[50] prepared what were probably water-insoluble complex indamines either by conjoint oxidation of di- or tetrathiosulfuric acids of *p*-phenylenediamine or *p*-aminophenol with an amine or a phenol or by conjoint oxidation of a *p*-diamine with a primary amine in the presence of sodium thiosulfate, after which the water-insoluble indamines were converted to their water-soluble *S*-aryl thiosulfate form with neutral Na_2SO_3.

[47] *DBP* 88,392; 91,720; 94,501.
[48] Moll and Vond, *USP* 3,372,167.
[49] C. Heid, *Melliand Textilber.* **45**, 648 (1964).
[50] A. G. Green and A. Meyenberg, *USP* 681,117.

2. *Azo Dyes*

Green and Meyenberg reported the first preparation of aniline-4-thiosulfuric acid, by saturating an aqueous slurry of 4,4′-diaminodiphenyl disulfide with sulfur dioxide.[50a] The product was diazotized and coupled with phenol.

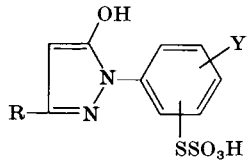
The stability of the thiosulfuric acid group to nitrous acid in diazotization processes as indicated by Green's observation is surprising in view of the ease with which such oxidants as iodine and peroxide convert alkyl and aryl thiosulfates to disulfides. As indicated by Schultheis *et al.*,[34] since nitrous acid liberates iodine from iodide it might be assumed that nitrous acid would oxidize thiosulfates to disulfides. However, under the conditions of diazotization oxidation is substantially absent. In the special case of *o*-amino-*S*-aryl thiosulfuric acids, reaction with nitrous acid gives the *o*-arylene -diazo sulfides. The intermediates of value therefore, are aniline-3- and 4-thiosulfuric acids and their derivatives. The following phenyl thiosulfuric acids have been cited as useful intermediates for azo dyes[51]: 3-amino-, 4-amino-, 3-amino-6-chloro, 3-amino-6-bromo-, 3-amino-4-methyl-, 3-amino-5-ethyl-, 3-amino-5-ethoxy-, 3-amino-6-sulfonamide-, 3-amino-4-hydroxy-, 3-amino-4-methoxy-, 3-amino-4-carboxy, 4-amino-3-fluoro-, 4-amino-3-methoxy, 4-amino-6-phenylsulfonamide-, 4-amino-5-hydroxy-, 4-amino-5-carboxy-, 4-amino-5-chloro-, 4-amino-6-chloro-.

Examples of such dyes, their color, and the fibers for which they are suitable are shown in Table I.[51–54]

a. Azo Dyes Bearing an S-Aryl Thiosulfuric Acid Group in the Coupling Component. The patent literature discloses two main types of coupler: 1-(thiosulfato)phenyl-5-pyrazolones and sulfonaphthylaminophenyl thiosulfates.

i. 1-(*Thiosulfato*)*phenyl*-5-*pyrazolones*. The 1-(thiosulfato)phenyl-5-pyrazolones[55] are derived by diazotization of unsubstituted or substituted aniline-3- or -4-thiosulfates, reacting with an alkali metal sulfite to form a phenylhydrazine sulfonate salt, acidifying the sulfonate with strong mineral acid at 0°–35° to form a hydrazine and condensing equimolar proportions of the hydrazine and an α-unsubstituted β-keto ester or an α-unsubstituted β-keto acid to form the pyrazolone by ring closure.

[50a] A. G. Green and A. Meyenberg, *BP* 4,792 (1900).
[51] G. A. Geselbracht and Martin Marietta Corp., *USP* 3,334, 085.
[52] G. A. Geselbracht and Martin Marietta Corp., *USP* 3,334,084.
[53] F. E. Barwick, *USP* 3,573,274.
[54] B. R. Fishwick, C. E. Vellins, A. H. Wyld, and ICI, *BP* 1,161,113.
[55] G. A. Geselbracht and Martin-Marietta Corp., *USP* 3,346,587.

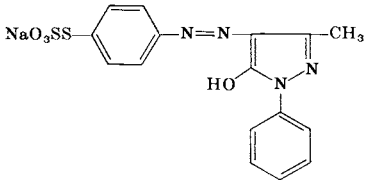
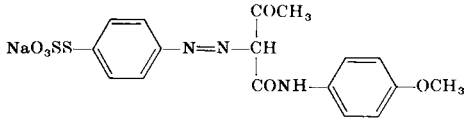
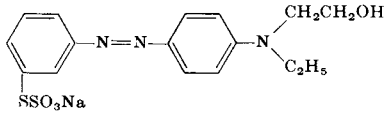
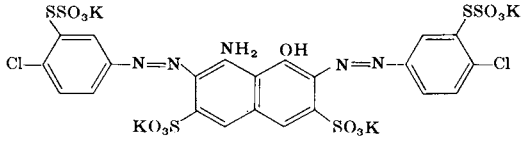
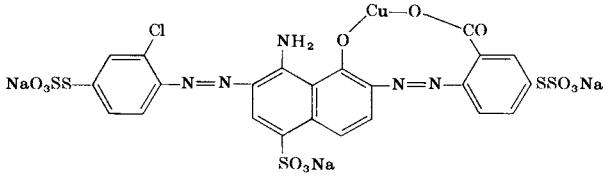


Couplers claimed are those in which R is H, phenyl, chlorophenyl, methoxyphenyl, carboxyl, lower alkyl, etc., and Y is H, halogen, lower alkyl, lower alkoxy, sulfonamido, etc.

Azo dyes derived from such couplers have been described[56] and are

TABLE I

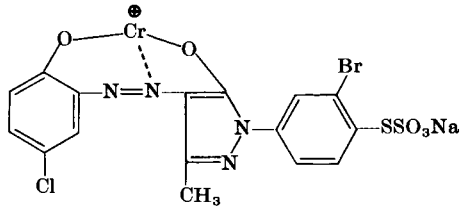
AZO DYES

Dye	*Hue*	*Fiber*	*Ref.*
	Reddish yellow	Cotton	52
	Yellow	Cotton	53
	Yellow	Cellulose triacetate	54
	Blue	Cotton	51
	Blue	Cotton	51

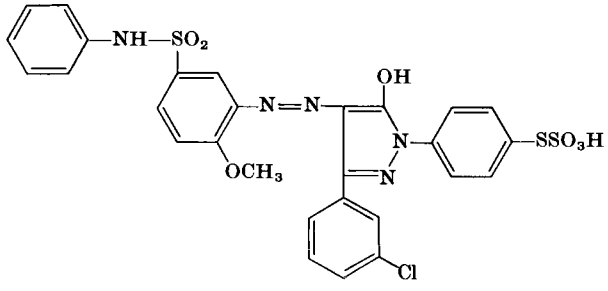
[56] G. A. Geselbracht and Martin-Marietta Corp., *USP* 3,346,550.

said to be suitable for the dyeing and printing of cotton fabrics by application with thiourea and curing; they may be used also for the dyeing of wool.

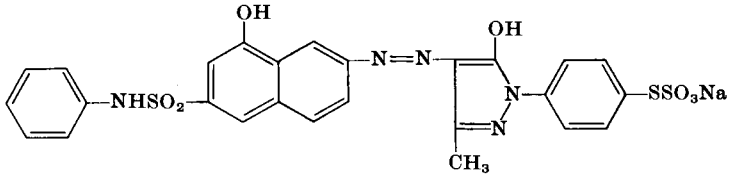
Diazonium salts used may contain metallizable groups such as –OH, or COOH; the following structure is typical of a 1:1 complex:



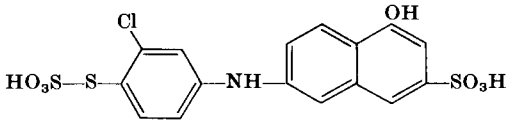
The unmetallized structures are typified by the following



ii. Sulfonaphthylaminophenyl thiosulfates. Sulfonaphthylaminophenyl thiosulfate couplers are prepared, for example, by heating together an



aminophenyl thiosulfate and an equivalent concentration of a naphthalenesulfonic acid, naphtholsulfonic acid, aminonaphthol sulfonic acid, or dihydroxynaphthalenesulfonic acid, with sodium bisulfite.[57] The preparation of sulfonaphthylaminoalkyl thiosulfates is also described. Typical of the couplers synthesized is

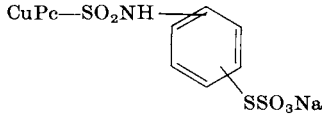


[57] F. E. Barwick, G. A. Geselbracht, and Martin-Marietta Corp., *USP* 3,496,207.

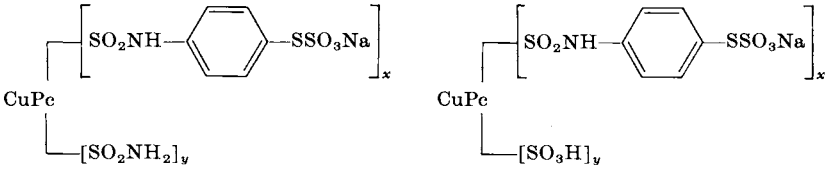
Diazonium salts are coupled[58] with such derivatives to give azo dyes (including examples of metallized dyes) said to be particularly useful for wet-fast dyeings on cotton and regenerated cellulose fabrics.

3. *Dyes Derived from Phthalocyanines*

The reactions of aminoaryl thiosulfates with chlorsulfonated metallized phthalocyanines have been disclosed[59]; such dyes carry functional groups such as



Compositions in which the final product carries, additionally, minor proportions of sulfonic, sulfonamide, and *N*-alkyl sulfonamide groups, etc., have been discussed.[60] Blue and green dyes especially suitable for the dyeing of cotton in fast shades are produced, typical of which are structures such as



Copper phthalocyanine dyes in which the divalent sulfur atom of the thiosulfuric acid group is bonded directly to the benzene rings of the chromophore are produced[61] by the treatment of tetrathiocyanophthalocyanines with mixtures of sodium sulfite and bisulfite in aqueous media. The dyes give intense green shades on cotton when reacted with sodium tetrasulfide.

IV. Properties and Dyeing Processes

A. *S*-Alkyl Thiosulfates

The *S*-alkyl thiosulfate dyes share with the *S*-aryl compounds generally high aqueous solubility and solution stability at neutral pH. Their *Colour Index* classification is "Condense Sulfur Dyes" for those types designed for application to cellulosic fibers.[46] The alkyl thiosulfates

[58] F. E. Barwick, G. A. Geselbracht, and Martin-Marietta Corp., *USP* 3,562,246.
[59] T. E. Leslie, W. J. Bryan, and Martin-Marietta Corp., *USP* 3,325,511.
[60] W. J. Bryan, T. E. Leslie, and Martin-Marietta Corp., *USP* 3,334,116.
[61] T. C. Crawford, *USP* 3,577,429.

structured suitably for the dyeing of wool, silk, polyamides, and polyurethanes may assume other *Colour Index* classifications.

1. *Dyes for Cotton Fibers*

The dyestuffs of this group (for example, Inthion Brilliant Blue I5G and other blue and green dyes) have been concisely reviewed by Schimmelschmidt *et al.*[17] Dye molecules containing several thiosulfuric acid groups are preferred so as to provide the optimum degree of polyfunctionality in fixation reactions. Further, it is a feature of dyes of this type that they are substantially free from other water-solubilizing groups. Notable in this group of dyes are those prepared by the condensation of dye sulfonyl chlorides and carbonyl chlorides with Bunte salts bearing reactive amine groups. In discussing the polycondensation processes with sulfur-bearing compounds suitable for use with Inthion dyes, the authors distinguish between the stability of alkyl disulfides and aryl disulfides with respect to Na_2S and point out that only the former resist reduction. Thus the treatment of *S*-alkyl thiosulfates with Na_2S (even in excess) proceeds only to the insoluble polycondensed disulfide dye.

$$2RSSO_3Na + Na_2S \longrightarrow RS_3R + 2Na_2SO_3$$

$$RS_3R + Na_2SO_3 \longrightarrow RSSR + Na_2S_2O_3$$

This is in sharp contrast to the behavior of *S*-aryl thiosulfates as represented by solubilized sulfur dyes and Dykolite dyes.

According to Schimmelschmidt *et al.* and Farbwerke Hoechst A.G., who have pioneered the modern technology of Bunte salt dyes, there are several approaches to pad-dyeing processes for cellulosics:

(a) According to Schultheis *et al.*[62] the dyes are fixed in cellulosic fibers by simultaneous or post application of alkalis or alkaline reducing agents. Na_2CO_3 or Na_3PO_4 may be used as alkalis, and NaCN or Na_2S as reducing agents, the latter being preferred. Final fixation by heat may be employed. Depending upon the substantivity of the dyestuff the process may be adapted to batch, pad-jig, etc., and continuous pad methods.

(b) The dyes may be fixed in cellulosic or protein fibers with elemental sulfur in the presence of bases followed by thermofixation or steaming.[63]

(c) The dyestuffs are fixed[64] on cellulose or protein fibers by heat

[62] W. Schultheis, K. Schimmelschmidt, H. Hoffmann, E. Baier, A. Bode, and FH, *USP* 3,088,790.

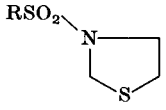
[63] K. Jellinck, T. Hezedus, H. Ulmer, H. Bartl, and FH, *USP* 3,097,908.

[64] H. Ulmer, P. Karacsonizi, K. Schimmelschmidt, E. Baier, and FH, *USP* 3,100,673.

treatment in the presence of compounds bearing thiocarbonyl or mercapto groups, e.g., thioacetamide, thiourea, mercaptobenzothiazole, dithiocarbamates. With thiourea the reactions may be carried out in alkaline media.[65]

(d) Fixation under acid conditions is achieved in the presence of substances that confer simultaneously crease- and shrinkproof characteristics, e.g., dihydroxydimethylol ethylene urea.[66]

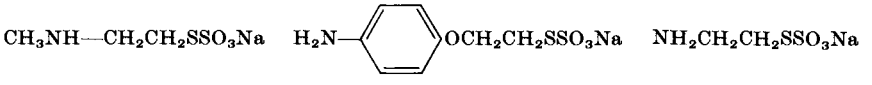
Schimmelschmidt *et al.* state that the dyes may be fixed in acid medium with formaldehyde or with substances evolving formaldehyde; thiol intermediates react with CH_2O to give thials [$(R—S)_2CH_2$] or cyclize to give heterocyclic compounds, e.g.,



Thus, Courtaulds North America, Inc.,[67] fix Inthion Brilliant Blue I5G to cotton using CH_2O, $Zn(BF_4)_2$, and heat.

(e) The *S*-alkyl thiosulfate dyes provide insoluble thiols $D—(SH)_x$ when boiled in mineral acid solution to give disperse dyes, pigments, or alkali-soluble mercaptides.[68] Thus the tetrasulfochloride from copper phthalocyanine condensed with 4 moles of aminoethyl thiosulfuric acid gives a stable green powder insoluble in water and soluble in dilute alkali when heated 30 minutes at 90° in strong HCl (16%).

Courtaulds North America, Inc.,[69] disclose a novel process for the insolubilization of dyes containing alkyl–SSO_3Na groups in fibers of cotton, polynosic rayon, nylon, and polyester by treatment at pH $\leqslant 5$ at $\geqslant 60°$ with aqueous $NaClO_2$. It is suggested that chlorine dioxide is the active entity in the insolubilization process. Two to three equivalents of $NaClO_2$ per $-SSO_3$ group are required. Twenty-three Bunte salt dyes have been studied including dyes derived from 3-aminobenzyl thiosulfate:



[65] H. Bressler *et al.* and FH, *FP* 1,527,396.
[66] FH, *BeP* 614,329 (1962).
[67] Courtaulds North America, Inc., *BP* 1,085,019.
[68] K. Schimmelschmidt, H. Hoffmann. E. Baier, H. Ulmer, and FH, *USP* 3,226,395.
[69] Courtaulds North America, Inc., *BP* 1,081,019.

It is suggested that the following reactions form the basis for the process:

$$2NaClO_2 + 2H^+ \longrightarrow 2HClO_2 + 2Na^+$$

$$2HClO_2 \longrightarrow ClO_2 + \tfrac{1}{2}ClO_3^- + \tfrac{1}{2}Cl^-$$

$$2DSSO_3^- + ClO_2 \longrightarrow DS{-}SD + 2SO_4^{2-} + \tfrac{1}{2}Cl_2$$

Courtaulds North America, Inc.,[70] also claim that *S*-alkyl thiosulfate dyes, e.g., Inthion Brilliant Green IB, may be fixed on cotton by padding the dyed fabric with a solution containing salts of Hg, Ag, Cu, e.g., $HgCl_2$, followed by drying and heating.

The *S*-alkyl thiosulfate dyes as represented by Inthion Dyes (FH) have been termed polycondensation dyes so as to denote the polymolecular character of the dye when fixed through treatment with, for instance, Na_2S. By the use of radioactively labeled S^{2-} the following reaction sequence has been confirmed[71]:

$$DSSO_3^- + \overset{*}{S}^{2-} \rightleftharpoons DS\overset{*}{S}^- + SO_3^{2-}$$

$$DS\overset{*}{S}^- + DSSO_3^- \longrightarrow DS{-}\overset{*}{S}{-}SD + SO_3^{2-}$$

$$DSSSD + SO_3^{2-} \xrightarrow{\overset{*}{S}^{2-}} DSSD + S\overset{*}{S}O_3^{2-}$$

A practical process for the dyeing and printing of cotton is very simple, e.g., (1) pad or print the dyestuff from aqueous solution; (2) dry the material; (3) pad with NaSH or Na_2S in salt solution; (4) air briefly; (5) rinse, wash, and dry.

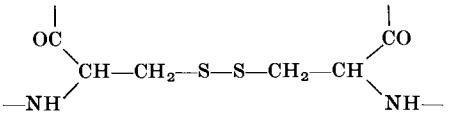
Such a process requires an extended sequence of steps; a "single-pad" process whereby the final dye fixation process is achieved by dry heat or steam has also been described. Here the reagent of choice is thiourea incorporated in the dye solution along with an alkali. It is proposed that an intermediate in the reaction sequence is the unsymmetrical dye–formamidine disulfide which then rearranges to the symmetrical disulfide.

2. *Dyes for Wool, Silk, and Polyamide Fibers*

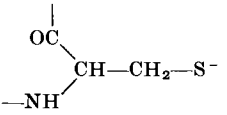
The product Wool Fast Turquoise Blue SW, reported to be a condensation product of CuPc sulfochloride with aminoethylthiosulfuric acid, exhibits excellent wet-fastness on wool fibers. Work by Osterloh[72] and by Milligan and Swan[30] indicates that *S*-alkyl thiosulfates undergo

[70] Courtaulds North America, Inc., *BP* 1,085,016.
[71] G. Kaufmann, *Melliand Textilber.* **44**, 1245 (1963).
[72] F. Osterloh, *Melliand Textilber.* **44**, 57 (1963).

reaction with the wool fiber, which contains the cystine group



and, in the presence of water, cysteine



The Bunte salt dye reacts directly with cysteine, and according to Osterloh, the following reactions ensue:

$$2CyS^- + 2DSSO_3^- \rightleftharpoons 2CySSD + 2SO_3^{2-}$$

$$2SO_3^{2-} + 2CySSCy \rightleftharpoons 2CyS^- + 2CySSO_3^-$$

$$2CySSD \rightleftharpoons DSSD + CySSCy$$

$$2DSSO_3^- + CySSCy \rightleftharpoons DS—SD + 2CySSO_3^-$$

This represents an overall $-SO_3^{2-}$ interchange between dye and fiber and is in agreement with the findings of Milligan and Swan. The actual structure of the dye in the fiber appears to be a function of the dyeing method and especially the pH of the dyebath, so that both symmetrical disulfides (e.g., DS—SD) and unsymmetrical (or CyS—SD) disulfides are likely to be present.

A considerable amount of work on synthesizing *S*-alkyl thiosulfate dyes for wool and polyamide fibers has appeared in the patent literature, but little is known concerning the merits of these dyes. Dyebaths for nylon fiber set with acetic acid appear to be employed for the products Inthion Brilliant Blue I3G and Inthion Brilliant Yellow 7G. For strong shades the dyebaths are exhausted with formic acid.[73] As with all phthalocyanine blues the lightfastness of Blue I3G is evidently poor; however, if the dyestuff is padded onto nylon along with thiourea and $(NH_4)_2SO_4$, dried, and steamed or cured, a great improvement in lightfastness is secured.[74]

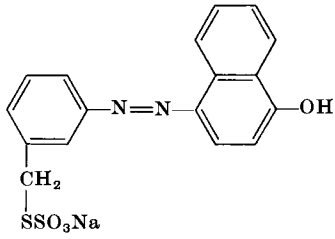
3. *Dyes for Human Hair*

Keratin-containing substances and especially live human hair are

[73] FH, "Dyeings on Polyamide Fibers."

[74] H. Luttringhaus, *Amer. Dyest. Rep.* **53**, 728 (1964).

dyed[75] with *S*-alkyl thiosulfate dyes by a process that involves first treating the hair with a reducing agent capable of forming a mercaptan group in the keratin, reacting the –SH group with a dye such as a Bunte salt dye to form covalent bonds between keratin and dye, and finally blocking the remaining –SH groups in the hair.[75] Thus reddish brown shades on a gray ground are obtained by treating the hair (a) for 15 minutes with 2% thioglycolic acid at pH 9, (b) with a 3% solution of the dye



at pH 9.0 for 30 minutes at 20°, and (c) finally for 15 minutes at 20° with a 2% solution of formaldehyde.

4. *Vat Dyes*

The reaction products of chlorsulfonated vat dyes and aminoethylthiosulfuric acid are applied as the vat and the leuco dye is oxidized in the fiber by known methods.[27] The principal advantages of the introduction of thiosulfuric acid groups into a vat chromophore would appear to be the value, in terms of application procedures, in working with a water-soluble product and possible enhancement in reduction rate of the quinonoid structure that arises from molecular subdivision. The destiny of the thiosulfuric acid group under caustic–hydrosulfite reduction is not clear.

B. *S*-Aryl Thiosulfates

1. *Solubilized Sulfur Dyes*

The derivatives of sulfur dyes, designated as "CI Solubilized Sulfur Dyes" in the *Colour Index*,[46] comprise 50 products of ill-defined chemical structure. It is generally assumed by all authors that, because of the preparative processes used, and the behavioral characteristics of the dyes, they are truly representative of the Bunte salt class. Several characteristics are shared in common: (a) As Bunte salts the dyes are in the chromophorically oxidized state. (b) They exhibit unusually high solubility in water (up to 200 gm/liter) and have excellent stability in

[75] R. E. Randebrock, *USP* 3,415,606.

neutral solution. (c) They are nonsubstantive, essentially, on cellulose fibers from aqueous solutions by contrast to the relatively high affinity of reduced sulfur dyes. (d) They are readily converted to a leuco or reduced state by NaSH, Na_2S, $Na_2S_2O_4$, $HOCH_2SO_2Na$, glucose, etc., and undergo insolubilizing reactions with a variety of mercaptans, thiols, and thiocarbonyl compounds.

The solubilized sulfur dyes are employed chiefly for the coloring of cellulosic fibers and have special value for fabrics and yarns blended from cotton or viscose rayon with fibers of secondary cellulose acetate or cellulose triacetate; methods for their use on nylon and cellulose-nylon blends have also been indicated.[76]

The modern literature on the technology of solubilized sulfur dyes is mainly in the names of C. Heid and Cassella Farbwerke Mainkur (CFM). Their patents and general publications on Hydrosol and Hydrosol Supra dyes provide a very substantial body of information in this field. The publications cover several broad areas such as the chemical mechanisms of dyeing with Hydrosol dyes, the practical dyeing processes, and the chemical techniques that may be applied in dyeing processes to improve the quality of the end product.

a. The Chemical Mechanisms. The reactions by which the *S*-aryl thiosulfate is converted into a substantive dye during the dyeing process have been studied carefully by Heid.[77] It is noted that the high degree of alkalinity resulting from the hydrolysis of sodium sulfide solutions, while necessary for the solubilization of sulfur dyes in the form DS—SD, may in some ways be either unnecessary or detrimental to the processing of aryl thiosulfates. Thus, high alkalinity is detrimental to the characteristics of many fibers and the reaction of OH^- ion on $DSSO_3Na$ often leads to undesirable by-products such as soluble sulfinates and sulfonates. Hence attention has been paid to the use of NaSH in place of Na_2S and it is covered by a patent.[78]

The reaction of sulfhydrate ion with $DSSO_3Na$ proceeds through intermediate stages of reaction in which insoluble dye polysulfides are formed, as illustrated by the following S-nucleophilic displacement equilibria:

$$2DSSO_3^- + HS^- \rightleftharpoons DS_3D + 2SO_3^{2-} + H^+$$

or

$$DSSO_3^- + HS^- \rightleftharpoons DSS^- + SO_3^{2-} + H^+$$

followed by

$$DSSO_3^- + DSS^- \rightleftharpoons DS_3D + SO_3^{2-}$$

[76] W. E. Wood, *Text. Mfr.* **84**, 423 (1958).

[77] C. Heid, *Teintex* **30**, No. 7, 465 (1965).

[78] C. Heid *et al.* and CFM, *DBP* 1,085,439; *BP* 874,151; *USP* 3,113,824; *FP* 1,252,299.

By the elimination of $S_2O_3^{2-}$, which is thermodynamically favorable, reactions such as the following ensue:

$$DS_3D + SO_3^{2-} \rightleftharpoons DS_2D + S_2O_3^{2-}$$

$$DS_3D + SO_3^{2-} \rightleftharpoons DSSSO_3^- + DS^-$$

$$DSSSO_3^- + DS^- \rightleftharpoons DS_2D + S_2O_3^{2-}$$

$$DS_2D + 2HS^- \rightleftharpoons 2DS^- + S_2^{2-} + 2H^+$$

$$S_2^{2-} + SO_3^{2-} \rightleftharpoons S_2O_3^{2-} + S^{2-}$$

$$S^{2-} + H^+ \rightleftharpoons HS^-$$

The sum total of all these equilibria representing the final stoichiometry of the reduction is

$$DSSO_3^- + HS^- \rightleftharpoons DS^- + S_2O_3^{2-} + H^+$$

In the presence of sufficient base, the equilibrium is forced to the right and the soluble leuco compound is efficiently formed. In the absence of added base H_2S is formed.

An interesting development has been the successful reduction of solubilized sulfur dyes by electrolytic means.[79] The thiol is taken up in $Na_2CO_3/NaHSO_4$ solution and used to dye cotton textiles. This would seem to be of special interest in relation to effluent disposal problems.

b. The Dyeing Processes. Because of high water solubility and minimal affinity for the fiber, solubilized sulfur dyes diffuse into and penetrate the fiber structures exceptionally well.[80] These factors are of special importance in high-speed continuous dyeing processes and in dyeing processes that require the circulation of dye solution through a relatively dense assembly of yarn or fabric. Cellulosic fibers so processed are then exposed to the reaction of selected reducing agents in order to transform the Bunte salt dye into the substantive leuco form, when true dyeing of the fibers ensues. Much has been written by the authors on the proper selection of reducing agents and particular stress has been laid on the suitability of low-alkalinity systems and the physical and chemical protection this offers to the lustre, handle, and winding properties of cellulose yarns and the chemical stability of cellulose ester fibers[81] in comparison with the higher alkalinity of classical sulfur dye systems and vat dyes. Thus with Hydrosol dyes according to Heid, "it is possible to coordinate the type of reducing agent with the material and the dyeing method," and through the use of hydrosulfite or glucose, to ease effluent problems.

[79] C. Heid *et al.* and CFM, *DBP* 1,906,083.

[80] C. Heid, *Can. Text. J.* **84**, 47 (1967).

[81] C. Heid, *Z. Gesamte Textilind.* **65**, 1028 (1964).

Yarns and fabrics may be dyed with solubilized sulfur dyes by all the usual batch and continuous techniques. For instance, the sequence for package dyeing is as follows[80]: The material is worked at 30° in a dyebath which contains dyestuff, salt, soda ash, and a wetting agent; after 10 minutes the reducing agent is added and the fabric or yarn processed a further 15 minutes at 30°, whereupon the bath is heated gradually to exhaust the leuco dye. After a total dyeing time of 60 minutes, the material is water-rinsed and oxidized in a conventional manner.

A semicontinuous "cold-pad-batch" process may be employed[82] in which the fabric is padded in a dye solution also containing soda ash, NaSH, a wetter, and a sequestering agent, together with ammonium hydroxide. The padded fabric is batched wet on a roll, stored for 2–3 hours at room temperature, washed, and oxidized. Several precautions are to be observed to prevent uneven migration of dye and bronzing at selvedges; the use of a dye stabilizer, Stabilisol S, is recommended.

The various pad processes for Hydrosol and Hydrosol Supra dyes have been discussed in detail by Heid[82] and are summarized in the tabulation below:

Process	*Pad liquor*
Pad-batch (cold) Pad-roll	Dyestuff, NaSH, Na_2CO_3, Stabilisol S, wetting agent, sequestrant, and salt
Pad (hot)-dry	Dye, glucose, thiourea, NaSH, NaOH or NaSH, Stabilisol S, NaOH
Pad-thermofix	Dye, urea, Hydrosol Fixing Agent KT

Wood[76] has treated the subject similarly in relation to Sulphosol dyes, for which Na_2S and $Na_2S_2O_4$–soda ash are recommended; the latter is particularly useful for viscose rayon fibers. Where Na_2S is used with Sulphosol colors the amount is considerably less than that required for conventional sulfur dyes.

c. Modified Processes. With few exceptions, the solubilized sulfur dyes lack the tinctorial purity and brilliance of other classes of cotton dyes, notably the fiber-reactive dyes. Heid *et al.* have devised methods for the post-application and simultaneous application of fiber-reactive dyes with solubilized sulfur dyes, for example: (a) CI Solubilized Sulfur Yellow 5 is dyed on cotton yarn with NaSH, Na_2CO_3, and Na_2SO_4; the dyeing is rinsed and overdyed with CI Reactive Red 9 by known methods and finally oxidized.[83] (b) Cotton is dyed from a bath contain-

[82] C. Heid, *Z. Gesamte Textilind.* **70**, 626 (1968).

[83] C. Heid, K. Holoubek, and CFM, *USP* 3,294,475.

ing together CI Solubilized Sulfur Yellow 5, CI Reactive Blue 7, NaSH, Na_2CO_3, and Na_2SO_4 at 90°; the dyeing is washed and oxidized.[84] Alternatively, a solubilized sulfur dye, a reactive dye, urea, and alkali ($NaHCO_3$) are applied and dried; the material is then treated with NaSH–Na_2CO_3, steamed, rinsed, and oxidized.[85]

The fixation of solubilized sulfur dyes in fibers *without the use of reducing agents* has been systematically studied by Heid *et al.* Thus, Hydrosol dyes are applied to fibers simultaneously with polyfunctional reactive compounds such as the following and fixation of dye is achieved by drying or curing at high temperatures[86]: ethylene glycol diglycidyl ether–Na_2CO_3; polymethoxy methylol melamines; 1,3,5-tris(acryloyl)-hexahydro-*s*-triazine; tris(aziridinyl)phosphine oxide; tris(chloroacetyl)-diethylenetriamine condensate; ethylene glycol bis(2-hydroxy-3-chloro-1-propyl) ether; bis(β-hydroxyethyl) sulfone[86a]; polyalkylene-imines.[86b]

Alternatively, Heid *et al.*[87] react the solubilized sulfur dye first in aqueous solution with, e.g., ethylene imine, followed by epichlorhydrin to give a dye for cotton not requiring the use of reducing agents.

A process not requiring high-temperature treatments is described by Heid *et al.*[88] The dyebath comprises $SC(NH_2)_2$, $OC(NH_2)_2$, and NH_4Cl; the fabric is dried and aftertreated with polyfunctional agents.

Fixation under acid conditions was achieved by Courtaulds North America, Inc.,[89] who used Lewis acids with formaldehyde; simultaneous coloring and cross-linking of cellulose fibers is achieved thereby.

Solubilized sulfur dyes are fixed on fibers[90] by dry heat in the presence of reactants such as thiourea, ammonium thiocyanate, urea, acetyl ureas, hexamethylenetetramine, 2-mercaptobenzothiazole, formic acid, and trisodium thiocyanurate.

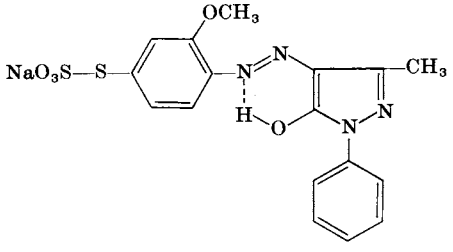
The fixation[91] of solubilized sulfur dyes without the use of post-oxidation is achieved by contacting the dyestuff with polycondensing agents such as Na_2S_2–Na_2S_4 at ambient temperatures. The simultaneous

[84] M. Stuhlmiller, G. Bottiger, C. Heid, and CFM, *USP* 3,294,474.
[85] CFM, *BP* 1,060,556.
[86] CFM, *BP* 1,034,606.
[86a] CFM, *BeP* 656,363.
[86b] CFM, *USP* 3,579,286.
[87] F. Engelhardt, C. Heid, W. Gunzert, E. Krusche, A. Meyer, and CFM, *DBP* 1,815,133.
[88] C. Heid *et al.* and CFM, *DBP* 1,178,513.
[89] Courtaulds North America, Inc., *BP* 1,097,466.
[90] L. Tigler, C. D. Weston, G. A. Geselbracht, L. A. Meszaros, R. R. Rupp, and Martin Marietta Corp., *USP* 3,387,913.
[91] C. D. Weston, W. S. Griffith, and Martin Marietta Corp., *USP* 3,419,343.

coloration of cotton-polyester blends with solubilized sulfur dyes and disperse dyes is similarly achieved.[92]

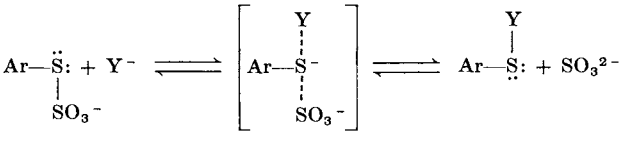
2. *Condense Sulfur Dyes*

The Bunte salts of azo and phthalocyanine dyestuffs designated *S*-aryl thiosulfates have been classified by the *Colour Index* as "Condense Sulfur Dyes."[46] (The grouping embraces also *S*-alkyl thiosulfates.) Seventeen products have been listed. The *S*-aryl thiosulfate dyestuffs (for example, Dykolite dyestuffs), which are of generally high water solubility and are basically nonsubstantive on cellulosic fibers, are designed for the high-speed continuous pad dyeing of cellulosic fabrics and their blends. The dyes are generally bright in shade and diffuse readily into cotton fiber from aqueous systems. The structure of CI Condense Sulfur Orange 2 has been disclosed.[15]



The fixation of such dyes on cellulosic fabrics has been achieved in a variety of ways, of which three techniques are important: (a) the spontaneous polycondensation of dye at ambient temperature with aqueous inorganic polysulfides, and especially Na_2S_4, in the presence of brine[91,92]; (b) the reduction of the thiosulfate group to a thiol ArSH or ArSNa, followed by polycondensation on the fiber using alkali metal polythionates[93]; (c) polycondensation of the Bunte salt through the use of thiocarbonyl compounds, e.g., thiourea with heat.

Techniques (a) and (b) have been studied in detail.[15] In all such processes the functional group in the dyestuff is the divalent sulfur atom. With nucleophiles or bases (Y^-) a displacement reaction takes place through a possible transition state, as shown.



[92] C. D. Weston, W. M. McAllister, and Martin Marietta Corp., *CP* 818,296.
[93] C. D. Weston and Martin Marietta Corp., *USP* 3,415,609.

The equilibrium is determined thermodynamically by the relative free energies of the two systems. When the sulfite ion is very much more stable (less basic) than the displacing nucleophile the reaction proceeds to product Ar—S—Y. Nucleophilic activity is related to a function of the two constants, pK_a and E°, for the nucleophile and it is shown on this basis that, for Bunte salts, nucleophiles such as S^{2-}, $NH_2CH_2CH_2S^-$, and S_4^{2-} are powerfully reactive, whereas SCN^- and 4-nitrothiophenolate ion are weak reagents.

An interesting alternative viewpoint is provided by the "hard and soft acids and bases" classification due to Pearson, which states that the most stable compounds result from the bonding of soft acids with soft bases, where softness is a function of the polarizability of an ion and is found usually in systems with vacant *d* orbitals; thus S^{2-} is a very soft base whereas the softness of S in 4-nitrothiophenolate ion is "hardened" by the electroinductive and mesomeric properties of the *p*-nitrophenyl group. The intermediate $D—S^{\delta+}$ is a soft acid.

Sulfite ion is an S nucleophile of moderate activity and is displaced with ease by inorganic sulfur species and by alkyl mercaptides especially; many thiolates, particularly those bearing electron-donor groups, also displace SO_3^{2-} readily.

The fixation of *S*-aryl thiosulfates with Na_2S_4 is almost spontaneous in brine solution (which diminishes adverse electrostatic effects). Following the pioneer publications of Foss, the successive equilibria are represented as follows:

$$DSSO_3^- + S_4^{2-} \rightleftharpoons DS_5^- + SO_3^{2-}$$
$$DSSO_3^- + DS_5^- \rightleftharpoons DS_6D + SO_3^{2-}$$
$$DS_6D + SO_3^{2-} \rightleftharpoons DS_5SO_3^- + DS^-$$
$$DS_5SO_3^- + DS^- \rightleftharpoons DS_5D + S_2O_3^{2-}$$
$$DS_5D + SO_3^{2-} \rightleftharpoons DS_4SO_3^- + DS^-$$
$$DS_4SO_3^- + DS^- \rightleftharpoons DS_4D + S_2O_3^{2-}$$
$$\overline{2DSSO_3^- + S_4^{2-} \rightleftharpoons DS_4D + 2S_2O_3^{2-}}$$

The reaction of 2 moles of *S*-aryl thiosulfate with 1 mole of S^{2-} gives the following:

$$2DSSO_3^- + S^{2-} \rightleftharpoons DSSD + S_2O_3^{2-} + SO_3^{2-}$$

Treatment with a further mole of S^{2-} gives

$$DSSD + S^{2-} \rightleftharpoons DSS^- + DS^-$$

which on aging may dissociate further to $2DS^- + \frac{1}{8}S_8$. Further excesses of S^{2-} take up S_8 to form S_x^{2-}.

Thus, most *S*-aryl thiosulfates when treated with excess Na_2S yield alkali-soluble dye thiolates or mixtures with hydrodisulfides, many of which have high stability to atmospheric oxidation. They are very suitable for pad application on textile materials and may be fixed spontaneously by treatment in an aqueous brine solution containing alkali metal polythionates at ordinary temperatures. The reactions proceed as follows:

With DS^-

With DS^-

$$DS^- + {}^-O_3SSSSO_3^- \longrightarrow DSSSO_3^- + S_2O_3^{2-}$$

$$DS^- + DSSSO_3^- \longrightarrow DSSD + S_2O_3^{2-}$$

With DSS^-

$$DSS^- + {}^-O_3SSSSO_3^- \longrightarrow DSSSSO_3^- + S_2O_3^{2-}$$

$$DSS^- + DSSSSO_3^- \longrightarrow DS_4D + S_2O_3^{2-}$$

Many of the *S*-aryl thiosulfate dyes, especially the metal complexes, find use as substantive dyes for polyamide and other fibers.

CHAPTER III

PHYSICAL CHEMISTRY OF DYEING: STATE OF DYE IN DYEBATH AND IN SUBSTRATE

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I. General Introduction

The physical chemistry of dyeing has been the subject of a very large number of research investigations, particularly over the last four decades. The establishment of reliable methods of dye purification has led to the collection of reasonably accurate quantitative data for the dyeing process, which is regarded as one of diffusion into the fiber phase and subsequent adsorption on the fiber surface. These studies were therefore mainly concerned with the overall rate of dyeing and the equilibrium sorption isotherms for various dye–fiber systems. The state of dyes in solution has also been investigated in some detail, notably diffusion and aggregation characteristics, since these factors also influence dye uptake by fibers. The electrochemical nature of sorption processes in the fiber with acid dyes was established, and the concepts of Donnan equilibria and the Gilbert-Rideal theory were applied with some limited success. The application of thermodynamics to dyeing systems led to the concept of "dyeing affinity," i.e., the free-energy

change accompanying a dye adsorption process, as a measure of the strength of dye–fiber bonding. It was also generally recognized that four kinds of binding forces influenced dye adsorption, viz., coulombic interactions, dispersion forces, hydrogen bonding, and π-electron interaction. The manufacture of synthetic dyes having a wide range of structures and properties and the development of newer synthetic fibers also led to several investigations on the relationships between dye structure and dye substantivity.

Advances in the physical chemistry of dyeing up to about the year 1954, dealing with all the above-mentioned aspects for the dyeing of cellulose with direct and vat dyes, of cellulose acetate with disperse dyes, of proteins and polyamide fibers with acid dyes, and to some extent of polyester fibers with disperse dyes have been excellently dealt with by Vickerstaff.[1]

The present review concerns itself mainly with the advances that have taken place in the physical chemistry of dyeing over the last 20 years, although, where relevant, adequate discussions have been included of the earlier work. The review is classified broadly into three sections: (a) the state of dye in the dyebath and the fiber; (b) the kinetics of dyeing; and (c) equilibrium sorption and mechanisms of dyeing.

Practically all the physicochemical investigations in dyeing phenomena have been concerned with the adsorption of dyes on fibers from aqueous baths. Physicochemical studies of dyeing from nonaqueous dyebaths and dyeing at high temperatures have not been extensively carried out so far. A few relevant features of vapor-phase dyeing are, however, included in this review. The physical chemistry of reactive dye systems has not been included here since this topic has already been discussed in great detail in a previous volume of this series (D. Hildebrand in *CSD VI*).

II. State of Dye in the Dyebath

Most dyes in aqueous solution show a tendency to associate and it is very likely that the forces that bring about aggregation in solution are also responsible for substantivity of some types of dye to fiber substances. This association in the aqueous phase may be between similar ions or molecules of individual dyes, or between unlike ions or molecules of different dyes when present in mixtures. Association of dye molecules in aqueous solutions in the presence or absence of additives has been

[1] T. Vickerstaff, "The Physical Chemistry of Dyeing," 2nd ed. Oliver & Boyd, Edinburgh, 1954.

studied extensively employing different methods of measurement, but seldom has there been agreement among the results of different methods. Aggregation is so common a phenomenon that it is now considered as a general property of the dye. Only in a very few cases, because of steric factors or electrical influences that prevent close association of dye molecules, is this behavior not observed. The study of dye aggregation is important in relation to dyeing kinetics since the dimensions of the diffusing molecules can influence dye uptake. Furthermore, the energy changes associated with the changes in the degree of aggregation during the dye adsorption process can also influence the energy and extent of adsorption at different temperatures. Formation of aggregates can also limit the saturation adsorption value under a given set of conditions. Each of the methods of measurement has certain merits and limitations, but none has been found to be entirely satisfactory.[2]

A. Methods of Measurement

Osmotic pressure measurements have received much less attention during recent years because of practical difficulties due to adsorption of dye by the membrane and the difficulty in calculating Donnan membrane equilibrium on account of the presence of diffusable counterions, while information obtained from sedimentation measurements is of limited value. With diffusion measurements, it has been found necessary to use excess electrolyte to counteract the accelerating effect of the diffusion potential due to the more rapid diffusion of the smaller counterions. Furthermore, when such measurements are carried out using the porous sintered disk apparatus of Northrop and Anson,[3] the presence of stagnant boundary layers near the surface of the sintered disk introduces serious errors in the measurement. It is, therefore, necessary to insert mechanical stirrers that sweep away these stagnant layers continuously by actually brushing against the surface of the sintered disk. The Stokes cell,[4] which is a considerable improvement on the Northrop and Anson cell, has the advantage that it is possible to remove such stagnant layers by mechanical stirring, both from the upper as well as lower surfaces of the sintered disk. Different types of cells have recently been reviewed.[5]

[2] E. Coates, *J. Soc. Dyers Colour.* **85**, 355 (1969).

[3] J. H. Northrop and M. L. Anson, *J. Gen. Physiol.* **12**, 543 (1929).

[4] R. H. Stokes, *J. Amer. Chem. Soc.* **72**, 763 (1950).

[5] R. Mills and L. A. Woolf, "The Diaphragm Cell: Theory and Practice of the Diaphragm Cell for Liquid Diffusion Measurements." Diffusion Res. Unit, Res. Sch. Phys. Sci., Australian National University, Canberra, 1968.

Craven *et al.*[6] have carried out a comparative study of the diffusion of a series of four azo dyes in stirred and unstirred glass sintered disk cells of the Northrop and Anson, and the Stokes types. Their results indicate that the presence of a stagnant layer in the unstirred cell gives rise to a considerable error at lower temperatures, but the error decreases with increasing temperature. In such methods, the main assumption is that dye aggregates are spherical and the average radius of aggregates is calculated from the Stokes-Einstein equation. A micromethod has been developed by Luck[7] wherein diffusion of dye into liquid or solid substrate can be measured. In the case of diffusion in a liquid, the measured decrease of the diffusion coefficient due to an increase in concentration of the dye has been attributed to the association of the dye molecules, and employing the diffusion technique, it has been shown that diffusion coefficients of CI Direct Red 7 and its triazole derivative under identical conditions of experiment are very nearly the same.[8]

With respect to conductivity measurements, interpretation is difficult due to the unknown number of counterions that are associated with dye aggregates, and therefore the method is restricted to highly purified dye solutions in the absence of extra electrolyte. Chaudhuri and Das Gupta[9] have found that with CI Acid Red 87, Acid Red 51, Basic Violet 1, and Cyanine in aqueous solution, molecular conductivity does not have a linear relation with the square root of concentration, and departure of the conductivity curve from linearity has been attributed to the formation of ionic micelles. This was substantiated by the fact that the critical concentration at which ionic micelles were formed coincided with the concentration at which rapid quenching of fluorescence commenced. Similar to the behavior observed in the case of colloidal electrolytes, Baddi and Sivaraja Iyer[10] noticed that at low concentrations of CI Direct Yellow 12 and Direct Blue 1, equivalent conductivity first decreased on increasing concentration, increased sharply within the subsequent narrow range of concentration, passed through a maximum, and then decreased again with an increase in concentration (Fig. 1). Maxima in the conductivity curve indicated that formation of ionic aggregates of high conductivity took place in the presence of added electrolyte. However, as the rise in conductivity was not very marked, the degree of aggregation was comparatively small. An interesting observation of these workers was that the values of critical concentra-

[6] B. R. Craven, A. Datyner, and J. G. Kennedy, *Aust. J. Chem.* **24**, 723 (1971).

[7] W. Luck, *Ber. Bunsenges. Phys. Chem.* **69**, 255 (1965); *CA* **62**, 13,302 ff. (1965).

[8] H. Uedaira and H. Uedaha, *Bull. Text. Res. Inst. Jap.* No. 65, p. 11 (1963); *J. Soc. Dyers Colour.* **80**, 266 (1964).

[9] K. D. Chaudhuri and D. R. Das Gupta, *Indian J. Phys.* **26**, 67 (1952).

[10] N. T. Baddi and S. R. Sivaraja Iyer, *Kolloid-Z. Z. Polym.* **210**, 132 (1966).

tions obtained from conductivity measurements for aggregate formation were closely related to the equilibrium dyebath concentrations at which adsorption isotherms of these dyes on different cellulosic fibers showed a marked change in slope leading to the formation of a plateau region. In this region, aggregate formation changed the simple equilibrium that existed between adsorbed dye and single ions of dye in the bath to a more complicated equilibrium wherein ionic aggregates, in addition to adsorbed dye and single ions, were involved. Furthermore, it was also

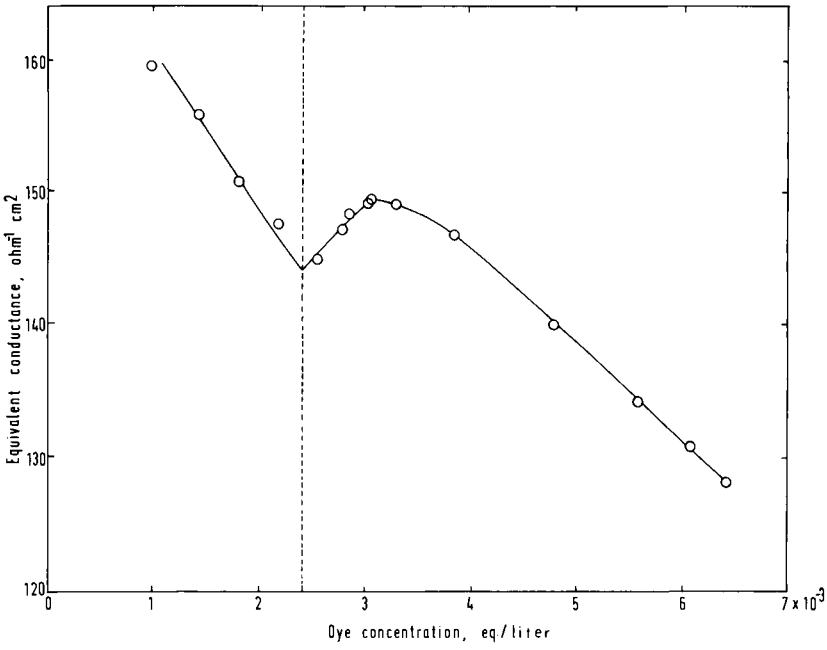

FIG. 1. Conductivity of CI Direct Blue 1 in 10 g/liter NaCl solution at 50°.

shown that the unaggregated single-ion concentration present at the critical concentration remained constant in all aggregated dye solutions. Frank's work[11] on CI Acid Orange 7 and Direct Red 2 reveals that conductance data for the former dye in water do not show any anomalies as compared with other strong electrolytes, and this dye behaves as a strong uni-univalent electrolyte. On the other hand, in case of CI Direct Red 2, reduction in conductance is much greater than could be explained by increased ionic strength due to addition of electrolyte, and with this

[11] H. P. Frank, *J. Colloid Sci.* **12**, 480 (1957).

dye larger particles are formed which retain a large fraction of counterions. At 25°, several acid dyes show a tendency for micellar formation, but at 90°, these dyes are more or less completely disaggregated.[12]

The optical properties of dye solutions have been examined and a characteristic spectrum for aggregates has been found in solutions of several dyes. According to Giles *et al.*,[13] a large number of dyes, including azo, anthraquinone, and reactive dyes, show two absorption bands in the visible region which could be tentatively identified as the x and y bands attributed by Lewis and Calvin to the electronic oscillation along the different axes of the molecule having a planar configuration. With an increase in dye concentration, the shorter wave band y increases in height relative to the longer band x as a result of aggregation of dye molecules. Both these bands persist in all media, but there is a solvatochromic shift of both bands with changes in the solvent. Precise determination of the state of aggregation could not, however, be carried out because the change in the height of y band relative to that of x band was small (5%) for a hundredfold increase in the concentration. The presence of these two bands has been detected in the leuco derivatives of most of the anthraquinone vat dyes and also with CI Acid Blue 43.[14] In the case of basic dyes the characteristic absorption spectrum also shows changes with the degree of dye aggregation.[15–18] Spectrophotometric as well as potentiometric data have been obtained for the study of aggregation effects with CI Mordant Violet 5 and the concentration of monomer and dimer at each dye concentration has been calculated.[19] With this dye, within a certain concentration range, best agreement with experimental results could be obtained when the aggregate was postulated to contain two ions. On the other hand, CI Mordant Black 11, because of a more extended π-electron system and the presence of a nitro group, shows a greater tendency to aggregate. With this dye, micelles are formed and these have associated gegenions.[20]

In the case of reactive dyes, aggregation in aqueous solutions has also been found to take place. Changes in absorption spectra of CI Reactive Violet 4 indicate that there exists a monomer–dimer equilibrium in the

[12] T. Iijima and M. Sekido, *Sen-i Gakkaishi* **16**, 571 (1960); *J. Soc. Dyers Colour.* **77**, 136 (1961).

[13] C. H. Giles, S. M. K. Rahman, and D. Smith, *J. Chem. Soc.*, p. 1209 (1961).

[14] M. Mitsuishi and G. Aida, *Bull. Chem. Soc. Jap.* **39**, 246 (1966).

[15] H. Dunken, D. Schmidt, and K. Palm, *Z. Chem.* **2**, 349 (1962).

[16] W. West and S. Pearce, *J. Phys. Chem.* **69**, 1894 (1965).

[17] M. E. Lamm and D. M. Neville, *J. Phys. Chem.* **69**, 3872 (1965).

[18] S. R. Palit and G. K. Saxena, *Nature (London)* **209**, 1127 (1966).

[19] E. Coates and B. Rigg, *Trans. Faraday Soc.* **57**, 1637 (1961).

[20] E. Coates, B. Rigg, R. Murton, and D. H. Smith, *J. Soc. Dyers Colour.* **79**, 465 (1963).

concentration range of 8–800 mg/liter of dye in the aqueous solution, but at a concentration of 4 g/liter larger aggregates are formed.[21] Padhye and Karnik,[22] from changes in the intensity of x and y bands in visible spectra of CI Reactive Blue 7, 18, and 21, have shown the presence of monomer and dimer, both in case of the parent dye as well as the hydrolyzed dye. In the case of dyes having phototropic tendencies, changes in the particle size of the dye are reflected in phototropicity. With phthalocyanine acids and some vat dyes, two peaks are observed in the spectrum corresponding to molecular and aggregated state of dye, and from these measurements a measure of the degree of dispersion has been obtained.[23]

Examination of luminescence spectra reveals the state of aggregation of CI Basic Red 1 and Basic Violet 11 in aqueous solutions.[24] Fluorescence intensity measurements have been carried out on solutions of thionine with a view to detecting the tendency of dye ions to associate into dimers in different solvent compositions. This dye has been found to be highly associated at concentrations of about 10^{-3} M in water, but the degree of association decreases upon addition of a small amount of methanol.[25]

The use of light-scattering measurements to study the molecular weight of solutes in solution is based on the following equation derived by Raman and Ramanathan[26] and Debye.[27] Debye showed that this equation can be used to determine the molecular weight of solutes in solutions from an experimental measurement of the various quantities used in the following equation:

$$\frac{Hc}{\tau} = \frac{1}{M} + 2Bc$$

where

$$H = \frac{[32\pi^3 n_0^2 (n - n_0)^2/c^2]}{3\lambda^4 N}$$

In this equation, c is the concentration in grams per milliliter, M is the solute weight-average molecular weight, B is a constant depending on

[21] R. Weingarten, *Melliand Textilber.* **48**, 301 (1967).

[22] M. R. Padhye and R. R. Karnik, in "Physicochemical Aspects of Interaction of Dyes in Solution and in Fibre Systems," p. 56. University of Bombay, 1969.

[23] G. Eigenmann and F. Kern, *Text.-Rundsch.* **16**, 167 (1961).

[24] V. L. Levshin, E. G. Baranova, L. D. Derkacheva, and L. V. Levshin, *Termodinam. Str. Rastvorov, Tr. Soveshch., 1958* p. 275 (1959); *CA* **54**, 21942d (1960).

[25] G. Haugen and R. Hardwick, *J. Phys. Chem.* **69**, 2988 (1965).

[26] C. V. Raman and K. R. Ramanathan, *Phil. Mag.* [6] **45**, 213 (1923).

[27] P. Debye, *J. Appl. Phys.* **15**, 338 (1944).

the solute, n is the refractive index of the solution and n_0 that of the solvent, λ is the wavelength of the incident light in vacuum, N is Avogadro's number, and τ is the absolute turbidity of the solution in excess of that of solvent, being measured at right angles to the incident beam. The molecular weight of the solute can be obtained by plotting Hc/τ versus c, and extrapolating the results to zero concentration, the intercept being $1/M$. This equation is valid for molecules in solutions and colloidal suspensions in which the longest dimension is not greater than $\lambda/10$, and the scattering is symmetrical about 90°. For large particles, the scattering will not be symmetrical about 90°, and it would be necessary to measure the angular variation of the intensity of the scattered light. Equations for the calculation of molecular weight in such cases have been derived by Debye,[28] Zimm,[29] and Zimm *et al.*[30] It is also possible, from light-scattering studies, to obtain information regarding the shape of the scattering particles.

The light-scattering technique has been employed by Alexander and Stacey[31] to study the colloidal behavior of CI Acid Orange 7 and 63, Acid Red 89, and Direct Red 2. These investigators observed that at 20°, CI Direct Red 2 formed stable micelles with molecular weights ranging from 10^5 to about 10^6, depending on the concentration of the electrolyte present in the system. At 60°, however, no aggregates could be detected. Similar behavior was also noted in case of CI Acid Red 89. Urea and phenol were found to be effective in preventing the aggregation of these dyes. CI Acid Orange 63 formed stable micelles with molecular weight of about 10^5, independent of electrolyte concentration at temperatures between 20° and 60°. CI Acid Orange 7 did not form stable micelles even at high concentrations. Light-scattering measurements along with conductance and viscosity have been used by Frank[11] to study the solution behavior of CI Direct Red 2 and Acid Orange 7, and micellar particles of large size could only be detected in the presence of added salt. In the case of the former, a particle weight of the order of 1,900,000 was obtained at 25° in the presence of 0.04 M KCl, while for CI Acid Orange 7 the particle weight varied with concentration and temperature, reaching a value of 37,000 at 5° in the presence of 0.04 M KCl. Frank concluded that micelles had the form of rodlike particles in which the dye ions were associated in the manner of a stack of coins. Light-scattering investigation on the variation in the micellar size of CI Direct Red 2 under actual dyeing conditions reveals that in the presence of electrolyte, micelles remain unaltered in size during dyeing,

[28] P. Debye, *J. Phys. Colloid Chem.* **51**, 18 (1947).

[29] B. H. Zimm, *J. Chem. Phys.* **16**, 1093 and 1099 (1948).

[30] B. H. Zimm, R. S. Stein, and P. Doty, *Polym. Bull.* **1**, 90 (1945).

[31] P. Alexander and K. A. Stacey, *Proc. Roy. Soc., Ser. A* **212**, 274 (1952).

but in pure dye solutions, as well as in the presence of a detergent, there is a change in micellar size.[32] The presence of micelles larger than spheres of 2820 Å radius has been detected in the case of CI Direct Yellow 4 from the measurements of the angular distribution of light intensities diffused from aqueous dye solutions, and large micelles increase in quantity with increased concentration of electrolyte present but decrease with an increase in temperature.[33] A detailed investigation of the association phenomena of aqueous solutions of Orange 8 and CI Acid Orange 7 has been carried out by Milićević and Eigenmann[34] employing osmometry, spectroscopy, viscometry, and response to a sodium ion-specific electrode. These measurements reveal that whereas solutions of Orange 8 contain monomeric anions of dye, even in concentrations near the limit of solubility, solutions of CI Acid Orange 7 at concentrations $> 10^{-2}$ M contain mainly dimeric anions.

The X-ray small-angle scattering method has been used to detect association of direct and acid dyes (CI Direct Red 83 and Acid Orange 7) in aqueous solutions, and this method has been found suitable for investigation of particles of molecular weight of about 1000.[35,36] With both these dyes in the absence of electrolyte, degree of association is very low, but aggregation is promoted by the presence of electrolyte when the particles grow uniformly in length as well as breadth, especially in case of the latter dye. NMR spectroscopic studies have been carried out on CI Basic Orange 14 to examine its aggregation behavior with increasing concentration in solution.[37] These studies indicate a shift to high field of proton resonance absorption, which can be interpreted in terms of the formation of sandwich-type dimers.

Solubilization is a fundamental phenomenon connected with colloidal aggregates, and measurements of solubilization by dye solubility methods have been found to be very convenient. Furthermore, solubility data can be used to obtain thermodynamic activity coefficients for the dye molecule using the solubility product principle. The solubilities of several direct and acid dyes have been determined at different temperatures, and it has been shown that addition of salts having no common ion increases solubility while addition of salts having a common ion decreases the solubility of some dyes.[38] Lipatov and Lipatov[39] have

[32] S. R. Sivarajan, *J. Indian Inst. Sci., Sect. A* **36**, 282 (1954).

[33] A. Banderet and P. Meyer, *Bull. Soc. Chim. Fr.* [5] p. 53 (1953); *CA* **47**, 4087 (1953).

[34] B. Milićević and G. Eigenmann, *Helv. Chim. Acta* **47**, 1039 (1964).

[35] O. Kratky, H. Ledwinka, and I. Pilz, *Monatsh. Chem.* **97**, 922 (1966).

[36] O. Kratky, I. Pilz, and H. Ledwinka, *Monatsh. Chem.* **98**, 227 (1967).

[37] D. J. Blears and S. S. Danyluk, *J. Amer. Chem. Soc.* **88**, 1084 (1966).

[38] B. N. Mel'nikov and P. V. Moryganov, *Kolloid. Zh.* **17**, 99 (1955); *CA* **49**, 9931b (1955).

[39] S. M. Lipatov and G. V. Lipatov, *Kolloid. Zh.* **14**, 52 (1952); *CA* **46**, 4349d (1952).

found that the solubility of several direct dyes increases slowly in the lower temperature range up to 31°, but rapidly in the temperature range of 31°–50°. In most cases, the theoretical heat of solution was greater than the calculated value at higher temperatures, which could be attributed to the increased flexibility of the dye molecules at these temperatures. The temperature dependencies of the heats of solution of CI Direct Red 28 and Direct Blue 1 in water in the presence of sodium chloride, pyridine, and ethanol are appreciably different.[40] With CI Direct Red 28, there is a sharp decrease in the heat of solution between 60° and 75° in all the solvents, while CI Direct Blue 1 shows a gradual decrease over the entire temperature range of 20°–90°.

A relationship has been found to exist between the heat of solubilization of several direct dyes and the ability of the dye to be adsorbed by cellulose. Baddi and Sivaraja Iyer[10] observed that the curves correlating solubility with salt concentration at a given temperature and solubility with temperature at a fixed salt concentration have the salient feature of an initial smooth rise followed by a rapid increase in solubility. Similar behavior has been observed in the case of paraffin chain salts and other colloidal electrolytes. A marked increase in solubility beyond a critical concentration of dye at a given temperature and salt concentration has been attributed to the formation of ionic aggregates which take up a considerable amount of solute in the solution phase. Micellar solubilization of phenylazonaphthols (water-insoluble dyes) can be effected in the aqueous phase in the presence of different types of surfactant, and in many cases log micellar solubilization vs. log concentration plots are linear with slopes varying with the nature of the dye.[41]

The aggregation tendency of CI Basic Blue 25 in electrolyte solutions has been examined by optical and solubility measurements, and results indicate a tendency for dimerization in very dilute solutions followed by higher polymerization as dye concentration is increased.[42] Aqueous solubilities of several disperse dyes have been measured,[43–45] and some of these dyes have been found to have significant solubility in water. In many cases, the aqueous solubility of a disperse dye has a bearing on the dyeing behavior of hydrophobic fibers.

[40] S. M. Lipatov and I. M. Movchovich, *Kolloid. Zh.* **21**, 522 (1959); *J. Soc. Dyers Colour.* **76**, 519 (1960).

[41] A. M. Mankowich, *J. Colloid Sci.* **14**, 131 (1959).

[42] G. R. Haugen and E. R. Hardwick, *J. Phys. Chem.* **67**, 725 (1963).

[43] C. L. Bird, *J. Soc. Dyers Colour.* **70**, 68 (1954).

[44] D. Patterson and R. P. Sheldon, *J. Soc. Dyers Colour.* **76**, 178 (1960).

[45] I. B. Blinicheva, B. N. Mel'nikov and L. V. Afinogenova, *Tr. Ivanov. Khim.-Tekhnol. Inst.* **11**, 43 (1969); *CA* **75**, 99193 (1971).

A polarographic technique which measures the diffusion coefficient of the dye in the presence of salt has been described by Hillson and McKay.[46] This method is claimed to be simple and quick and also to permit the degree of aggregation to be measured over a wide range of concentrations. The polarographic method can be described briefly as follows. At the dropping mercury electrode, when the potential is gradually increased, the current does not show much variation with the potential applied until a value of the potential is reached corresponding to the reduction potential for the diffusing ionic species which then gets reduced at the dropping mercury electrode. A polarographic wave is obtained. The limiting current i_D, corresponding to the height between the two horizontal portions of the polarographic wave, is related to the diffusion coefficient of the dye ion and its concentration in the solution by the well-known Ilkovič equation,[47]

$$i_D = KnD^{1/2}c$$

where n is the number of electrons transferred per ion reduced, D is the diffusion coefficient, c is the molar concentration, and K a constant that depends on the capillary characteristics and that can conveniently be obtained from calibration experiments using standard solutions of cadmium chloride, since the diffusion coefficient of cadmium ions is accurately known. Knowing the diffusion coefficient of the standard ion, the diffusion coefficient D of the dye ion can be calculated. The value of n can be determined independently by controlled potential coulometry. The molecular weight M of the dye can then be determined using the following empirical linear relationship[47a]:

$$\log_{10} D = -0.3773 \log_{10} M - 4.392$$

This equation has been found valid over a wide range of molecular weights and diffusion coefficients for various types of molecule. Knowing the molecular weight of monomeric dye and the aggregated dye, aggregation number can be calculated. Polarographic measurements offer several advantages. Each drop presents a new surface of mercury to the solution and products of electrolysis are not allowed to accumulate. Since hydrogen has a high overvoltage on pure mercury, i.e., it requires a more negative potential than on a platinized platinum electrode before discharge of H^+ occurs, the method is useful in acid solutions. If small drops of mercury are used, the extent of electrolysis

[46] P. J. Hillson and R. B. McKay, *Trans. Faraday Soc.* **61**, 374 (1965).

[47] D. Ilkovič, *Collect. Czech. Chem. Commun.* **6**, 498 (1934); *CA* **29**, 28588 (1935).

[47a] S. R. Sivaraja Iyer and G. S. Singh, *in* "Physicochemical Aspects of Interaction of Dyes in Solution and in Fibre Systems," p. 32. University of Bombay, 1969.

is small and little loss of species from solution takes place, i.e., the concentration remains effectively constant.

With CI Basic Blue 9 and Direct Red 28, agreement between the results obtained by this technique and by other investigators employing different methods is found to be good. In the presence of salt, definite aggregates are formed, in the case of CI Basic Blue 9, which do not grow further with an increase in concentration. On the other hand, CI Direct Red 28 has been found to aggregate very strongly in the presence of salt. The behavior shown by CI Basic Violet 3 and Acid Blue 25 was intermediate between that of CI Basic Blue 9 and Direct Red 28. Recent polarographic studies by Sivaraja Iyer and Singh[48] reveal that CI Direct Yellow 12, Direct Blue 1, Acid Orange 7, and Basic Blue 9 are only slightly aggregated. Aggregation with these dyes seems only to proceed to the dimerization stage at concentrations up to 10^{-3} M in the presence of 0.2 M NaCl. On the other hand, as has been observed when other techniques of measurements are used, CI Direct Red 2 and 28 are highly aggregated in solution even at low salt and dye concentrations, and the degree of aggregation is strongly temperature-sensitive. In the case of vinyl sulfone reactive dyes, polarographic studies reveal that the sulfuric ester form of the dye is more aggregated than the corresponding vinyl sulfone[49] (Table I). Polarographic studies have also been carried out on the solubilization of disperse dyes in ethylene oxide condensates, and it has been observed that diffusion coefficients as well as particle sizes of disperse dyes obtained by this technique agree well with the corresponding values for the micelles of nonionic surfactants.[50]

TABLE I

AGGREGATION NUMBER OF SULFURIC ESTER AND VINYL SULFONE FORMS OF REACTIVE DYES AT 40°

	CI Reactive Blue 19		*CI Reactive Yellow 14*		*CI Reactive Orange 7*	
Concentration (M)	*Sulfuric ester*	*Vinyl sulfone*	*Sulfuric ester*	*Vinyl sulfone*	*Sulfuric ester*	*Vinyl sulfone*
1×10^{-4}	1	1	4	2	1	1
5×10^{-4}	3	1	9	4	5	3
1×10^{-3}	8	3	16	6	12	10

[48] S. R. Sivaraja Iyer and G. S. Singh, *Kolloid-Z. Z. Polym.* **242**, 1196 (1970).

[49] E. H. Daruwalla and V. N. Sharma, unpublished work.

[50] S. Hayano and N. Shinozuka, *Bull. Chem. Soc. Jap.* **42**, 1469 (1969).

In recent years, some novel methods have been developed to measure the aggregation of both anionic and nonionic dyes. Aggregation of four anionic dyes has been studied spectrophotometrically at high concentrations using a cell of very small path length. The deviation from Beer's law observed has been explained on the basis of stacking of individual dye molecules behind one another to form aggregates.[51] A maximum slope method has also been suggested for the determination of aggregation constant and degree of aggregation in dye solutions.[52,53] A novel experimental approach to the study of association of dye molecules, based on the partition between solvents (isoextraction) has been developed by Mukerjee and Ghosh.[54] From surface tension measurements, Giles and Soutar[55] have been able to demonstrate that both anionic and cationic dyes tend to concentrate at a water interface. Association in the gas phase of disperse dyes of widely differing chemical constitution has been measured by the application of Knudsen effusion and torque effusion methods.[56] Green and Jones[57] have measured the vapor pressures of a number of aromatic azo compounds over a range of temperature and found that most of these compounds appeared to be dimeric in the vapor, although with some, a higher degree of association could be detected.

It has been observed that quantitative agreement between the results of different methods is of a low order. This is mainly due to the fact that the results of different methods provide answers in different forms and also the environmental conditions for the satisfactory working of these methods are different. Interpretation of the results of absorption characteristics of dyes in terms of dye aggregation should be carried out with caution because with several dyes it is not possible to correlate spectral changes with dye aggregations.[58] Normally in the case of dyes where the charge is localized, spectral changes are small, while dyes which have delocalized charge within the chromophore system show pronounced spectral changes. However, in some cases good agreement has been obtained between the results of spectral and potentiometric methods,[19] spectroscopic or conductometric and osmometric studies,[34,59]

[51] D. Pugh, C. H. Giles, and D. G. Duff, *Trans. Faraday Soc.* **67**, Part 2, 563 (1971).

[52] M. Hida, A. Yabe, H. Murayama, and M. Hayashi, *Bull. Chem. Soc. Jap.* **41**, 1776 (1968).

[53] M. Hida and T. Sanuki, *Bull. Chem. Soc. Jap.* **43**, 229 (1970).

[54] P. Mukerjee and A. K. Ghosh, *J. Amer. Chem. Soc.* **92**, 6403 (1970).

[55] C. H. Giles and A. H. Soutar, *J. Soc. Dyers Colour.* **87**, 301 (1971).

[56] H. Kojima, *Sen-i Gakkaishi* **25**, 540 (1969); *CA* **73**, 16244a (1970).

[57] H. S. Green and F. Jones, *Trans. Faraday Soc.* **63**, 1612 (1967).

[58] R. B. Mckay, *Trans. Faraday Soc.* **61**, 1787 (1965).

[59] B. Farhadieh, *Diss. Abstr. B* **27**, 126 (1966).

and spectral and fluorescence measurements.[60] Similarly, recent polarographic studies by Sivaraja Iyer *et al.*[61] on the diffusion coefficients for a series of unaggregated acid dyes CI Acid Red 13, 18, 41, and 88 at 40° reveal a good agreement between the diffusion coefficient data and those obtained by Craven *et al.*[6] for the same dyes under identical conditions employing the stirred Stokes cell.

Aggregation of a dye in an aqueous phase is governed by, among other factors, the chemical constitution of the dye. Nishida[62] observed the following order of decreasing solubility for direct dyes: CI Direct Red 28, Direct Blue 15, Direct Blue 6, Direct Blue 14, Direct Blue 8, Direct Red 2, Direct Violet 39, and Azoorseillin. The abnormally low solubility of the last two dyes has been attributed to chelation within the dye molecule. However, in many cases the relationship between constitution and tendency to dissociate in water is not positive. Differences in micelle formation between CI Direct Red 2, 7, and 28 can be explained on the basis of steric effects.[63] From the results of measurements of the diffusion coefficients in aqueous solution of a series of direct dyes with regular changes in the structure, Mel'nikov *et al.*[64] observed that a break in conjugation led to a reduction in the aggregating tendency. Amphoteric ion-forming direct dyes have a greater tendency for micellar formation due to mutual attraction of two dye molecules through acidic and basic groups.[65] As hydrophobic bonding plays an important part in the association of dyes, differences in the association of dyes with similar molecular structures can be explained on the basis of differences in such bonding.[66] In the case of acid dyes with related structures but differing in the number of sulfonic acid groups, the fewer of these groups present in the molecule, the greater is the tendency for ionic micelle formation.[67]

B. Nature of Bonding in Dye Aggregates

Aqueous dye solutions show behavior similar to colloidal electrolytes, and in solution are likely to exist completely dissociated and also as

[60] J. Lavorel, *J. Phys. Chem.* **61**, 1600 (1957).

[61] S. R. Sivaraja Iyer, K. Subramanian, and A. S. Ghanekar, *in* "Diffusion of Solutes in Solution and in Fibre Systems," p. 19. University of Bombay, 1973.

[62] K. Nishida, *Sen-i Gakkaishi* **14**, 865 (1958); *CA* **53**, 1724i (1959).

[63] Y. Tanizaki and N. Ando, *Nippon Kagaku Zasshi* **78**, 343 (1957); *CA* **51**, 10230d (1957).

[64] B. N. Mel'nikov, B. Krasovitskii, and P. V. Moryganov, *Tech. Text. Ind. USSR* No. 1, p. 126 (1960).

[65] A. Schaeffer, *Text.-Rundsch.* **12**, 59 (1957).

[66] H. Uedaira and H. Uedaira, *Kolloid-Z.* **194**, 148 (1964); *CA* **60**, 14658c (1964).

[67] T. Iijima and M. Sekido, *Sen-i Gakkaishi* **16**, 571 (1960); *J. Soc. Dyers Colour.* **77**, 136 (1961).

nonionic micelles together with ionic micelles having occluded unionized dye. Various mechanisms have been put forward to explain aggregation of dyes in aqueous solutions, and the nature of forces involved in aggregation is not clear. It is very likely that van der Waals forces, hydrogen bonding, interaction of π-electrons, or coupling of electron oscillators may be operative in several cases. In view of the fact that aggregation does not stop at the dimer stage, the phenomenon has been associated with additive forces of the van der Waals type.[68] In the case of direct dyes, zwitterion-forming dyes have a particular tendency to micellar formation which can be ascribed to mutual attraction of two dye molecules through acidic and basic groups and subsequent increase in tiny micelles through intermolecular forces.[65] London dispersion forces are also responsible when stacking of dye molecules in the polymer in parallel orientation is likely to take place. Intermolecular hydrogen bonding or bonding by sandwich water molecules is possible, depending on the chemical structure of the dye and the nature of the additive present in the aqueous system.[69,70]

Comparatively very few attempts have been made regarding the study of the thermodynamics of dye aggregation. This may partly be due to the difficulties in the accurate determination of the equilibrium constant of the system due to successive polymerization of the dye leading to micellar formation. In many cases, investigations have been confined to the preliminary stage of dimerization only. This is probably because, as discussed by Coates,[2] the formation of quadramers from dimers takes place at a very slow rate. Bonding energies for associated molecules of different basic dyes have been found to vary between 5 and 13 kcal/mole.[71,72] Values for the energy of dimerization and entropy change for fluorescein are normal values expected when two monomers combine to form a dimer through hydrogen bonding. On the other hand, in the case of CI Basic Violet 10, hydrophobic bonding appears to be the main cause for dimerization, and there is a positive entropy change with this dye.[73]

The concept of hydrophobic bonding arises from the special structure of water and changes in the structure of water due to dissolved solutes. Although it was recognized long ago that hydrogen bonding was responsible for many unusual properties of water, it is only recently that

[68] T. Forster and E. König, *Z. Electrochem.* **61**, 344 (1957).
[69] V. L. Levschin and I. S. Lonskaya, *Opt. Spectrosc. (USSR)* **11**, 148 (1961).
[70] V. L. Levschin and L. V. Krotova, *Opt. Spectrosc. (USSR)* **13**, 457 (1962).
[71] L. V. Levschin and V. K. Gorshkov, *Opt. Spectrosc. (USSR)* **10**, 401 (1961).
[72] E. Rabinowitch and L. F. Epstein, *J. Amer. Chem. Soc.* **63**, 69 (1941).
[73] K. K. Rohatgi and G. S. Singhal, *J. Phys. Chem.* **70**, 1695 (1966).

some basic understanding has been obtained of the nature of liquid water. No exact theory of liquid water, accounting for all its properties, has appeared so far. Bernal and Fowler[74] proposed a model for liquid water based on a broken-down ice structure with most of the hydrogen bonding still in existence. This theory has been criticized due to the apparent rigidity of the model, as the more dense molecular arrangement would involve undue bending strain on the hydrogen bonds. Frank and Wen[75] postulate the existence of a spatially extended network structure (flickering clusters) by means of intermolecular hydrogen bonds. However, Némethy and Scheraga[76] suggested that mainly large clusters are formed.

The structure of water near an ion has been described by Frank and Wen,[75] Samoilov,[77] and Bockris and Reddy[78] by referring to three regions. In the primary or structure-enhanced region next to the ion, water molecules are immobilized and oriented by the ionic field, and they move along with the ion. The secondary or structure-broken region is the one in which the normal bulk structure of water breaks down to varying degrees. Finally, at a sufficient distance from the ion, water structure is unaffected by the ion and displays the tetrahedrally bonded network characteristic of bulk water. The cause of structure breaking is supposed to be the approximate balance in the secondary region between two competing orienting influences which act on the water molecule, i.e., the normal structural orienting influence of neighboring water molecules and the orienting influence upon the dipole of the spherically symmetrical ionic field. Introduction of a nonpolar solute in water creates a structural disturbance resulting in a rearrangement of the water molecules in their lowest energy configuration in relation to themselves as well as the new alien molecules. This dissolution of nonpolar molecules in water causes an increase in heat capacity and a decrease in the randomness of the system. Némethy and Scheraga[76] have shown that the presence of highly hydrogen-bonded water molecules is favored in the immediate vicinity of the nonpolar solute. This is because of the increase in the attractive intermolecular (van der Waals) contacts which results in the stabilization of hydrogen-bonded water clusters. Thus, the probability of finding a cluster is much greater

[74] J. D. Bernal and R. H. Fowler, *J. Chem. Phys.* **1**, 515 (1933).

[75] H. S. Frank and W.-Y. Wen, *Discuss. Faraday Soc.* **24**, 133 (1957).

[76] G. Némethy and H. A. Scheraga, *J. Chem. Phys.* **36**, 3401 (1962); *J. Phys. Chem.* **66**, 1773 (1962).

[77] O. Ya. Samoilov, "Structure of Aqueous Electrolyte Solutions and the Hydration of Ions." Consultants Bureau, New York, 1965.

[78] J. O'M. Bockris and A. K. N. Reddy, "Modern Electrochemistry." Plenum, New York, 1970.

in the neighborhood of a nonpolar solute than anywhere in bulk water. Nonpolar solutes thus increase the mean reorientation time of the water molecules and lead to structuring of water. This more ordered region of water round a nonpolar solute has been referred to as "iceberg" because of its greater icelike character.[79]

With CI Basic Blue 9, Mukerjee and Ghosh,[80] from the results of the effect of urea on dye association, have postulated the presence of structured regions of low entropy termed "icebergs" around the dye molecule, and have indicated the thermodynamic relationship of the association of dyes to hydrophobic bonding where the primary driving force is the gain in entropy when part of the icebergs disappear. Results of the recent polarographic studies with CI Direct Yellow 12, Direct Blue 1, Direct Red 2 and 28, Acid Orange 7, and Basic Blue 9 also support the formation of hydrophobic bonds between dye molecules during aggregation.[48] Heats of aggregation are 5.08, 8.31, 4.96, and 2.97 kcal/mole in the case of CI Direct Yellow 12, Direct Blue 1, Acid Orange 7, and Basic Blue 9, respectively, under specific concentrations of KCl. The unitary entropy values, obtained after making allowance for the cratic term, have been found to be positive, which supports the concept that the bonds between dye molecules arise from the tendency for the desolvated nonpolar groups to associate with each other rather than with neighboring molecules. Such formation is favored by the large entropy gain which is associated with the breakdown of "icebergs" around the nonpolar parts of the dye molecules.

Recent studies by Sivaraja Iyer and Singh[81] on the dimerization of a series of acid dyes having the general formula 1-amino-4-alkylamino-anthraquinone-2-sulfonic acid, sodium salt (where the alkyl group is a methyl, ethyl, propyl, or butyl) and employing a polarographic technique, reveal positive values for unitary entropy change ΔS which increases with an increase in nonpolar character of the parent dye, indicating that hydrophobic bonding plays an important role in the aggregation of dyes. A comparison of the theoretical values of the thermodynamic parameters for pairwise hydrophobic bond formation between alkyl side chains using Némethy and Scheraga's model[76] and the experimental values for these dyes emphasizes the role of hydrophobic bonding in the aggregation process (Table II). Using calorimetric measurements, Derbyshire[82] has arrived at the value of 10.48 kcal/mole

[79] H. S. Frank and M. W. Evans, *J. Chem. Phys.* **13**, 507 (1945); H. S. Frank, *Proc. Roy. Soc., Ser. A* **2** 47, 481 (1958).

[80] P. Mukerjee and A. K. Ghosh, *J. Phys. Chem.* **67**, 193 (1963).

[81] S. R. Sivaraja Iyer and G. S. Singh, *J. Soc. Dyers Colour.* **89**, 128 (1973).

[82] A. N. Derbyshire, *Trans. Faraday Soc.* **51**, 909 (1955).

TABLE II

COMPARISON OF EXPERIMENTAL AND CALCULATED THERMODYNAMIC PARAMETERS OF PAIRWISE HYDROPHOBIC BOND FORMATION BETWEEN SIDE CHAINS AT 30°

	Experimental			*Calculated*		
Alkyl side chain	ΔF (*kcal/mole*)	ΔH (*kcal/mole*)	ΔS (*e.u.*)	ΔF (*kcal/mole*)	ΔH (*kcal/mole*)	ΔS (*e.u.*)
Methyl	−0.75	−0.49	4.0	−0.69	−0.56	4.1
Ethyl	−0.99	−1.29	7.6	−1.03	−1.46	7.3
Propyl	−1.38	−1.63	10.2	−1.46	−1.94	9.8

as the heat of dimerization of CI Acid Orange 7. This value is relatively high, and as there is little likelihood of polar bonds being formed between dye molecules, nonpolar bonding between aromatic nuclei has been favored. The mean heat of dissociation for the dimers in the case of CI Mordant Violet 5 in the range of 25°–60° has been found to be 9.2 kcal/mole.[19] With reactive dyes, values obtained for energy of association are quite different for the original and hydrolyzed dyes.[21,22] Whereas for both CI Reactive Violet 4 and Reactive Blue 7, free energy for dimerization is of the order of 5–6 kcal/mole, with hydrolyzed CI Reactive Blue 7 the corresponding value is 14–16 kcal/mole in the temperature range of 30°–90° (Table III).

TABLE III

THERMODYNAMIC FUNCTIONS OF PARENT AND HYDROLYZED REACTIVE DYES

Dye		*Heat of association* (*kcal/mole*)	*Free energy of dimerization* (*kcal/mole*)	*Entropy* (*e.u.*)
CI Reactive Blue 7	Unhydrolyzed	−6.22	−7.77	+5.15
	Hydrolyzed	−15.18	−6.81	−27.90
CI Reactive Blue 18	Unhydrolyzed	−4.61	−7.35	+9.14
	Hydrolyzed	−7.10	−8.02	+1.25
CI Reactive Blue 21	Unhydrolyzed	−8.33	−6.88	−4.88
	Hydrolyzed	−8.37	−9.06	+2.32

C. EFFECT OF ADDITIVES IN THE DYEBATH

Association of a dye in aqueous solution is markedly dependent on electrolyte concentration, temperature, and nature of the additives used. Different types of additive, viz., solvents or solubilizing agents, swelling agents or carriers, surfactants of different types, high polymeric com-

pounds, and hydrotropic agents, are commonly used during dyeing to effect wetting, dispersion, solubilization, or leveling of the dye. The main resistance which the forces of association have to overcome are the electrostatic repulsion forces due to charged dye ions. Addition of electrolytes results in creating an oppositely charged ionic atmosphere around the dye anions, and therefore the energy necessary for the approach of dye anions is reduced. Concentrated salt solutions weaken the hydration of the dipoles, thereby facilitating intermolecular bonding. As the electrolyte cation is capable of forming an ionic atmosphere of opposite charge around the dyestuff anion, only the cations are responsible for association of anionic dyes. This is found to be so, as shown by Bubser and Eichmanns,[83] who observed that in the case of different sodium halides with similar cations and dissimilar anions, the same extent of aggregation is observed with CI Direct Blue 1. The effect of electrolyte cations has been found to depend on the true ionic concentration, which in turn is dependent on the ionic mobility. Sivaraja Iyer and Singh[48] from recent polarographic studies of several direct dyes have also shown that when comparison is made of the aggregation behavior in the presence of NaCl and KCl, the latter electrolyte has been found to have greater influence on dye aggregation than the former, as the critical dye concentration for aggregate formation is shifted to a much lower value when KCl is added to the dye solution. This has been attributed to the greater solvating and water-structure-breaking effect of potassium as compared with that of sodium. In case of CI Acid Orange 52, however, solubility is also influenced by the electrolyte anions, the order of the decreasing effect being Cl, Br, NO_3, and CNS. Studies in the aggregation tendencies of acid dyes in ammonium nitrate solution reveal that the salting-out tendency is not related essentially to the proportion of solubilizing group in the dye molecule.[84]

The aggregating effect resulting from the addition of electrolyte to bath can be nullified by use of alcohols. The hydrotropic effect of alcohol has been attributed to the fact that the hydrophobic portion of the molecule gets oriented towards the dyestuff aggregates while the hydrophilic portion gets projected into the solvent water where it gets solvated and brings about dissolution of the aggregates. The hydrotropic effect of alcohols increases with the length of the hydrophobic portion in the molecule.[83]

Evidence of interaction between direct dyes and hydrophilic organic compounds, viz., pyridine, monoethanolamine, triethanolamine, diethylamine, Cellosolve, etc., has been put forth from the results of

[83] W. Bubser and H. Eichmanns, *Palette* **20**, 20 (1965).

[84] K. N. Davies and J. Whetstone, *J. Chem. Soc., London* p. 865 (1954).

refractive index measurements, electrophoretic mobility, and diffusion.[85–88] The presence of these solvents in the bath causes an appreciable decrease in the degree of aggregation of direct dyes. However, at higher concentrations of these compounds, there is an increase in the particle size of the dye due to increased solvation. These compounds bring about a reduction in the electrophoretic mobility of direct dyes which has been attributed to a decrease in the negative charge on the dye anions resulting from the solvation of the molecules by these hydrophilic compounds and not to an increase in the degree of aggregation. In the case of basic dyes, the degree of association depends to a great extent on the nature of the solvent.[89–91] Considerable aggregation is observed with ethylene glycol and glycerol, but aggregation in formamide, acetonitrile, and acetone is comparatively lower. Simple alcohols show intermediate behavior between these two groups of compounds, and it is suggested that the hydroxyl group in the solvent molecule plays a substantial role in the aggregation of the dissolved dye. Urea decreases the self-association of basic dyes,[80] and in the case of a homologous series of carboxylic acid amides, association of basic dyes has been found to decrease with increasing molecular weight of the hydrotropic compound.[91] Addition of polymeric compounds like starch and hydroxyethyl cellulose brings down the effective dyebath concentration due to adsorption of direct dyes, for which these compounds have considerable substantivity.[92,93] Although CI Acid Red 88 dissociates to anionic monomer and dimer in dilute acetic acid solution, aggregation of dye anion increases by addition of vinyl polymers, viz., polyvinyl pyridine, polymethylvinyl pyridine, and polyvinyl pyrrolidone, to the bath, the extent of aggregation depending on the basicity of the vinyl polymer.[94] At low concentrations, urea, formamide, and

[85] B. N. Mel'nikov and P. V. Moryganov, *Kolloid. Zh.* **21**, 618 (1959); *CA* **55**, 4965d (1961).

[86] B. N. Mel'nikov and I. B. Kotova, *Izv. Vyssh. Ucheb. Zaved., Tekhnol. Tekstil'noi Prom.* No. 4, p. 89 (1962); *CA* **58**, 3525g (1963).

[87] B. N. Mel'nikov and N. A. Batunova, *Izv. Vyssh. Ucheb. Zaved., Tekhnol. Tekstil'noi Prom.* No. 3, p. 107 (1966); *J. Text. Inst.* **57**, A892 (1966).

[88] N. A. Batunova and B. N. Mel'nikov, *Kolloid. Zh.* **32**, 650 (1970); *CA* **73**, 132244z (1970).

[89] Kh. L. Arvan, *Izv. Akad. Nauk SSSR, Ser. Fiz.* **20**, 443 (1956); *CA* **51**, 725h (1957).

[90] Kh. L. Arvan and N. E. Zaitseva, *Opt. Spectrosk.* **10**, 272 (1961); *CA* **55**, 15064f (1961).

[91] D. Wagner, *Text.-Prax.* **24**, 310 and 383 (1969).

[92] Y. Suda and T. Shirota, *Sen-i Gakkaishi* **17**, 414 (1961); *CA* **55**, 17014a (1961).

[93] Y. Suda, H. Ujikawa, and T. Shirota, *Sen-i Gakkaishi* **19**, 51 (1963); *CA* **63**, 1931e (1965).

[94] Y. Takase and T. Ogawa, *Sen-i Gakkaishi* **18**, 720 (1962); *CA* **62**, 13304a (1965).

methanol decrease the tendency of acid dyes to aggregate in aqueous solution due to loosening of the hydrophobic bonding, but at higher concentrations, these compounds affect the solvent properties and cause precipitation of the dyes.[95]

Considerable interest has been shown in the study of interaction of surface-active agents with dyes, as such an interaction modifies the dyeing behavior considerably. Evidence of interaction between dyes belonging to different classes and different types of surfactant is available from studies in viscosity,[96] diffusion,[97] conductivity,[98,99] surface tension,[100] surface-film formation,[101] and spectroscopy.[102–107] Sodium lauryl sulfate forms complexes with azo dyes, as is observed from the shift in the absorption maxima to longer wavelengths. Such complex formation is not observed in the case of highly hydrophilic dyes.[106] However, with some dyes this compound acts as an electrolyte causing association of the dye. Direct dyes have been found to interact with nonionic surface-active agents, and in each case absorption maxima shift to longer wavelength on increasing concentration of the surfactant. For effective complex formation, the hydrophobic group in the surfactant should be oleyl or nonylphenol and the hydrophilic portion should contain 15–25 moles of ethylene oxide.[108] Most pronounced changes on addition of nonionic surfactants are observed with CI Acid Blue 15, 116, and 120; Direct Violet 12; and Acid Violet 17.[102] With CI Direct Red 2, interaction with poly(oxyethylene) oleyl ether takes place at a concentration lower than the critical micelle concentration (c.m.c.), while with sodium lauryl sulfate, this dye interacts at the c.m.c. In the case of poly(oxyethylene) nonylphenol ether sulfate, interaction is possible at concentrations both above and below c.m.c.[96]

[95] W. U. Malik and S. M. Saleem, *J. Oil Colour Chem. Ass.* **52**, 551 (1969).

[96] S. Kuwabara and T. Shirota, *Kogyo Gijutsuin Sen-i Kogyo Shikensho Kenkyu Hokoku* **81**, 27 (1967); *CA* **68**, 79815e (1968).

[97] B. R. Craven and A. Datyner, *J. Soc. Dyers Colour.* **79**, 515 (1963).

[98] K. Yamaki, *Kogyo Kagaku Zasshi* **65**, 1854 (1962); *CA* **58**, 9272a (1963).

[99] M. Mitsuishi, G. Aida, and E. Kosuge, *Sen-i Gakkaishi* **20**, 720 (1964); *CA* **63**, 5810c (1965).

[100] M. J. Schwuger, *Kolloid-Z. Z. Polym.* **240**, 872 (1970).

[101] M. Muramatsu, *Bull. Chem. Soc. Jap.* **32**, 114 (1959).

[102] Y. Nemoto, *Kogyo Kagaku Zasshi* **62**, 542 (1959); *CA* **57**, 13919f (1962).

[103] Y. Nemoto and T. Imai, *Kogyo Kagaku Zasshi* **62**, 1286 (1959); *CA* **57**, 14460f (1962).

[104] R. Haque and W. U. Malik, *J. Phys. Chem.* **67**, 2082 (1963).

[105] K. Tori and T. Nakagawa, *Kolloid-Z.* **191**, 42 (1963).

[106] M. Mitsuishi, *Sen-i Gakkaishi* **20**, 656, 662, and 715 (1964); *CA* **63**, 5809d (1965).

[107] W. U. Malik and S. P. Verma, *J. Phys. Chem.* **70**, 26 (1966).

[108] M. Sekido and Y. Tanaka, *Sen-i Gakkaishi* **19**, 974 (1963); *CA* **63**, 5808a (1965).

Interaction between CI Acid Blue 120 and nonionic surfactants, polyethylene glycol, and polyvinyl alcohol has been explained on the basis of stoichiometric reversible equilibrium, and several thermodynamic parameters have been calculated. This equilibrium has been found to be governed by the molecular weight of surfactant, temperature, and the hydrophil–lypophil balance in the surfactant.[103] In all cases, the extent of interaction has been shown to decrease with an increase in temperature.[96] Complex formation between acid or basic dyes and different types of surfactant has been studied employing conductivity measurements and various parameters, viz., equilibrium constant, chemical potential of complex formation, heat of reaction, and entropy change.[98]

Cationic dyes suitable for polyacrylonitrile fibers form addition products with different types of anionic surfactant.[109] During the study of interaction between anionic surfactants with alkyl chains of different length and CI Basic Red 1 and also between an anionic dye, CI Acid Red 87, and cationic surfactants having gegenions of different degrees of hydration, Kondo *et al.*[110,111] have observed the phenomena of flocculation and resolubilization of dye by the surfactant. In the case of anionic surfactants and cationic dye, the concentration range of surfactant over which flocculation takes place decreases with increasing length of alkyl chain, while with anionic dye and cationic surfactants, the flocculation limit increases with an increasing degree of hydration of the surfactant ion. Deflocculation has been shown to result from micellar formation between dye–surfactant complex and the ions of the surfactant. Flocculation and deflocculation behavior depends on the reasonance structure of the dye.

The interaction between dye and soap has been explained on the basis of the formation of double salts or complexes and the presence of soap can lead to a restraint on the tendency for molecular rearrangement in the dye solution so that the specific nature of dye in changing its color is not realized. Generally, the amount of dye bound per molecule of soap is less than that in the case of protein–dye interaction, which can be attributed to interaction with ionic micelles.[107] In the case of leuco vat dyes, aggregation behavior is considerably modified by addition of cetyl octadeca(oxyethylene),[112] and effects of pyridine, triethanolamine, and octylphenol–ethylene oxide condensate additions

[109] W. Beckmann, *Vortr. Orig. Int. Kongr. Grenzflaechenakt. Stoffe. 3rd*, 1960, Vol. 4, p. 20 (1961); *CA* **57**, 12754b (1962).

[110] T. Kondo and K. Meguro, *Bull. Chem. Soc. Jap.* **32**, 267 (1959).

[111] T. Kondo, K. Meguro, and H. Nito, *Bull. Chem. Soc. Jap.* **32**, 857 (1959).

[112] J. Wegmann, *Melliand Textilber.* **48**, 183 (1967).

to the leuco vat dyebath are similar to those observed with direct dyes.[113]

Regarding the mechanisms underlying interaction of ionic dyes and surface-active agents, there exist certain controversies. It has been suggested by some workers that the interaction is between the anionic sulfonate groups in the dye molecule and the hydrophilic portion of the surfactant (charged oxonium groups in ethylene oxide chain).[114,115] On the other hand, from spectrophotometric and diffusion measurements, Craven and Datyner[116,117] have given evidence of interaction between acid anthraquinone wool dyes and nonionic surfactants, and have observed that the extent of interaction increases with the hydrophobicity of the dye molecule, particularly when nonpolar aliphatic side chains are present, but decreases due to the presence of solubilizing sulfonate groups in the dye molecule. Complex formation, according to these workers, has been attributed to the interaction of the hydrophobic part of the dye molecule with the hydrophobic part of the surfactant, giving rise to mixed micelles. This deduction has been further substantiated from the measurements of cloud points and c.m.c. of the surfactants in aqueous solution containing acid dyes. The contribution of dipole forces and van der Waals attraction forces has been emphasized in the interaction of CI Direct Red 28 and monolayers of octadecylamine, cetyl alcohol, ethyl stearate, etc., spread on substrate containing the dye. Under certain conditions where the dye molecules acquire a negative charge and the film a positive charge, the possibility of ion–ion attraction can also exist.[101]

In the dyeing of hydrophobic fibers with nonionic dyes from aqueous dispersion, the presence of dispersing agent in the dyebath is inevitable. These agents stabilize the dye suspension in the bath, and also in some cases act as restraining and retarding agents.[118] These compounds influence the kinetics of the dyeing process more than the thermodynamic aspects.[119] Attempts have also been made to study mixtures of water-soluble anionic polymers with the conventional wetting and dispersing agents as dispersing systems for the formulation of monoazo disperse dyes, and the results obtained are highly specific with respect

[113] M. I. Artym and P. V. Moryganov, *Tekhnol. Tekst. Prom.* No. 6, p. 107 (1959); *CA* **54**, 11485d (1960).

[114] W. Luck, *Melliand Textilber.* **41**, 315 (1960).

[115] K. Shinoda, T. Nakagawa, B. Tamamushi, and T. Isemura, "Colloidal Surfactants," p. 155. Academic Press, New York, 1963.

[116] B. R. Craven and A. Datyner, *J. Soc. Dyers Colour.* **77**, 304 (1961).

[117] B. R. Craven and A. Datyner, *J. Soc. Dyers Colour.* **83**, 41 (1967).

[118] C. L. Bird, P. Harris, and F. Manchester, *J. Soc. Dyers Colour.* **71**, 139 (1955).

[119] W. McDowell and R. Weingarten, *Melliand Textilber.* **52**, 716 (1971).

to a given combination of a dispersing agent and an anionic polymer.[120] Different mechanisms seem to be operative in solubilization of disperse dyes in the bath depending on the constitution of the dye and the dispersing agent, the quantity of the hydrotropic agent present in the bath, and the temperature.[121–123] With several nonionic surfactants, practically molecular dispersion of the dye can be obtained when the content of the dispersing agent in the aqueous phase exceeds the c.m.c., and it is postulated that the dye is stabilized molecularly and stoichiometrically in the hydrophilic portion of the surfactant micelle. At concentrations below c.m.c., an equilibrium exists between the dye, surfactant, and compounds produced by the interaction of the surfactant both with the dissolved dye and fine crystalline dye in the aqueous medium. Solubility values obtained experimentally for several nonionic dyes in solutions of hydrotropic agents at different concentrations are in conformity with the standard solubility rules governing the relationship between constitution and efficiency of hydrotropic compounds.[124] Different types of solubilization of nonionic dyes can be operative depending on the structure of the dye and the electrical character of the surfactant. In some cases, successive solubilization is observed where two dyes are solubilized step by step in the surfactant solution after the saturation of either of the two, while in others, simultaneous solubilization of the two dyes can take place.[125] As in the case of anionic dyes, urea also exerts a hydrotropic action on nonionic dyes and it penetrates the clusters of water around the disperse dye molecule.[126] With aromatic sulfonic acids, solubilization is effected through hydrophobic as well as van der Waals interactions.[127] The mechanism of solubilization appears to be different with different disperse dyes at various temperatures.[128]

[120] F. Wolf, U. Koch, and W. Homeister, *Melliand Textilber.* **52**, 456 (1971).

[121] S. Kuroiwa and H. Komiyama, *Sen-i Gakkaishi* **20**, 183 (1964); *CA* **62**, 13327a (1965).

[122] S. Kuroiwa and Y. Nakamura, *Sen-i Gakkaishi* **21**, 386 (1965); *CA* **64**, 11363e (1966).

[123] S. Kuroiwa and S. Ogasawara, *Sen-i Gakkaishi* **24**, 536 (1968); *CA* **71**, 42621z (1969).

[124] D. Wagner, *Melliand Textilchem.* No. 5, p. 131; No. 6, p. 163 (1965).

[125] F. Tokiwa, *Bull. Chem. Soc. Jap.* **43**, 939 (1970).

[126] A. Katayama, T. Matsuura, K. Konishi, and N. Kuroki, *Kolloid-Z. Z. Polym.* **202**, 157 (1965).

[127] A. Katayama, T. Takagishi, K. Konishi, and N. Kuroki, *Kolloid-Z. Z. Polym.* **206**, 162 (1965).

[128] S. Kuwabara, *Kogyo Gijutsuin Sen-i Kogyo Shikensho Kenkyu Hokoku* **83**, 19 (1968); *CA* **70**, 38869y (1969).

D. Behavior of Dyes in Mixtures

The behavior of dyes when applied from a mixture is in many cases quite different from their behavior when applied as individual components. One of the reasons for the additive or nonadditive behavior of dye mixtures appears to be the formation of complexes of dyes in the aqueous phase, and spectrophotometric as well as chromatographic techniques have been used to detect such complex formation. In the case of nonadditive mixtures, some relations appear between the nonadditive properties with respect to the amounts of dye adsorbed by the substrate and those in the absorption spectra of the dyes in aqueous solution. Interaction of dyes in mixtures is predominantly noticed with direct cotton dyes, and spectra of mixtures of several direct azo dyes in aqueous solution are different from the spectra that could be predicted on the basis of additivity of the spectra of individual dyes.

A critical evaluation of the interaction of direct, acid, and basic dyes in aqueous solution in terms of chemical constitution of dye has been carried out by Inscoe *et al.*[129] In general, features which are desirable in dyes to be substantive to cellulose also tend to favor interaction of dyes in mixtures. Disazo dyes derived from benzidine, *o*-dianisidine, or *o*-tolidine appear to be most active in dye interactions. In the case of mixtures in which both dyes are of this type or those in which one dye is of this type and the second is a direct dye which is a bisazostilbene, or a urea derivative, no interaction appears to take place. In benzidine-type dyes, the presence of amino groups in the end component is less favorable to dye interaction than hydroxyl groups. Lack of interaction in mixtures of dyes having sulfonic acid groups near the center of the molecule seems to arise from the electrical repulsion or the bulkiness of these groups producing a steric effect. Most of the monoazo dyes show no interaction with each other, and spectra obtained for mixtures of these dyes are generally additive. However, several of these dyes give slightly nonadditive spectra with direct bisazo dyes. Absorption spectra of mixtures of CI Direct Blue 1 and acid dyes from *p*-X-aniline → γ-acid where X is H, CH_3, OCH_3, Cl, or NO_2, have been found to be nonadditive, which is explained on the basis of interaction between the corresponding bands of the components.[130]

Mixtures of CI Direct Blue 1 and *p*-nitroniline → γ-acid show nonadditive behavior, and it is postulated that although a 1:1 complex is

[129] M. N. Inscoe, J. H. Gould, M. E. Corning, and W. R. Brode, *J. Res. Nat. Bur. Stand.* **60**, 65 (1958).

[130] Y. Tanizaki, T. Kobayashi, and N. Ando, *Bull. Chem. Soc. Jap.* **32**, 119 (1959).

formed at low concentration of the acid dye, higher complexes are possible in the presence of an excess of the acid dye. A mixture of CI Direct Blue 1 and sulfanilic acid → cresidine shows an additive spectrum when the ratio of the two dyes is about 1:5, but not when it is 1:40. On the other hand, a mixture of CI Direct Blue 1 and sulfanilic acid → cresidine → cresidine shows a nonadditive spectrum which is explained on the basis of formation of 1:1 complex over the range of concentrations studied.[131] Complex formation in the case of mixtures of CI Direct Yellow 12 with certain bisazo dyes can be considerably reduced by having terminal residues in the dye molecule which impart noncoplanarity to the structure and thereby sterically hinder complex formation.[132]

Among direct dyes, interaction between molecules of CI Direct Yellow 12 and Direct Blue 1 has been examined critically employing spectroscopic, chromatographic, and calorimetric methods.[133,134] From the changes in the absorption spectra of these dyes in aqueous solution, it has been proved that 1:1 and 1:2 complexes are formed, and the charge-transfer spectra from CI Direct Blue 1 and Direct Yellow 12 appear in the complexes. Free-energy change, enthalpy change, and entropy change for the complex formation of CI Direct Blue 1 and dyes of similar structure with CI Direct Yellow 12 have been obtained employing a special method of calculating the equilibrium constant. In the complex state, *o*-tolidine dyes appear to be more coplanar than in the free state. The effective auxochromic NH_2 and OH groups contribute to greater changes in the enthalpy and entropy, while the sulfonic acid dye has been found to contribute to entropy changes depending on the location of the group. The heat of formation of a 1:1 complex of CI Direct Yellow 12 and CI Direct Blue 1 has been measured by direct calorimetry, and the value found to be 15 kcal/mole. The formation of the 1:1 complex takes place to an appreciable extent in the aqueous solution even at 90°. Indirect evidence for the formation of 1:1, 1:2, 1:3, and 1:4 complexes between these two dyes is available, the formation of higher complexes being favored in the presence of excess of CI Direct Yellow 12 in the aqueous phase.[135] The strength of interaction between mixtures of specific direct dyes differs depending on the structure of the

[131] T. Kobayashi, Y. Tanizaki, and N. Ando, *Bull. Chem. Soc. Jap.* **32**, 675 and 680 (1959).

[132] T. Kobayashi, K. Saito, Y. Tanizaki, and N. Ando, *Bull. Chem. Soc. Jap.* **35**, 935 (1962).

[133] A. N. Derbyshire and R. H. Peters, *J. Soc. Dyers Colour.* **72**, 268 (1956).

[134] Y. Tanizaki, T. Hoshi, and N. Ando, *Bull. Chem. Soc. Jap.* **38**, 264 (1965).

[135] E. H. Daruwalla and G. G. Kulkarni, *Bull. Chem. Soc. Jap.* **37**, 1250 (1964).

dye, equilibrium constants in complex formation decreasing in the order: CI Direct Yellow 12–Direct Blue 1, Direct Red 2–Direct Blue 1, Direct Red 81–Direct Blue 71, Direct Red 81–Direct Blue 1 or Direct Red 2–Direct Blue 71, and Direct Yellow 12–Direct Blue 71.[136]

Mixtures of CI Basic Violet 1, Basic Blue 9, and Basic Red 13 with each other show interaction in aqueous solution, but in acetate buffer there is no evidence of interaction.[129] Interaction of CI Basic Yellow 4 and CI Basic Blue 9 in various solvents has been studied and the spectrum of such a mixture has been found to deviate from that expected from the summation of the two individual spectra of the dyes. The heat of formation of the complex between these dyes has been found to be 9 kcal/mole, which is higher than the energy of dimerization of CI Basic Blue 9 — about 6 kcal/mole.[137] Mixtures of cationic dyes CI Basic Blue 66 and 67 or CI Basic Orange 21 and 22 show evidence of interaction resulting from the formation of 1:1 complexes.[138]

With respect to reactive dyes, study of the absorption spectra of aqueous solutions of CI Reactive Orange 12 and Reactive Blue 2 in the presence or absence of alkali reveals an additive behavior indicating that these dyes do not react with each other.[139] Evidence of interaction of disperse dyes in ethanol, hexane, or solutions of dispersing agents is also available. Bird and Rhyner[140] found that absorption spectra of solutions of certain disperse dye mixtures in Ultravon W were additive while with other mixtures, the spectra were markedly different from those of the individual components. Interaction between certain pairs of disperse dyes in an aqueous bath has also been noticed by Andreeva and Belen'kii.[141] Recent studies employing solubility, mixed melting point, conductivity, and chromatographic measurements reveal that in the case of several disperse dyes, interaction does take place and this is reflected in the uptake of these dyes by synthetic fibers when they are applied as binary mixtures.[142–144] Interaction between certain disperse

[136] Z. Morita and M. Sekido, *Bull. Chem. Soc. Jap.* **38**, 2041 (1965).

[137] Kh. L. Arvan, *Dokl. Akad. Nauk SSSR* **121**, 123 (1958); *CA* **54**, 25835e (1960).

[138] C. Yatome and Y. Takase, *Gifu Daigaku Nogakubu Kenkyu Hokoku* No. 19, p. 53 (1969); *CA* **71**, 31251c (1969).

[139] T. A. Kon'kova and L. I. Belen'kii, *Tech. Text. Ind. USSR* No. 1, p. 99 (1967).

[140] C. L. Bird and P. Rhyner, *J. Soc. Dyers Colour.* **77**, 12 (1961).

[141] L. G. Andreeva and L. I. Belen'kii, *Tech. Text. Ind. USSR* No. 3, p. 109 (1964).

[142] K. Hoffmann, W. McDowell, and R. Weingarten, *J. Soc. Dyers Colour.* **84**, 306 (1968).

[143] W. McDowell, R. Weingarten, and K. Hoffmann, *Melliand Textilber.* **50**, 1340 (1969).

[144] E. H. Daruwalla, R. M. Patel, and K. S. Tripathi, *Proc. Hung. Text. Conf., 18th, 1970* Vol. 2, p. 173 (1971).

dyes has also been observed in the presence of benzoic acid or benzyl alcohol as carriers.[145,146] Although it has been suggested that interaction is most favorable with disperse dyes of similar structure,[145,146] recent studies[144] reveal that there is no correlation between chemical constitution of disperse dyes and their behavior regarding interaction with each other. In some cases, dyes of similar structure as well as those with dissimilar structure show interacting behavior, and the case is the same with noninteracting dyes also.

Interacting or noninteracting behavior of dyes in aqueous solution is largely governed by several factors, viz., the presence of additives in the bath, dye concentration, etc. The presence of alcohol, pyridine, or nonionic compounds (polyethylene oxide condensates) prevents interaction, while the presence of inorganic electrolytes or an increase in dye concentration appears to increase the extent of interaction. Mixtures of CI Basic Violet 1, Basic Blue 9, and Basic Blue 13 with each other give nonadditive spectra in aqueous solutions, but in acetate buffers, the spectra are additive. On the other hand, the spectra of mixtures of these basic dyes with certain direct azo dyes show marked effects of interaction, both with and without acetate buffer.[129] With certain mixtures, with an increase in the concentration, one component brings about changes in the absorption band associated with the other component and these changes are similar to those produced by increasing the concentration of electrolyte in the bath. The action of nonionic surface-active agents on mixtures of dyes is supposed to be one of deflocculation of the complex of dyes and formation of complex between dye and surfactant.[147] The interaction of dyes in mixtures can also have a marked influence on the kinetics of dyeing.

E. Determination of Activity Coefficients

In ionic solutions, departure from ideal behavior due to electrostatic interactions between the charged ionic species is usually discussed in terms of Debye-Hückel theory, which relates the activity coefficient of an ion in a given solution to the additional free-energy contribution resulting from electrical effects. The following expression can be derived for the activity coefficient, f_i of a given ionic species

$$\log f_i = -\frac{AZ_i^2\mu^{1/2}}{1 + Ba_i}$$

[145] M. J. Schuler and W. R. Remington, *Discuss. Faraday Soc.* **16**, 201 (1954).

[146] J. Rais and M. Jagrova, *Int. Kolorostenkongr., 4th, 1962* p. 68 (1962); *CA* **59**, 15428e (1963).

[147] M. Mitsuishi, *Sen-i Gakkaishi* **20**, 665 (1964); *CA* **63**, 5809g (1965).

In this expression, A and B are constants, Z_i is the valency of the ion, μ is the ionic strength of the solution, and a_i is the ion size parameter representing the average distance of the closest approach between two ions. At infinite dilution, the above equation can be approximated to a limiting form given by the expression

$$\log f_i = -AZ_i^2\mu^{1/2}$$

The determination of activity coefficients for dyes is of considerable importance in relation to treatment of the thermodynamic parameters for dye aggregation and dye adsorption processes.

Ionic activity coefficients of several direct and acid dyes (CI Direct Yellow 12, Direct Blue 168, Acid Orange 7 and 52, and Acid Red 18) under different conditions of dye and electrolyte concentration have been determined employing solubility[10,148,149] and isopiestic measurements[150] and through use of a reversible sodium ion electrode[34] or semipermeable membranes.[151] Attempts have been made to correlate the experimentally obtained values with those calculated theoretically employing appropriate forms of the Debye-Hückel equation mentioned above. CI Direct Yellow 12 has been found to behave in dilute solutions as a normal 1:2 valence type of strong electrolyte,[10] while CI Direct Blue 1 behaves as a normal electrolyte between 1:2 and 1:4 valence type.[151] From the results of the comparison of the measured and calculated values of activity coefficients, it is concluded that the monobasic dye Orange 8 behaves as a typical 1:1 valence type of electrolyte up to the solubility limit. On the other hand, CI Acid Orange 7, which also is a monobasic dye, behaves in a similar manner only at concentrations below $10^{-2}\,M$, but at concentrations above this, dimers are formed, and it behaves as a typical 1:2 electrolyte.[34] In the thermodynamic treatment of dyeing, when mean activities are substituted for concentrations, a significant improvement in the evaluated thermodynamic quantities for dye adsorption is observed.[152]

III. State of Dye in Substrate

A. Monomolecular and Multilayer Adsorption

The state of dye in fiber substances has received considerably less attention than other aspects of the chemistry of dyeing, and only a few

[148] M. Mitsuishi and G. Aida, *Sen-i Gakkaishi* **17**, 665 (1961); *J. Soc. Dyers Colour.* **77**, 580 (1961).

[149] M. Mitsuishi and G. Aida, *Sen-i Gakkaishi* **18**, 288 (1962); *CA* **57**, 12753i (1962).

[150] S. Chadwick and S. M. Neale, *J. Polym. Sci.* **28**, 355 (1958).

[151] D. R. Hardisty and S. M. Neale, *J. Polym. Sci.* **46**, 195 (1960).

[152] S. R. Sivaraja Iyer and N. T. Baddi, *in* "Contributions to Chemistry of Synthetic Dyes and Mechanism of Dyeing," p. 36. University of Bombay, 1967.

direct approaches have been made at quantitative evaluation of the state of dye aggregation in the substrate. In many cases, the results are controversial, particularly due to differences in the techniques of measurement or to the interpretation being of a very generalized nature. Direct evidence is lacking because the state of aggregation is too low to be detected by established methods. Using regenerated cellulose and gelatin as models for cellulosic and protein fibers, Campbell *et al.*[153] found that in the case of CI Direct Red 2, the single absorption peak corresponding to the monodisperse state shifted to shorter wavelength with an increase in association of dye, indicating that the dye was present partly as monodisperse and partly in an aggregated state in both these substrates. The disaggregating effect of phenol could readily be observed in these optical studies.

Similar to the behavior in solution, most of the dyes show absorption bands when adsorbed in cellulose or polyvinyl alcohol, and the shorter waveband increases in height relative to the longer with an increase in concentration.[154] A spectrophotometric investigation on films of regenerated cellulose and polyvinyl alcohol with dyes belonging to different classes lends support to the presence of dye in the substrate as aggregates situated as micelles.[13,155–158] Weissbein and Coven[159] have developed a technique whereby ultrathin dyed film could be obtained for electron-microscopic examination. At a resolution limit of 30° Å, no aggregate formation could be detected with CI Direct Blue 1 in viscose, while with CI Direct Blue 78, dense clusters of minute dye particles could be observed, although the former dye did show aggregation behavior in spectroscopic studies. X-Ray evidence with respect to CI Direct Blue 1 is also in favor of nonaggregation even at high concentration of the dye in the substrate.[160] It is, however, very likely that in both electron-microscopic and X-ray studies, crystallites under a certain particle size may not be detected.

X-Ray investigations have also been carried out on acidic oxy-

[153] D. S. E. Campbell, D. Cathcart, C. H. Giles, and S. M. K. Rahman, *Trans. Faraday Soc.* **55**, 1631 (1959).

[154] K. Fujino and F. Fujimoto, *Sen-i Gakkaishi* **17**, 172 (1961); *CA* **55**, 9881g (1961).

[155] H. Bach, E. Pfeil, W. Philippar, and M. Reich, *Angew. Chem.* **75**, 407 (1963).

[156] B. N. Mel'nikov, I. B. Blinicheva, and S. A. Zabrodin, *Tech. Text. Ind. USSR* No. 2, p. 93 (1968).

[157] K. Tsuda, K. Nishida, H. Watanabe, and T. Hirata, *Kolloid-Z. Z. Polym.* **240**, 827 (1970).

[158] T. Ohtsu, K. Nishida, K. Nagumo, and K. Tsuda, *Kolloid-Z. Z. Polym.* **249**, 1077 (1971).

[159] L. Weissbein and G. E. Coven, *Text. Res. J.* **30**, 58 and 62 (1960).

[160] E. I. Valko, *Text. Res. J.* **27**, 883 (1957).

celluloses and alginic acid dyed with basic dyes.[161] In the case of dyed acidic oxycellulose, the diffraction pattern of undyed oxycellulose appeared in addition to a line which was identical with the one which appeared in the diffraction pattern of the dye itself. An explanation of this extra pattern has been put forth which assumes that the dye cation bound to the carboxyl groups on adjacent chains can associate through van der Waals forces forming a diffraction system. Electron-microscopic studies of the physical state of reactive dyes in cellulose and disperse dyes in hydrophobic fibers lend support to the presence of aggregates of dyes in these substrates.[162] Indirect evidence of the presence of acid dyes as micelles in wool has been obtained from adsorption of vapors of water and lower aliphatic alcohols.[163] The possibility of aggregates of leuco vat dyes being present in cellulose substrate has been indicated by Wegmann.[164] Kratky *et al.*,[165] employing a low-angle X-ray scattering technique, have shown that 1,4-diaminoanthraquinone exists as compact aggregates in cellulose acetate films. On the other hand, absorption spectra of films of high polymers containing CI Disperse Violet 1 indicate that dye located in the interior of the film is molecularly dispersed while in some substrates that located near the outer surface is aggregated.[166] When a nonionic surfactant is used in the bath during dyeing, the resultant dyed films show the presence of molecularly dispersed dye. Similarity in the absolute heat of association of a disperse dye with cellulose acetate and in the dye crystal has been interpreted as evidence for dye aggregation in the substrate.[167]

Recently, direct evidence for the aggregated state of dye molecules in a cellulose substrate in the case of direct, reactive, azoic, and vat dyes became available from the results of the measurement of the specific surface of dyes when present in cellulose through selective adsorption of *p*-nitrophenol from water.[168] Direct cotton dye of low lightfastness and a reactive dye gave specific surface values which were much too low for a monolayer. They appeared to exist in the substrate in the form of multilayers, and a direct dye of high lightfastness appeared to be present as a three-dimensional aggregate of dimers. Indirect evidence for the

[161] J. O. Warwicker, *J. Text. Inst.* **49**, T148 (1958).

[162] D. P. Johari, *Text. Res. J.* **40**, 575 (1970).

[163] C. H. Giles, H. M. Elder, and A. H. Tolia, *Text. Res. J.* **34**, 839 (1964).

[164] J. Wegmann, *Amer. Dyest. Rep.* **51**, P276 (1962).

[165] O. Kratky, P. Mittelbach, and A. Sekora, *Kolloid-Z.* **200**, 1 (1964).

[166] S. Kuroiwa, A. Horiguchi, and H. Komiyama, *Sen-i Gakkaishi* **20**, 52 (1964); *CA* **62**, 13326g (1965).

[167] T. G. Majury, *J. Soc. Dyers Colour.* **70**, 442 (1954).

[168] C. H. Giles, R. Haslam, A. R. Hill, and A. S. Trivedi, *J. Appl. Chem. Biotechnol.* **21**, 5 (1971).

state of dye in a substrate could be obtained from light-fading behavior; such behavior of a dye in a specific fiber substance, among other factors, has been found to depend to a considerable extent on the state in which the dye exists in the fiber. Giles and Shah[169] have made a critical evaluation of the fading behavior of a large number of dyes belonging to different classes when they are present in a variety of substrates. Except in a very few cases, the results of these studies reveal that dyes are present in an aggregated state with a wide range of particle-size distribution. These deductions have been substantiated by the results of studies in which disaggregating agents are used to effect breakdown of the aggregates in the substrate and also when aggregation is controlled by changes in the substrate structure. In several cases, a correlation between the results obtained by the use of electron microscopy and those from fading rate curves, regarding the state of dye in a substrate, could be established.[170] Fading rate curves also reveal that several fluorescent brightening agents are present in cellulose in the aggregated form.[171] CI Basic Blue 9 in films of ethyl methyl cellulose or in a dyed film of gelatin is present as molecularly dispersed and aggregated dye, as is evident from the fact that during irradiation, the aggregated dye fades more slowly than the molecularly dispersed dye.[172]

Contrary to the concept of dye aggregation in substrate, several investigators are in favor of assuming monomolecular adsorption of dye in the fiber substance, at least at lower dye concentrations, and they have put forward direct and indirect evidence for considering such a state. Daruwalla *et al.*[173,174] have shown from heat of dyeing measurements that over an appreciable range of dye concentration in different cellulosic fiber substances and in polyvinyl alcohol fiber, values of heats of dyeing remain constant (Fig. 2). This behavior has been explained on the basis that within this range the dye formed a monomolecular layer in the substrate, but when the concentration of adsorbed dye was increased further, multimolecular layers were formed, and as the energy released in sorbate–sorbate interaction is less than that released in sorbate–sorbent interaction, the heat of dyeing decreased subsequently. It could, however, be argued that such behavior could be explained equally satisfactorily by assuming adsorption of specific dye aggregates rather than single dye ions. In view of the fact that the ratio of the

[169] C. H. Giles and C. D. Shah, *Trans. Faraday Soc.* **65**, 2508 (1969).
[170] D. P. Johari, *Amer. Dyest. Rep.* **58**, 33 (1969).
[171] M. Hayashi, *Kogyo Kagaku Zasshi* **63**, 118 (1960); *CA* **56**, 3676c (1962).
[172] D. S. E. Campbell and C. H. Giles, *J. Soc. Dyers Colour.* **74**, 164 (1958).
[173] E. H. Daruwalla and A. P. D'Silva, *Text. Res. J.* **33**, 40 (1963).
[174] E. H. Daruwalla and A. S. Trivedi, unpublished work.

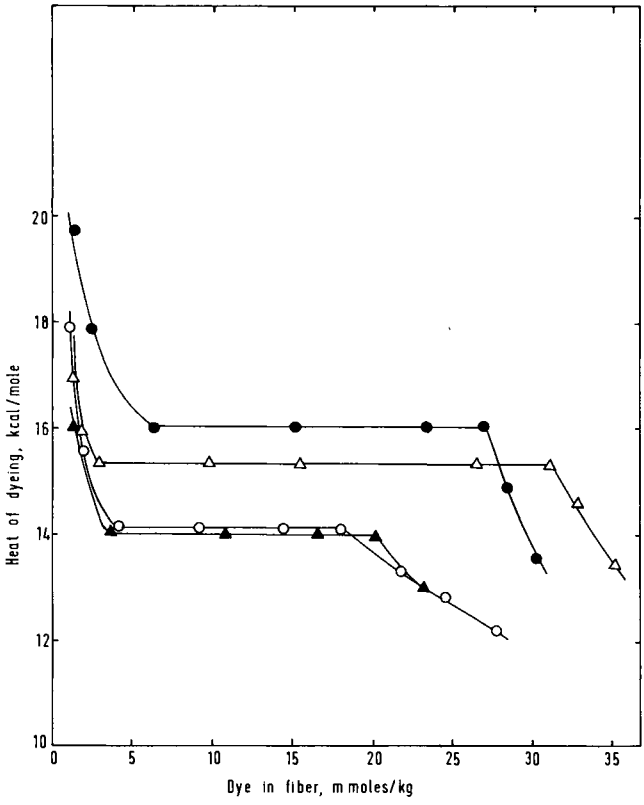

FIG. 2. Heats of dyeing of CI Direct Yellow 12 on different fibers: ○, cotton; ●, viscose; △, cuprammonium; ▲, polyvinyl alcohol.

saturation values in different fiber substances are practically the same irrespective of the dye used, it is difficult to expect the same state of aggregation in the case of all the dyes, as the dyes examined have dissimilar structures and are known to have different aggregation tendencies.

Positive evidence is forthcoming from the results of adsorption of CI Direct Blue 1 on formaldehyde cross-linked cellulosic fibers. When the limiting co-area per adsorbed dye molecule is calculated from the saturation value and the specific area determined from nitrogen adsorption of cross-linked fiber, a value of 370 $Å^2$ is obtained which is in close agreement with the value of 366 $Å^2$ per dye molecule, calculated from the projected area of the model of the dye with the dye molecule lying flat on a plain surface.[175] With disperse dyes in hydrophobic fibers and

[175] S. R. Sivaraja Iyer and L. S. Rao, *Text. Res. J.* **38**, 668 (1968).

several reactive dyes in cellulose, fading rate curves support the concept of monomolecular dispersion.[176] Wegmann,[177] on the basis of spectrophotometric measurements of CI Disperse Violet 1 in solution and on hydrophobic fibers, also favors unimolecular distribution of dye in the substrate, especially when dyeing from a monomolecular solution of the dye. However, when dyeing is carried out from an oversaturated solution or from the vapor phase, a multilayer aggregated state of dye is possible. Merian[178] has shown that certain specially synthesized disperse dyes exhibit phototropism on irradiation when applied to polyamide fiber and has concluded that disperse dyes are monomolecularly distributed in hydrophobic fibers, although there might be differences in the mode of physical adsorption. Measurements of the dichroic constants of films of regenerated cellulose and polyvinyl alcohol dyed with certain direct dyes reveal that up to a certain stage of dye concentration in the substrate, dye molecules appear to be adsorbed tightly and regularly on the polymer film in a state of monomolecular layer formation.[179,180] Comparison of the absorption spectra of CI Direct Red 28, Direct Red 7, Basic Violet 10, and Basic Green 4 in aqueous solution and on regenerated cellulose film reveals that in the film these dyes are adsorbed to form a monomolecular layer until concentrations are about 150 times higher than those at which aggregation in solution is detected.[181] Points arising from the First Perkin Discussion[182] on recent advances in dyeing theory favor the concept, based on most of the experimental data available at present, that the prime process in the dyeing of cellulosic fibers is the adsorption of single dye molecules.

If the dye molecules are adsorbed on accessible surfaces in fiber substances, the next problem is to obtain values for such accessible surfaces in different substrates, and also to assess whether all the sites on surfaces have equal activity or whether there are present in the fiber sites of differential energy. Very few attempts have been made to determine the total surface area of cellulose from dyeing experiments. Daruwalla and his colleagues[173,174] have obtained from heat of dyeing measurements values of 21–38, 32–58, 38–66, and 35–45 m^2/g as the

[176] E. H. Daruwalla and R. M. Patel, unpublished work.

[177] J. Wegmann, *Can. Text. J.* **79**, 37 (23 Nov. 1962).

[178] E. Merian, *Text. Res. J.* **36**, 612 (1966).

[179] K. Fujino, F. Fujimoto, and K. Fujita, *Sen-i Gakkaishi* **15**, 490 (1959); *CA* **53**, 15571h (1959).

[180] Y. Kobayashi and S. Okajima, *Kogyo Kaguku Zasshi* **62**, 1905 (1959); *CA* **57**, 12715g (1962).

[181] O. Manabe, K. Nanjo, S. Matsubara, M. Nakagaki, and H. Hiyama, *Kagaku To Kogyo (Osaka)* **35**, 12 (1961); *CA* **55**, 21792i (1961).

[182] First Perkin Discussion, *J. Soc. Dyers Colour.* **86**, 454 (1970).

surface available for dyeing in cotton, viscose, cuprammonium and polyvinyl alcohol fibers, respectively, depending on the size and shape of the dye molecules adsorbed, and assuming dye molecules lying flat with the aromatic rings on top of monomer units in the polymer chain molecules. Baddi and Sivaraja Iyer[10] obtained values of 116, 205 and 192 m^2/g for cotton, viscose, and cuprammonium rayon, respectively, employing the nitrogen adsorption technique for water-swollen uncollapsed fibers. Calorimetric studies of heats of wetting of cotton and viscose,[183] measurement of zeta potentials for cotton and viscose in electrolyte solutions,[184] and studies in the negative adsorption of chloride ions on cotton and viscose[185] also gave surface area values similar to those obtained by nitrogen adsorption technique. Thus, although the surface areas available for adsorption of dyes are lower than those for adsorption of nitrogen or water vapor, possibly due to the differences in the size and shape of adsorbent, appreciable surface in cellulosic fiber is available for dye adsorption provided the fibers are in a swollen state in the aqueous phase. Zeta-potential measurements and surface charge density have also been employed to calculate the area covered by adsorbed dye molecules in different fiber substances, and also to detect changes in available surface area in the substrate after different physical and chemical treatments.[186–190] The effective surface in dyeing of wool has been found to be considerably greater than that for several synthetic fibers, and in general, values of surface area for dye adsorption lie intermediate between those for adsorption of nitrogen and water vapor.[190]

The results of several investigators reveal that all the sites in cellulosic fiber substances are not of equal activity and there are present a limited number of sites for which dye has very high affinity. Daruwalla and D'Silva[173] found that values of heats of dyeing at low concentrations of dye in substrate were always higher than the normal values, irrespective of the direct dye used. Similar behavior was also observed in

[183] S. R. Sivaraja Iyer and N. T. Baddi, *Cell. Chem. Technol.* **3**, 561 (1969).

[184] S. R. Sivaraja Iyer and R. Jayaram, *J. Soc. Dyers Colour.* **87**, 338 (1971).

[185] B. I. Nemade, S. R. Sivaraja Iyer, and R. Jayaram, *Text. Res. J.* **40**, 1050 (1970).

[186] T. Suzawa and H. Takahashi, *Kogyo Kagaku Zasshi* **72**, 906 (1969); *CA* **71**, 113995n (1969).

[187] T. Suzawa and N. Kitagawa, *Kogyo Kagaku Zasshi* **73**, 1858 (1970); *CA* **75**, 37709n (1971).

[188] T. Suzawa and T. Mukai, *Kogyo Kagaku Zasshi* **73**, 2451 (1970); *CA* **74**, 65382y (1971).

[189] T. Suzawa, K. Nagareda, and Y. Mizuhiro, *Sen-i Gakkaishi* **27**, 108 (1971).

[190] V. F. Androsov, V. S. Bondarenko, K. I. Andreeva, and I. D. Tugai, *Tech. Text Ind. USSR* No. 2, p. 95 (1970).

case of polyvinyl alcohol fiber.[174] Katayama *et al.*[191] observed that Vinylon contained a small number of active sites and a large number of weaker sites for adsorption. It has been observed that the amount of a direct dye desorbed from a fiber varies with the microstructure of the fiber, the type of dye present, and the conditions of dyeing.[192] Different explanations, viz., the presence of small amounts of nitrogeneous matter, sites of ionic character, etc., have been put forward to explain the differences in activity of sites for adsorption, but it appears that in pure cellulose substance all the sites available for adsorption or reaction are not of equal energy. However, no conclusive evidence is at present available to indicate the presence of sites of unequal activity in cellulose, because it is possible to explain these results on the basis of nonuniform distribution of pores in the fiber structure, thereby leading to differences in the diffusion rate during desorption for the outer and inner regions of the fiber.[193]

B. Location of Dye and State of Orientation

Another controversy exists with respect to the state of the dye in the fiber substance. Some investigators are of the opinion that adsorption of dye, either as single ions or as aggregates, takes place on specific surfaces in the substrate, while others have put forward the view that dyes are present in the fiber in voids or empty spaces without any specific attachment with the molecules of the polymer.

Considerable evidence is available to reveal that dye–fiber attachment does take place. Giles and his co-workers,[194–198] from refractive index measurements, studies in monolayer formation, and surface viscometry, have shown that interaction between dyes and polymers does take place depending on the structure of the dye and the polymer. Changes in the internal energy of dye molecules as a result of their sorption by fibers have been observed in case of several dye–polymer systems.[199] Results of IR-spectroscopic measurements also reveal inter-

[191] A. Katayama, N. Kuroki, and K. Konishi, *Sen-i Gakkaishi* **15**, 1007 (1959); *CA* **54**, 7153d (1960).

[192] Y. Watanabe and K. Miyasaka, *Sen-i Gakkaishi* **13**, 765 (1957); *J. Text. Inst.* **49**, A679 (1958).

[193] P. W. Lange and E. Lindvall, *Sv. Papperstidn.* **57**, 235 (1954).

[194] M. M. Allingham, C. H. Giles, and E. L. Neustädter, *Discuss. Faraday Soc.* **16**, 92 (1954).

[195] F. M. Arshid, C. H. Giles, and S. K. Jain, *J. Chem. Soc.*, pp. 559 and 1272 (1956).

[196] A. Camaron, C. H. Giles, and T. H. MacEwan, *J. Chem. Soc.*, p. 1224 (1958).

[197] C. H. Giles, *Text. Res. J.* **31**, 141 (1961).

[198] C. H. Giles, V. G. Agnihotri, and A. S. Trivedi, *J. Soc. Dyers Colour.* **86**, 451 (1970).

[199] L. I. Belen'kii, T. V. Bromberg, and M. E. Kazanskaya, *Tr. Tsent. Nauch.-Issled. Inst. Khlopchatobumazh. Prom.* p. 115 (1958); *CA* **55**, 20440b (1961).

molecular bonding between hydroxyl groups in disperse dye molecules and functional groups in certain polymers.[200] Heats of dyeing are quite high so long as dye molecules are adsorbed on specific surfaces of the substrate, but subsequently when dye gets adsorbed on the already existing layer of dye on the polymer, the values decrease at a rapid rate.[173] The viscosity of aqueous solutions of polyvinyl alcohol increases on addition of CI Direct Red 28[201] and also the X-ray diagram of polymer is remarkably altered in presence of this dye, indicating interaction between the dye and the polymer.[202] Interaction between dyes and hydroxylated polymers in solution has been detected by Centola,[203] who suggested coordination complexes between dye and polymer through hydrogen bonding. The presence of methyl cellulose or polyvinyl alcohol displaces the height and position of the maximum wavelength of light adsorption of several direct dyes and the extent of displacement has been found to depend on the forces of attraction between polymer and dye.[204] Addition of direct dyes to aqueous solutions of methyl cellulose increases the viscosity of solution, and in some cases can even bring about flocculation of methyl cellulose. Schwertassek[205] observed that in the case of cellulose dyed with substantive or reactive dyes, the fiber substance adsorbed less iodine due to the hydroxyl groups in cellulose taking part in dye–fiber bonding. No such effect was observed in the case of cellulosic fibers dyed with vat dyes, as these dyes are not likely to be bonded to cellulose.

On the other hand, Bach *et al.*[155] have put forward evidence to show that no forces between direct dyes and cellulose are operative and dye is present in the substrate only as micelles. According to these investigators, dye penetrates cellulose in a monomolecular state, but subsequently is deprived of its water of solvation, and aggregation takes place. These aggregates are stable in the absence of water, and once aggregate formation has taken place, movement of dye inside cellulose is hindered. Brody[206] is of the opinion that in nylon 66 fiber, where the structural elements are similar to those of an imperfect crystal, dyeing with disperse and acid dyes takes place by filling the vacancies caused primarily due to chain folding, and the maximum dye-absorbing

[200] I. A. Naumova, B. E. Zaitsev, and L. I. Belen'kii, *Izv. Vyssh. Ucheb. Zaved., Khim. Khim. Tekhnol.* **12**, 314 (1969); *CA* **71**, 4409d (1969).

[201] K. Fujino and F. Fujimoto, *Sen-i Gakkaishi* **15**, 483 (1959); *CA* **53**, 15631h (1959).

[202] N. Okada and I. Sakurada, *Kobunshi Kagaku* **15**, 671 (1958); *CA* **54**, 14761h (1960).

[203] G. Centola, *Ann. Sci. Text. Belg.* **3**, 157 (1955); *CA* **52**, 21121f (1958).

[204] G. Centola, *Tinctoria* **52**, 341 (1955); *CA* **50**, 10413e (1956).

[205] K. Schwertassek, *Faserforsch. Textiltech.* **11**, 159 (1960).

[206] H. Brody, *Text. Res. J.* **35**, 895 (1965).

capacity of the fiber is governed by the packing requirements for a fixed free volume within the fiber. Very recent studies reveal that polyester fibers have structure with rodlike morphological units separated by narrow voids, and the accessibility as well as the location of disperse dye molecules is mainly governed by the size, amount, and tortuosity of these voids.[207] It is not certain whether the microscopic discontinuities or void spaces are initially present in synthetic fibers or are formed during dyeing under thermal treatments. In the case of polyamide and polyester fibers when these fibers are subjected to thermal treatments, specific changes take place in the substrate due to evolution of adsorbed gases as well as low polymeric compounds, and the voids thus produced in the surface layers of the fiber can accommodate dye molecules within them.[208] According to Forward and Simmens,[209] the availability of the spherulitic regions in nylon 66 is not entirely governed by steric hindrance because rate of uptake of the same dye is governed also by the dyebath conditions.

The location of a dye molecule inside a substrate has been found to be governed by the morphological characteristics of the fiber, the amount of dye present, and the conditions of dyeing. Crystalline regions of natural and regenerated cellulose fibers show characteristic dye uptake depending on crystalline structure and spatial distribution as well as size and uniformity of crystallites,[210] and, in general, direct dyes are adsorbed at the surfaces of micelles.[211] Alignment of direct dye molecules on cellulose chain can vary depending on the state of the hydrated surface of the fiber.[212,213] When electrolyte concentration in the dyeing system is increased, the presence of the inorganic cations in the neighborhood of the hydrated cellulose surface results in "structure-breaking effects" on the iceberg-like arrangement of water molecules on cellulose and around hydrated cations, leading to closer packing of dye molecules on the cellulose surface. This is reflected in an increase in the heat of dyeing of direct dyes with increasing electrolyte concentration in the system. With respect to polyvinyl alcohol fibers, initially direct dye molecules are adsorbed on the high-order regions in the substrate,[214] and the location and state of aggregation of these dye molecules differ

[207] J. O. Warwicker, *J. Soc. Dyers Colour.* **88**, 142 (1972).
[208] Y. Matsunaga and M. Kato, *Sen-i Gakkaishi* **17**, 144 (1961); *CA* **55**, 8866d (1961).
[209] M. V. Forward and S. C. Simmens, *J. Text. Inst.* **46**, T671 (1955).
[210] K. Miyasaka and T. Namiki, *Sen-i Gakhaishi* **11**, 141 (1955); *CA* **51**, 17169g (1957).
[211] H. Ruck, *Kolloid-Z.* **199**, 66 (1964).
[212] E. H. Daruwalla, *Palette* **25**, 30 (1967).
[213] S. R. Sivaraja Iyer, G. Srinivasan, and N. T. Baddi, *Text. Res. J.* **38**, 693 (1968).
[214] Y. Kobayashi, S. Okajima, and K. Fukuda, *Sen-i Gakkaishi* **27**, 102 (1971).

depending on the conditions under which these dyes have been applied.[215]

In the case of reactive dyes for cellulosic fibers, it has been assumed that with dyes having bifunctional reactive groups (dichlorotriazine), the molecules in the substrate get so oriented that they bring about cross-linking of adjacent cellulose chains. As evidence it has been shown that in case of cotton dyed with these dyes, even a very small amount of the reacted dye is sufficient to render the fiber insoluble in the conventional cellulose solvents.[216] However, these deductions are not very convincing because even in the case of monofunctional reactive dyes, the dyed substrate can be rendered insoluble in the solvent, and with bifunctional dyes even if both the reactive groups were to react with the hydroxyls of the same cellulose chain, insolubilization can equally well be effected. The only conclusive evidence of cross-linking is available in the case of CI Reactive Black 5 by Mack *et al.*[217] from electron-microscopic studies. However, very recent dichroic studies by Gulrajani and Padhye[218] in the orientation of this reactive dye reveal that interchain bonding is not predominently involved in dyeing of cellulose with this dye, and changes in dichroism observed on stretching the substrate are of the same order of magnitude as those for monofunctional dyes, where the possibility of cross-linking does not exist.

Several investigations have been carried out with a view to examining the orientation of vat dye molecules on the cellulose substrate during dyeing and also during subsequent oxidation and soaping, but the results obtained are conflicting. According to Sumner *et al.*[219] during the dyeing process the leuco vat dye molecules first get oriented with the long axis of the molecule parallel to the cellulose chains for van der Waals binding forces to operate to a maximum extent, but on oxidation, attachment with cellulose chains gets disrupted and the molecules get randomly arranged. Subsequent treatment with a detergent solution results in the crystallization of the dye and alignment of the dye molecules at right angles to the axis of the needle-shaped crystals. These deductions have been supported by results of the experiments on unsoaped and soaped cellulosic fiber dyed with anthraquinone vat dyes carried out by Warwicker[220] employing X-ray diffraction techniques.

[215] V. E. Nepomnyashchii, A. P. Ershov, and A. A. Kharkharov, *Tech. Text. Ind. USSR* No. 1, p. 91 (1968).

[216] T. Vickerstaff, *Melliand Textilber.* **39**, 765 (1958).

[217] C. H. Mack, H. H. McGregor, Jr., and S. R. Hobart, *Text. Res. J.* **35**, 353 (1965).

[218] M. L. Gulrajani and M. R. Padhye, unpublished work.

[219] H. H. Sumner, T. Vickerstaff, and E. Waters, *J. Soc. Dyers Colour.* **69**, 181 (1953).

[220] J. O. Warwicker, *J. Text. Inst.* **50**, T404 (1959).

According to Bender and Foster,[221] association of vat dye anions takes place within cellulose substrate during dyeing, and this tends to hinder diffusion of dye ions from cellulose when it is placed in a solution medium. On the other hand, Wegmann[222] suggested that the changes in shade observed in the case of vat dyes after soaping were not primarily due to crystallization of the dye, but were a result of the changes in the polarization of the dye brought about by soaping and subsequent stabilization by attraction of dye molecules to the substrate. Thus, both after oxidation and soaping, the dye molecules not only remain in the same state of distribution but also in the same position. To resolve the ambiguity, Gulrajani and Padhye[223] recently examined

TABLE IV

IR AND VISIBLE DICHROISM OF VAT DYES BEFORE AND AFTER SOAPING TREATMENTS

Group	*Dye*	*Treatment*	C=O	C—H	*Visible*
A	CI Vat Blue 20	Before	∥	⊥	∥
		After	⊥	∥	⊥
	CI Vat Yellow 2	Before	∥	—	∥
		After	⊥	—	⊥
B	CI Vat Orange 9	Before	⊥	∥	Nil
		After	⊥	∥	⊥
	CI Vat Green 1	Before	⊥	∥	∥
		After	⊥	∥	⊥
	CI Vat Red 12	Before	⊥	∥	∥
		After	⊥	∥	⊥
C	CI Vat Orange 15	Before	∥	—	∥
		After	∥	—	∥
D	CI Vat Blue 5	Before	⊥	—	∥
		After	⊥	—	⊥

the dichroism of several vat dyes in the UV, visible, and IR regions before and after soaping treatments, and concluded that the behavior of vat dyes could be classified into four distinct classes on the basis of their visible and IR dichroism, dyes belonging to each class showing characteristic orientation before and after the soaping treatment (Table IV).

Recent studies in the location and orientation of disperse dyes in synthetic fibers are very interesting and throw more light on the mechanism of dyeing of these fibers. Blacker and Patterson[224] are of the

[221] M. Bender and W. H. Foster, Jr., *Trans. Faraday Soc.* **64**, 2549 (1968).

[222] J. Wegmann, *J. Soc. Dyers Colour.* **76**, 282 (1960); *Melliand Textilber.* **41**, 1110 (1960); *Amer. Dyest. Rep.* **51**, P276 (1962).

[223] M. L. Gulrajani and M. R. Padhye, *in* "Dye-Polymer Interactions," p. 4. University of Bombay, 1971.

[224] J. G. Blacker and D. Patterson, *J. Soc. Dyers Colour.* **85**, 598 (1969).

opinion that disperse dye molecules first occupy the least-oriented regions of the polyamide and polyester fibers, and when these zones are occupied, adsorption then takes place on the more oriented regions. When the quantity of dye in the substrate increases, so does the orientation factor. When the amorphous polymer structure does not change irreversibly during dyeing, dichroism developed by disperse dyes

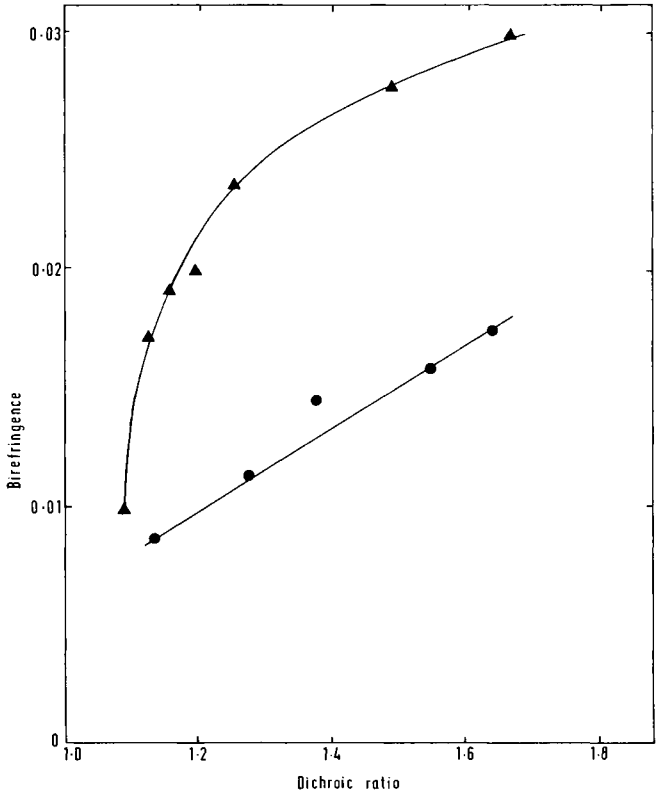

FIG. 3. Relation between dichroic ratio and birefringence of nylon 6 films containing fluorescent brightener: ▲, application of brightener prior to stretching; ●, application of brightener subsequent to stretching.

changes reversibly with temperature, and the dye molecules tend to return to the original state of combination, which is defined by the structure of the polymer and the dye molecules.[225] Lightfastness studies by Giles *et al.*[226] using certain disperse dyes also indicate physical changes in the substrate (porosity) produced by these dyes during

[225] K. Nakayama, S. Okajima, and Y. Kobayashi, *J. Appl. Polym. Sci.* **13**, 659 (1969).
[226] C. H. Giles, A. Yabe, and C. D. Shah, *Text. Res. J.* **38**, 467 (1968).

dyeing. Very recently, Gulrajani and Padhye[227] have examined the orientation of a disperse fluorescent brightener of the coumarin type in nylon 6 films at different stages of stretching. The dichroic behavior of the brightener in the unoriented film was characteristically different from that in the prestretched film, and on stretching the film, there was a pronounced increase in birefringence without a corresponding increase

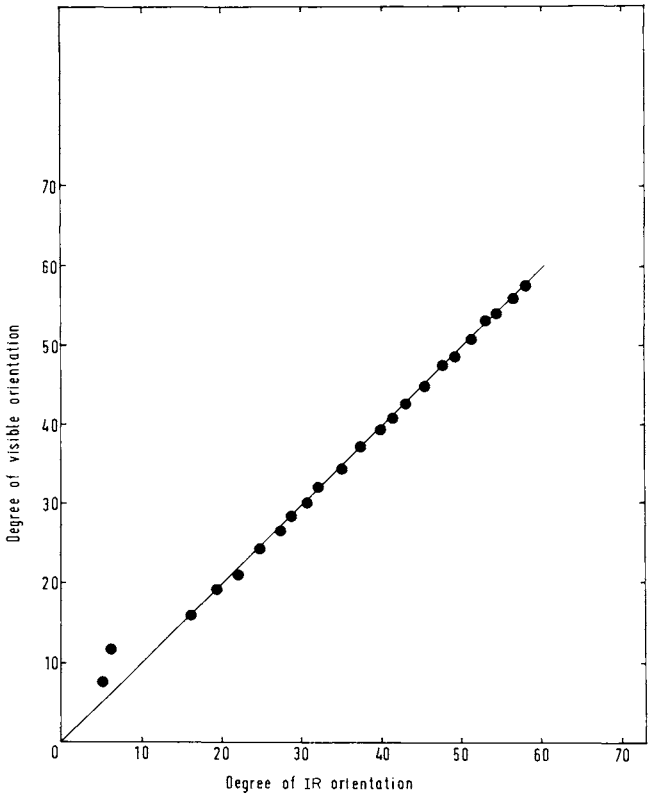

FIG. 4. Relation between degrees of IR and visible orientation for prestretched and poststretched nylon 6 films dyed with CI Reactive Orange 3 (unreacted and reacted).

in dichroism during the initial stages. On the other hand, in case of stretched film, birefringence and dichroism showed a linear relationship (Fig. 3). In the case of reactive disperse dyes on nylon, however, correlation between polymer orientation in the amorphous region and dye orientation has been found to be linear with unit slope, which goes to show that in this system, the dye molecules occupy amorphous regions

[227] M. L. Gulrajani and M. R. Padhye, unpublished work.

in the polymer and they follow completely the polymer orientation[228] (Fig. 4).

C. Dye–Dye Interaction in Substrate

The existence of specific sites in a homopolymer like cellulose is rather difficult to visualize. However, recent work on the morphology of cotton reveals that the fiber substance does have areas of varying activity. Saturation values for direct dyes indicate that cellulose is very selective in providing sites for different dye molecules, and there is a likelihood of a quantitative correlation between the morphology of cellulosic fiber and dyeing equilibrium. As the number of sites available for direct dye adsorption is dependent on the concentration of electrolyte in the system, it appears that the sites are relatively close to one another in the substrate. In such a state, one dye can lower the sorption of another dye when the two are applied in a mixture, although the two dyes may not occupy mutually common sites or surface areas. In most of the investigations connected with the dyeing of cellulose with mixtures of direct dyes, the nonadditive properties in the amounts of dye adsorbed when a binary or tertiary mixture of dyes is used have been attributed to formation of complexes between dyes in solution rather than in the substrate.[135,229–231] However, Derbyshire and Peters,[133] from spectral studies of dyed cellophane, have come to the conclusion that CI Direct Blue 1 was partly adsorbed as single dye molecules and partly as a 1:1 complex with CI Direct Yellow 12 when dyeings were carried out from a bath containing mixtures of these dyes. With respect to reactive dyes, Kon'kova and Belen'kii[139] have indicated the absence of interaction in the fiber with several pairs of dyes and have concluded that the mixture components influence each other only during the exhaustion and subsequent chemical reaction with cellulose.

Dye–dye interaction on soluble substrates can lead to changes in the configurational form of the polymer. Such behavior has been observed in the case of flexible protein molecules and polyelectrolytes. For instance, if to a very dilute solution of CI Basic Orange 14, where the dye is present in monomer form, is added a soluble protein, there is an immediate color change consistent with the dimerization of the dye.[232] What actually happens in such a case is that adsorption of the dye on the protein takes place and there is simultaneous interaction between

[228] M. R. Padhye and A. W. Palekar, unpublished work.

[229] Y. Horiki, Y. Tanizaki, and N. Ando, *Bull. Chem. Soc. Jap.* **33**, 163 (1960).

[230] Y. Horiki, *Bull. Chem. Soc. Jap.* **33**, 974 (1960).

[231] J. J. Porter and W. S. Perkins, *Text. Res. J.* **40**, 81 (1970).

[232] See Chapter V in this volume for discussions of metachromasy and dye-stacking.

adsorbed dye molecules resulting in stacking of the adsorbed dye. The stacking tendency is mainly governed by steric factors, although it is not the only one to be considered.[232] In the case of application of mixtures of dyes, competitive effects in dye adsorption have been mainly attributed to direct competition for the same sites in the substrate. However, if dye–substrate interaction involves conformational effects as mentioned above, conformational competitive effects are possible because in such a case one dye adsorbed can at least conceptually produce a conformational change in the substrate that would discourage the adsorption of the second dye. Such conformational competitive effects can be considered as "allosteric competition," which is quite distinct from direct competition for active sites (isosteric inhibition). With hydrophobic fibers and disperse dyes, there is some evidence that water molecules may act as allosteric competitors in an aqueous atmosphere of low concentration. For instance, it has been observed that much higher apparent saturation values are obtained with dry dye vapors, but when water vapor is released in the system, dye gets desorbed and the saturation value is reduced to the level expected in aqueous dyebath systems.[233] Although it is possible to explain this behavior in terms of competition for hydrogen-bonding sites between dye and water, certain evidence is available which supports the concept that water binding could create a configurational change in the polymer that is unfavorable for binding of the dye.

Studies on the dyeing behavior of mixtures of disperse dyes reveal that some of the mixtures are additive, with each dye behaving independently of the other, while with certain dye mixtures, one dye component interferes with rate of dyeing, partition between aqueous phase and hydrophobic fibers, and the saturation value of the second dye. Although such a behavior has been explained on the basis of interaction between certain dyes in the aqueous dispersion,[141,143] recent studies[144] based on light absorption characteristics of disperse dyes and fading behavior when present in hydrophobic fiber substances suggest that interaction between certain disperse dyes does take place in the substrate. Those dyes that interact with each other in the aqueous dispersion phase also have a strong tendency for interaction in the fiber phase. However, dyes that interact in aqueous dispersion or in substrate when applied to synthetic fibers from an organic solvent, perchloroethylene, fail to show any interaction in the bath or in the fiber phase.[234] It appears that in the case of some disperse dyes, there is an interaction

[233] I. D. Rattee, *in* "Dye-Polymer Interactions," p. 23. University of Bombay, 1971.
[234] K. V. Datye, S. C. Pitkar, and U. M. Purao, *Textilveredlung* **6**, 593 (1971).

between the dye and certain organic solvents, which results in the prevention of dye–dye interaction in the bath.[235] With respect to simple acid dyes when applied to polyamide fibers as binary mixtures, no interaction is observed in the substrate and it is generally assumed that the ratio of the surfaces occupied by the two dyes does not alter substantially during dyeing.[236] With respect to cationic dyes on polyacrylonitrile fibers, Rosenbaum[237] observed deviations of experimental results from a Langmuir-type relation for competition between different cations and a limited number of anionic sites (sulfonic groups), and this behavior has been explained on the basis of electrostatic repulsive interactions between the sites.

[235] E. H. Daruwalla and J. R. Patel, unpublished work.

[236] E. Atherton, D. A. Downey, and R. H. Peters, *J. Soc. Dyers Colour.* **74**, 242 (1958).

[237] S. Rosenbaum, *Text. Res. J.* **33**, 899 (1963).

CHAPTER IV

PHYSICAL CHEMISTRY OF DYEING: KINETICS, EQUILIBRIUM, DYE–FIBER AFFINITY, AND MECHANISMS

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I. Kinetics of Dyeing

The dyeing of textiles from a dyebath in which the textile fabric or yarn is immersed consists of three main processes. These are, in sequence, the diffusion of dye through the dye solution to the textile fiber surface, dye adsorption at the surface, and, finally, dye diffusion from the surface to the interior of the textile substrate. All three factors can influence or control the kinetics of dyeing.

The first process, namely, diffusion of dye molecules through the dye solution to the fiber surface, is influenced by the hydrodynamic flow of the dye solution which carries the dye to the fiber surface. Therefore, factors such as rate of stirring of the dyebath, geometry of the textile substrate, and the design of the dyeing machine itself, which influence

the hydrodynamic flow pattern, will also influence the process of dye diffusion to the fiber surface. This process of dye diffusion through the dye solution to the fiber surface is called convective diffusion.

Very little is known about the second stage of the dyeing process, i.e., dye adsorption at the fiber surface. Experimentally, it is not possible, using known techniques, to study directly this adsorption process at the fiber surface. Therefore, it is commonly assumed that there exists an instantaneous equilibrium between adsorbed dye and the dye in the solution phase adjacent to the fiber surface. Lack of direct experimental evidence on this point is a drawback, since it is logical to suppose that the equilibrium distribution of dye between the fiber and solution phases, which varies considerably from dye to dye, would also influence the dye diffusion process by modifying the boundary conditions.

The third and final stage in the dyeing process involves the diffusion of dye from the fiber surface to the interior of the fiber. Most of the experimental work on dyeing kinetics and dye diffusion in textile substrates has been carried out on this particular aspect of the dyeing process, for reasons that will be discussed in subsequent sections. This third and final stage of the dyeing process is found to depend on several factors. These are, for example, the affinity of the dye for the fiber, the size and structure of the dye molecule, notably its hydrophobic character, dye aggregation in the solution and fiber phases, the influence of electrical factors due to charges on the fiber surface and on the dye if both fiber and dye contain ionizable groups, and the polymeric structure of the fiber.

The following sections deal with the three main processes of dyeing for various dye–textile substrate systems.

A. Convective Diffusion

Several studies have shown that the rate of dye uptake by fibers reaches a maximum value when the agitation in the dyebath is vigorous. Hanson *et al.*[1] showed that increasing the agitation of cellulose in the form of cellophane sheet, cotton cloth, and viscose yarn by increasing the oscillation of the material suspended in a dyebath containing the dye CI Direct Blue 1 resulted in a maximum leveling-off value for adsorbed dye. However, the actual extent of maximum dye uptake was least for cotton cloth and greatest for cellophane sheet. These results show that the geometry of the system affects the hydrodynamic factors which in turn affect dye uptake. Because the cellophane sheet has the

[1] J. Hanson, S. M. Neale, and W. A. Stringfellow, *Trans. Faraday Soc.* **31**, 1718 (1935).

simplest geometry, it is least affected by the hydrodynamic flow patterns and shows the largest dye uptake. Studies by Alexander and Hudson[2] on the times of half-dyeing (time for 50% exhaustion) of the acid dye CI Acid Orange 7 in wool fibers have shown that dye uptake reaches a maximum value beyond a certain rate of agitation of the dyebath. Similarly, Fern[3] has shown that for a series of direct dyes on cotton yarn, dye uptake is a maximum independent of the rate of circulation of dye liquor, at high rates of circulation. These results, along with several others of a similar nature, suggest that there is a surface barrier to the uptake of dye by the fiber which is reduced as the rate of agitation and, hence, the rate of flow of the dye solution past the fiber surface is increased.

In normal practice, the flow patterns in dyeing systems are very complicated. The surface barrier concept has therefore been analyzed only in terms of a simple model. In this model, it is assumed that the dye liquor has a streamlined flow pattern parallel to a sheet or film of the substrate material immersed in the dyebath. The rate of dye uptake by the film or sheet is influenced by diffusion and convective mass transport of the dye. It is thus necessary to know also the flow pattern of dye liquor before analyzing the rate of dye uptake.

Levitch[4] has calculated the flow velocity for streamline flow of an incompressible fluid parallel to a plane sheet or film. His results show the existence of a thick hydrodynamic boundary layer δ_H and a thin diffusional boundary layer δ_D adjacent to the film surface, the latter layer being about one-tenth δ_H. Figure 1 shows the hydrodynamic and diffusional boundary layers in steady-state laminar flow parallel to a plane slab, the main-stream velocity being V_0. In the case of dye solution–textile substrate systems, Peters[5] defines this boundary layer as the region in which the dye concentration changes its value at the surface to 99% of that in the bath. The main barrier to transport of solute to the surface is this diffusional boundary layer δ_D, which, as mentioned earlier, is very much thinner than the hydrodynamic boundary layer δ_H. If it is assumed that at all times the solute concentration $C = 0$ (very rapid absorption of solute by the sheet) at the surface, then the distribution of fluid velocity (a) and solute concentration (b) can be described theoretically by Fig. 2 (Levitch[4]), where C_0 is

[2] P. Alexander and R. F. Hudson, *Text. Res. J.* **20**, 481 (1957).

[3] A. S. Fern, *in* "The Physical Chemistry of Dyeing" (T. Vickerstaff), p. 145. Oliver & Boyd, Edinburgh, 1954.

[4] V. G. Levitch, "Physicochemical Hydrodynamics." Prentice-Hall, Englewood Cliffs, New Jersey, 1962.

[5] R. H. Peters, *in* "Diffusion in Polymers" (J. Crank and G. S. Park, eds.), Chapter 9, p. 315. Academic Press, New York, 1968.

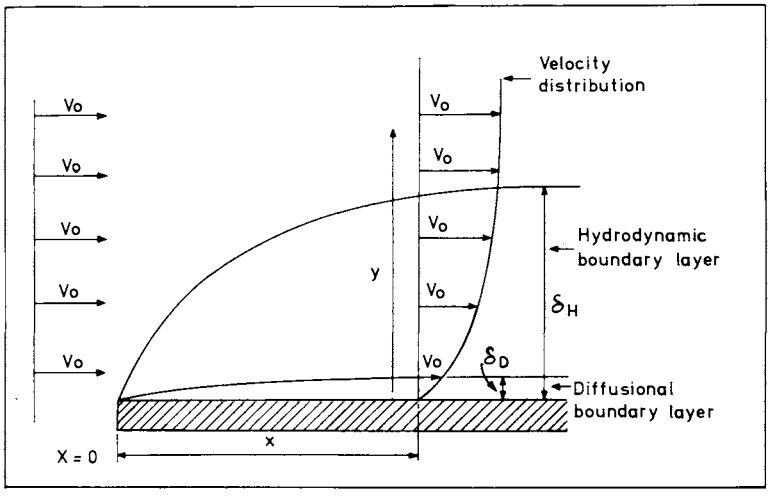


FIG. 1. Hydrodynamic and diffusional boundary layers in steady-state laminar flow parallel to a plane slab. Horizontal arrows represent fluid velocity, in magnitude and direction, at point of origin of arrows. Diffusional boundary layer has a thickness (δ_D) about one-tenth that of the hydrodynamic boundary layer (δ_H). Both layers are parabolic in shape; V_0 is the main-stream velocity. Reproduced by permission from Levitch and Prentice-Hall, Inc.[4]

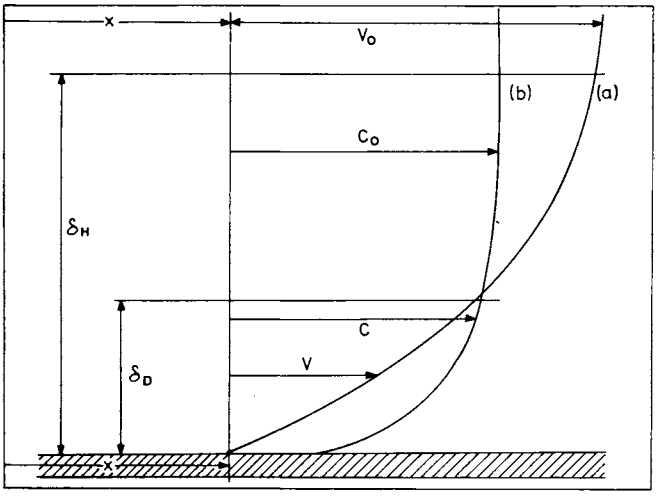


FIG. 2. Theoretical distribution of fluid velocity (a) and of solute concentration (b) at surface of plane sheet immersed in solution of uniform concentration C_0 flowing with a steady main-stream velocity V_0 parallel to the surface of the sheet. Sheet adsorbs solute so rapidly that at all times $C = 0$ at the surface. Reproduced by permission from Levitch and Prentice-Hall, Inc.[4]

the uniform concentration of solute in the solution flowing with a steady main-stream velocity V_0 parallel to the surface of the sheet or film. This very simple model shows that $\delta_D \ll \delta_H$ and also the important fact that the sheet is not uniformly accessible. When the flow velocity increases, the diffusional boundary layer decreases and hence mass transfer to the surface of the film is increased. Applying this simple model to the system aqueous CI Direct Blue 1–cellophane sheet at 90°, McGregor and Peters[6] have shown that $\delta_D \simeq 30\ \mu m$ which is in reasonable agreement with the values obtained under practical dyeing conditions for a well-stirred system.[7–9]

Assuming, as discussed earlier, that the main barrier to transport is the diffusional boundary layer, McGregor and Peters[6] set up a quasi-steady-state model for the dye diffusion process across a plane sheet of thickness $2l$ from a dye solution of constant concentration c. This model is illustrated in Fig. 3. Equating the flux just within the sheet, i.e.,

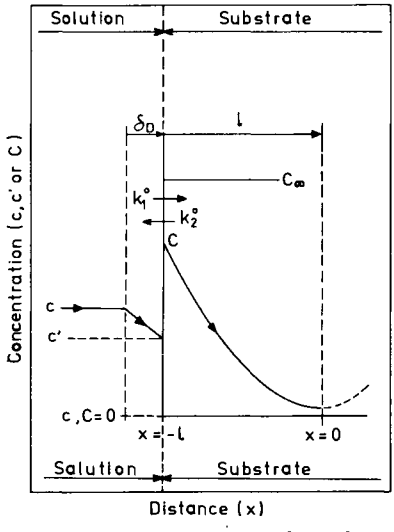


FIG. 3. Diffusional boundary layer model for a plane sheet; c is solution concentration; C is concentration in sheet; k_1^0 is velocity constant for passage of dye from solution to substrate through the interfaces at $x = \pm l$; k_2^0 is the velocity constant for passage in the opposite direction through the interfaces at $x = \pm l$; l is the half-thickness of the sheet. Reproduced by permission from McGregor and Peters.[6]

[6] R. McGregor and R. H. Peters, *J. Soc. Dyers Colour.* **81**, 393 (1965).

[7] P. Alexander, D. Gough, and R. F. Hudson, *Trans. Faraday Soc.* **45**, 1058 (1949).

[8] L. Bircumshaw and A. Riddeford, *Quart. Rev., Chem. Soc.* **6**, 157 (1952).

[9] P. Alexander and R. F. Hudson, *in* "Wool, its Chemistry and Physics" (C. Earland, ed.), 2nd ed., p. 152. Chapman & Hall, London, 1963.

$-D_f(\partial C/\partial x)_{x=-l}$ with the rate of supply of dye through the diffusional boundary layer δ_D they obtained the following equation

$$\frac{D_s}{\delta_D} = (c' - c) = D_f\left(\frac{\partial C}{\partial x}\right)_{x=-l} \tag{1}$$

where D_s is the diffusion coefficient within δ_D. With the further assumption of instantaneous equilibrium between dye concentrations on both sides of the interface, i.e., resistance to dye diffusion is negligible at the interface as compared to the diffusional boundary layer, the following equation can be written for the system,

$$Kc' = C_{x=-l} \tag{2}$$

When the whole system is in equilibrium, $c' = c$ and the dye concentration in the fiber substrate is equal to C_∞ and hence

$$Kc = C_\infty \tag{3}$$

Combining Eqs. (1), (2), and (3), the boundary condition at $x = -l$ is given by

$$\left(\frac{\partial C}{\partial x}\right)_{x=-l} + h(C_\infty - C) = 0 \tag{4}$$

where $h = D_s/(KD_f\delta_D)$. The numerical solution for Fick's second diffusion equation

$$\frac{\partial C}{\partial t} = D_f\frac{\partial^2 C}{\partial x^2} \tag{5}$$

using the boundary conditions given by Eq. (4) and the condition that when $t = 0$, $C = 0$ in $-l < x < l$, has been given earlier by various workers in connection with other transfer processes.[10-12] The effect of flow rate on rate of dyeing can now be expressed quantitatively by the parameter $L = D_s l/(KD_f\delta_D)$. Since the flow rate is inversely proportional to δ_D, the effect of flow rate on rate of dyeing can be expressed in terms of the variation in the thickness of the diffusional boundary δ_D. An increase in flow decreases δ_D and hence increases L. The numerical solutions of Eq. (5) discussed earlier can be expressed graphically as functions of M_t/M_∞ versus $D_f t/l^2$ for plane sheets or M_t/M_∞ versus $D_f t/a^2$ for cylinders, where M_t and M_∞ refer to the dye uptake at time t

[10] A. B. Newman, *Trans. Amer. Inst. Chem. Eng.* **27**, 203 (1931).

[11] H. S. Carslaw and J. C. Jaeger, "Conduction of Heat in Solids." Oxford Univ. Press, London and New York, 1947.

[12] J. Crank, "Mathematics of Diffusion." Oxford Univ. Press (Clarendon), London and New York, 1956.

and after infinite time, and l and a represent the half-thickness of the sheet and radius of the cylinder, respectively. Figure 4 illustrates such rate-of-dyeing curves for various values of L. It can be seen that as $L \rightarrow \infty$, $\delta_D \rightarrow 0$, i.e., as the stirring rate becomes more efficient (high flow rate) the dyeing rate is controlled only by diffusion in the solid substrate. A limit to the rate of dyeing is thus reached at some high value of the flow rate and further stirring will not have any more effect on dyeing rates.

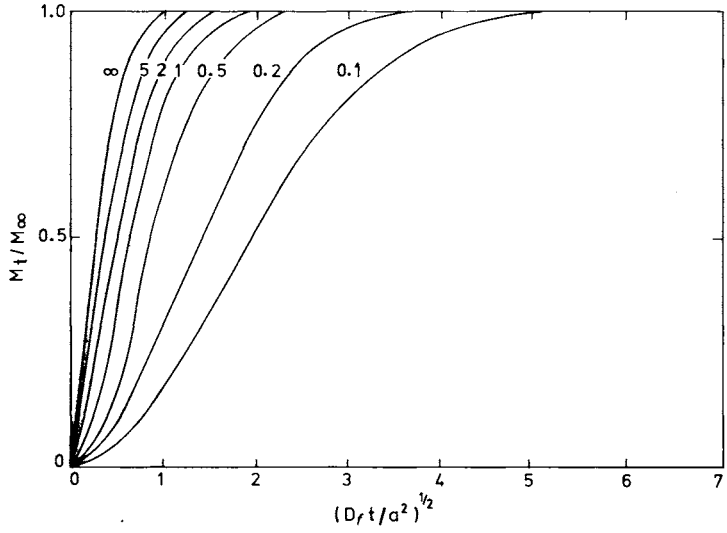


FIG. 4. Theoretical variation of M_t/M_∞ with $(D_f t/a^2)$ for cylinder. M_t is total dye content of the sheet at time t and M_∞, the equilibrium dye content, i.e., for $t \rightarrow \infty$. D_f is diffusion coefficient in a cylinder and a the cylinder radius. Numbers on curves are values of the parameter $L(=h^0a)$; high values of L correspond to low values of δ_D and to high values of V_0, i.e., to efficient stirring.[10,12] Reproduced by permission from McGregor and Peters.[6]

The parameter L can be expressed as a function of flow rate V_0 by the equation $L = V_0^n$ where n is an arbitrary constant $\leqslant 1$. The value of n is ill-defined and uncertain, and cannot be obtained directly from dyeing kinetic studies, although Alexander *et al.*,[7] from their studies on wool dyeing, state that $n \simeq 1$. It is thus convenient to assume for simplicity that n is equal to unity. Hence L is directly proportional to V_0. McGregor and Peters,[6] from plots of the experimental values of dye adsorbed as a function of V_0 for the diffusion of CI Acid Red 18 in nylon fabrics at 75° for different times of dyeing (Fig. 5), have shown that there is a good

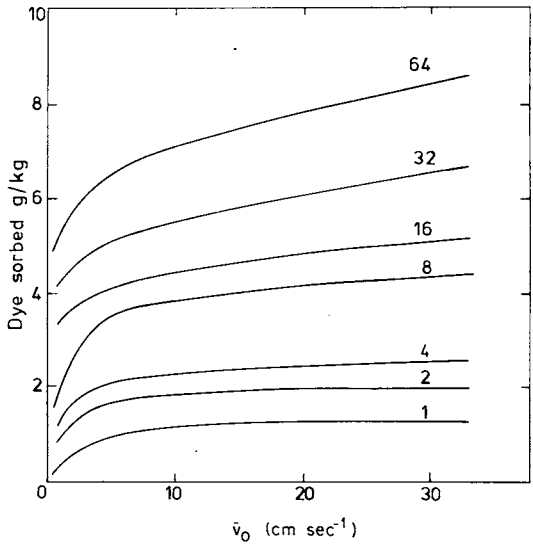


FIG. 5. Experimental variation of M_t (dye sorbed) with mean flow velocity V_0 for sorption of acid dye by nylon fabric at 75°. Dye: CI Acid Red 18. (0.02 g/liter) at pH 3.0. Numbers on curves are times of dyeing (minutes); $\bar{V}_0$ is mean fluid velocity through a tube across the opening of which fabric was mounted. Reproduced by permission from McGregor and Peters.[6]

qualitative agreement between such data and the theoretical data plotted from the results described in Fig. 4.

These workers have also shown that rate-of-dyeing curves for CI Direct Blue 1 on cotton fabrics at 90° for an unstirred and stirred dye-bath are similar to the theoretical curves for a low value of L (unstirred) and a high value of L (stirred), respectively, i.e., the curve is sigmoidal

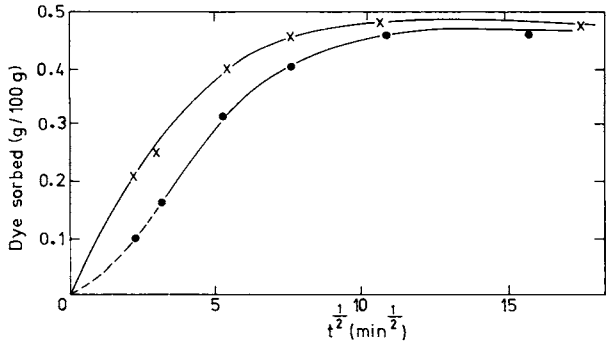


FIG. 6. Experimental variation of M_t/M_∞ with $t^{1/2}$ for sorption of direct dye by cotton fabric at 90°. Dye: CI Direct Blue 1 (0.05 g/liter), in the presence of 5.0 g/liter of NaCl; ×, stirred; ●, unstirred. Reproduced by permission from McGregor and Peters.[6]

for unstirred baths and becomes exponential for stirred baths (see Fig. 4). Furthermore, the values of time of half-dyeing $t_{1/2}$ tend to reach a constant value as L increases, i.e., with increasing stirring rate (Fig. 6). This result is in agreement with experimental data of Alexander and Hudson[2] for the dyeing of wool with CI Acid Orange 7. This model also reveals the important fact that the rate of dyeing as a function of flow rate is affected by dye–substrate interactions, since L can be modified by the partition function K.

TABLE I

BEHAVIOR OF DISPERSE DYES IN CELLULOSE ACETATE AT 80°[a]

Dye	K	D_f ($cm^2\ sec^{-1}$)	$L = D_s l/(KD_f\delta_D)$
2-Methoxy-4-nitroaniline → *N*-bis-(β-hydroxyethyl)aniline	450	1.7×10^{-11}	430
2,4-Dinitro-4′-hydroxydiphenylamine	400	1.6×10^{-10}	52
p-Aminoazobenzene	380	1.8×10^{-9}	4.6
4-Nitroaniline → diethylaniline	12,500	0.63×10^{-10}	4.2

[a] Values of D_f and K are taken from D. F. Scott (M.Sc. Thesis, Leeds University, 1956) and J. S. Jadhav (M.Sc. Tech. Thesis, Manchester University, 1964). Values assumed are $l = 1.0 \times 10^{-3}$, $D_s = 1.0 \times 10^{-5}$; $D = 3.0 \times 10^{-3}$; solutions are well stirred. Reproduced by permission from McGregor and Peters.[6]

The calculations of L by McGregor and Peters[6] for disperse dye–cellulose acetate systems given in Table I show that when K (a constant for such systems independent of dye concentration) is high, L is very low, i.e., the system is sensitive to stirring rate and vice versa. These results show that for a rapidly diffusing dye L is low and the system is sensitive to rate of stirring. In ionic dye–substrate systems where K is not constant, but varies with dyebath conditions, L varies in a complex manner with dye and salt concentration, pH, etc. Further complications regarding a quantitative theoretical approach to describe dye uptake in terms of convective diffusion are the nonzero concentration of dye at the substrate surface and the very complex geometries of the textile materials in practical dyeing systems. Recently, McGregor *et al.*[13] studied the rate of uptake of anionic dyes by methoxymethylnylon 66 film as a function of the rate of flow of liquid. The experimental conditions were such that the film was located parallel to the flow of dye liquor, the flow pattern conforming to that for a liquid with a constant

[13] R. McGregor, R. H. Peters, and K. Varol, *J. Soc. Dyers Colour.* **86**, 437 and 442 (1970).

bulk velocity flowing over a flat plate. When the kinetics was controlled by the diffusion in the liquid phase, i.e., at low pH and low dye concentration and a relatively high temperature, the rate data were in agreement with the simple model for diffusion discussed earlier and shown in Fig. 2. The calculated values of the thickness, δ_D, of the diffusional boundary layer from the flux equation were in good agreement with theory. In an extension of these studies using disperse dyes, it is shown that the kinetics can be described by a model in which the rate is controlled by diffusion through the liquid and into the film itself. An application of Levitch's model shows that the parameter L determines the dyeing properties. Since $L = D_s l/(KD_f\delta_D)$, its value for any dye at any given temperature is only a function of δ since D_f, D_s, and K are constants for a disperse dye. This situation is therefore different from that for the anionic dye–methoxymethylnylon 66 system discussed earlier, where L is a function not only of δ but also of dyebath conditions.

Some attempts to study actual dyeing systems have been made by Boulton and Crank,[14] Olofsson,[15] McGregor,[16] and Burley *et al.*[17] Burley *et al.*[17] studied mass-transfer processes in hank dyeing machines by comparing practical dyeings in such machines with a mathematical model of a computer-simulated dyeing process. They found that a limiting rate of flow was required to eliminate color differences between hanks in practical dyeing machines. This limiting flow rate condition can be established using the computer-simulated model dyeing process. Reasonably good agreement was obtained between theory and experiment for the ratio of dye concentration at the surface of a nylon carpet yarn to the concentration at the center for different times of dyeing of the yarn with CI Acid Red 13 under practical conditions in a small-scale dyeing machine.

B. Rates of Dyeing and Diffusion

The kinetics of dyeing can be studied in two ways: (a) by measuring overall rates of dyeing and (b) by measuring dye concentration–distribution curves in the substrate as a function of time. Overall rates of dyeing give information on the average diffusion coefficient of the dye in the subtrate, also known as the apparent diffusion coefficient. Such

[14] J. Boulton and J. Crank, *in* "The Physical Chemistry of Dyeing" (T. Vickerstaff), p. 156. Oliver & Boyd, Edinburgh, 1954.

[15] B. Olofsson, *J. Chim. Phys.* **55**, 285 (1958); "Grundena for Textil Vatberedning." Svensk Textilforskringsinstitut, Göteborg, Sweden, 1962.

[16] R. McGregor, *J. Soc. Dyers Colour.* **81**, 429 (1965).

[17] R. W. Burley, I. D. Rattee, and J. R. Flower, *J. Soc. Dyers Colour.* **85**, 187 and 193 (1969).

studies give no information on the molecular mechanism of dyeing. Concentration–distribution studies give more information on dyeing mechanisms since the data show the variation of the diffusion coefficient in the textile substrate with the nature of the dye adsorption process.

1. *The Overall Rate*

Rate-of-dyeing data can be obtained by measuring in an isothermal dye solution–substrate system the change in dyebath concentration as a function of time, provided the system is efficiently and strongly agitated under reproducible conditions so that effects due to the diffusion of dye to the fiber surface and the presence of diffusional boundary layers are minimal. As discussed earlier, the rate-of-dyeing data can be interpreted in terms of the diffusion of dye inside the textile substrate. The diffusion coefficients of dyes inside textile substrates are generally several thousand times less than the dye diffusion coefficients in the dyebath solution phase and hence rate of dyeing is mainly governed by dye diffusion in the substrate.[18] Furthermore, as pointed out by Peters,[5] the range of behavior shown by different dyes is so wide that rate-of-dyeing data can give significant information for comparison of the dyeing behavior of a given class of dyes on a particular textile substrate. These wide differences in rates are due mainly to the different affinities of dyes for the fiber, although other factors such as molecular size, dye structure, or degree of aggregation in the dyebath can also influence dyeing rates. Studies on overall rates of dyeing can be classified into three broad sections according to the substrate, which can be cellulose, hydrophobic fibers, or wool.

a. Cellulose. Most of the work on rates of dyeing has for several years been confined to cellulose due to its ready availability in simple geometric sheet form where the influence of stirring is minimal and rates of dyeing are related to dye diffusion inside the cellulose substrate. From sorption rates the diffusion coefficient D_a, generally called the apparent diffusion coefficient, can be calculated by applying Eq. (6)[19] for a plane sheet or Eq. (7)[20] for the case of a fiber.

$$\frac{C_t}{C_\infty} = 1 - \frac{8}{\pi^2}\left[\exp\left(\frac{-\pi^2 D_t}{b^2}\right) + \frac{1}{9}\exp\left(\frac{-9\pi^2 D_t}{b^2}\right) + \frac{1}{25}\exp\left(\frac{-25\pi^2 D_t}{b^2}\right) + \cdots\right] \quad (6)$$

[18] T. Vickerstaff, "The Physical Chemistry of Dyeing." Oliver & Boyd, Edinburgh, 1954.

[19] J. W. McBain, *Z. Phys. Chem.* **68**, 471 (1909).

[20] A. V. Hill, *Proc. Roy. Soc., Ser. B* **104**, 39 (1928).

$$\frac{C_t}{C_\infty} = 1 - 0.692\left[\exp\left(\frac{-5.785D_t}{r^2}\right) + 0.190\exp\left(\frac{-30.5D_t}{r^2}\right) + 0.0775\exp\left(\frac{-74.9D_t}{r^2}\right) + 0.0415\exp\left(\frac{-139D_t}{r^2}\right) + 0.0258\exp\left(\frac{-223D_t}{r^2}\right) + \cdots\right] \quad (7)$$

The use of these equations presupposes that the dyebath is of constant composition and the diffusion process obeys Fick's law. In the case of fibers, it is further assumed that the fiber cross-section is uniformly circular, but this is not always true, particularly for natural fibers or even viscose and cellulose acetate. Neale,[21] from studies in cellophane sheet–direct dye solution systems, obtained for several dyes a wide range of apparent diffusion coefficients D_a, from 300×10^{-10} cm^2 sec^{-1} for CI Direct Orange 12 to 1.2×10^{-10} cm^2 sec^{-1} for Chlorazol Fast Orange AG. However, it is important to note that D_a represents only an average value of the diffusion coefficient, since direct dye diffusion in a cellulose substrate is a function of the dye concentration in the substrate. This was first demonstrated by Garvie and Neale[22] for the system CI Direct Blue 1–cellophane sheet using the single and multiple membrane methods. Standing *et al.*[23] have suggested that D_a can be approximately correlated with the average integral diffusion coefficient $\bar{D}$, i.e., the coefficient obtained by integrating some suitable form of an equation as, for example, Eq. (8).

$$\bar{D} = \frac{1}{C_\infty}\int_0^{C\infty} D(C)\,dC \quad (8)$$

This equation expresses the true diffusion coefficient $D(C)$ as a function of dye concentration C in the substrate at time t and equilibrium adsorbed dye concentration C. Crank and Henry[24] tested the validity of this idea theoretically for ten different relationships between $D(C)$ and C involving power, linear, exponential, and error functions of C, calculating the overall rate-of-dyeing curve in each case using Eq. (9).

$$\frac{\partial C}{\partial t} = \frac{\partial}{\partial x}\left\{D(C)\frac{\partial C}{\partial x}\right\} \quad (9)$$

[21] S. M. Neale, *J. Soc. Dyers Colour.* **52**, 252 (1936).
[22] W. M. Garvie and S. M. Neale, *Trans. Faraday Soc.* **34**, 335 (1938).
[23] H. A. Standing, J. O. Warwicker, and J. Willis, *J. Text. Inst.* **38**, 335T (1947).
[24] J. Crank and M. E. Henry, *Trans. Faraday Soc.* **45**, 639 and 1119 (1949).

The D_a values were obtained in each case from the same half-dyeing times for each of the calculated rate-of-dyeing curves. A comparison of D_a and $\bar{D}$ values calculated for each of the ten functions showed that they were only approximately equal, with variations up to 20% for some of the relationships tested. The shape of the rate-of-adsorption curve was also not very sensitive to the way in which $D(C)$ varied with C. Crank[25] showed, for example (Fig. 7), that the calculated curve of

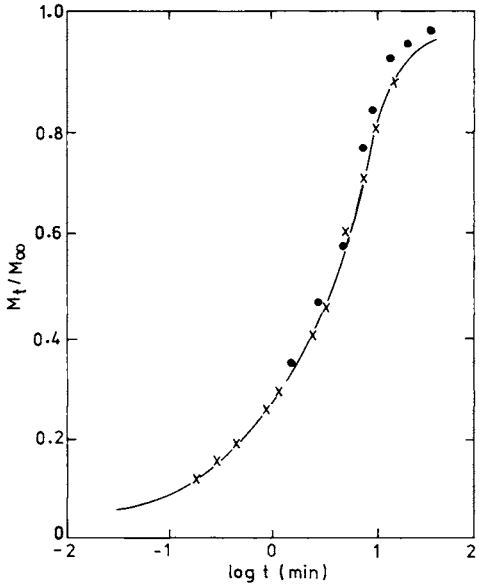


FIG. 7. Insensitivity of rate of dyeing to variations in diffusion coefficient: ●, experimental data of Neale for CI Direct Blue 1 on viscose sheet; ——, best-fitting curve assuming a constant diffusion coefficient; ×, assuming diffusion coefficient to be a linear function of concentration. Reproduced by permission from Crank.[25]

M_t/M_∞ versus $\log t$, assuming either a constant diffusion coefficient or a linear variation of D with C, was almost identical with the experimental curve obtained by Garvie and Neale[22] for CI Direct Blue 1 on viscose sheet. It is thus apparent that while the experimental rate-of-dyeing curves agree well with the theoretical behavior to be expected for a solute obeying Fick's law, the results on variation of $D(C)$ with C in the fiber phase as well as the insensitivity of D_a to the manner in which the true diffusion coefficient varies with concentration in the fiber suggest that D_a has little theoretical significance in relation to the true

[25] J. Crank, *J. Soc. Dyers Colour.* **64**, 386 (1948).

molecular mechanisms of dyeing. Further aspects of this problem are discussed at a later stage.

Direct dyes are known to aggregate in solution and aggregation markedly influences dye adsorption. Nevertheless, practically no work has been carried out on the influence of dye aggregation on kinetics of dyeing. Recently Ghanekar,[26] using a polarographic method, studied changes in the average molecular weight of CI Direct Red 28 in a viscose fiber dyebath system as a function of time of dyeing. The results show that there is no change in the average degree of aggregation as a result of dye uptake. This suggests that the dye most probably diffuses into the fiber as single molecules from the dyebath, a dynamic equilibrium being maintained between single dye molecules and dye aggregates in solution during the dyeing process (see also Vickerstaff,[18] Boulton and Morton,[27] and Sivarajan[28]).

b. Hydrophobic Fibers. Vickerstaff[18] found that the rate of dyeing of secondary cellulose acetate fibers with disperse dyes obeys Hill's equation, i.e., Fick's law, and the diffusion coefficient is a constant. Similar results have been obtained for disperse dyes on other hydrophobic fibers such as cellulose triacetate and cellulose acetate,[29] nylon,[30] and polyethylene terephthalate.[31] Several recent studies on the dyeing of polyester substrates with disperse dyes from infinite dyebaths by Sekido and Kojima,[32] Cegarra and Puente,[33] and Datye and Rajendran[34] show that the dye diffusion inside the substrate obeys Fick's law with a constant diffusion coefficient independent of dye concentration. Cegarra and Puente[33] also showed that while Hill's equation[20] fits the experimental rate-of-dyeing curve fairly well, a better fit is obtained by using Eq. (10), derived by them taking into account the heterogenous nature of the reaction and a desorption rate. These factors become more important during the later stages of dye uptake by the fiber system.

$$C_t = C_\infty(1 - e^{-kt})^{1/2} \tag{10}$$

Waters[35] found that the diffusion coefficients in Terylene fibers were much less at the normal dyeing temperature of 85°–100° than in the

[26] A. S. Ghanekar, Ph.D. Thesis, University of Bombay, India (1972).

[27] J. Boulton and T. H. Morton, *J. Soc. Dyers Colour.* **56**, 145 (1940).

[28] S. R. Sivarajan, *J. Indian Inst. Sci.* **36**, 282 (1954).

[29] T. G. Majury, *J. Soc. Dyers Colour.* **70**, 442 and 445 (1954).

[30] C. L. Bird and D. F. Scott, *J. Soc. Dyers Colour.* **72**, 56 (1956).

[31] D. Patterson and R. P. Sheldon, *Trans. Faraday Soc.* **55**, 1254 (1959).

[32] M. Sekido and H. Kojima, *J. Soc. Fiber Sci. Technol. Jap.* **21**, 644 (1965); **22**, 33 (1966).

[33] J. Cegarra and P. Puente, *Text. Res. J.* **37**, 343 (1967).

[34] K. V. Datye and R. Rajendran, *Indian J. Technol.* **4**, 101 (1966).

[35] E. Waters, *J. Soc. Dyers Colour.* **66**, 609 (1950).

case of nylon or secondary cellulose acetate. In general, it may be concluded that for all types of hydrophobic fibers the mechanism of disperse dye uptake is governed by a constant diffusion coefficient in the fiber phase independent of dye concentration. The half-dyeing times for disperse dyes on cellulose acetate vary within wide limits from about 1 to 30 minutes.[36] Vickerstaff[37] and Bird and Scott[30] observed a similar wide range of $t_{1/2}$ values from 3 to 30 minutes for cellulose acetate and nylon, respectively.

One important aspect of dyeing with disperse dyes which is not apparent from the above-mentioned studies is the influence of the state of dye in the dyebath on the dyeing process. Disperse dyes in the bath are present as a very finely divided dispersion of dye particles which acts as a reservoir of dye which is released as the dye dissolved in the solution is used up. Hence, the rate of dyeing will be affected by rate of dissolution of dye. Vickerstaff and Waters,[38] for example, from studies on the dyeing of cellulose acetate with CI Disperse Red 11 found that the diffusion coefficient calculated using Hill's equation[20] varied with dyebath concentration by as much as $2\frac{1}{2}$ times, although this should not be the case for a system having a constant diffusion coefficient. In dye dispersions it is evident that changes in dispersion properties occur with changes in concentration and/or degree of dispersion. The occurrence of crystal growth in practical dyebaths is known to reduce rate of dyeing. Since dispersing agents change the equilibrium between dye dispersion and dye in true solution in dyebaths, the rate of dyeing is also affected by changes in concentration of the dispersion agent. The solubility of disperse dyes, though small, is a very important factor in dyeing. Bird and Manchester[39] have shown that for dyes which are fairly insoluble, increasing the concentration of a dispersing agent reduces equilibrium dye uptake, but increases the rate of dyeing. Conversely, for the more soluble disperse dyes the rate of dyeing is decreased.

As mentioned earlier, the rate of dye uptake of Terylene fibers is very small up to 100°. Hence dyeing time is speeded up either by increasing the temperature or by adding a "carrier," which can be an aromatic hydrocarbon, ether, ester, etc. For example, Walls[40] has shown that the addition of 8% Tumescal D at 100° considerably increases the rate of dyeing of Terylene by Dispersol Fast Scarlet B150. Vickerstaff[41] has

[36] C. L. Bird, F. Manchester, and D. F. Scott, *J. Soc. Dyers Colour.* **72**, 49 (1956).
[37] T. Vickerstaff, *J. Soc. Dyers Colour.* **59**, 592 (1949).
[38] T. Vickerstaff and E. Waters, *J. Soc. Dyers Colour.* **58**, 116 (1942).
[39] C. L. Bird and F. Manchester, *J. Soc. Dyers Colour.* **71**, 604 (1955).
[40] I. M. S. Walls, *J. Text. Inst.* **45**, 258P (1954).
[41] T. Vickerstaff, *Hexag. Dig.* **20**, 7 (1954).

suggested that the organic compounds which increase dyeing rate do so by forming a complex with the dye which dyes more readily and hence "carries" the dye into the fiber. Other mechanisms of carrier action which have been proposed are, for example, dissolution of the dye in the particles of the insoluble carrier,[42,43] which enables a more rapid transport of dye to the fiber surface than in the absence of the carrier. Sufficient proof for these ideas has not been obtained.

Alternatively, Schuler,[44] Remington and Schroeder,[45] and Salvin and Walker[42] suggest that carriers are adsorbed by the fiber and modify the amorphous region of the fiber. For example, Schuler[44] has shown that equimolar quantities of carrier enhance dyeing rate to the same extent. According to Glenz *et al.*[46] and Remington and Schroeder,[45] the chemical structure of carriers influences only the extent of adsorption by the fiber. For example, Vickerstaff[41] has shown that phenol, which has a low affinity, must be applied at a concentration of 20 g/liter to give the same carrier effect as biphenyl having a concentration of 0.75 g/liter. Such evidence, as well as the influence of carriers on swelling[35,42,47] and on fiber mechanical properties,[41,47,48] indicate that carriers influence fiber structure by a plasticizing effect which enhances dye penetration by increasing the mobility of polymer chain segments. Since dyes have different chemical structures and hence various shapes and sizes, this factor also can influence carrier action.[48] McGregor *et al.*,[49] using the microdensitometric technique, have recently shown that when films of cellulose triacetate are immersed in a dyebath (CI Disperse Red 15) containing carrier LB, both dye and carrier diffuse simultaneously into the film, which also exhibits a marked volume change if not already swollen completely by a pretreatment. In this situation, as discussed by these authors, Fick's equations were found to be inapplicable since these equations are not valid for a strongly swelling system or where bulk flow may be a major factor. On the other hand, if the film is already fully swollen by pretreatments in carrier LB, the diffusion data could be described by Fick's equations. When a carrier such as trichlorobenzene was used, the diffusion behavior more or less conformed to

[42] V. S. Salvin and R. A. Walker, *Amer. Dyest. Rep.* **48**, No. 14, 35 (1959).

[43] F. Fortess, W. J. Myles, V. S. Salvin, and W. A. Schoenberg, *Amer. Dyest. Rep.* **45**, P88 (1956).

[44] M. J. Schuler, *Text. Res. J.* **27**, 352 (1957).

[45] W. R. Remington and H. E. Schroeder, *Text. Res. J.* **27**, 177 (1957).

[46] O. Glenz, W. Beckmann, and W. Wunder, *J. Soc. Dyers Colour.* **75**, 141 (1959).

[47] F. M. Rawicz, D. M. Cates, and H. A. Rutherford, *Amer. Dyest. Rep.* **50**, 320 (1961).

[48] A. Wurtz, *Melliand Textilber.* **42**, 913 (1961).

[49] R. McGregor, R. H. Peters, and C. R. Ramachandran, *J. Soc. Dyers Colour.* **84**, 9 (1968).

Fick's equations with or without pretreatment of the film, although this carrier markedly accelerated the rate of dyeing as compared to dyeing rates with aqueous dispersions in the absence of carrier. Since trichlorobenzene, unlike carrier LB, does not significantly swell the polymer film, it is likely that the differences in behavior between these carriers is related to marked differences in their swelling properties, ease of penetration into the film, and their interaction with the various other components in the system. The validity of Fick's law in all cases for both carriers where the films were pretreated and equilibrated in blank dyebaths shows that the diffusion of dyes could be described by Fick's equations provided the system is in mechanical equilibrium, there are no significant volume changes during diffusion, and bulk flow is negligible.

The various theories discussed above regarding carrier action have recently been reviewed and summarized by Murray and Mortimer.[50] They point out that the available evidence is in agreement with the conclusions reached in a paper presented at the Piedmont Section of the AATCC[51] and the work of Rochas and Courmont.[52] These conclusions are that carrier mechanism involves action within the fiber rather than in the dyebath, the carrier loosening the internal structure of the hydrophobic fiber and also partially replacing interchain bonds with more readily disrupted fiber–carrier bonds. The increase in mobility in the fiber structural elements increases the number of openings for the same thermal energy conditions as compared to a dye–fiber system in the absence of a carrier. Consequently there will be greater dye diffusion in the more permeable structure which results from the carrier action.

Very few studies have been carried out on the relation between the size of the disperse dye molecule and its rate of diffusion. Bird *et al.*[36] found that dye diffusion coefficients in cellulose acetate decreased with increasing molecular weight. A similar correlation was observed by Vickerstaff[18] for aminoazobenzene derivatives on nylon. Giles[53] has plotted the data from the paper of Bird *et al.*[36] as shown in Fig. 8, which very clearly illustrates the relationship between disperse dye molecular weights and their corresponding diffusion coefficients.

Sekido and Kojima[32] using the roll-film method for the dyeing of

[50] A. Murray and K. Mortimer, "Review of Progress in Coloration and Related Topics," Vol. 2, p. 67. Soc. Dyers Colour., Bradford, England, 1971.

[51] AATCC, Piedmont Section, *Amer. Dyest. Rep.* **48**, No. 22, p. 23; No. 23, p. 37 (1959); see also Murray and Mortimer.[50]

[52] P. Rochas and M. Courmont, *IFATCC Congr.* (1959); see also Murray and Mortimer.[50]

[53] C. H. Giles, *Brit. Polym. J.* **3**, 279 (1971).

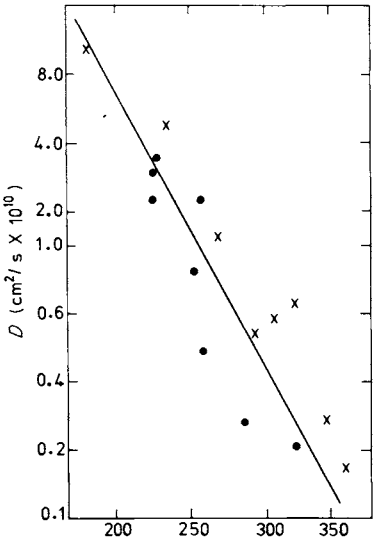


FIG. 8. Relation between molecular weight of disperse dyes and their diffusion coefficient (log) in cellulose acetate at 80°: ●, anthraquinone dyes; ×, azo dyes. (Plotted from data of Bird *et al.*[36]) Reproduced by permission from Giles.[53]

polyester film found that with increasing molecular size the dye diffusion coefficient in the substrate decreases. Since the affinity of the dye for the polymer chain is a function of the size of the dye molecule for such systems and since with increasing molecular size diffusion into void spaces within the polymer substrate will also decrease, the results mentioned above can be expected. However, in addition to molecular size the shape factor can also be important, as shown by Vickerstaff[18] in the dyeing of nylon 66 with aminoazobenzene derivatives, which gave a decreasing diffusion coefficient with increasing molecular size whereas anthraquinone derivatives showed no such correlation. Glenz *et al.*[46] observed a general linear relationship between a parameter related to the molecular volume of the dye and the reciprocal of its diffusion coefficient. However, they also point out that for those dyes where there is greater affinity of the dye for the fiber due to the presence of –OH or –NH groups which can form H bonds with the fiber, this chemical factor is more important than the size factor.

Merian[54] has obtained a linear relation (Fig. 9) between log D and a function of molecular size and molecular weight of the dye based on the equation

$$\log D = \log K - AV_m - Bd \tag{11}$$

[54] E. Merian, *Text. Res. J.* **36**, 612 (1966).

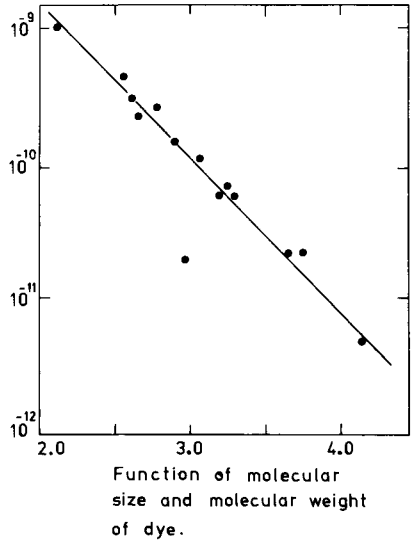


FIG. 9. Illustrating the influence of the molecular structure of disperse dyes upon their rate of dyeing on cellulose acetate. Reproduced by permission from Giles.[53] The x axis = [1/110][2 × molecular cross section (in Å^2) + molecular weight].

In this equation, which was originally derived by Park[55] for diffusion of organic vapors in polystyrene, K, A, and B are constants, V_m is the molar volume, and d the minimum molecular diameter of the diffusing molecule.

c. Acrylic and Wool Fibers. The kinetics of dyeing acrylic fibers with basic dyes has not been as extensively investigated as those of cellulose–direct dye and synthetic fibers–disperse dye systems. Practical difficulties in studying dyeing kinetics for the acrylic fiber–basic dye system are related to (a) the very rapid adsorption of dye on the fiber, which leads to errors in precise determination of the start of a diffusion process; (b) the strong adsorption of dye on sites, which influences the diffusion of the dye molecules dissolved inside the fiber phase and complicates the theoretical treatment of the diffusion process, and (c) the irregular nature of fiber cross sections, which makes the use of Hill's equation for cylindrical fibers [Eq. (7)] somewhat inaccurate. Nevertheless, sufficient data are now available to give a reasonable description of the kinetics of dyeing and the dye diffusion process.

Vogel *et al.*[56] showed that there is an inverse relation between dye

[55] G. S. Park, *Trans. Faraday Soc.* **47**, 1007 (1951).

[56] T. Vogel, J. M. A. DeBruyne, and C. L. Zimmerman, *Amer. Dyest. Rep.* **47**, P581 (1958).

substantivity and diffusion rates in the fiber. Highly substantive dyes have low diffusion rates and vice versa. Beckmann[57] derived the following expression for the rate of dyeing V in terms of the saturation concentration of dye S, the fiber and dyebath concentrations C_f and C_l, respectively, and two dye-dependent factors z and a, which are related to the influence of the ζ potential.[57,58]

$$V = a(C_l + z/a)(S - C_f) \tag{12}$$

The ζ potential studies showed that for a given fiber having a finite negative ζ potential in water, basic dye adsorption neutralizes this potential at a particular concentration and any further increase in dye concentration does not significantly change the potential, i.e., the concentration of adsorbed dye remains practically constant. However, it is difficult to evaluate these parameters precisely and Eq. (12) is largely empirical.

Rosenbaum[59] calculated the apparent diffusion coefficient D_a from experimental studies for various cationic dye–polyacrylonitrile fibers containing sulfonate sites. Equation (13) was used to calculate D_a, where S is the site content (based on % sulfur expressed in moles per kilogram), A_w is the outer surface area in square centimeters per gram and D_ϕ in moles per kilogram is the adsorbed dye concentration at time t in minutes.

$$\frac{D_\phi}{S} = \frac{2}{\pi^{1/2}} A_w(D_a t)^{1/2} \tag{13}$$

Figure 10 shows that D_a increases with increasing site content. Rosenbaum also showed, by carrying out rate-of-dyeing studies for an oxazine dye on fibers of different site content but having the same denier as well as surface and equilibrium dye concentrations, that this increase in D_a due to an increase in sulfonate content was not due to an increase in surface dye concentration. These results, shown in Fig. 11, indicate that the decrease in $t_{1/2}$ with increasing site content can only be due to a change in site content since at no stage did surface concentration increase with site content.

Rosenbaum[59] and Goodwin and Rosenbaum[60] showed that the rate of dye uptake was independent of pH and salt concentration. They also observed that D_a was independent of the concentration of dye on the fiber surface. Cegarra[61] has shown that rate of dyeing is independent of

[57] W. Beckmann, *J. Soc. Dyers Colour.* **77**, 616 (1961).
[58] O. Glenz and W. Beckmann, *Melliand Textilber.* **38**, 296 and 783 (1957).
[59] S. Rosenbaum, *Text. Res. J.* **34**, 291 (1964).
[60] F. L. Goodwin and S. Rosenbaum, *Text. Res. J.* **35**, 439 (1965).
[61] J. Cegarra, *J. Soc. Dyers Colour.* **87**, 149 (1971).

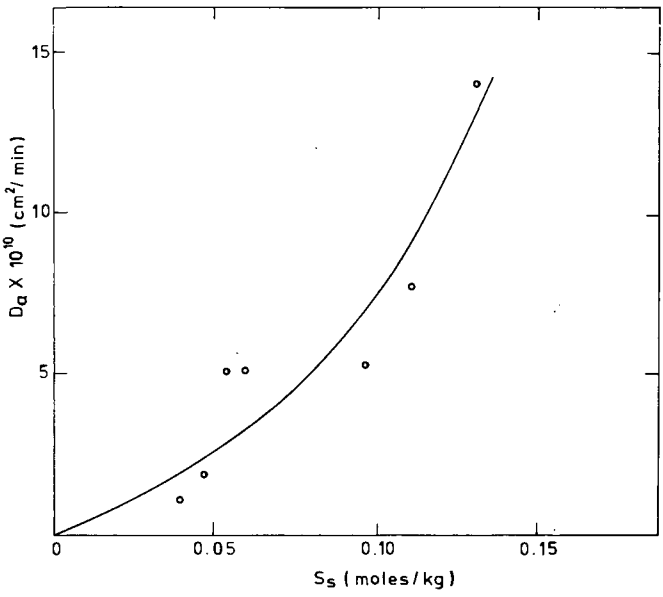


FIG. 10. Variation of apparent diffusion coefficient D_a with site content. Temperature, 95.1°; pH, 4.20; NaOAc, 0.07 *M*. Reproduced by permission from Rosenbaum.[59]

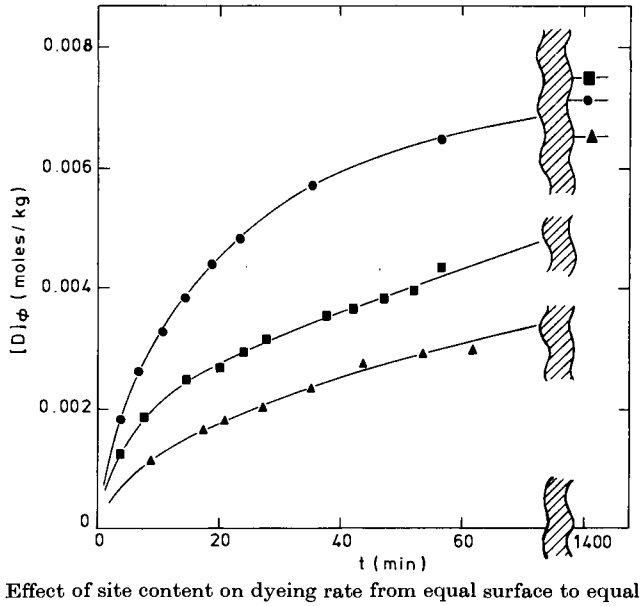


FIG. 11. Effect of site content on dyeing rate from equal surface to equal equilibrium concentrations: oxazine dye; sites in moles/kg, ▲, 0.044; ■, 0.050; ●, 0.109; pH 4.20; NaOAc 0.07 *M*; temperature, 95.1°. Reproduced by permission from Rosenbaum.[59]

pH for acrylic fibers having both strong and weak acidic groups (Liacryl 16), but for Courtelle, which has only weak acid groups, pH influences dyeing rate. Sand,[62] from more recent studies on sorption of basic dyes by Orlon 42 yarn, postulated a definite concentration dependence of D_a which is contrary to Rosenbaum's results. Cegarra[61] in a recent review suggests that these differences may only be due to the different simplifications used by both workers in calculating D_a.

Beckmann[57] observed that the rate of dyeing was independent of dyebath concentration for the initial stages of dye uptake. Rosenbaum[63] also came to a similar conclusion for dyes such as CI Basic Green 4 and CI Basic Green 1, but for an oxazine dye which gives surface saturation only at high dyebath concentrations, the dye concentration in solution at low concentrations influences the rate of dyeing (see also Cegarra[61]).

An analysis by Carbonell *et al.*[64] of the experimental data for basic dye–acrylic fibers has shown that the kinetic equations of Hill [Eq. (7)] and Cegarra and Puente [Eq. (10)] appear to describe the data quite well throughout the dyeing process, while Patterson and Sheldon's[31] equation, namely, $C_t = kt^{1/2}$ gave good agreement for short dyeing times.

It is generally concluded from the various studies reported that diffusion of dyes in acrylic fibers is based on a site-to-site mechanism with the fiber element playing an important role. A free-volume model of dye diffusion relating the diffusion coefficient to polymer segment relaxation time appears to be well established and is discussed later.

Rate-of-dyeing studies on wool fibers have been carried out by Speakman and Smith[65] for the dyeing of wool with CI Acid Orange 10 as a function of pH, temperature, and fiber structure. A linear relationship exists between dye uptake and the square root of dyeing time in the early stages of dyeing, diffusion occurring from a surface layer of constant dye concentration. Therefore, the slope of this linear curve varies as D, where D is the diffusion coefficient. Lemin and Vickerstaff[66] obtained similar results for the dyeing of wool with CI Acid Orange 7. Since wool fibers contain a membrane on the outer surfaces, called the epicuticle, that offers a great resistance to dye diffusion, any damage to this membrane increases dye uptake.[67] Lindberg[68] observed that short

[62] H. Sand, *Kolloid-Z. Z. Polym.* **218**, 30 (1967).

[63] S. Rosenbaum, *J. Appl. Polym. Sci.* **7**, 1275 (1963).

[64] J. Carbonell, J.P. Merminod, and R. Hasler, *Bol. Inst. Invest. Text.* p. 23 (1968); see Cegarra.[61]

[65] J. B. Speakman and S. G. Smith, *J. Soc. Dyers Colour.* **52**, 121 (1936).

[66] D. Lemin and T. Vickerstaff, *J. Soc. Dyers Colour.* **63**, 405 (1947).

[67] J. Lindberg, B. Philip, and N. Gralen, *Nature (London)* **162**, 458 (1948).

[68] J. Lindberg, *Text. Res. J.* **20**, 381 (1950).

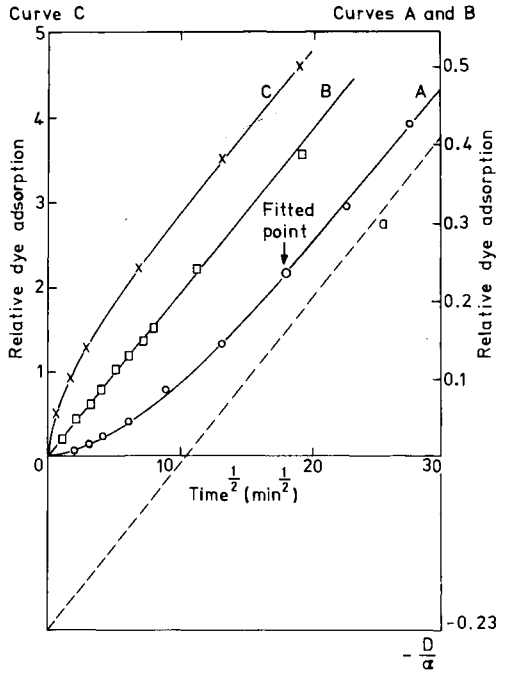


FIG. 12. (A) Theoretical curve for uptake through surface barrier, with experimental points for virgin Merino wool; a, asymptote to A. (B) Theoretical curve for uptake without surface barrier, with experimental points for abraded Merino wool. (C) Experimental curve for rough keratin (cow's horn) membrane. Reproduced by permission from Medley and Andrews.[69]

treatments of wool fibers with alcoholic potash considerably increase the rate of uptake of hydrochloric acid, and this is due to the severe degradation of the epicuticle by the treatment with alcoholic caustic potash. Medley and Andrews[69] have studied the influence of the epicuticle as a surface barrier to wool dyeing. They set up a simple model of a homogeneous solid in the form of a plane sheet with a barrier covering the surface immersed in an infinite dyebath. The barrier was assumed to be nonabsorbent with a linear concentration gradient of dye across its thickness. From the corresponding equation for the flux, assuming a diffusion coefficient D_b in the barrier, the equations for the boundary condition for flow of dye into the solid with the normal diffusion coefficient D were set up and solved as in the case of a semi-infinite solid using a method due to Crank.[12] The result is an equation giving relative dye uptake as a function of $t^{1/2}$. A plot of relative dye uptake versus $t^{1/2}$, shown in Fig. 12, has an initially concave upward

[69] J. Medley and M. W. Andrews, *Text. Res. J.* **29**, 398 (1959).

trend which then becomes linear with increasing dye uptake. The initial concavity is attributed to an effect of the surface barrier that is a maximum in the initial stages of dye diffusion and that becomes progressively less important as sorption increases. The theoretical curve for the same system in the absence of a surface barrier was linear throughout. This model has been tested experimentally for the dyeing of virgin and abraded merino wool with CI Acid Orange 7 from a well-stirred infinite dyebath. The experimental points for the virgin wool (epicuticle is present) could be fitted into the theoretical curve for uptake through a surface barrier. The experimental points for the abraded wool lie on the theoretical curve for dye uptake in the absence of a surface barrier.

Medley and Andrews[70] extended these studies to wool fiber assemblies which could be regarded as a set of cylinders. They also showed that when alcohol was present in the aqueous dyebath, the dyeing rate was increased. Medley and Ramsden,[71] from studies of dye uptake by Soxhlet-extracted nongreasy water mixtures, have suggested that the increased dyeing rates in the presence of various alcohols may be due to interaction between the dye and alcohol that reduces adsorption forces with the fiber and hence increases dye mobility. From a more recent study of the structure of water-swollen keratin in relation to the diffusion of various types of molecules including dyes, Medley[72] suggests that since the percentage of water in swollen keratin at saturation is about 33%, the diffusion of various types and sizes of charged and uncharged molecules is inconsistent with the pore theory of diffusion of Standing *et al.*,[23] which is usually used to describe the diffusion of dyes in water-swollen cellulose fibers. In this theory, which is discussed in Section I,C, it is assumed that the adsorbed dye is relatively immobile, and diffusion of free dye occurs mainly through the liquor in the pores. According to Medley, the adsorbed ions are also mobile and migration is not due to solution in the internal water. The affinity of the dye for the wool fiber is due to hydrophobic interactions which are of a nonlocalizing nature and hence dye migration is not restricted by dye affinity.

Since wool has both an unusual heterogeneous structure and high chemical reactivity, any damage caused to wool in normal wet processing, such as, for example, carbonizing, alkaline scouring, or bleaching, that modifies its chemical structure will also affect dye diffusion and adsorption.[5] Changes in the sulfur content of the fibers also affect rate

[70] J. Medley and M. W. Andrews, *Text. Res. J.* **30**, 855 (1960).
[71] J. Medley and D. K. Ramsden, *Proc. Int. Wool Text. Res. Conf., 2nd*, p. 707 (1960).
[72] J. Medley, *Proc. Int. Wool Text. Res. Conf., 3rd*, p. 117 (1965).

of dyeing since such changes are related to changes in the number of disulfide cross-links. An increase in the number of cross-links decreases the dye diffusion rate.[65,66] Pretreatments of the fiber which break cross-links increase the rate of dyeing.

Very little work has been done on the effect of molecular size and shape in relation to the diffusion of dyes in wool fibers. Giles *et al.*[73] observed a decrease in the diffusion coefficient with increasing molecular volume for the diffusion of a given series of acid dyes in wool and gelatin. It was also observed that for two dyes having the same molecular weights, volumes, and ionic charge, the dye having the lesser minimum cross-sectional area, i.e., the slimmer dye molecule, diffused more rapidly. Cockett *et al.*[74] from studies on the effect of urea on dyeing rates of acid dyes in wool conclude that urea increases the rate of dyeing by disaggregation of the dye in solution as well as reduction of dye–protein hydrophobic interactions. Holmes[75] measured the diffusion constants D_w and D_h, respectively, for five dyes both in aqueous solutions and in human hair. The dyes had effective molecular radii ranging from 2.0 Å (azobenzene *p*-sulfonic acid) to about 6.5 Å (Neolan Black). A correlation between the relative diffusion rates in hair and in water and the effective molecular radius was used to calculate the average size of holes in human hair. The method, although it is based on assumptions, some of which are of doubtful validity, is interesting and could be applied to other fiber or membrane systems also.

2. *Practical Significance of Rate-of-Dyeing Curves*

The rate of dye diffusion is of great interest particularly in relation to penetration and leveling properties. The rate of dyeing is of equal or greater importance in practice because it is the main factor that determines the inequalities in dye distribution in the textile substrate during the initial stages of dyeing. Therefore, several studies on dyeing rates have been carried out by various workers under practical dyeing conditions to give an index of comparative dyeing behavior of any given class of dyes for a particular textile fiber. The basic experimental methods of studying dyeing rate are well described by Vickerstaff.[18] In general, dyeing rate is best defined in terms of the time of half-dyeing $t_{1/2}$, where $t_{1/2}$ is defined as the time required to adsorb half as much dye as it will in the equilibrium state. Since in practice the affinities of different dyes for any given textile substrate under any given set of

[73] C. H. Giles, A. P. Montgomery, and H. A. Tolia, *Text. Res. J.* **32**, 99 (1962).
[74] K. R. F. Cockett, I. D. Rattee, and C. B. Stevens, *J. Soc. Dyers Colour.* **85**, 461 (1969).
[75] A. W. Holmes, *Proc. Int. Wool Text. Res. Conf., 3rd*, p. 79 (1965).

conditions differ considerably, to eliminate this effect, Boulton[76,76a] and Boulton and Reading[77] obtained $t_{1/2}$ values for direct dyes on viscose not under the conditions of an infinite dyebath, but with the electrolyte concentration adjusted to give a final exhaustion of 50%. A wide range of $t_{1/2}$ values was obtained. Under these conditions, rate of dyeing is approximately related to diffusion rate in the fiber. Neale confirmed this result by comparing his D_a (apparent diffusion coefficient) values for 19 dyes on cellophane with the $t_{1/2}$ values obtained by Boulton and Reading[77] for the same dyes on viscose rayon. Boulton[76a] in later work compiled data on $t_{1/2}$ for almost 200 dyes. Values of $t_{1/2}$ cover a very wide range, as can be seen from Table II for a selected list of dyes, and this factor is of practical importance.

Because of its practical significance, several measurements of dyeing rate over the wide range of dyebath exhaustion (5–100%) met with under practical conditions have been carried out by Lemin *et al.*[78] for direct dyes on cotton at liquor ratios of 5:1 and 60:1 in the presence and absence of salt. The $t_{1/2}$ values differ considerably from those of Boulton[76,76a] and cover only a fairly narrow range of rates unlike the very wide range of rates obtained by Boulton.[76,76a] Furthermore, the rates are much more at the higher liquor ratio of 60:1 (low exhaustion) as compared to the rates for the lower liquor ratio of 5:1 (high exhaustion). In such dyeings from finite dyebaths, the rate is a function of dye affinity, and the boundary condition of constant surface dye concentration for infinite dyebaths as used in Eq. (7) is invalid. Rate-of-dyeing equations for finite dyebaths assuming constant D which have been solved by Wilson[79] and Crank[80] for a plane sheet and for an infinite cylinder, respectively, are given in Eqs. (14) and (15).

$$\frac{M_t}{M_\infty} = 1 - \sum_{n=1}^{\infty} \frac{2\alpha(1 + \alpha)}{1 + \alpha + \alpha^2 q_n^2} \exp\left[\frac{-4Dq_n^2 t}{l^2}\right] \tag{14}$$

where the q_n's are the nonzero positive roots of

$$\tan q_n = -\alpha q_n$$

and α is the ratio of the volumes of solution and sheet or, if there is a partition factor K, then α is the ratio divided by K.[79]

$$\frac{M_t}{M_\infty} = 1 - \sum \frac{4\alpha(1 + \alpha)}{4 + 4\alpha + 4\alpha^2 q_n^2} \exp\left[\frac{-q_n^2 Dt}{a^2}\right] \tag{15}$$

[76] J. Boulton, *Trans. Faraday Soc.* **31**, 276 (1935).
[76a] *J. Soc. Dyers Colour.* **60**, 5 (1944).
[77] J. Boulton and B. Reading, *J. Soc. Dyers Colour.* **50**, 381 (1934).
[78] D. Lemin, E. J. Vickers, and T. Vickerstaff, *J. Soc. Dyers Colour.* **62**, 132 (1946).
[79] A. H. Wilson, *Phil. Mag.* [7] **39**, 48 (1948).
[80] J. Crank, *Phil. Mag.* [7] **39**, 362 (1948).

TABLE II

RATE OF DYEING OF DIRECT DYES ON VISCOSE RAYON[a]

Dye	*Concentration of sodium chloride used* (%)	*Time of half-dyeing* (*min*)
Chlorazol Fast Orange GS	5.0	0.07
Icyl Orange R	28.0	0.10
Diazo Brilliant Orange GR	4.0	0.22
Benzo Fast Yellow 4GL	8.5	0.26
Chrysophenine G	13.0	0.26
Chlorazol Violet R	5.0	0.50
Durazol Red 2B	5.0	0.84
Pyrazol Orange G	2.4	0.88
Primuline AS	2.0	1.00
Chlorazol Fast Yellow 5GKS	2.0	1.1
Benzo Fast Helio 4BL	5.4	1.2
Rosanthrene Pink	16.0	1.4
Benzo Fast Yellow RL	8.5	1.5
Chlorazol Dark Green PL	0.4	4.5
Benzopurpurine 4B	0.3	8.9
Oxyphenine GG	2.0	9.6
Chlorazol Sky Blue FF	2.0	15.9
Melantherine BH	1.0	19.0
Chlorazol Brown MS	0.0	21.0
Trisulphon Brown B	2.0	25.2
Chlorazol Green G	1.2	27.7
Benzopurpurine 10B	0.2	27.7
Diazo Black OT	1.0	42.8
Direct Fast Scarlet SE	0.0	100
Solar Orange 4G	10.0	126
Chlorazol Fast Orange AGS	3.3	156
Chlorantine Fast Scarlet BNLL	9.0	260

[a] Reproduced from Boulton.[76a]

where α is related to the fractional equilibrium exhaustion ϵ by $\alpha = (1 - \epsilon)/\epsilon$ and the q's are the nonzero roots of Eq. (16)

$$\alpha q_n J_0(q_n) + 2J_1(q_n) = 0 \tag{16}$$

where $J_0(q_n)$ and $J_1(q_n)$ are the Bessel functions of the first kind.[80]

When the rate curves for different percentage exhaustions are plotted using these equations it is found that as exhaustion decreases from 90% to 30%, $t_{1/2}$ increases from about 2 to 60 minutes, i.e., the rate of dyeing increases with increasing exhaustion. The calculated curves are shown graphically in Fig. 13. It is best to compare dyeing rates under

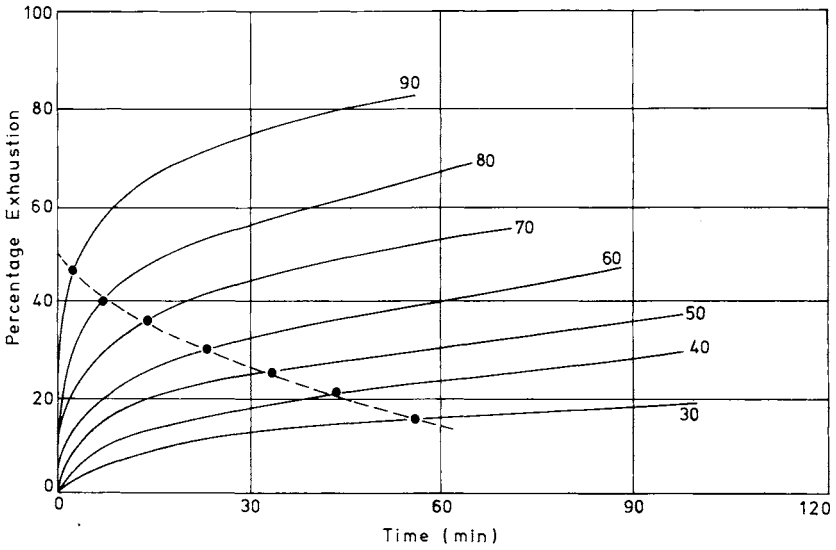


FIG. 13. Rate-of-dyeing curves from a finite dyebath leading to different percentage exhaustion, as indicated on curves. Reproduced by permission from Imperial Chemical Industries, Ltd., Organic Division, Manchester, England.

conditions of the same percentage exhaustion as was done by Boulton.[76,76a] Since the overall dyeing rate is dependent on a wide variety of factors under technical dyeing conditions (affinity, liquor ratio, electrolyte concentration, agitation rate, pH, temperature, substrate geometry), it is practically impossible to study dyeing rates separately for each set of conditions. Since diffusion rate is a fundamental property of the dye for a particular substrate independent of most of the variables mentioned above and, furthermore, it markedly influences the dye sorption process, the diffusion rates of individual dyes may be the most useful criteria as a guide to dyeing behavior. Rapidly diffusing dyes show good leveling and penetration and are easily applied. Contrary behavior is shown by slowly diffusing dyes.[18,76,76a]

Under conditions of very high affinity, i.e., high exhaustion, dyeing and diffusion rates are not correlated.[18] For example, cross-sections of cellulose fibers dyed with CI Direct Red 2 and CI Vat Green 1, respectively, under conditions of rapid and very high ($\sim 100\%$) exhaustion showed that all the dye was present only in a narrow annular ring at the fiber surface.[81] It can thus be inferred, since the penetration is very small, that the very rapid rate of dyeing is controlled, not by dye diffusion in the fibers, but by the rate at which dye reaches the fiber surface from the solution. As discussed in Section I,B,1,c, similar

[81] J. Boulton and T. H. Morton, *J. Soc. Dyers Colour.* **55**, 481 (1939).

results have been reported by Beckmann,[57] Rosenbaum,[63] and Cegarra[61] for basic dyes on polyacrylonitrile fibers, the initial portion of the rate curve being independent of dyebath concentration except at low dye concentrations.

Kilby[82] has shown that over a wide range of fractional exhaustion values ϵ ranging from 0.30 to 0.999 it is possible, assuming dye adsorption is controlled by a simple diffusion process, to calculate a quantity $Dt_{1/2}/\alpha$ for every value of the quantity $\alpha = (1 - \epsilon)/\epsilon$. The quantity α is thus the ratio of equilibrium dye liquor concentration to the equilibrium concentration of adsorbed dye. If α is a constant, as for example in dyeing with disperse dyes (constant partition coefficient) or in the case of direct dyes on cellulose at a fixed salt–dye ratio,[83] then $\alpha_2 V_1 = \alpha_1 V_2$, where V_1 and V_2 correspond to two different liquor ratios. It is now possible to calculate the $t_{1/2}$ value corresponding to any given V and α either from a graph of log $Dt_{1/2}/\alpha$ versus log α or from a simple equation. Alternatively, if $t_{1/2}$ is known, D can be calculated. Such calculations are useful at the low liquor ratios and high exhaustions that are often met with in practice and where dyeing rates are too rapid to be measured experimentally. The results obtained for disperse dyes on cellulose acetate are in very good agreement with the above-mentioned theoretical approach. However, when exhaustion is very high and rapid as discussed earlier, D is not the controlling factor for dyeing rate and Kilby's equation cannot be used readily in such circumstances.

The adsorption of vat dyes on cellulose is very high and rapid (the so-called strike) due to the high electrolyte content which is necessary for dyeing. Under such conditions, the initial dyeing rate is generally found to be proportional to the equilibrium exhaustion.[84] Vickerstaff[18] has suggested that the strike is inversely proportional to the antilogarithm of the dye affinity from the initial dyebath and $D^{1/2}$. This equation may be used as a guide to the compatibility of vat dyes. This is also in agreement with the data of Clark and McCleary.[84] A similar approach has been found to be useful for disperse dyes on nylon.[18]

Since mixture dyeing is of practical importance, many studies have been made to correlate rates of dyeing and rates of diffusion of individual dyes with their compatibility in mixture dyeing. In the case of mixtures of two direct dyes, Vickerstaff[18] has suggested, on the basis of earlier studies by several workers, that the two dyes will be compatible if their diffusion rates in the fiber are the same, i.e., Boulton's rates of dyeing

[82] W. F. Kilby, *J. Soc. Dyers Colour.* **76**, 479 (1960).

[83] H. F. Willis, J. O. Warwicker, A. R. Urquhart, and H. A. Standing, *Trans. Faraday Soc.* **41**, 506 (1945).

[84] O. W. Clark and H. R. McCleary, *Amer. Dyest. Rep.* **38**, P828 (1949).

should be approximately the same for both dyes and, in addition, both dyes should have the same charge and standard affinity. However, in general, for direct dye adsorption on cellulose the equilibrium absorption of one dye is reduced in the presence of a second dye.[85,86] Dye interaction in solution and on the fiber to form complexes can be a reason for such behavior since such interactions are known to occur.[87–89] Sekido and Morita[90] studied the rate of dyeing of cellulose with a mixture of CI Direct Red 2 and CI Direct Blue 1 and with the individual dyes using Sekido's roll-film method (see Section I,D). From an analysis of the concentration–distribution curves for the single dyes and the mixtures at various electrolyte concentrations, they conclude that dye interaction occurs in the cellulose substrate and increases with increasing salt concentration, but decreases with decreasing temperature. Since these two dyes have widely different affinities, the surface concentration of one dye is reduced in the presence of the other dye. Similar studies[91] using mixtures of CI Direct Yellow 12 and CI Direct Red 81, which gave approximately the same surface concentration, showed that the concentration distributions were similar and agreed with theoretical distribution curves in one dimension[92] assuming constant diffusion coefficients for the individual dyes which are independent of the presence of the other. Figure 14 shows the satisfactory agreement between the calculated and experimental dye distribution curves. A similar analysis for the interacting mixture of CI Direct Red 2 and CI Direct Blue 1 showed that diffusion of CI Direct Blue 1 is accelerated by the presence of CI Direct Red 2.

In mixture dyeing with disperse dyes, since the dye diffusion process obeys Fick's law with a constant diffusion coefficient and dye uptake by the fiber is a constant partition process, when the uptake of dyes in a mixture occurs, the presence of one dye should not markedly affect the other and in general the rates of dyeing of disperse dyes on most hydrophobic fibers are the same whether dyed singly or from a mixture of dyes. However, this is true only in the cases of additive dye mixtures, i.e., where no interaction between the dyes occurs. Johnson *et al.*[93] have

[85] J. Boulton, A. E. Delph, F. Fothergill, and T. H. Morton, *J. Text. Inst.* **24**, 113P (1933).

[86] S. M. Neale and W. A. Stringfellow, *Trans. Faraday Soc.* **27**, 1167 (1933).

[87] A. N. Derbyshire and R. H. Peters, *J. Soc. Dyers Colour.* **72**, 268 (1958).

[88] Y. Horiki, Y. Tanizake, and N. Ando, *Bull. Chem. Soc. Jap.* **33**, 163 (1960).

[89] Y. Youki, *Bull. Chem. Soc. Jap.* **33**, 974 (1960).

[90] M. Sekido and Z. Morita, *Bull. Chem. Soc. Jap.* **35**, 1375 (1962).

[91] M. Sekido and Z. Morita, *Bull. Chem. Soc. Jap.* **36**, 1602 (1963).

[92] H. Uedaira, *J. Soc. Text. Cell. Ind. Jap.* **14**, 967 (1958).

[93] A. Johnson, R. H. Peters, and A. S. Ramadan, *J. Soc. Dyers Colour.* **80**, 129 (1964).

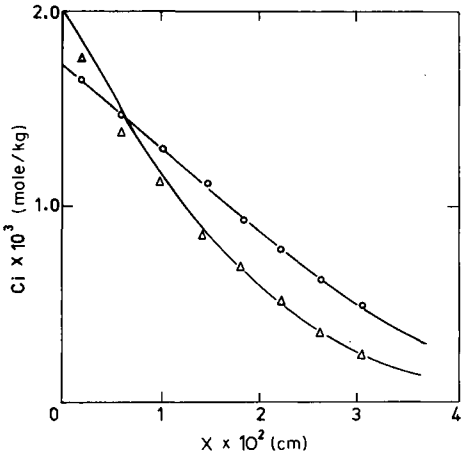


FIG. 14. Dye distribution in mixture dyeing: ○, CI Direct Yellow 12; △, CI Direct Red 81; ——, theoretical curve; $[D_1]_\sigma = 9.40 \times 10^{-5}$, $[D_2]_\sigma = 6.35 \times 10^{-5}$; NaCl, 3.0×10^{-2} mole/liter; 90°; 500 minutes. Reproduced by permission from Sekido and Morita.[91]

shown that the dyes in such mixtures yield normal melting point diagrams with a eutectic point. Mixtures of interacting dyes show no eutectic. Interaction was supposed to occur in the fiber phase. Hoffmann *et al.*[94] explain the departure from ideal behavior for binary disperse dye mixtures in terms of the formation of mixed crystals in aqueous dispersions, the adsorbed dyes in the fiber phase always being present in a monomolecular form. It is interaction in the dyebath and not in the fiber phase which results in deviations from ideal behavior (see also McDowell *et al.*[95]). More recently, Daruwalla *et al.*[96] have obtained evidence for interaction in both fiber and dyebath for certain disperse dye mixtures.

In the case of mixtures of acid dyes extreme interaction between the dyes in the mixture leads to incompatibility in dye uptake on nylon. Competition of the dyes in the mixture for the limited number of sites in the substrate can lead to the exclusion of the dye having the weaker affinity.

However, it was observed by Meggy[97] that in some cases the order of dyeing rate in mixtures was not the same as the order of affinities. For predicting compatibility, Meggy[97] considered a dyebath containing

[94] K. Hoffmann, W. McDowell, and R. Weingarten, *J. Soc. Dyers Colour.* **84**, 306 (1968).
[95] W. McDowell, R. Weingarten, and K. Hoffmann, *Melliand Textilber.* **50**, 1340 (1969).
[96] E. H. Daruwalla, R. M. Patel, and K. Tripathi, *Magy. Textiltech.* **23**, 86 (1971).
[97] A. B. Meggy, *J. Soc. Dyers Colour.* **66**, 510 (1950).

equimolar quantities of the two dyes and a fiber which can be divided into two regions having fractions a_1 and a_2 of the total fiber surface area through which dyeing takes place. He then proposed that the ratio a_1/a_2 is proportional to the relative affinities μ_1° and μ_2° of the dyes. The rates of adsorption were defined by the products a_1D_1 and a_2D_2, respectively, and the following equation for relative rates of adsorption was obtained:

$$\frac{a_1D_1}{a_2D_2} = \frac{\exp(-\Delta\mu_1^\circ/RT)}{\exp(-\Delta\mu_2^\circ/RT)}\frac{D_1}{D_2} \tag{17}$$

The compatibility factor $k = D\exp(-\Delta\mu^\circ/RT)$ and hence all dyes having the same k value will be compatible. In practice this is supported by experimental evidence. Atherton and Peters,[98] however, pointed out that while Meggy[97] assumes competition at the fiber surface, no interaction is assumed inside the fiber. This is because the division of the fiber surface into two separate portions, in each of which only one component of the binary mixture is dyeing, implies that each dye diffuses inside the fiber independently under its own concentration gradient. This assumption is difficult to understand in view of the large degree of incompatibility in mixture dyeing.

Atherton and Peters[98] proposed therefore that the driving force for diffusion is related to the activity gradient of the dye in the fiber phase, i.e.,

$$S = -\frac{D}{z}\frac{\partial}{\partial x}\left(\frac{\theta}{1-\theta}\right) \tag{18}$$

where S is diffusion per unit area of fiber, z the dye basicity, x the penetration in the fiber, θ the fraction of dyeing sites occupied, and $\theta/(1-\theta)$ the activity of the adsorbed dye. Hence the flux dQ/dt for such dye across the surface can be written as

$$S_1 = \frac{dQ_1}{dt} = -D_1\frac{\partial}{\partial x}\left[\frac{\theta_1}{1-\theta_1-\theta_2}\right] \tag{19}$$

$$S_2 = \frac{dQ_2}{dt} = -D_2\frac{\partial}{\partial x}\left[\frac{\theta_2}{1-\theta_1-\theta_2}\right] \tag{20}$$

In these equations Q_1 and Q_2 refer to the dye uptake of the two dyes, the quantities $\theta_1/(1-\theta_1-\theta_2)$ and $\theta_2/(1-\theta_1-\theta_2)$ being the respective activities of dye 1 and dye 2 in the presence of each other. Hence for

[98] E. Atherton and R. H. Peters, *Text. Res. J.* **26**, 497 (1956).

dyebaths in which all dye concentrations are constant throughout dyeing, the relative rates of uptake of each dye are proportional to each other, as shown by Eq. (21) obtained by dividing Eq. (19) and Eq. (20) after differentiating with respect to x.

$$\frac{S_1}{S_2} = \frac{(dQ_1/dt)}{(dQ_2/dt)} = \frac{D_1}{D_2}\left[\frac{(1-\theta_2)\left(\frac{\partial\theta_1}{\partial x}\right) + \theta_1\left(\frac{\partial\theta_2}{\partial x}\right)}{(1-\theta_1)\left(\frac{\partial\theta_2}{\partial x}\right) + \theta_2\left(\frac{\partial\theta_1}{\partial x}\right)}\right]$$

$$= \frac{D_1\theta_1}{D_2\theta_2}\left[\frac{\left(\frac{1-\theta_2}{\theta_1}\right)\left(\frac{\partial\theta_1}{\partial x}\right) + \frac{\partial\theta_2}{\partial x}}{\left(\frac{1-\theta_1}{\theta_2}\right)\left(\frac{\partial\theta_2}{\partial x}\right) + \frac{\partial\theta_1}{\partial x}}\right]$$

$$= \frac{D_1 z_1 C_{1,s}}{D_2 z_2 C_{2,s}} \exp\left[\frac{-(\Delta\mu_1^\circ - \Delta\mu_2^\circ)}{RT}\right] \qquad (21)$$

The quantities $C_{1,s}$ and $C_{2,s}$ refer to dyebath concentrations. This constant proportionality between Q_1 and Q_2 is shown in Fig. 15, which is typical of results obtained for 9 acid dyes in 21 binary mixtures. However, these results (see also Meggy[97]) are valid only for infinite dyebath conditions. In practical dyeing, finite baths where changes in dye concentration occur are commonly used. Hence, compatibility under

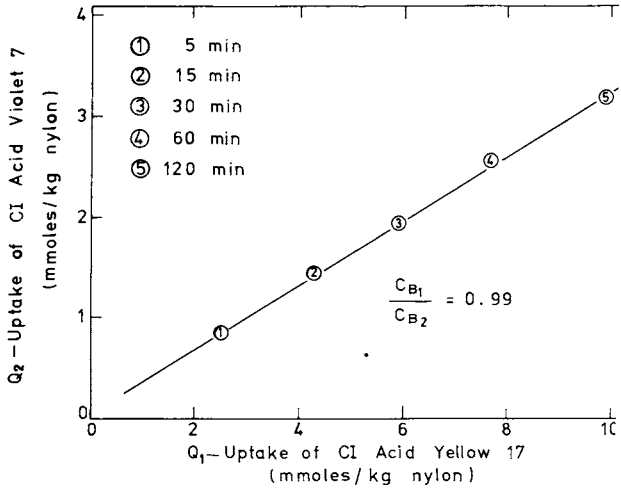


FIG. 15. Relative dye uptake from infinite dyebath at 75° from binary mixtures of CI Acid Yellow 17 and CI Acid Violet 7. Reproduced by permission from Atherton and Peters.[98]

such conditions should be defined. In a subsequent paper, Atherton *et al.*[99] derived the equation

$$\frac{dC_{1,s}/C_{1,s}}{dC_{2,s}/C_{2,s}} = \frac{d \ln C_{1,s}}{d \ln C_{2,s}} = \frac{D_1 z_1}{D_2 z_2} \exp\left[\frac{-(\Delta\mu_1^\circ - \Delta\mu_2^\circ)}{RT}\right] \tag{22}$$

for finite dyebaths. Logarithmic plots of $C_{1,s}$ and $C_{2,s}$ should therefore be linear and this was found to be true for several binary mixtures. It should, however, be borne in mind that Eq. (22) is valid only if the assumption made in the earlier equation, namely $\theta_1 + \theta_2 \simeq 1$, is valid, i.e., the fiber surface is saturated with dye at all times. If this is not valid, i.e., the total amount of dye is insufficient to saturate the external surface, Eq. (22) will still be valid if θ_1/θ_2 is constant. Hence in finite dyebaths where exhaustion is high, both ratios $C_{1,s}/C_{2,s}$ and θ_1/θ_2 should be constant throughout dyeing for a compatible mixture. The compatibility ratio k is given by Eq. (23),

$$k = \frac{D_1 z_1}{D_2 z_2} \exp\left[\frac{-(\Delta\mu_1^\circ - \Delta\mu_2^\circ)}{RT}\right] \tag{23}$$

which is another form of Eq. (17). Hence if $C_{1,s}/C_{2,s}$ is to be constant,

$$D_1 z_1 \exp(-\Delta\mu_1^\circ/RT) = D_2 z_2 \exp(-\Delta\mu_2^\circ/RT) \tag{24}$$

which is similar to Meggy's criterion for compatibility. If $k = 1$, the two dyes will be compatible since each side of Eq. (24) is characteristic of one dye in the mixture. Deviation of k from unity will be a measure of the extent of incompatibility. Experimental values of k were obtained from the slopes of the $\log C_{1,s}$ versus $\log C_{2,s}$ plots for several binary mixtures. Comparison of these values with k values calculated from experimental data for finite dyebaths is found to be reasonably satisfactory, as shown in Table III. Diffusion equations for mixtures of acid dyes in which there is an interference between the diffusing dye molecules have also been discussed by Tsuda.[100]

Meyer *et al.*[101] have shown from studies on Basacryl dyes that compatibility of binary mixtures of these dyes in fibers could be defined by a compatibility index Z, where

$$Z = \tan\alpha \times \tan\beta = kK_{\text{ads}}D^{1/2} \tag{25}$$

In this equation $\tan\alpha$ is the slope of the D_f (dye adsorbed) versus $t^{1/2}$

[99] E. Atherton, D. A. Downey, and R. H. Peters, *J. Soc. Dyers Colour.* **74**, 242 (1958).

[100] K. Tsuda, *Bull. Text. Res. Ind. Jap.* No. 9, p. 47 (1961); see *J. Text. Inst.* **53**, 515A (1962); *J. Soc. Text. Cell. Ind. Jap.* **18**, 710 (1962); see *J. Soc. Dyers Colour.* **79**, 40 (1963).

[101] U. Meyer, W. Ender, and A. Wurz, *Melliand Textilber.* **47**, 653 and 772 (1966).

TABLE III

COMBINATION OF DIFFUSION COEFFICIENT AND AFFINITY DATA IN FORM REQUIRED FOR CALCULATION OF COMPATIBILITY INDEX[a]

Dye	*Colour Index No.*	D/r^2 ($\times 10^5$)	z	$\exp(-\Delta\mu^\circ/RT)$ ($\times 10^3$)	$\frac{Dz\exp(-\Delta\mu^\circ/RT)}{r^2}$ ($\times 10^8$)
Tartrazine N	Acid Yellow 23	1.43	3	1.18	5.08
Solway Blue A	Acid Blue 69	3.28	2	0.794	5.21
Lissamine Red 6B	Acid Violet 7	1.19	2	2.75	6.57
Azo Geranine 2G	Acid Red 1	3.18	2	1.59	10.1
Lissamine Yellow 2G	Acid Yellow 17	2.50	2	2.09	10.45
Solway Blue BN	Acid Blue 45	4.38	2	1.41	12.35
Lissamine Red 7BP	Acid Violet 5	0.78	2	10.0	15.6
Naphthalene Fast Orange 2G	Acid Orange 10	7.00	2	1.35	18.9
Naphthalene Scarlet 48	Acid Red 18	1.94	3	4.47	26.1

[a] Reproduced by permission from Atherton *et al.*[99]

plot and $\tan\beta = K_{ads}/M$ is related to the substantivity of the dye where K_{ads} is the adsorption equilibrium constant. Basacryl dyes have Z values $\simeq$ 350 and Basacryl X dyes have $Z \simeq 150$.[102] These studies also show that compatibility as in the case of nylon–acid dye mixtures is a function of both the dye affinity and diffusion coefficient. Exhaustion curves and $t_{1/2}$ values for single dyes are not of much use in selecting compatible dyes, and it is necessary to consider the adsorption isotherm also. Recently Harwood *et al.*[103] have shown that for the uptake of the two basic dyes CI Basic Yellow 13 and CI Basic Blue 22 in acrylic films from mixtures, the conditions being such that the fiber surface is saturated with the dye,

$$\frac{(dQ_1/dt)}{(dQ_2/dt)}$$

is proportional to the ratio of concentrations of the yellow and the blue dye in solution.

[102] N. G. Morton and M. E. Kracht, *J. Soc. Dyers Colour.* **85**, 639 (1969).

[103] R. J. Harwood, R. McGregor, and R. H. Peters, *J. Soc. Dyers Colour.* **88**, 288 (1972).

3. *Effect of Temperature on Dyeing and Diffusion Rates*

The rate of dyeing increases with temperature, but the dye uptake at equilibrium decreases, since dyeing is an exothermic process. Since practical dyeing is carried out at shorter time intervals than required for equilibrium dyeing, the amount of dye taken up is influenced by the dyeing time. Figure 16 shows that in the case of slow-dyeing dyes, dye uptake can be a maximum within normal dyeing time (time A) at about 100°, whereas rapid dyeing dyes will have maximum adsorption at about 40°. Experimental data for disperse dyes on nylon give curves similar to those shown in Fig. 16.[18] Several studies have shown that dye

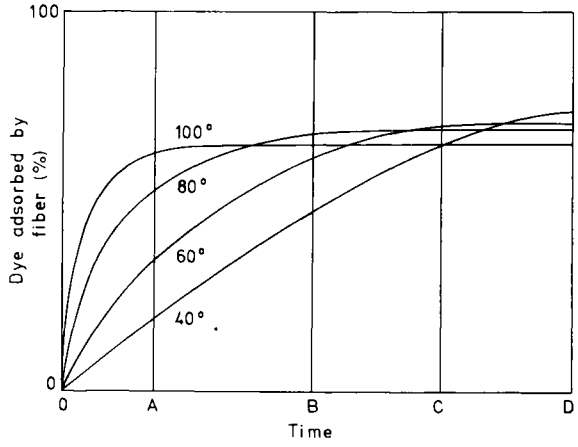


FIG. 16. Effect of temperature on dyeing rate and equilibrium. Reproduced by permission from Imperial Chemical Industries, Ltd., Organic Division, Manchester, England.

diffusion inside the textile substrate has a greater temperature coefficient than dye diffusion in the dyebath. Quantitatively, the effect of temperature on dyeing rate may be expressed by a suitable form of the Arrhenius equation, namely,

$$D_T = D_0 \exp(-E/RT) \tag{26}$$

where D_T is the observed diffusion coefficient, D_0 is a constant, and E is the energy of activation. Diffusion in the fiber is thus regarded as an activated process, and hence only that fraction of the total number of dye molecules having an activation energy E in excess of the average energy of the diffusing molecules will be able to overcome the restraints to diffusion and move inside the fiber. Logarithmic plots of ln D_T versus $1/T$ are usually linear provided T is below the glass transition temperature and E can be calculated from the slope of the linear plot.

The results of Garvie *et al.*[104] for the diffusion of CI Disperse Violet 36 into cellophane sheets gave a value of 14 kcal for the activation energy of diffusion. Similarly, from rate-of-dyeing studies of 22 disperse dyes on nylon yarn[18] as a function of temperature an average value of 22 kcal was obtained for the activation energy. Vickerstaff[18] has also discussed the use of such data in practical dyeing systems to calculate for a range of similar dyes their temperature properties.

The higher values of E for nylon as compared with cellulose may to some extent be correlated with the fact that nylon has a more compact structure than cellulose. Speakman and Smith[65] obtained a value of 22 kcal for the activation energy of diffusion of CI Acid Orange 10 in wool fibers. The modern synthetic fibers such as Terylene and the acrylics have a highly compact structure with restricted chain mobility and, as discussed earlier, their dyeing rates at ordinary temperatures (60°–100°) are very low. Practical dyeing in short time intervals is possible only by raising the temperature. Above 100°, the dye uptake for both types of fibers increases considerably. For example, Waters[35] observed a 48-fold increase in the relative diffusion rate of CI Disperse Orange 3 into Terylene for a 15° rise in temperature. The calculated value of E for such a change is very high, being 68 kcal. Patterson and Sheldon[31] have also obtained a value of about 60 kcal for disperse dyes on Terylene. Lyle *et al.*[105] have obtained a 3- to 16-fold increase in dye uptake for various disperse dyes on Orlon acrylic filaments and fibers in the temperature range 95° to 120° for a dyeing time of 1 hour. Tokasaka[106] has obtained the high value of 60 kcal for the activation energy of dyeing Orlon with disperse dyes. The correlation between activation energies and fiber structure is discussed in Section I,C.

C. Influence of Fiber Structure

Comparatively little work has been carried out on the influence of fiber structure on dyeing kinetics. Diffusion of dyes in fibers is affected by adsorption of dye in the fiber substrate, purely geometrical effects, anisotropy of structural elements, electrical charges on the fiber surface, extent of order and disorder in the fiber matrix, and physical changes in fiber structure due to temperature and/or mechanical effects such as stretch. Chemical changes in fiber structure such as, for example, acetylation of cellulose fibers, which can change the extent of crystallinity as well as the hydrophobic or hydrophilic nature of the fiber, can also

[104] W. M. Garvie, L. H. Griffiths, and S. M. Neale, *Trans. Faraday Soc.* **30**, 271 (1934).
[105] C. G. Lyle, J. J. Iannarone, and R. J. Thomas, *Amer. Dyest. Rep.* **40**, P585 (1951).
[106] A. Tokasaka, *J. Soc. Text. Cell. Ind. Jap.* **20**, 100 (1964).

influence rate of dye uptake. For example, Bird and Tabbron[107] observed a decrease in dyeing rate of disperse dyes with increasing acetyl value for cellulose acetate fibers.

An example of geometrical effects due to swelling is the more rapid diffusion of direct dyes in "never-dried" highly swollen cellulose gel films as compared to the usual commercial films.[108–110] Other geometrical effects such as surface area or pore size will be discussed at a later stage. Warwicker[110] from his studies of edgewise diffusion in swollen cellulose gel films observed that the diffusion of a direct dye was anisotropic, being more rapid along the axis of extrusion than perpendicular to it. However, as pointed out by McGregor and Peters,[111] this type of anisotropy is not so important for diffusion in fibers, which is a radial process. The important elements for diffusion in fiber structures are the variation in diffusion path lengths and tortuosity in the accessible amorphous regions due to the density of molecular packing and orientation in these regions. The ease of formation of "holes" of a suitable size for the passage of dye in the polymer matrix is another factor of importance. This factor can be significant in relation to the diffusion of dyes in hydrophobic synthetic fibers at higher temperatures.

For fibers that are almost identical as regards the internal physical structure (crystallinity, orientation, etc.) it has been observed that the rates of dyeing for both natural and synthetic fibers are related to the fiber denier, i.e., the rate of dyeing is proportional to the specific external surface area of the fiber, increasing with increasing surface area. Boulton and Morton[27] observed a rough correlation between $t_{1/2}$ and the fineness of hairs or filaments for various natural cellulosic fibers. Preston and Pal[112] correlated dyeing rates with $d^{1/2}$ for CI Direct Blue 1 on viscose fibers having a circular cross-section over a wide range of fiber denier d. Relative dye adsorption after 5 minutes was found to be a linear function of $1/d^{1/2}$. The fiber denier is defined as the weight in grams of 9000 meters and hence for fibers otherwise identical in all respects, the specific external surface area varies inversely as d.

An increase in fiber orientation with a decrease in d was also observed by these workers and this factor can also influence dyeing rates.

[107] C. L. Bird and G. Tabbron, *J. Soc. Dyers Colour.* **76**, 217 (1960).

[108] R. McGregor, R. H. Peters, and J. H. Petropoulos, *Trans. Faraday Soc.* **58**, 1045 (1962).

[109] K. J. Heritage, *Trans. Faraday Soc.* **54**, 1902 (1958).

[110] J. O. Warwicker, *J. Polym. Sci., Part A* **1**, 3105 (1963).

[111] R. McGregor and R. H. Peters, *J. Soc. Dyers Colour.* **84**, 267 (1968).

[112] J. M. Preston and P. Pal, *J. Soc. Dyers Colour.* **63**, 430 (1947).

Preston and Kapadia[113] showed that when the relative dye adsorption after 15 minutes normalized for specific surface area effects by multiplying dye adsorption by $d^{1/2}$ was plotted as a function of double refraction, a marked decrease in dyeing rate was observed with increasing fiber orientation.

Munden and Palmer[114] showed that an increase in draw ratio reduces the dyeing rate of CI Acid Blue 45 in nylon 66, $t_{1/2}$ changing from 1 minute at 0% of draw to 32 minutes at 260% draw. Since the process of drawing changes the fiber crystallinity and orientation, these factors would also influence the rate of dyeing in addition to geometrical factors such as the denier. Cegarra and Puente,[115] from rate-of-dyeing studies of polyester fibers by disperse dyes at 100° in the absence of a carrier, conclude that the rate is mainly influenced by the specific surface area of the polyester filament and that filaments with a high specific surface area dye more rapidly. Rosenbaum[63] obtained similar results for the diffusion of CI Basic Green 4 into polyacrylonitrile fibers. Davis and Taylor[116] from studies on the diffusion of CI Acid Orange 7 into nylon 66 found that each integral unit of draw ratio reduced the diffusion coefficient to about half its previous value, as shown in Fig. 17, although the equilibrium adsorption was practically unaffected.

Drawing increases the fiber orientation, density, and crystallinity. The activation energy for diffusion was found to increase with draw ratio from about 22 to 28 kcal/mole, as could be expected. Changes in density and crystallinity due to a preliminary treatment with boiling water also influenced the diffusion coefficient, and the activation energy was reduced to about 18 kcal/mole independent of draw ratio. This indicates a more accessible structure for the boiled fibers and hence more rapid dyeing. However, no simple correlation could be observed between density and diffusion rate. For example, a comparison of diffusion rates relative to the rates for an untreated sample showed that at 102° the treated sample that had a higher density was slower dyeing, whereas at 60° it was dyed more rapidly. Thus the advantage of a more open structure in the amorphous regions due to boiling appears to have been lost at 102° due to enhanced crystallinity. If, however, the more open structure (holes) is frozen in rapid cooling to 60° (as was done in the present case), the accessibility would be relatively higher than for the untreated sample at 60° even though the crystallinity of the boiled sample is higher, and hence dye diffusion would be faster in the treated

[113] J. M. Preston and A. H. Kapadia, *J. Soc. Dyers Colour.* **63**, 434 (1947).
[114] A. R. Munden and H. S. Palmer, *J. Text. Inst.* **41**, 609P (1950).
[115] J. Cegarra and P. Puente, *Text. Res. J.* **36**, 134 (1966).
[116] J. Davis and H. S. Taylor, *Text. Res. J.* **35**, 405 (1965).

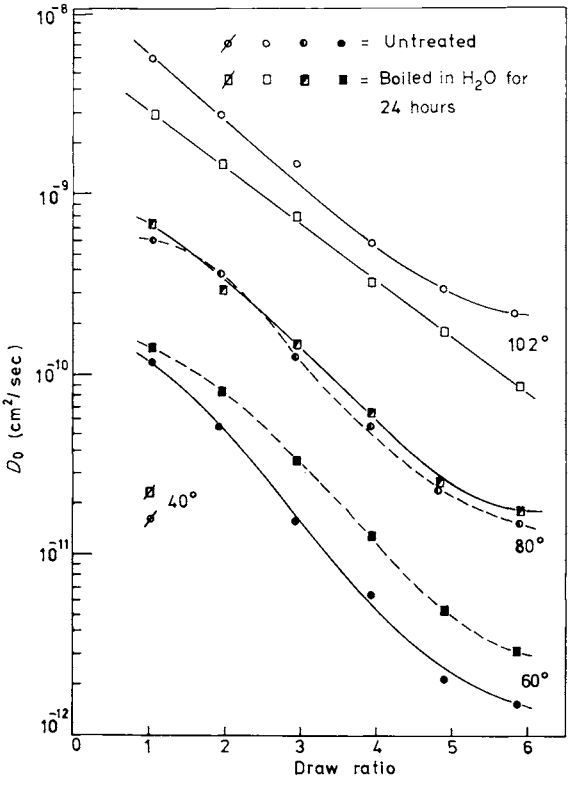


FIG. 17. Variation of diffusion coefficient at zero concentration with draw ratio of nylon 66. Reproduced by permission from Davis and Taylor.[116]

sample at 60°. It is therefore important to consider the relative temperature of dyeing and treatment as a significant variable in addition to diffusion when correlating structural changes with changes in diffusion rate.

Speakman and Smith[65] found that the rate of dyeing in wool fibers was almost proportional to the fiber surface area for unit weight. The presence of outer scales in wool was found to offer considerable resistance to dyeing since descaling appreciably increased the dyeing rate. Disulfide cross-links in wool which increase with increasing sulfur content were also found to decrease dyeing rates by reducing the dye permeability.[65,66] Giles *et al.*[73] correlate marked differences in rate of dye uptake of various dyes by wool and gelatin with differences in porosity of the substrates, the rate being four times higher in gelatin for almost all the dyes. Sivaraja Iyer *et al.*[117] have recently shown that cross-linking of viscose

[117] S. R. Sivaraja Iyer, L. S. Rao, and A. S. Ghanekar, *Textilveredlung* **5**, 345 (1970).

fiber with formaldehyde from nonaqueous baths (Form D Process of Reeves *et al.*[118]) leads to a reduction in the accessibility and average pore size. Studies on the kinetics of dyeing with direct dyes for blank and Form D cross-linked viscose fibers by Sivaraja Iyer and Ghanekar[119] also show that the activation energy for diffusion increases from 19 kcal/mole for blank viscose to 32.7 kcal/mole for the cross-linked material (% bound formaldehyde = 0.29). The corresponding diffusion coefficient is reduced approximately by a factor of 10. These results illustrate the marked influence on dye uptake due to changes in fiber structure as a result of cross-linking.

Peters and White[120] observed that heat-setting treatments of fibers using saturated steam increased the dyeing rate for nylon, but Yasuda[121] found an opposite effect. Since the conditions of heat-setting (wet or dry) modify the degree of crystallinity and also the accessibility of the amorphous regions of the polymer, these conditions of treatment also influence the rate of dyeing. In the case of nylon 6, both dry and saturated steam heat-set fibers have a higher degree of crystallinity compared to the untreated sample.[122–124] Tsuruta and Koshimo[122] and Koshimo and Kakishuta[125] observed that the rate of dyeing for steam-set nylon using direct and acid dyes of varying sizes and molecular weights is higher than that for the untreated material. However, the rate of dyeing for dry heat-set nylon is lower than that for the untreated material. These results hence show that an increase in density and crystallinity need not always result in a decrease in dyeing rate. The differences in behavior for the steam-set and the dry-set materials can be attributed to changes in molecular packing in the amorphous regions of nylon fibers, which are partly broken by steam-setting, leading to greater mobility of molecular chains and a more open structure which facilitates dye diffusion.[122,123,125]

Merian *et al.*[126] observed that the saturation values and average diffusion coefficients for disperse dyes on polyester fibers showed a minimum at a particular heat-setting temperature of about 150°. More

[118] W. A. Reeves, R. M. Perkins and L. H. Chance, *Text. Res. J.* **30**, 179 (1960); L. H. Chance, R. M. Perkins and W. A. Reeves, *ibid.* **31**, 366 (1961).

[119] S. R. Sivaraja Iyer and A. S. Ghanekar, unpublished work.

[120] H. W. Peters and T. R. White, *J. Soc. Dyers Colour.* **77**, 601 (1961).

[121] T. Yasuda, *J. Soc. Text. Cell. Ind. Jap.* **17**, 601 (1961).

[122] M. Tsuruta and A. Koshimo, *J. Appl. Polym. Sci.* **9**, 3 (1965).

[123] A. Koshimo, *J. Appl. Polym. Sci.* **9**, 81 (1965).

[124] H. J. Rau and U. A. Schwair, *Melliand Textilber.* **46**, 1423 (1966).

[125] A. Koshimo and T. Kakishuta, *J. Appl. Polym. Sci.* **9**, 91 (1965).

[126] E. Merian, J. Carbonell, U. Lerch, and V. Sanahuja, *J. Soc. Dyers Colour.* **79**, 505 (1963).

recently, Drumbleton *et al.*[127] have shown that diffusivity of a disperse dye in polyethylene terephthalate fibers exhibits a minimum at about 175°. X-Ray studies showed that heat-setting results in changes in crystallinity and crystallite size, and changes in diffusivity are attributed to this change in fiber properties. Sprague[128] studied the average diffusion coefficients of disperse dyes at various temperatures for a series of cellulose acetate yarns representing a wide range of orientation and molecular aggregation. He concluded that dye diffusion rate is a more sensitive index of small structural differences in fibers than wet mechanical properties. For example, the activation energy for diffusion gave evidence for an unreported transition in the acetate structure in the presence of water at 87°–95°, whereas a study of wet mechanical properties gave no evidence for this transition.

The activation energies E for dye diffusion have already been listed for various dye–fiber systems in Section I,B,3. As mentioned earlier, provided the range of temperature does not include the glass transition temperature T_g, the simple Arrhenius equation [Eq. (26)] can be used to calculate E. This activation energy can be regarded as the energy required by the diffusing molecule to "jump" from one adsorption site to an adjacent vacant site. The preexponential constant D_0 in Eq. (26) can be expressed by Eq. (27),

$$D_0 = e\lambda^2 \frac{kT}{h} \exp\left[\frac{\Delta S^*}{RT}\right] \tag{27}$$

where λ is the characteristic length for a diffusion jump. The Arrhenius equation can now be written as

$$D = e\lambda^2 \frac{kT}{h} \exp\left[\frac{\Delta S^*}{RT}\right] \exp\left[\frac{-E}{RT}\right] \tag{28}$$

where k and h have their usual significance and ΔS^*, the activation entropy, can be regarded as a measure of the changes in order or disorder in a diffusion jump. Equations (26) and (27) are in accordance with the theory of absolute reaction rates.[129] Majury[130] has pointed out that large contributions to ΔS^* and E can also arise from the influence of temperature on the internal sorption process. Thus the thermodynamic parameters ΔG_{dif}, ΔH_{dif}, and ΔS_{dif} for the overall diffusion

[127] J. H. Drumbleton, J. P. Bell, and T. Murayama, *J. Appl. Polym. Sci.* **12**, 2491 (1968).

[128] B. S. Sprague, *J. Polym. Sci., Part C* **20**, 159 (1967).

[129] S. Glasstone, K. J. Laidler, and H. Eyring, "The Theory of Rate Processes." McGraw-Hill, New York, 1941.

[130] T. G. Majury, *J. Soc. Dyers Colour.* **70**, 445 (1954).

process can be written as

$$\Delta G_{dif} = -\Delta G + \Delta G' \tag{29}$$

$$\Delta H_{dif} \simeq \Delta E_{dif} = -\Delta H + \Delta H' \tag{30}$$

$$\Delta S_{dif} = -\Delta S + \Delta S' \tag{31}$$

where the terms ΔG, ΔH, and ΔS, and $\Delta G'$, $\Delta H'$, and $\Delta S'$ refer, respectively, to the changes in these parameters due to overall sorption behavior and penetration of the fiber structure by the dye molecules.

The thermodynamic parameters ΔG (free energy of dyeing) and ΔH (heat of dyeing) for the equilibrium overall sorption process can be obtained from isotherm data. The total free energy ΔG_{dif} and heat of activation ΔH_{dif} for the overall diffusion process are calculated from the experimental data on temperature coefficients of diffusion. Thus the values of $\Delta G'$ and $\Delta H'$ can be calculated from Eqs. (29)and (30). Table IV

TABLE IV

COMPARISON OF ACTIVATION ENERGIES (KCAL/MOLE) OF DIFFUSION IN SECONDARY ACETATE AND TRIACETATE[a]

	Secondary Acetate			*Triacetate*		
Dye	ΔH_{dif}	$-\Delta H$	$\Delta H'$	ΔH_{dif}	$-\Delta H$ (approx.)	$\Delta H'$
p-Nitroaniline	15	7	8	26	7	19
N,*N*-Dimethyl-*p*-nitroaniline	13	8	5	20	8	12
Aminoazobenzene	18	9	9	31	9	22

[a] Reproduced by permission from the Society of Dyers and Colourists.

gives a comparison of these activation energies for diffusion. These results indicate that the activation energies of diffusion are much higher for cellulose triacetate than for the secondary acetate. Majury[130] concludes that these differences are related to the higher degree of obstruction to diffusion in the triacetate. The experimental values of the apparent diffusion coefficient D_a can be correlated with the measured values of ΔG, the free energy of sorption by the linear relationship

$$\ln D_a = \text{constant} + (\Delta G/RT) \tag{32}$$

Since $\Delta G = -RT \ln K$ where K is the partition coefficient

$$\ln D_a = \ln D_0 - \ln K \tag{33}$$

If D_0 is identified with the diffusion coefficient of the free dye in the fiber system, i.e., unadsorbed dye, then Eq. (33) reduces to

$$D_a = D_0/K \tag{34}$$

which is similar to the equation

$$D_a = V \frac{d[D]_i}{d[D]_\phi} D_0 \tag{35}$$

derived by Standing *et al.*,[23] for the apparent diffusion coefficient in terms of the gradient $d[D]_i/d[D]_\phi$ of the internal equilibrium isotherm existing in the pores of the fiber and the diffusion coefficient D_0 of the free dye molecules in the aqueous solution present in the fiber pores. V is the fractional volume of the pores in liters per kilogram of the fiber. McGregor and Peters[111] have more recently studied this model to include nonlinear internal sorption isotherms in order to explain the concentration dependence of D. All these models are oversimplified to the extent that the dye molecules in the fiber phase are regarded as either free or immobilized, and this arbitrary division has not been clearly substantiated experimentally. While it is experimentally known that equilibrium sorption takes some time inside the fiber phase, it is supposed that there is an instantaneous equilibrium between the concentrations of free and bound dye molecules. There is no experimental proof for this hypothesis. Nevertheless, these ideas are very useful in emphasizing the important role of sorption processes when a dye diffuses into a fiber system. The work on sorption diffusion processes is discussed in Section II.

Patterson and Sheldon,[31] assuming that for dye diffusion, λ in Eq. (28) can be regarded as the distance a dye molecule has to cover to pass a polymer chain, calculated the values of diffusion coefficients, entropies, and activation energies for the diffusion of two nonionic dyes in polyethylene terephthalate fibers having different draw ratios. These results are shown in Table V. The higher activation energies for the fibers having the higher draw ratio are consistent with the larger energy barrier, which can be expected in the more-oriented fiber sample. If it is further assumed that the activation energy is a measure of the energy requirements to form a "hole" of sufficient size to enable passage of the dye molecule, then the activation entropy ΔS^* can be regarded more specifically as a measure of the resulting structure disorientation during "hole" formation. The values of ΔS^* which have been calculated by Patterson and Sheldon[31] are also shown in Table V. Since these values of ΔS^* are large and positive, the authors conclude that a considerable disordering of molecular chains occurs over a fairly wide region during

TABLE V

VALUES OF DIFFUSION COEFFICIENTS, ENTROPIES, AND ACTIVATION ENERGIES OF DIFFUSION[a]

	CI Disperse Red 1			*CI Disperse Red 15*		
	N type	*F type*	*Staple*	*N type*	*F type*	*Staple*
Diffusion coefficients ($cm^2\ sec^{-1}$)						
70°	0.088×10^{-14}	0.39×10^{-14}	0.005×10^{-14}	0.10×10^{-14}	0.36×10^{-14}	—
80°	1.17	1.16	0.239	25.7	2.88	0.99×10^{-14}
85°	—	—	—	63.9	19.2	14.2
90°	7.72	8.9	2.1	190	46.2	69.5
95°	22.9	28.8	8.5	602	315	132
Activation energy (kcal/mole)	47	56	61	46	77	80
D_0 ($cm^2\ sec^{-1}$)	10^{18}	10^{21}	10^{23}	10^{22}	10^{28}	10^{29}
Entropies of activation (cal/mole deg)	85	98	107	100	130	135

[a] Reproduced by permission from Patterson and Sheldon.[31]

"hole" formation. The much lower values of the diffusion coefficient at 70° in all the fibers studied, coupled with the fact that these values are well below the linear Arrhenius plots of log diffusion coefficient versus $1/T$ for the higher temperatures, have been explained in terms of the known existence of a glass–rubber transition point at about 80° in such fibers. The motions of molecular chains below this temperature reduce considerably the frequency of "hole" formation and hence the diffusion coefficient. Recently, Blacker and Patterson[131] studied the optical dichroism of dyed polyester fibers as a function of the dye concentration in the fiber. The orientation factor was found to increase with increasing dye concentration even after allowing for any decrease in orientation due to factors such as shrinkage and hydrolysis. The apparent diffusion coefficient was found to decrease markedly with increasing dye concentration in the fiber phase. They conclude that the dye molecules first occupy the less-oriented zones in the fiber and only at higher dye concentrations are the more-oriented zones in the accessible region occupied. The decrease in the apparent diffusion coefficient with increasing dye concentration is explained by assuming that the increase in the orientation factor with increasing dye concentration also implies an increase in the ΔS^* value.

However, it should be pointed out that with increasing dye concentration, the greater crowding of the molecules in the fiber substrate and their more ordered arrangement should lead to a decrease in the entropy. Therefore, if the other factors in Eq. (28) remain unaltered, only a decrease in entropy can explain the decrease in the apparent diffusion coefficient D_a. It should also be pointed out that in the case of diffusion processes which are interpreted in terms of a movement of the free volume, the apparent activation energy E would be expected to decrease with increasing temperature above the glass transition temperature T_g, as has been observed for such kinetic phenomena in polymers.[132–135] The temperature range of 80°–95° ($T_g \simeq 80°$) in the work of Patterson and Sheldon[31] is perhaps not sufficiently wide to indicate possible variations in the energy of activation with temperature. Cohen and Turnbull[136] even suggest that diffusion can occur by a

[131] J. G. Blacker and D. Patterson, *J. Soc. Dyers Colour.* **85**, 598 (1969).

[132] J. B. Wilkens and F. A. Long, *Trans. Faraday Soc.* **53**, 1146 (1957).

[133] M. E. Williams, R. F. Landel, and J. D. Ferry, *J. Amer. Chem. Soc.* 77, 3071 (1955).

[134] H. Fujita, *in* "Diffusion in Polymers" (J. Crank and G. S. Park, eds.), Chapter 3, p. 75. Academic Press, New York, 1968.

[135] C. A. Kumins and T. K. Kwei, *in* "Diffusion in Polymers" (J. Crank and G. S. Park, eds.), Chapter 4, p. 107. Academic Press, New York, 1968.

[136] M. H. Cohen and D. Turnbull, *J. Chem. Phys.* **31**, 1164 (1959).

redistribution of the total free volume in such a way that no activation energy is involved.

Tagaki and Hattori[137] showed from studies on the diffusion of CI Acid Blue 82 into nylon 6 monofilaments at various draw ratios that the diffusion coefficient increased to a maximum at a draw ratio of about 1.6 and then rapidly decreased with increasing draw ratio. The activation energy for dye diffusion remained almost constant at about 20 kcal/mole up to a draw ratio of 3 and then increased with further drawing to about 30 kcal/mole at a draw ratio of about 5. It is suggested that in the intermediate region of draw ratios between 1.6 and 3 the polymer structure passes through an unstable phase and becomes stabilized above a draw ratio of 3. In the intermediate stage of drawing, the diffusion of dye decreases rapidly and hence the entropy of diffusion according to Eq. (28) decreases since E remains constant, i.e., the polymer structure is changed in such a way that the diffusing dye molecule partly loses its degree of freedom in the activated state of diffusion. Similarly, Morizane *et al.*[138] also observed that the apparent diffusion coefficient of an acid dye in nylon 6 increases with draw ratio to a maximum value at a draw ratio of 2 and then decreases with further increase in draw ratio. These results are explained by changes in the physical fine structure of nylon 6 with drawing, in particular the degree of swelling which reaches a maximum in the vicinity of a draw ratio of 2 as observed by Kanetsuna[139] from a study of lateral order distribution in nylon 6.

Rosenbaum[140] has developed a free-volume model of dye diffusion to give a relation between diffusion coefficient of dye and relaxation time of polymer segments in the fiber structure. His model was developed for diffusion of basic dyes in acrylic fibers above the glass transition temperature T_g where enough free volume is available for motion of the polymer segments. This free volume can be regarded in terms of units of molecular size which are called "holes," the orientation of these holes being about the same as that of a polymer segment. The average polymer segment is supposed to have about the same order of size as a dye molecule. The movement of a hole to a position adjacent to a dye molecule enables the dye molecule to jump into it with greater ease than a polymer segment. Dye diffusion is thus explained in terms of a movement in the free volume. As discussed earlier, above the glass transition temperature T_g, the activation energy for diffusion can then decrease

[137] Y. Tagaki and H. Hattori, *J. Appl. Polym. Sci.* **9**, 2167 (1965).

[138] H. Morizane, Y. Suda, and F. Nakajima, *Colourage (Annu.)* p. 26 (1970).

[139] H. Kanetsuna, *J. Soc. Text. Cell. Ind. Jap.* **18**, 800 (1962).

[140] S. Rosenbaum, *J. Polym. Sci., Part A* **3**, 1949 (1965).

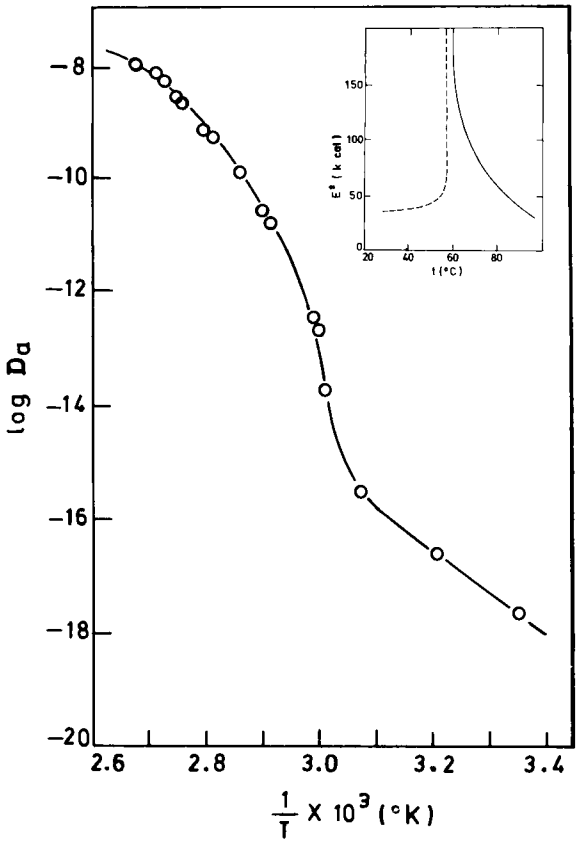


FIG. 18. Arrhenius plot of apparent diffusion coefficient D_a of CI Basic Green 4 in experimental polyacrylonitrile fibers. Inset plot shows variation with temperature of apparent activation energy. Reproduced by permission from Rosenbaum.[140]

with increasing temperature,[132,133] i.e., the Arrhenius equation with constant activation energy E need not be valid. Figure 18, which shows the data obtained by Rosenbaum[140] for the diffusion of CI Basic Green 4 in polyacrylonitrile fibers, clearly indicates the marked change in E near T_g and the subsequent decrease in E with temperature at all temperatures above T_g (see also Goodwin and Rosenbaum[60]).

The diffusion data could be fitted into the well-known Williams *et al.*[133] equation

$$\log a_T = -[c_1^g(T - T_g)]/[c_2^g + T - T_g] \tag{36}$$

where a_T is the ratio of relaxation times τ at temperatures T_g and T or in the present case the ratio D_{T_g}/D_T, since D is proportional to τ. How-

ever, the constants used by Rosenbaum [140] in this equation were obtained from his experimental data and are not the same as those used in the original WLF equation given above, the modified equation being

$$\log a_T = -[10.75(T - T_g)]/[28.5 + T - T_g] \quad (37)$$

The marked changes in polymer structure in the neighborhood of the glass transition temperature T_g are also reflected in marked changes in diffusion coefficients and dye uptake. Such marked changes in dye uptake in a narrow range of temperature near T_g have also been reported by Kitamura *et al.*[141] for polyester–disperse dye systems. Such results have led to the use of the term "transition temperature for dyeing" to describe this phenomenon.[5] The dye molecules, which due to their size require large free volumes, apparently take full advantage of any volume fluctuations which lead to an increased probability of formation of larger voids above T_g.

D. Concentration–Distribution Curves

The rate-of-dyeing studies which were discussed earlier in Section I,B give data only on the apparent diffusion coefficients D_a and the overall rates of dyeing. This is because these data are based on macroscopic measurements of dyeing kinetics in terms of changes in concentration of the dyebath and correlations between these concentration changes and the diffusion coefficient of the dye molecules penetrating the fiber matrix using, for example, Fick's law of diffusion. Since the phenomenon of dyeing involves interactions at the molecular level between dye molecules themselves, the fiber substrate and dye molecules, and dye–solvent interactions, such macroscopic studies do not provide much information on the molecular nature of the dyeing process or the particular peculiarities of dyeing behavior for different dye–substrate systems. Studies on the extent and rate of penetration of dyes in the polymer substrate as a function of the distribution of dye throughout the polymer give revealing information on the above-mentioned two aspects of dyeing. It is generally assumed in these experiments that the dyebath concentration is constant.

The experimental methods used by several workers are (a) the multiple membrane method of Garvie and Neale,[22] (b) the edgewise distribution method of Boulton and Morton,[81] later developed by Warwicker [110] for studying concentration dependence of diffusion rates,

[141] K. Kitamura, I. Iko, and M. Matsui, unpublished results referred to in Peters.[5]

and (c) the more recently developed microdensitometric technique of Peters and co-workers.[108,142–144]

In the multiple membrane method a block of polymer is simulated by forming a sandwich of 14 sheets of cellophane clamped together. The sandwich is immersed in the well-stirred dye solution and after a known time interval it is removed, chilled in cold water, and the dye content in the individual layers analyzed. It is thus possible to construct concentration–distribution curves for the dye which has diffused into the polymer system.

The roll-film method used by Sekido is a modification of the sandwich method and has been used by Sekido and Kojima[32] to study the diffusion of disperse dyes in polyester films. In this method, a roll of film is wound about 16–17 times around a Pyrex glass tube of about 1 cm diameter and 10 cm long. This method can also be used in a modified form for studying diffusion in polyester fabrics at high temperatures.[34]

In the edgewise diffusion method used by Warwicker,[110] a film of material is clamped in a frame between two suitably aligned flat blocks of stainless steel. When this assembly is immersed in the dye solution for a known time interval the dye diffuses from the edge of the film. A microdensitometer is used to determine the concentration gradient in the plane of the fiber. In the earlier work of Boulton and Morton,[81] only the extent of dye penetration for a given time interval was measured and not the concentration vs. distribution. Similar studies on penetration have been carried out by several other workers for the diffusion of disperse dyes in nylon,[145,146] and cationic dyes in acrylic fibers.[60]

The multiple membrane method has the disadvantage that the interfaces between the several sheets of film in the sandwich can influence the results due to factors such as entractment of dye liquor or air bubbles. Furthermore, the method is laborious and has the disadvantage of being somewhat inaccurate owing to the mathematical analysis of the results, which involves a double graphical differentiation of the data for use in the equation

$$\frac{ds}{dt} = -A\frac{dc}{dx} \tag{38}$$

where ds/dt is the weight of dye diffusing in unit time, A is the area in

[142] R. H. Peters and J. H. Petropoulos, *Bull. Inst. Text. Fr.* **84**, 49 (1959).

[143] R. McGregor, R. H. Peters, and J. H. Petropoulos, *Trans. Faraday Soc.* **58**, 771 (1962).

[144] R. McGregor, R. H. Peters, and J. H. Petropoulos, *Trans. Faraday Soc.* **58**, 1054 (1962).

[145] B. Kramer, *Melliand Textilber.* **35**, 518 (1954).

[146] W. Luck, *Melliand Textilber.* **36**, 927 and 1028 (1955).

square centimeters, x is the thickness of the membrane, and c is the dye concentration in the membrane at any given distance of penetration. In the edgewise diffusion method, a possible source of error is dye migration along the surface of the film. Moreover, neither of these methods is very sensitive to variations in concentration dependence, for very short time intervals and dyeing times are required which are relatively much longer than those used in practice.

In the microdensitometric method of Peters and co-workers,[108,142-144] which was developed to measure the concentration vs. distribution in a single sheet of polymer film, these errors are mostly eliminated. The general procedure used is to mount the film in a suitable frame and immerse the assembly in the well-stirred dye solution. After a predetermined time interval, the film is removed from the dyebath plunged into ice water to remove adhering solution, and then freeze-dried to immobilize the dye. Cross-sections of the films are then cut, suitably mounted on microscopic slides in a medium having the requisite refractive index, and scanned with a microdensitometer. This method is very accurate and reproducible, but various precautions have to be taken to obtain reliable data. Full details of this method are discussed in the various papers of Peters and co-workers referred to earlier. Olofsson,[147] Jelly and Pontius,[148] and Luck[146] have developed microdensitometric methods for studying very short dye penetration of the order of 10^{-3} cm. Melnikov *et al.*[149] have also used a microphotometric method to study the concentration versus penetration distance curves of direct dyes into cellulose films at high temperatures in the range 100°–130°. Their results show that the diffusion coefficient changes with distance of penetration from the surface, decreasing with increasing depth of penetration for the dye Direct Blue K, whereas for the dye Direct Diazo Black S, the diffusion coefficient is independent of the path of the dye. The addition of electrolytes is found to increase the rate of diffusion of dye into the film.

It is interesting to note that Brody[150] has more recently developed a method for the determination of dye diffusion in fibrous substances as a function of dye concentration in the fiber, based on the approximation method of Crank in which D corresponding to any time interval t can be calculated from measured adsorption isotherms and rate-of-dyeing

[147] B. Olofsson, *Medd. Sv. Textilforskringsinst.* Göteborg, Sweden. p. 29 (1953).

[148] E. E. Jelly and R. P. Pontius, *J. Photogr. Sci.* **2**, 15 (1954).

[149] B. N. Melnikov, M. N. Kirillova, and P. V. Moryganov, *Tekhnol. Tekst. Prom.* No. 6, p. 118 (1963); see *J. Soc. Dyers Colour.* **80**, 397 (1964); No. 2, p. 108 (1966); see *J. Text. Inst.* **57**, 641A (1966).

[150] H. Brody, *J. Soc. Dyers Colour.* **82**, 14 (1966).

curves. His results for the shape of the diffusion versus concentration curves for diffusion of CI Direct Blue 1 in rayon fiber at 90° are in good agreement with the results discussed earlier, which were obtained by McGregor *et al.*[108] for the same system. However, his absolute D values are lower than those of McGregor *et al.*,[108] probably because the latter used cellophane film and not rayon fibers, and hence differences due to orientation in fibers as well as skin effects can influence the values of D. This method is more simple than the microdensitometric method and warrants further study.

More recently, Blacker and Patterson[131] have developed a method for determining concentration distance profiles without cross-sectioning. In this method a microspectrophotometer is used for measuring the continuous changes in the transmitted monochromatic light when a dyed film of circular cross-section and about 10 μm thick is scanned across its long axis by moving the filament across a narrow slit. The dye distribution across the fiber is determined from computer calculations of the recorded data. It is also possible using this method to study the dichroism of the dyed filaments and hence the orientation of the dye molecules.

The methods (a), (b), and (c) mentioned above are all non-steady-state diffusion methods. Steady-state diffusion of dyes through single membranes can also be studied using the method of Garvie and Neale[22] (see also Barrer[151] and Peters[5]). In this method, a membrane divides two solutions containing different concentrations of the dye or an appropriate dye solution and a blank solution, and, after allowing a sufficient time interval for establishing a steady concentration gradient in the membrane, the rate at which the dye is transferred from the concentrated to the dilute solution is measured. The conditions of the experiment are so chosen that no significant changes in concentration take place in either solution during the experiment. The diffusion coefficient is calculated from the equation

$$\frac{ds}{dt} = -D_a \frac{\Delta C}{x} \tag{39}$$

where ΔC is the difference in concentration between the two sides of the membrane and $\Delta C/x$ refers to the gradient of dye concentration in the membrane. This method, which also gives information on the variations, if any, of dye diffusion coefficients with dye concentration in the membrane, is fully discussed by Vickerstaff.[18]

The results of concentration–distribution studies for various dye–fiber

[151] R. M. Barrer, *Fibrous Proteins, Proc. Symp.*, p. 108 (1946).

systems using the above mentioned methods are discussed in the following sections.

1. *Cellulose Fibers*

Garvie and Neale[22] found, using the multiple membrane method and the steady-state single membrane method, that the diffusion coefficient for direct dyes in cellophane was not constant, but varied with dye and salt concentration. Crank[152] using Matano's method[153] of analysis calculated the diffusion coefficient D at any period on single dye concentration–distance curves constructed from the data of Garvie and Neale.[22] He obtained a fairly linear concentration dependence of D.

McGregor *et al.*[108] obtained similar results using their microdensitometric method over a reasonably wide range of dye and salt concentrations for the diffusion of CI Direct Blue 1 at 90°. The observed departure from linearity at high dye concentration is attributed to changes in properties of the dye solution such as aggregate formation. Their results are shown in Fig. 19 and can be reasonably explained for most of the dye concentration range by the equation

$$D(c) = D_0(1 + \beta C) \tag{40}$$

where β is a constant and is a function of the square root of the electrolyte concentration. Analysis of the data in terms of the variation of $D(c)$ with salt concentration for different fixed concentrations of adsorbed dye gave the results shown in Fig. 20. It can be observed that in general $D(c)$ decreases rapidly with increasing salt concentration at low salt concentrations. The initial maxima observed at very low salt concentrations are consistent with the suggestion that the rapid rise in D_0 at low salt concentrations is due to the lowering of the electrical potential barriers to diffusion as the salt concentration increases. The apparent diffusion coefficient $\bar{D}$ calculated from all the above-mentioned results passes through a maximum with increasing salt concentration. This result is in qualitative agreement with similar data obtained by Neale and Stringfellow[86] from experimental overall rate-of-dyeing curves. However, this maximum occurs at a salt concentration of about 10 g/liter. These results therefore show that the measurement of the apparent diffusion coefficient $\bar{D}$ can give rise to misleading results regarding the influence of salt on the diffusion process in the fiber phase. McGregor and Peters[154] from a study of the diffusion of CI Direct Red 2

[152] J. Crank, *J. Soc. Dyers Colour.* **63**, 412 (1947).
[153] C. Matano, *Jap. J. Phys.* **8**, 109 (1932–1933).
[154] R. McGregor and R. H. Peters, *Trans. Faraday Soc.* **60**, 2062 (1964).

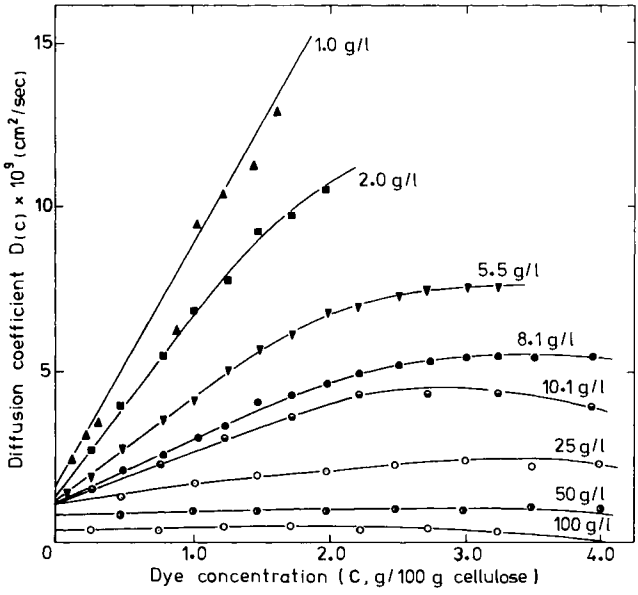


FIG. 19. $D(c)$ versus C relationships at 90° for different salt concentrations. Reproduced by permission from McGregor *et al.*[108]

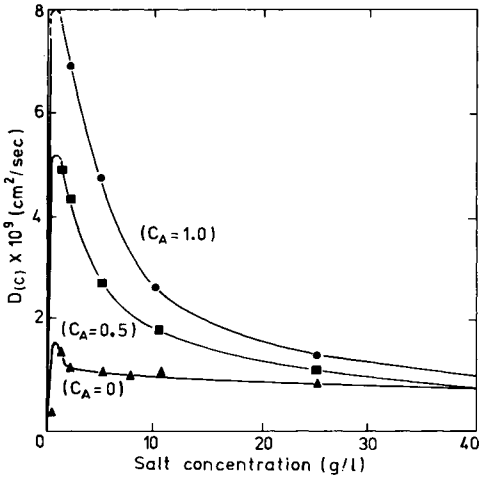


FIG. 20. Variation of $D(c)$ with salt concentration. Numbers on curves represent values of C_A (g/100 g). Reproduced by permission from McGregor *et al.*[108]

and its *meta* isomer into cellulose films conclude that the overall rate-of-dyeing curves agree well with the simple diffusion theory. However, a microdensitometric analysis of concentration-distribution during dyeing showed great deviations from simple theory, as can be seen from a comparison of Figs. 21 and 22, emphasizing again the fact that D_a has little theoretical value.

The anomalous diffusion behavior may arise from factors such as a variable surface concentration, internal sorption processes, the presence of deadend pores in the film, or aggregation of dye within the film. In the heterogeneous phase consisting of cellulose and the dye solution in the swollen amorphous regions, part of the dye is present in aqueous solution in the pores of the fiber and is also simultaneously adsorbed on the walls.[155–157] The dye may also diffuse along the cellulose chains, but

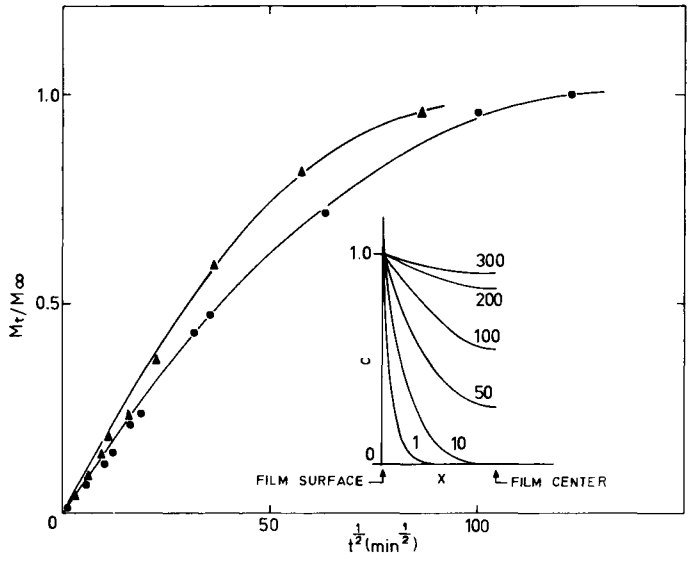


FIG. 21. Experimental rate-of-dyeing curves. [M_t is the amount of dye sorbed by unit weight of film at time t and M_∞ is the amount of dye sorbed by unit weight of film at equilibrium ($t \rightarrow \infty$).] The inset shows type of concentration distribution (schematic) to be expected in the films during dyeing, on the assumption that the rate-of-dyeing curves truly represent the "Fickian sorption" of dye. In the inset, the ordinate is dye concentration (C) (arbitrary units) and the abscissa is penetration distance (x) (arbitrary units). The numbers on the curves represent times of dyeing (arbitrary units). ●, Benzopurpurine 4B (0.25 g/liter dye; 5.0 g/liter salt; 50°, PT 600 cellophane); ▲, *m*-benzopurpurine 4B (0.50 g/liter dye; 5.0 g/liter salt; 25°, PT 600 cellophane). Reproduced by permission from McGregor and Peters.[154]

[155] T. H. Morton, *Trans. Faraday Soc.* **31**, 262 (1935).

[156] S. M. Neale, *J. Soc. Dyers Colour.* **52**, 252 (1936).

[157] E. Valko, *Trans. Faraday Soc.* **31**, 278 (1935).

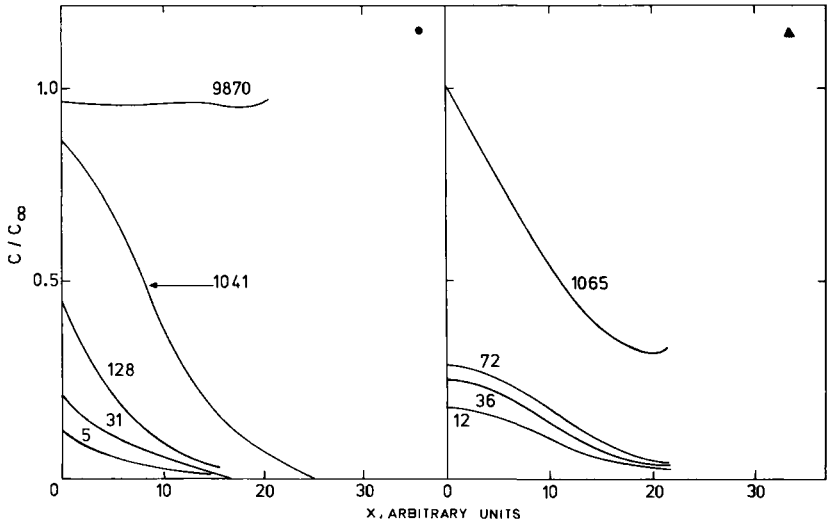


FIG. 22. Concentration distributions during dyeing. The symbol (● or ▲) in the upper righthand corner of each diagram shows to which of the curves in Fig. 21 the concentration distributions correspond. The numbers on the curves are the times of dyeing (t, min). [C is the concentration at a point inside the film, a distance x from the surface of the film. C_∞ is the equilibrium concentration in the film ($t \rightarrow \infty$)]. Reproduced by permission from McGregor and Peters.[154]

this is a very slow process compared to diffusion in the solution. Furthermore, since the dye is in general strongly bound to the surface, diffusion of this type is negligible.

If now in Eq. (35), which has been derived by Standing *et al.*[23] on the basis of the above-mentioned model, the assumption is made that the dye concentrations $[D]_i$ and $[D]_\sigma$ in the internal and external solutions, respectively, are the same, the observed decrease of D_a with increasing salt concentration at higher salt concentrations can be explained since the gradient of the equilibrium isotherm, namely, $d[D]_\phi/d[D]_i$ increases with increasing salt concentration. More recently, Warwicker,[110] obtained from his measurements of the diffusion of CI Direct Yellow 12 in cellulose sheets using the edgewise diffusion method a parameter α that is dependent on salt and dye concentration and is the ratio of the free volume for diffusion to the total volume. On introducing the assumption,[158] that the equilibrium isotherm is of the Freundlich type with an exponent $m = 0.5$, he showed that Eq. (41) for the concentration

$$D(C) = \frac{\alpha D_0}{m} \frac{1}{K^{1/m}} C^{(1-m)/m} \tag{41}$$

[158] F. H. Holmes, *Trans. Faraday Soc.* **54**, 1172 (1958).

dependence of the diffusion coefficient describes the nature of the dependence fairly well.

This model of the heterogeneous diffusion adsorption process of dye uptake, however, does not take into account the presence of a Donnan equilibrium between the dye in the internal solution phase and the external bulk solution phase, which means that the dye concentration in the cellulose pores is not the same as in the external solution as assumed in the model, but is a function of the Donnan distribution of ions, i.e., it depends on the dye and electrolyte concentration and the charge on the cellulose surface (see also Crank[152]). Moreover, recent studies by Sivaraja Iyer and co-workers[159,160] have shown that the internal adsorption isotherm, i.e., the isotherm expressing the variation of D_i and D_ϕ, is of the Langmuir type and not the Freundlich type, D_i being calculated from the measured values of the equilibrium dyebath concentration D_σ using the equations for a Donnan equilibrium. Recent studies on Donnan potentials and Donnan distribution of ions in the cellulose–aqueous dye + electrolyte solution system by Sivaraja Iyer and Jayaram,[161] and Sivaraja Iyer and Kalbag[162] have also shown that the so-called "volume term" used to express the dye concentration in the internal cellulose phase is not constant, but varies with electrolyte concentration. Warwicker's assumption of a variable value of α is in agreement with the above-mentioned discussion on the use of a variable volume term, since he correlates the value of α with that part of the free volume in the pores of the fiber which does not form part of the diffused double layer on the charged cellulose surface. Any quantitative theory of such diffusion adsorption processes should thus also take these factors into account.

McGregor and Mahajan,[163] using the steady-state method for studying the diffusion of acid dyes in cellulose films, have shown that the electrostatic repulsions between dye anions and the negative charge on the cellulose surface give rise to a surface barrier which influences dye diffusion.

Weisz and Zollinger[164] have recently presented an analysis of

[159] S. R. Sivaraja Iyer, G. Srinivasan, N. T. Baddi, and M. R. Ravikrishnan, *Text. Res. J.* **34**, 807 (1964).

[160] S. R. Sivaraja Iyer and N. T. Baddi, *in* "Contribution to the Chemistry of Synthetic Dyes and Mechanism of Dyeing," p. 36. Dept. Chem. Tech., Bombay University, Bombay, 1967.

[161] S. R. Sivaraja Iyer and R. Jayaram, *J. Soc. Dyers Colour.* **86**, 398 (1970).

[162] S. R. Sivaraja Iyer and V. N. Kalbag, unpublished results.

[163] R. McGregor and I. Y. Mahajan, *Trans. Faraday Soc.* **58**, 2484 (1962).

[164] P. B. Weisz and H. Zollinger, *Trans. Faraday Soc.* **64**, 1693 (1968).

sorption–diffusion behavior in dye–fiber systems based on the following model. The dye molecules are mobile in the solvent (water) within that volume fraction of the fiber capable of containing them and are immobilized in hydrophobic regions of the fiber. They derived the equation

$$D_a = \gamma\left(\frac{P}{b}\right)\left(\frac{c_0}{c_f}\right)nD \tag{42}$$

for the apparent diffusion coefficient where γ is a constant having a range of values from 1.0 to 1.6 depending on the form of the adsorption isotherm, P is the accessible fraction of the fiber, b is the tortuosity factor, c_0/c_f is the ratio of external dye concentration c in equilibrium with adsorbed dye concentration c_f, n is the equilibrium partition coefficient for mobile dye solution in the fiber phase in equilibrium with the external dye solution, and D is the true diffusion coefficient. The pore models of Standing *et al.*[23] and Warwicker[110] for the cellulose–direct dye solution system discussed earlier are thus seen to be a special case of the above equation where $n = 1$ and dye is adsorbed on the fiber surface. The equation can also be used for the disperse dye–hydrophobic polymer system. The model does not take into account electrical potential gradients, and, furthermore, the use of empirical factors such as γ does not really take into account the basic nature of the equilibrium sorption process. However, this model is capable of modification to include such additional factors. The calculated values of the diffusion coefficients for dye diffusion in cellulose as well as hydrophobic polymer substrates are found to yield diffusion coefficients for the mobile solute species that are equal in magnitude to the classical free diffusion coefficients for dye diffusion in aqueous solutions. These results suggest that for all fibers diffusion takes place through water-filled pores, but is considerably influenced by other factors such as adsorption, aggregation, and solution of dye in the polymer matrix.

2. *Hydrophobic Fibers*

a. Disperse Dye–Hydrophobic Fiber Systems. In the dyeing of hydrophobic fibers with the sparingly soluble nonionic disperse dyes from aqueous dispersions, the dye is considered to be transferred to the fiber through the solution phase. The dye dispersion solubilized in a suitable dispersing agent acts as a reservoir which readily supplies more dye to the true solution phase to maintain the strength of dye in aqueous solution. Thus, although the dye is sparingly soluble, sufficient dye is available in the solution for diffusion and dye uptake in the fiber phase. In general, the distribution of dye between the fiber and dyebath follows

a linear isotherm up to a saturation limit. This constant partition of dye between the solution and fiber phases is usually interpreted in terms of an ideal solution of dye in the fiber. Since the heats of dyeing in such systems are small, the forces of adsorption are not strong and do not very much restrict the movement of dye through the polymer. The possibility of a Langmuir-type dye adsorption isotherm as opposed to the solid solution hypothesis mentioned above cannot, however, be ruled out.

Concentration–distribution data for disperse dyes on cellulose triacetate, secondary cellulose acetate, nylon, Acrilan, and Terylene show that there is good agreement between the experimental data and the results expected on the basis of Fick's law for a constant diffusion coefficient.[29,30,49] A typical example of such agreement is the data obtained by McGregor *et al.*[49] for the diffusion of CI Disperse Red 15 from aqueous dispersion into nylon and secondary cellulose acetate. Their results are shown in Fig. 23. Deviation from Fick's law due to lack of pretreatment of the films of cellulose has already been discussed in Section I,B,1,b.

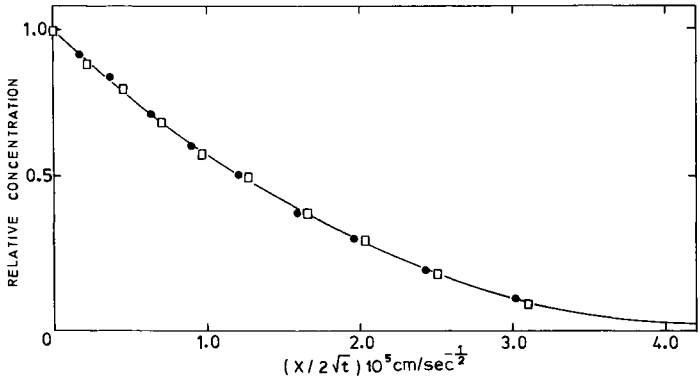


FIG. 23. Nylon 66 dyed with an aqueous dispersion of CI Disperse Red 15 at 55°: ●, 4 minutes; □, 9 minutes; ——, Fick's law curve. Reproduced by permission from McGregor *et al.*[49]

b. Ionic Dye–Hydrophobic Fiber Systems. Nearly all the investigations for such systems have been confined to a study of the diffusion of acid dyes in nylon. Many workers have shown that there is a close correlation between the number of terminal amino groups and the uptake of acid dyes by nylon, the relationship being nearly stoichiometric. McGregor *et al.*,[144] using the microdensitometric method, observed that the distribution of the trisulfonated dye CI Acid Red 18

in nylon 66 indicated a marked concentration dependence of the diffusion coefficient when the dye is adsorbed at a pH corresponding to a close stoichiometric relation between dye uptake and the number of terminal amino groups in nylon 66. A plot of the relative dye concentration in the fiber versus $x/2t^{1/2}$, where x is the distance of penetration and t is the time, gave a sigmoid type of curve as shown in Fig. 24. A plot of

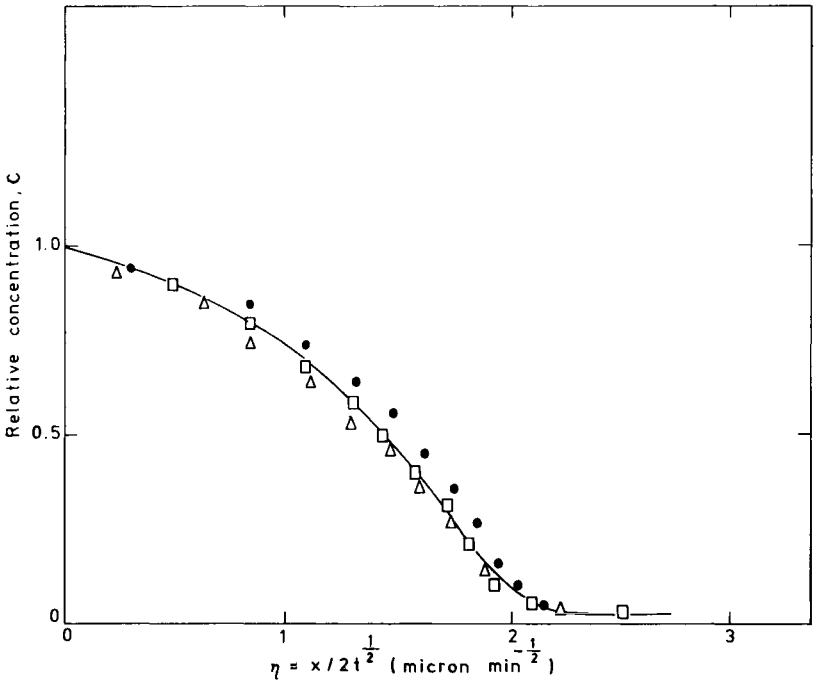


FIG. 24. Relative dye concentration C against $x/2t^{1/2}$ plot for CI Acid Red 18 in nylon 66: ●, 25 minutes; □, 50 minutes; △, 82 minutes. Reproduced by permission from McGregor *et al.*[144]

the variation of $D(c)$ versus C obtained from an analysis of the data in Fig. 24 using the equation

$$D_{(C=C_1)} = -\frac{1}{2t}\frac{\partial x}{\partial C}\int_0^{C_1} x\,dC \tag{43}$$

is shown in Fig. 25, where C_1 is any value of C between 0 and ∞. This method is the same as that used by Matano[153] for studying diffusion coefficients in metal systems and has been successfully employed in many cases for the dye–fiber systems. These results indicate that close

to the saturation value the diffusion coefficient $D(c)$ increases very sharply with dye concentration as shown by the common curve at pH 6 and pH 3.2. The change in $D(c)$ is more gradual at pH 1.95. At low pH values, Atherton *et al.*[165] have shown that nylon is overdyed, i.e., the dye is adsorbed in excess of the amino group content. Ferrini and Zollinger[166] suggest that together with the usual site-adsorption

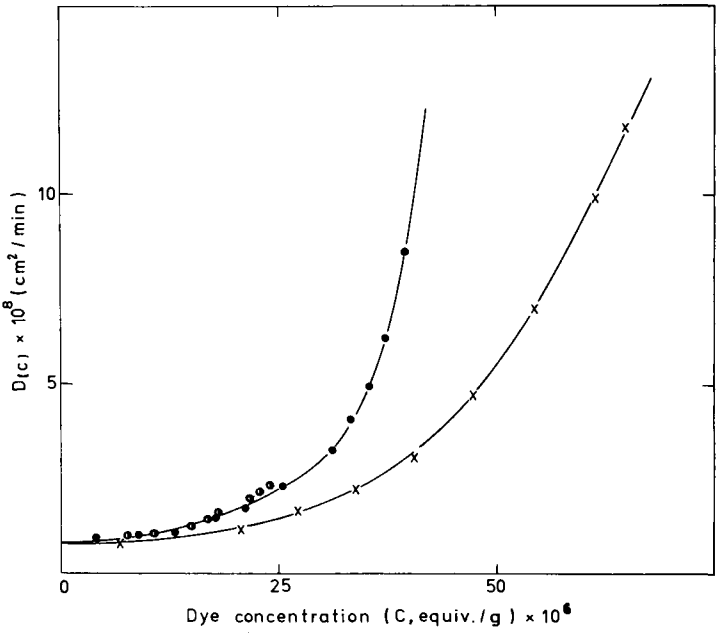


FIG. 25. Variation of $D(c)$ with C at 60°: ◑, pH 6.0, ● = pH 3.2, × = pH 1.95. Reproduced by permission from McGregor *et al.*[144]

mechanism there exists an additional solution mechanism of dye adsorption which is responsible for the phenomenon of overdyeing. However, McGregor and Harris[167] and Rattee[168] suggest that overdyeing can occur with fully dissociated dye anions of high affinity, electrical neutrality being maintained by simultaneous sorption of additional cations. The more gradual change in $D(c)$ with dye concentration seen in Fig. 25 at the low pH of 1.95 is attributed by McGregor

[165] E. Atherton, D. A. Downey, and R. H. Peters, *Text. Res. J.* **25**, 977 (1955).

[166] B. G. Ferrini and H. Zollinger, *Helv. Chim. Acta* **50**, 897 (1967).

[167] R. McGregor and P. W. Harris, "A Chemical Engineering Approach to Dyeing Problems: The Sorption of Acid Dyes by Polyamides," Research Monograph M-20. Allied Chem. Fibers Divi., Petersburg, Virginia, 1969.

[168] I. D. Rattee, *Chem. Soc. Rev.* **2**, 145 (1972).

et al.[144] to the additional effect of overdyeing. Furthermore, they observe that the diffusion coefficient in the overdyeing region is a constant and independent of dye concentration. This result is in agreement with the existence of a linear adsorption isotherm in this region, as reported by Atherton *et al.*[165] There is a fairly good agreement between the experimental results and the theoretical profile for diffusion with a constant diffusion coefficient of $\sim 6.5 \times 10^{-8}$ cm^2/minute. Brody[169] also observed that the dye Alizarine Sky Blue BS.FS, which exhibits considerable overdyeing in nylon at pH 6, has a diffusion coefficient independent of concentration in this region. The additional sorption of dye in the overdyeing region occurs in the same way as for the dye at concentrations below and up to the site-saturation value. The lack of dependence of the dye diffusion coefficient on adsorbed dye concentration above the site-saturation value can perhaps be explained by the fact that the additional cations which accompany the dye anions lower any resistance to diffusion due to electrical potential gradients. Such potential gradients are believed to be partially responsible for the concentration dependence of the dye diffusion process in the site-dyeing regions.[144,170]

The analysis of the diffusion data in terms of a concentration or activity gradient as the driving force for diffusion can be carried out using either the Gilbert and Rideal[171] theory or the theory of Atherton *et al.*[165] for the amine dyeing of polyamide fibers with acid dyes. In the former theory, the dye anion activity for monobasic dye is expressed by the equation $a_A = \theta/(1 - \theta)$ where $\theta = C/S$ is the fractional saturation of the available sites, C being the dye concentration and S the total available content of fixed sites, both expressed in terms of gram-ions per kilogram. For dye anions of basicity z the corresponding expression for the activity is

$$a_A = \theta/(z - \theta) \tag{44}$$

C being now expressed in gram-equivalents. This theory of Gilbert and Rideal[171] therefore implies that one dye anion can only occupy one site. However, Atherton *et al.*[99] argue that since a stoichiometric adsorption of polybasic acid dyes on nylon is possible even when dyes of different basicities are simultaneously adsorbed, the concept of each dyeing site being a region in the fiber in the vicinity of a single charged amino group is untenable. For example, for a dibasic dye this region will have to be extended to include two charged groups and so on. A rigid lattice of

[169] H. Brody, *Text. Res. J.* **35**, 844 (1965).

[170] R. H. Doremus, *J. Polym. Sci., Part B* **4**, 755 (1966).

[171] G. A. Gilbert and E. K. Rideal, *Proc. Roy. Soc., Ser. A* **182**, 335 (1944); **183**, 167 (1944).

sites for dyeing is thus precluded, and Atherton *et al.*[99] assume that the total number of available sites (terminal amino groups) can be grouped at will in numbers equal to the adsorbed dye basicity. The activity of a polybasic dye can therefore be given by the equation

$$a_A = \frac{1}{z}\frac{\theta}{(1-\theta)} = \left[\frac{C}{S - zC}\right] \tag{45}$$

In this equation C is expressed in gram-ions per kilogram and the fractional saturation value is $Cz/S = z\theta$. A replacement of the concentration gradient in the flux equation $J = -D\,\partial C/\partial x$ by the activity gradient or the chemical potential gradient of the dye anion leads to the following equations for the variation of $D(c)$ with θ:

$$D(c) = D_0\frac{1}{(1-\theta)^2} \qquad \text{(activity gradient model)} \tag{46a}$$

or

$$D(c) = D_0\frac{1}{(1-\theta)} \qquad \text{(chemical potential gradient model)} \tag{46b}$$

or

$$D(c) = D_0 z(z-\theta)^2 \tag{46c}$$

or

$$D(c) = D_0 z(z-\theta) \tag{46d}$$

where $D_0 = D(C)_{C=0}$. The equations (46c) and (46d) are based on the Gilbert and Rideal's[171] expression for the activity.[98] Using these equations the results shown in Fig. 26 were obtained by McGregor *et al.*[144] for the diffusion of CI Acid Red 18 in nylon 66. The results at pH 3.2 correspond to an experimental situation where the value of C_∞ is practically equal to the terminal amino group content S in the fiber, i.e., $C_\infty/S \to 1$. Experimentally, however, it is difficult to evaluate accurately the ratio C_∞/S. Furthermore, the relationship between the amino end group content in the fiber and the sulfonic acid groups in the dye need not be exactly stoichiometric because some of the amino end groups in the complex fiber structure may not be accessible to the dye. In general, therefore, in Eqs. (46c) and (46d) the factor $\theta = C/S$ is replaced by the term $\alpha\theta$ where $\alpha = C_\infty/S$. The calculations of the results for pH 3.2 are based on the assumption that $\alpha = 1$, whereas the results at pH 6 correspond to a condition of underdyeing, i.e., $C_\infty < S$ and $\alpha = 0.583$. The two sets of curves A and B are the theoretical limiting

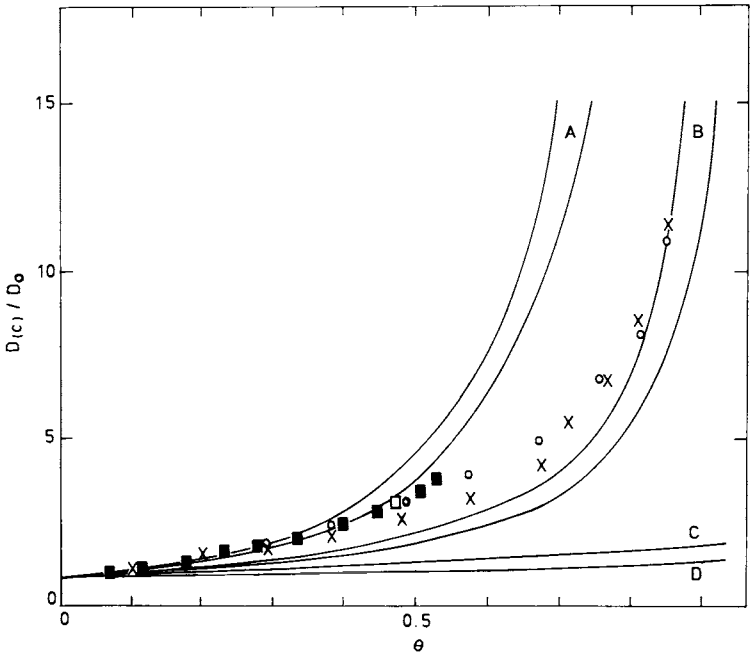


FIG. 26. Variation of $D(c)/D_0$ with θ: ■, pH 6.0; ○, pH 3.2 at 80°; ×, pH 3.2 at 60°. Curves calculated from, A, Eq. (46a); B, Eq. (46b); C, Eq. (46c); D, Eq. (46d). Reproduced by permission from McGregor *et al.*[144]

curves for an error $\pm 2\%$ in α for $\alpha = 1$. These results also indicate that the Gilbert and Rideal model (curves C and D) cannot explain the observed behavior. With the further assumption that the results are more reliable at the lower and intermediate values of θ, these authors point out that the variations of $D(c)$ with θ are in better agreement with the activity gradient model, i.e., Eq. (46a). The differences between the theoretical and experimental values at higher values of θ are probably associated with the phenomenon of overdyeing which occurs simultaneously with amine dyeing.

A comparison of the experimental diffusion profiles with the theoretical profiles which have been calculated by Atherton *et al.*[172] also conforms to the activity gradient model with $\alpha = 0.9$, i.e., C_∞ is 90% of the terminal amino group content S. Their results are illustrated in Fig. 27. The results of Sekido *et al.*,[173] using the roll-film method for the

[172] E. Atherton, R. McGregor, and R. H. Peters, unpublished work referred to in McGregor *et al.*[144]

[173] M. Sekido, T. Iijima, and A. Takahashi, *J. Chem. Soc. Jap., Ind. Chem. Sect.* **68**, 524 (1965).

diffusion of acid dyes in nylon 6, and the more recent results of Morizane *et al.*,[138] using the microspectrophotometric method for the diffusion of CI Acid Orange 10 in nylon 6 samples having high draw ratios, are in agreement with the activity gradient model.

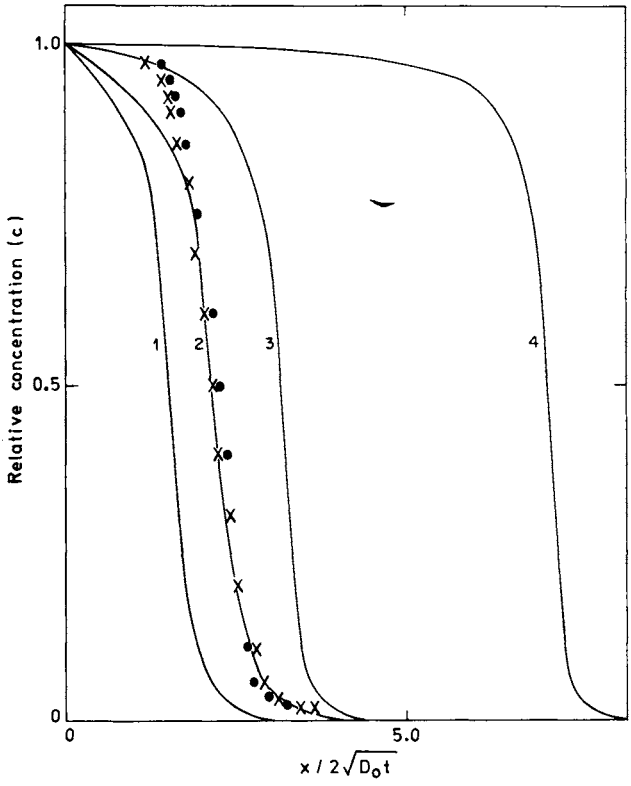


FIG. 27. Comparison of calculated and observed profiles: ●, pH 3.2 at 60°; ×, pH 3.2 at 80°. 1, Curve calculated from Eq. (46b) for $\alpha = 1.00$; 2, from Eq. (46a) for $\alpha = 0.90$; 3, from Eq. (46a) for $\alpha = 0.95$; 4, from Eq. (46a) for $\alpha = 0.99$. Reproduced by permission from McGregor *et al.*[144]

Hopper *et al.*[174] have recently studied the diffusion of acid dyes in nylon 66 films prepared by different methods. Their results show that the shape of the distribution curve is more or less independent of the physical state of the substrate provided it is homogeneous, although the actual rates of diffusion depend on the polymer structure, notably the degree of orientation. Assuming the Standing *et al.*[23] model for the diffusion process which was discussed earlier for the cellulose–direct dye

[174] M. E. Hopper, R. McGregor, and R. H. Peters, *J. Soc. Dyers Colour.* **86**, 117 (1970).

system (see Section I,B,1,a), that the diffusion of free dye occurs in the liquor in the pores and the diffusion of adsorbed dye occurs along the pores, the following equations are derived for the diffusion coefficient $D(c)$:

$$D(c) = D_{0,i}(1 - \alpha_1\theta)^2 \tag{47}$$

and

$$D(c) = D_{0,a}(1 - \alpha_1\theta) \tag{48}$$

In these equations $D_{0,i}$ and $D_{0,a}$ refer, respectively, to dye diffusion coefficients in and along the pores, and these diffusion coefficients are assumed to be independent of dye concentration. The factor α_1 ($\leqq 1$) allows for the departure from stoichiometry of the relation between amino end group content and sulfonic acid group content in the dye. As discussed earlier, McGregor *et al.*[144] have shown that equations of this type describe approximately the increase in the measured diffusion coefficient with concentration. However, such an approach does not take into account the existence of an electrical potential gradient $\partial\psi/\partial x$ set up in the fiber as a result of the difference in mobilities between the dye ions and the Cl^- or $HCOO^-$ ions with which they are exchanged in the internal fiber phase. Hopper *et al.*[174] have therefore derived the following flux equations taking into account the influence of the electrical potential gradient:

$$J_D = -D_{0,D_i}\left\{C_{D_i}\frac{\partial\mu_{D_i}}{\partial x} + C_{D_i}z\frac{F}{RT}\frac{\partial\psi}{\partial x}\right\} \tag{49}$$

for the dye anion and

$$J_{Cl} = -D_{0,Cl_i}\left\{C_{Cl_i}\frac{\partial\mu_{Cl_i}}{\partial x} + C_{Cl_i}z\frac{F}{RT}\frac{\partial\psi}{\partial x}\right\} \tag{50}$$

for the chloride ion, where the terms D_{0,D_i}, D_{0,Cl_i}, C_{D_i}, and C_{Cl_i} refer to the diffusion coefficients and concentrations of the dye and the chloride ions, respectively, in the internal fiber phase. With the further assumption that the dye behaves ideally, that the fiber remains electrically neutral at all times, and that there is a simple linear relationship between C_{D_i} and C_{D_a}, the following equation is obtained for the diffusion coefficient $D(c)$:

$$D(c) = D_{0,D_i}\frac{1 + (z - 1)\theta}{1 - \alpha_2\theta} \tag{51}$$

where

$$\alpha_2 = 1 - [(z - 1)D_{0,D_i}/D_{0,Cl_i}] \tag{52}$$

One important feature of such an analysis is that the adsorption process is assumed to be one in which the large dye molecule is adsorbed on the available polymer surface, the charged groups of the surface acting not as sites, but influencing the adsorption only by virtue of their electrical charge. The adsorption of the large anionic direct dyes by a charged cellulose surface has been explained on a similar basis by

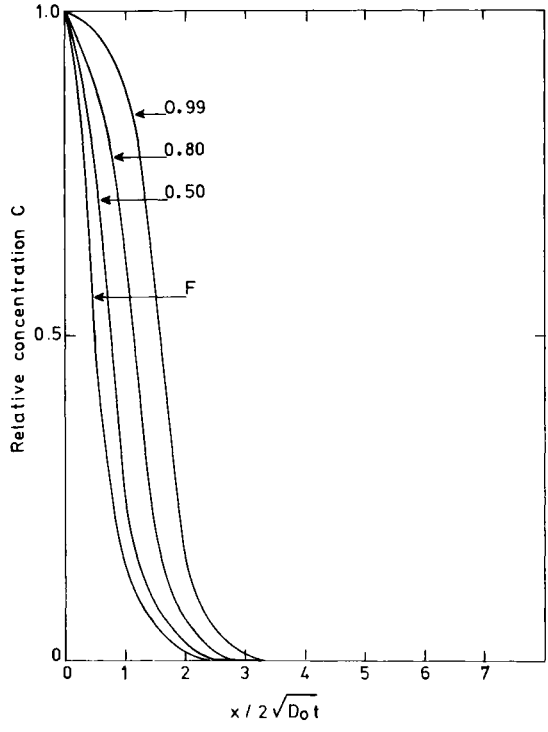


FIG. 28. Plot of relative concentration versus $x/2(Dt)^{1/2}$ at different values of $\alpha\theta$. (Numbers on curves represent values of $\alpha\theta$. For $\alpha = 1$, the numbers represent surface concentrations. If α is constant the numbers are proportional to surface concentration.) $D = D_0\{1/(1 - \alpha\theta)\}$; F, Fick's law. Reproduced by permission from Hopper *et al.*[174]

Sivaraja Iyer and co-workers[159–161] and has been discussed more recently by McGregor and Harris[167] for the acid dye–polyamide fiber system. The concentration-dependence of the diffusion coefficient analyzed in terms of Eqs. (47), (48), and (51) gives similar shapes, as shown in Fig. 28 where Eq. (48) is used. These results indicate that as $\alpha\theta \to 0$, the ideal curve for Fick's law is obtained, but as θ approaches unity the curves become sigmoidal and deviate from the ideal curve.

The curve profiles are thus seen to be dependent only on the dye concentration. Hence, for dyes having the same level of surface saturation even if their basicities are different, the shapes of the curves will be the same, as shown in Fig. 29. These results are in accordance with the

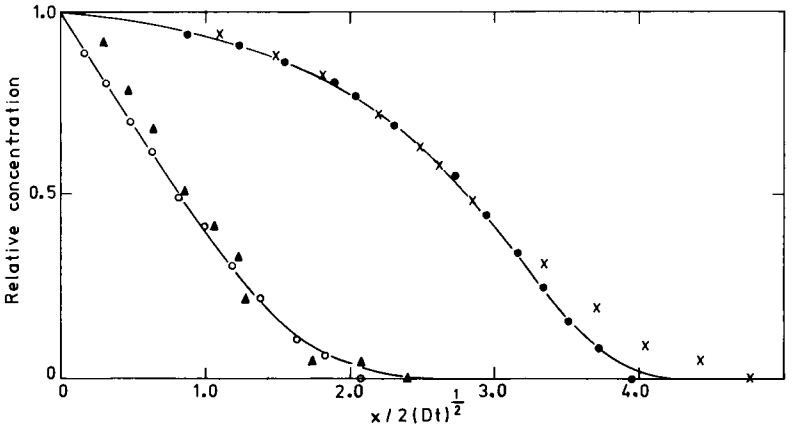


FIG. 29. Relative concentration versus $x/2(Dt)^{1/2}$ for two acid dyes applied under different conditions. Reproduced by permission from Hopper *et al.*[174]

	CI Acid Red	*Dye concn. (g/liter)*	*Time (min)*	*pH*	
●	18	0.446	4–9	3.2	Equilibrium uptake assumed equiv. to amine end group content
×	8	0.201	16	4.8	
○	18	0.443	64	6.5	Equilibrium uptake 19 mEq/kg
▲	8	0.200	25	8.4	Equilibrium uptake 21 mEq/kg

predictions of Eqs. (47) and (48), but not of Eq. (51), since this equation shows that the concentration–distance profile is a function of the dye basicity. However, a further analysis of Eqs. (47), (48), and (51) shows that a plot of $1/[D(c)]^{1/2}$ versus θ should be linear if Eq. (47) is valid, whereas a plot of $1/D(c)$ versus θ should be linear if Eq. (48) or (51) for $z = 1$ is used. Such an analysis of the data shown in Fig. 29 gives curves for both CI Acid Red 8 and CI Acid Red 18 using Eq. (47), whereas Eq. (48) or (51) yields a straight line for the monobasic dye CI Acid Red 8, but not for the tribasic dye CI Acid Red 18. However, substituting $z = 3$ in the equation for the tribasic dye and rearranging the terms

shows that a plot of $(1/D\theta)[(1/\theta) + 2]$ versus $1/\theta$ should be a straight line, and this is confirmed for the tribasic dye CI Acid Red 18. According to Hopper *et al.*,[174] Eq. (51), which also takes into account the effect of the electrical potential gradient on dye diffusion, appears to be more satisfactory than Eq. (47) or (48) in explaining all the experimental data.

Sand,[175] using the microdensitometric technique, compared the concentration profiles in nylon 6 of the monobasic acid dye CI Acid Orange 7 and its sulfonamide derivative, which, being nonionic, can be regarded as a disperse dye. As expected, the concentration profiles for the latter corresponded to diffusion with a constant diffusion coefficient, whereas the former gave profiles similar to that for acid dyes in nylon 66. He observed that his results could be satisfactorily explained by taking into account in the activity gradient model of Atherton and Peters[98] the equilibrium between the adsorbed dye and the free dye in the system, using a pore model similar to that discussed for cellulose–direct dye systems.

In a recent study of the diffusion of cationic dyes in acrylic films, Harwood *et al.*,[103] using similar arguments to those of Hopper *et al.*,[174] derived flux equations for the diffusion of dye and hydrogen ions. The model used for the sorption process is one in which the hydrogen ions associated with the acid groups in the polymer (SO_3H) are exchanged for dye ions, an allowance being made in the flux equations for the electrical potential gradient due to the difference in the mobilities of these ions. The concentration–distance profile for the diffusion process is found to be similar to that for acid dyes in nylon 66 shown by McGregor *et al.*[144] and that obtained by Hossain *et al.*[176] for the diffusion of CI Basic Green 4 in polyacrylonitrile. Hossain *et al.*[176] conclude that the concentration dependence of the diffusion coefficients of CI Basic Green 4 could be better explained on the basis of the chemical potential gradient model of Atherton and Peters,[98] which was discussed earlier in this section. The results of Harwood *et al.*[103] are also in agreement with this model since a plot of $1/D(c)$ versus the relative dye concentration is linear for both CI Basic Blue 22 and CI Basic Yellow 13 in acrylic film over most of the concentration range.

E. Kinetics of Dyeing from the Vapor Phase

The kinetics of dyeing from the vapor phase have not so far been extensively investigated, although such studies are of interest in relation to the Thermosol process, i.e., when a fibrous material is padded

[175] H. Sand, *Ber. Bunsenges. Phys. Chem.* **69**, 333 (1965).

[176] M. A. Hossain, H. Maeda, T. Iijima, and Z. Morita, *J. Polym. Sci. Part B* **5**, 1069 (1967).

with a dispersion of a nonionic dye, dried, and then heated to a high temperature of about 200° for a short period of time. Jones[177] studied the adsorption of saturated azobenzene vapor by cellulose acetate, the rate of adsorption being measured over a wide range of temperature using a completely enclosed silica spring balance. An analysis of the kinetic data using McBain's equation [Eq. (6)] gave sigmoidal curves which are nonlinear in the initial portions, in contrast to the linearity predicted by Fick's law for constant boundary conditions and a concentration-dependent diffusion coefficient. Further kinetic studies by Jones and Seddon[178] on the rates of absorption of some model compounds and disperse dyes by secondary cellulose acetate films show that the initial curvature in the rate curves occurs only for absorption from saturated vapors and is absent for absorption from unsaturated dye vapor. The curvature in the graphs for absorption of saturated vapors is explained by a two-stage process in which the initial surface curvature is due to a surface concentration which increases with time, whereas subsequent linear portions of the curve correspond to a steady-state diffusion down a concentration gradient within the film, the surface concentration being maintained constant. Datye and Rajendran[34] and Datye *et al.*,[179] from a study of the diffusion of disperse dyes in polyester substrates at elevated temperatures using Sekido's roll-film method, conclude that the dyeing occurs from the vapor phase and that the rate-determining step is the diffusion of dye in the fiber phase, Fick's law being obeyed corresponding to a constant surface concentration and a constant diffusion coefficient. These results are in agreement with the results reported by McGregor *et al.*,[49] Majury,[29] and Bird and Scott[30] for the diffusion of disperse dyes in hydrophobic fibers such as cellulose secondary acetate, cellulose triacetate, and nylon from aqueous dyebaths (see also Section I,B,1,b).

F. Application of Nonequilibrium Thermodynamics to Dyeing Kinetics

The application of the methods of classical thermodynamics to describe dyeing processes is limited to analyses of equilibrium systems, and the thermodynamic parameters obtained cannot give information regarding the rates of such processes. The application of the thermodynamics of irreversible processes, however, makes it possible to link together the topics of kinetics and thermodynamics. Attempts have therefore been made by a few workers to discuss the kinetics of dyeing

[177] F. Jones, *J. Soc. Dyers Colour.* **77**, 57 (1961).
[178] F. Jones and R. Seddon, *Text. Res. J.* **35**, 334 (1965).
[179] K. V. Datye, S. C. Pitkar, and R. Rajendran, *Indian J. Technol.* 4, 202 (1966).

processes using the principles of irreversible thermodynamics.[180–183] Irreversible phenomena in dyeing kinetics are related, for example, to temperature and concentration gradients and to chemical affinity or forces. The rate of dye transport is also influenced by parameters related to the structural features of the fiber which can resist penetration of the dye molecules in the fiber matrix. Other features of this parameter which control the amount of flux for a given set of forces are the properties of the total system at equilibrium. Generally, therefore, the equation for flux can be written as

$$J_i = \sum L_{ij}X_j \tag{53}$$

where $i = 1, 2, 3, \ldots n$ and $j = 1, 2, 3, \ldots n$. In this equation, which is the mathematical expression of the first principle of irreversible thermodynamics, it is assumed that the systems are near-equilibrium systems and only the linear terms have to be retained in the equations expressing the fluxes in powers of forces. The flux equations are called phenomenological equations and the coefficients L_{ij} are called phenomenological coefficients. From the thermodynamic standpoint, the phenomenological coefficients are experimental quantities just like, for example, virial coefficients in the virial expansion of the pressure. For a dyeing system consisting of three components, namely, water (1), dye (2), and substrate (3), the following equations can be written[184–189]:

$$J_1 = (L_{1,1}X_1 + L_{1,2}X_2 + L_{1,3}X_3)/T \tag{54}$$

$$J_2 = (L_{2,1}X_1 + L_{2,2}X_2 + L_{2,3}X_3)/T \tag{55}$$

$$J_3 = (L_{3,1}X_1 + L_{3,2}X_2 + L_{3,3}X_3)/T \tag{56}$$

Terms such as $L_{1,1}X_1$, $L_{2,2}X_2$, and $L_{3,3}X_3$ obviously represent a flux arising in response to its own force. The other terms, which are referred to as cross or coupling coefficients, link the superimposed effect of one

[180] L. Onsager, *Phys. Rev.* **37**, 405 (1931).

[181] I. Prigogine, "Thermodynamics of Irreversible Processes." Wiley (Interscience), New York, 1961.

[182] S. R. DeGroot and P. Mazur, "Non-equilibrium Thermodynamics." Wiley (Interscience), New York, 1962.

[183] T. L. Hill, "Thermodynamics for Chemists and Biologists." Addison-Wesley, Reading, Massachusetts, 1968.

[184] B. Milicevic and R. McGregor, *Helv. Chim. Acta* **49**, 1302 and 1319 (1966).

[185] R. McGregor, *J. Soc. Dyers Colour.* **82**, 450 (1966).

[186] W. McDowell and R. Weingarten, *Melliand Textilber.* **50**, 59 (1969).

[187] B. Milicevic, *Text. Res. J.* **39**, 677 (1969).

[188] H. Uedaira, *Sen-i-Gakkaishi* **16**, 403 (1960); *CA* **54**, 16836c (1960).

[189] G. E. Krichevskii and I. M. Movoshovich, *Izv. Vyssh. Ucheb. Zaved., Tekhnol. Tekstil'noi Prom.* No. 2, p. 107; No. **4**, p. 108 (1968).

force on a different flux. The second important fundamental postulate of irreversible thermodynamics due to Onsager[180] which can now be applied to Eq. (53) is given by the equation

$$L_{ij} = L_{ji} \tag{57}$$

This equation therefore states a reciprocal relationship between the phenomenological coefficients, i.e., the L matrix is symmetrical. The reciprocity relationship is of great help in simplifying the kinetic equations involving the phenomenological coefficients.

The simplest model of a dyeing system to which these concepts can be applied is the discontinuous model system, which can be pictured as follows. The system as a whole, i.e., the system containing fiber immersed in a dye solution, is regarded as a closed system at constant temperature and pressure, the pressure usually being atmospheric pressure. Within this closed system, the dyebath phase "s" and the fiber phase "f" can be described as open subsystems in which there is an exchange of chemical components during sorption and desorption processes. Exchange of heat energy between the subsystems is also permitted, the subsystems always being in thermal equilibrium with each other. In this model system therefore, all the important parameters of state, such as temperature and concentration, in both the solution and fiber phases remain uniform throughout each phase, although these parameters will in general have different values in each phase. When these parameters change in passing from one phase to another, the change will be stepwise and abrupt, i.e., discontinuous. Such a model of the dyeing process was first discussed by Uedaira.[188] He obtained the suitable phenomenological equations and calculated the entropy of the process. He concluded that the diffusion coefficient of the dye within the fiber is a function of the ζ potential and increases with increasing concentrations of foreign electrolytes added to the dyebath. The rate of dye uptake by the fiber was shown to be a function of the gradient of the chemical potential under steady-state conditions. Milicevic and McGregor[184] and McGregor,[185] considered the simplest case of dyeing where the fiber is regarded as a water-swollen isotropic gel and the fiber phase has a uniform constant total density at any moment, i.e., there is no movement of local centers of mass in the fiber phase regarded as a continuous medium. At constant temperature and pressure, and assuming that in the total system only the dye concentration changes during dyeing, the rate of change of Gibbs free energy of the whole discontinuous system during a dye sorption process under a specified set of dye

concentrations in the dyebath and the fiber, can be expressed by the equation

$$\frac{\partial G}{\partial n^f} = \Delta\mu = \Delta\mu^0 - RT \ln \frac{C^f}{C^s} \tag{58}$$

where n^f is the number of moles of dye within the fiber.

During a spontaneous dyeing process, therefore, the quantity $\Delta\mu$ is a measure of the decrease in the slope of the graph expressing the change in Gibbs free energy with extent of dye sorption. Hence, it is suggested that $-\Delta\mu$ (by convention, a positive quantity for a sorption process) can be regarded as the instantaneous affinity of dyeing in this discontinuous model system. These ideas also give a kinetic significance to $-\Delta\mu^0$, since $-\Delta\mu^0$ can be regarded as the value of $-\Delta\mu$ for the discontinuous model system at the point where $C^f/C^s = 1$, i.e., the standard affinity of dyeing now appears as the driving force for dye adsorption in this model when dye concentrations in the fiber and the dyebath are the same. It is thus unnecessary to regard $-\Delta\mu^0$ as being solely an equilibrium parameter. The standard affinity of dyeing is, however, generally determined from measurements at equilibrium using the equation

$$-\Delta\mu^0 = RT \ln \frac{(C^f)_\infty}{(C^s)_\infty} \tag{59}$$

where $(C^f)_\infty$ and $(C^s)_\infty$ are, respectively, the equilibrium dye concentrations in the fiber and solution phases. By combining Eqs. (58) and (59), and with the further assumption of an infinite dyebath, i.e., $(C^s)_\infty = C^s$, the following equation is obtained

$$-\Delta\mu = -RT \ln c^f \tag{60}$$

where $c^f = C^f/(C^f)_\infty$ is the relative dye concentration in the fiber phase.

If the reasonable assumption is now made that the instantaneous rate of dyeing is directly proportional to $-\Delta\mu$, then the thermodynamic and kinetic data can be linked together by the equation

$$\text{rate of dyeing} = \frac{dc^f}{dt} = B(-\Delta\mu) \tag{61}$$

where B is a rate parameter, i.e.,

$$\frac{dc^f}{dt} = -BRT \ln c^f \tag{62}$$

Under conditions near equilibrium, i.e., $c^f \rightarrow 1$, Eq. (62) can be simplified as

$$\frac{dc^f}{dt} = BRT(1 - c^f) \tag{63}$$

Figure 30 shows the rate-of-dyeing curves plotted using Eq. (63) and the conventional diffusion equation $J = D_0(-\partial C_f/\partial x)$ which is based on Fick's law. The most interesting aspect of this result is that identical curves are obtained using two entirely dissimilar models for the dyeing system. This result therefore indicates that interpretations of the dyeing mechanism from the shapes of rate-of-dyeing curves alone should be

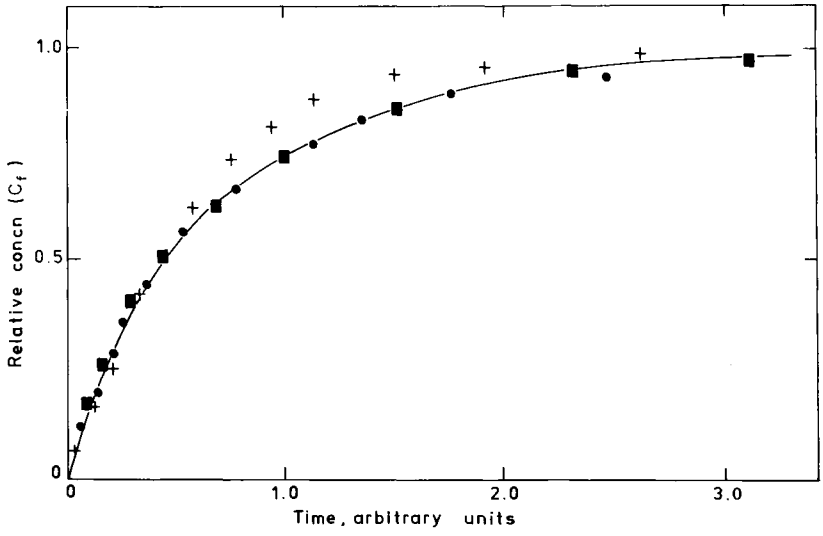


FIG. 30. The rate-of-dyeing curves for two model dyeing systems: ●, discontinuous model; ■, diffusion equation; +, limiting equation. Reproduced by permission from McGregor.[185]

made with a great deal of caution, since the concentration–distributions in the fiber phase according to these models give different profiles, as shown in Fig. 31. Furthermore, as discussed earlier, the reduction of the thermodynamic equations to one similar to the simple Fick's diffusion equation ignores the influence of the complex physicochemical nature of the fibrous polymer and hence the interaction of cross-coefficients in the phenomenological equations. Another assumption which may not be strictly correct is the correlation between concentration and chemical potential. These studies thus indicate that Fick's

equation for describing diffusion processes has only an empirical basis. A complete understanding of dyeing kinetics therefore requires a rigorous solution of the various thermodynamic equations in which all the molecular parameters of each chemical component in the system and their interactions with each other and the complex physicochemical parameters of the fiber itself have to be considered.

In a further extension of these ideas to the transport of dye molecules within the continuous fiber phase using the methods of nonequilibrium thermodynamics and the same restrictive conditions discussed above, Milicevic and McGregor[184] and McGregor[185] have pointed out that the driving force for dye transport is the local gradient of chemical potential.

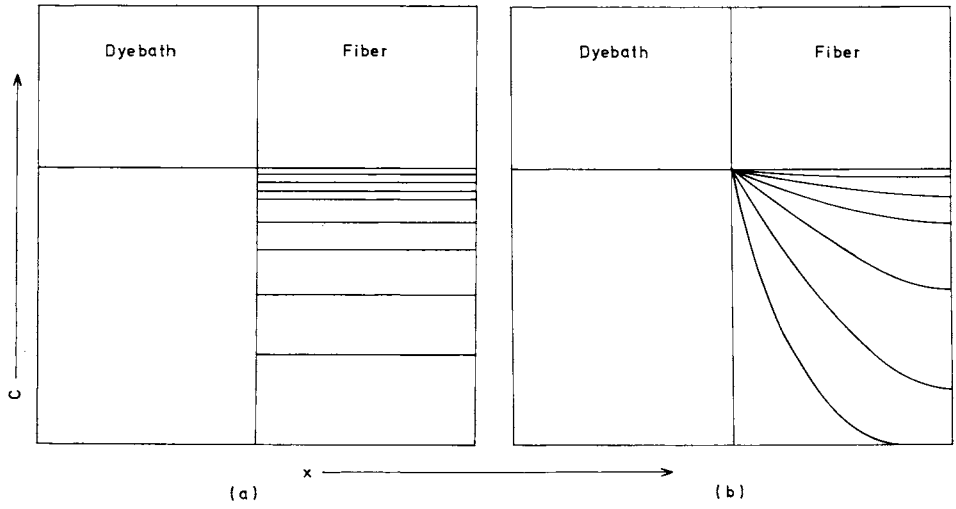


FIG. 31. The concentration distributions (a) for the discontinuous model during dyeing and (b) during a diffusion-controlled dyeing process (schematic). Reproduced by permission from McGregor.[185]

The flux can therefore be described as follows:

$$\text{Local rate of dye transport} = \left\{\frac{1}{\text{local resistance to dye transport}}\right\}\left\{\text{local gradient of chemical potential}\right\} \tag{64}$$

i.e.,

$$J = L\left[\frac{-\partial\mu_f}{\partial x}\right] \tag{65}$$

where $(-\partial\mu_f/\partial x)$ is defined as the affinity of dye transport within the fiber phase, i.e., the local variation of the chemical potential with distance during the dye transport process. L, the rate parameter = D_0C^f/RT.

McDowell and Weingarten[186] have discussed the significance of these developments in relation to the dyeing of polyester fiber with disperse dyes from aqueous suspensions containing solid dye. They have pointed out that Eq. (65) should be applied to each component in the system in arriving at the actual rate of dye transport inside the fiber phase. Hence expressing Eq. (65) in the form

$$v = kAD(-\Delta\mu) \tag{66}$$

where A is the surface parameter, D is the diffusion coefficient, and k is a constant, the following separate equations can be written:

$$v_l = kA_dD_L(-\Delta\mu_l) - v_{ul} \tag{67}$$

and

$$v_{ul} = k'A_dD_L(-\Delta\mu_{ul}) \tag{68}$$

where v_l refers to the rate of solution of the dye, v_{ul} to the rate of recrystallization of the dye, A_d to the area parameter for the solid dye, and D_L to the diffusion coefficient of the dye in solution. The equation

$$v_L = k''A_FD_L(-\Delta\mu_F) \tag{69}$$

refers to the rate of dye transport through the laminar boundary layer around the fiber. The rate of dye transport within the fiber is given by

$$v_F = k'''A_FD_F\left[\frac{-\partial\mu_F}{\partial x}\right] \tag{70}$$

The final rate-determining step for diffusion in the fiber phase will then depend on the interplay of these several parameters mentioned in Eqs. (67)–(70). For example, if the dye is present as large crystals or it has a low solubility, Eq. (67) can be the rate-determining step. The main value of such a treatment, however, lies in the fact that it emphasizes the importance of taking into account all the molecular parameters in the dyebath and the fiber phase before formulating any mechanism of the dyeing process.

Breuer[190] has discussed the kinetics of dyeing of keratin fibers on the basis of the thermodynamics of irreversible processes. He has also emphasized that the rate of dyeing depends on the molecular parameters

[190] M. M. Breuer, *Proc. Int. Wool Text. Res. Conf., 3rd*, Sect. III, pp. 58 and 91 (1965).

of all the components present in the system. Krichevskii and Movoshovich[189] have developed equations for the description of the kinetics of dyeing from a binary mixture using the principles of nonequilibrium thermodynamics.

II. Equilibrium Dyeing Processes, Dye–Fiber Affinity, and Mechanisms of Dyeing

The final stage of a dye adsorption process is usually reached when a state of equilibrium is attained in the system. This equilibrium process can be described in terms of dye adsorption isotherms under a given set of conditions. These isotherms can be represented in different ways depending on the nature of the dye–fiber system. For example, the equilibrium adsorption of anionic dyes on cellulose fibers at a given electrolyte concentration can be represented as an isotherm expressing the amount of dye adsorbed on the fiber as a function of the equilibrium external dyebath concentration or the equilibrium dye concentration in the water-swollen internal fiber phase. In the case of disperse dye–hydrophobic fiber systems, the equilibrium isotherms can be expressed in a similar manner, both for dye uptake from a solution and from the vapor phase. In the case of acid dye–nylon fiber or acid dye–wool fiber systems, however, the equilibrium isotherms are generally expressed as functional relationships between the extent of dye adsorption and the pH of the system at a given dyebath concentration or the extent of dye adsorption as a function of equilibrium dyebath concentration at a fixed pH. If only the adsorption of the free dye acid is considered in the absence of electrolytes, isotherms representing variation in adsorbed dye with varying dyebath pH and dye concentrations are also obtained. In such systems it is necessary to employ these different ways of expressing equilibrium dye uptake since these fibers are amphoteric in nature and hence the sorption of ionic acid dyes is influenced by the pH of the dyebath. In the adsorption of basic dyes on acrylic fibers where the extent of adsorption depends on the number of acidic groups in the fiber, the isotherms are usually expressed as a relationship between equilibrium dye uptake and equilibrium dyebath concentration at a given pH.

The mechanisms of dye adsorption in ionic dye–cellulose fiber or ionic dye–ionic fiber systems are based on an electrochemical approach to the equilibrium distribution of all the ions in the system between the fiber and the dyebath under a given set of conditions, such as for example, the electrolyte concentration and/or the pH of the dyebath. While different theories of dyeing have been developed to account for the

equilibrium sorption behavior for the different types of dyeing systems mentioned above, more recent approaches to the problem suggest that it should be possible to develop a unified theory of dyeing. In this theory, the differences between the different dyeing systems arise primarily from differences in the conditions for electroneutrality in the internal fiber and external solution phases at equilibrium. Such an approach discards the concept of charged sites, which is frequently used to explain the sorption of acid dyes on nylon and wool fibers or the sorption of basic dyes on acrylic fibers. The main driving force for dye adsorption is the thermodynamic affinity of the dye for the given fiber, the extent of dye uptake being governed by a balance between the free-energy change for such a process and a generally unfavorable surface potential effect towards dye adsorption.

In the case of disperse dye–hydrophobic fiber systems, the mechanism of dyeing differs from those of the other dyeing systems since no electrical phenomena are involved. Experimental dye adsorption isotherms in such systems are mostly of the linear partition type, but it is still a matter of some controversy as to whether the results can be interpreted satisfactorily in terms of adsorption on a surface or in terms of a solid solution of the dye in the fiber substrate.

An important recent trend in the study of equilibrium sorption is an awareness of the relevance to dyeing theory of information on the state of aggregation of dyes in solution, the energies of such processes, the nonideal behavior of dye molecules in the internal fiber phase and in the substrate, the structure of water in aqueous dye solutions and the fiber phase, and the surface area available for dye adsorption.

From the different types of dye adsorption discussed earlier, it is possible to obtain the different thermodynamic parameters for such processes, such as the free energies, enthalpies, and entropies of dyeing. Such data give valuable information on the magnitude of the driving force behind the dyeing process, the interplay of forces between all the components in the system resulting from molecular interactions in both the fiber and dyebath, and the extent of orientation of the adsorbed dye molecules. Studies on entropy changes are also useful in relation to the extent of hydrophobic bonding that plays an important part in some dye adsorption processes. Since, as discussed in the section on the kinetics of dyeing, an interpretation of the kinetics involves a model for the dye sorption process, the study of the physical chemistry of equilibrium dye adsorption is of great importance from this point of view.

In this section the equilibrium sorption processes, the dyeing mechanisms, and the thermodynamics are discussed separately for the different important dye–fiber systems. A brief discussion is also included on the nature of dye–fiber bonding.

A. Ionic Dye–Cellulose Fiber System

When cellulose is immersed in a solution of a direct dye, which is usually the sodium salt of a high molecular weight azo dye containing sulfonic groups to confer solubility in water, the dye is adsorbed by the cellulose at a decreasing rate until an equilibrium is reached between the concentration of the dye adsorbed on the cellulose and the concentration of the dye present in the dyebath. At this stage, the adsorbed dye is distributed uniformly throughout the fiber and is not confined to the superficial layer of the fiber. Furthermore, the adsorption process is completely reversible.[18,27,76a,83,85,191] The amount of dye adsorbed at equilibrium increases with increasing amounts of salt added to the dyebath. Therefore, addition of salt to the dyebath is an important factor in dyeing cellulose with direct dyes. In early theories, the effect of added electrolytes was attributed to the formation of dye aggregates of just the right dimension so that they are taken up by the cellulose and are retained in the fiber pores—"the optimum degree of dispersion theory."[192–194] As Valko[195] has pointed out, the presence of aggregates in direct dye solutions is of no importance in itself, but merely indicates the associating tendency of the dye molecules. He further states that the intermolecular forces which lead to aggregation in solution are the same as those which attract the dye to the fiber. This idea has received considerable support from the work of Boulton and Morton,[27] and Kruger and Rudow[196] on the optical behavior of dyed fibers and dye solutions. More recently, Bach *et al.*[197] from optical adsorption studies of dyed cellulose fibers conclude that the dye molecules penetrate the cellulose singly and then coagulate after being deprived of their water of solvation. Thus the dye molecules are present in the aggregated form inside the cellulose phase.

Gee and Harrison[198] demonstrated that the negative ζ potential of cotton with respect to water was considerably reduced by the addition of inorganic salts. They suggested that this negative ζ potential was responsible for the increase of equilibrium adsorption with increasing amounts of salts added to the dyebath. Although the hypothesis is qualitative, it was the first attempt to explain the action of the added

[191] E. H. Daruwalla and A. P. D'Silva, *Text. Res. J.* **33**, 40 (1963).

[192] R. Haller and H. Russia, *Kolloid-Z.* **30**, 249 (1922); see Boulton and Morton.[27]

[193] R. E. Rose, *Ind. Eng. Chem.* **25**, 1265 (1933).

[194] A. Schaeffer, *Melliand Textilber.* **14**, 598 (1933).

[195] E. I. Valko, *Trans. Faraday Soc.* **31**, 230 (1935); *in* "Colloid Chemistry" (J. Alexander, ed.), Vol. VI, p. 594. Van Nostrand-Reinhold, Princeton, New Jersey, 1946.

[196] D. Kruger and H. Rudow, *Ber. Deut. Chem. Ges.* **71**, 707 (1938); see Boulton and Morton.[27]

[197] H. Bach, E. Pfeil, W. Phillipar, and M. Reich, *Angew. Chem.* **75**, 407 (1963).

[198] W. W. H. Gee and W. Harrison, *Trans. Faraday Soc.* **6**, 42 (1910).

salt in terms of the electrochemical nature of dyeing, rather than the then popular "optimum degree of dispersion theory." The first quantitative relationship between the amount of dye adsorbed at equilibrium and the concentration of the electrolytes present in the equilibrium dyebath was developed by Hanson *et al.*[1] in terms of an ionic equilibrium of the Donnan membrane type. They postulated that the dye anions are first adsorbed by the cellulose and attract the sodium ions near them in order to maintain electrical neutrality in the cellulose phase. As a result, the concentration of sodium ions in the cellulose phase becomes greater than in the dyebath. The negative surface charge on the cellulose surface which increases with adsorption of dye anions tends to oppose further adsorption of dye anions. The effect of adding sodium chloride to the dyebath is threefold. It increases the activity of the dye in solution, reduces the concentration gradient of gegenions between the surface layer of the cellulose and the external solution, and thus reduces the osmotic work which must be done in bringing gegenions into the fiber during the adsorption; the negative electrical charge on the surface is screened by the Na^+ ions. Dye ions can thus approach more closely before any electrical repulsion becomes operative. All these effects of the addition of salt reduce the activation energy for the dyeing process. The theory also accounts semiquantitatively for the decrease in the amount of equilibrium dye adsorption as the carboxyl content of the cellulose increases.[199]

The kinetic, thermodynamic theory of dyeing put forward by Willis *et al.*[83] presents essentially the same Donnan equilibrium picture of the dye adsorption process, but it does not lend itself to the evaluation of the energetics of dyeing. In the development of this theory, an assumption is made for simplicity that the forces of attraction between dye and cellulose are constant over a small volume near the active centers in the fiber and are zero elsewhere. This simplifying assumption is equivalent to the division of the solution into an internal cellulose phase and an external aqueous solution phase. Peters and Vickerstaff[200] have shown that the theories of both Willis *et al.*[83] and Hanson *et al.*[1] can be derived using a conventional thermodynamic method in which it is assumed that the activity of an electrolyte may be represented as the product of the dye and electrolyte ionic activities in the fiber phase, and that these are proportional to the ionic concentrations, the proportionality constant having the dimensions of a reciprocal volume. The introduction of this arbitrary constant is also equivalent to the division of the solution phase into an internal cellulose phase and an external aqueous solution phase.

[199] C. E. F. Fishwick and S. M. Neale, *Trans. Faraday Soc.* **43**, 336 (1947).
[200] R. H. Peters and T. Vickerstaff, *Proc. Roy. Soc., Ser. A* **192**, 292 (1948).

Another theory essentially similar to that of Hanson *et al.*[1] has been suggested by Crank[152] on the basis of a diffusion adsorption model. He regards direct dyeing as a process of activated diffusion with adsorption, the energy of activation arising from the fact that the diffusing ions have to penetrate a surface potential barrier due to the combined electric charge of the cellulose itself and the dye ions adsorbed on its surface.

In the development of these theories, no serious account was taken of the effect of carboxyl groups in cellulose on the equilibrium adsorption. Standing and Warwicker[201] applied the Donnan theory of membrane equilibrium to the adsorption of CI Direct Yellow 12, taking into account the carboxyl group in viscose sheet, and suggested that the number of carboxyl ions which directly affect the equilibrium adsorption may be less than the total number of carboxyl groups in the viscose sheet.

The main postulates employed in all these theories of dyeing may be summarized as follows: (1) Donnan equilibria exist between the ions in the internal cellulose phase and the ions in the bulk external solution phase. (2) Electrical neutrality is maintained in the internal cellulose phase and the bulk external solution phase. (3) The adsorption of dye on fiber is directly proportional to the concentration of the free dye ions in the internal solution phase. (4) Since activities of dye ions are not known, the activity coefficients of all ions involved in the dyeing equilibrium are considered to be equal to unity. (5) The volume used for the internal cellulose phase, the so-called "volume term" V, is generally constant for a given fiber and is related to the percentage of water vapor taken up by the fibers at 100% relative humidity. Values of 0.22, 0.37, and 0.44 liter/kg of dry fibers have been used extensively for cotton, cuprammonium rayon, and viscose fibers, respectively. The cellulose fiber with its volume V of imbibed solution is regarded as an equipotential volume.

The existence of Donnan equilibria in cellulose–aqueous dye + electrolyte solution systems has been established by Neale and coworkers[202–204] from direct measurements of the Donnan potentials in such systems using cellophane sheets. The Donnan distribution of simple electrolyte ions in such a system was also studied analytically by Usher and Wahbi,[205] and Neale and Farrar.[206] However, the agreement between the measured Donnan potentials or Donnan distributions and the theoretical values of these quantities was only qualitative.

[201] H. A. Standing and J. O. Warwicker, *J. Text. Inst.* **40**, T175 (1949).
[202] S. M. Neale, *Trans. Faraday Soc.* **43**, 325 (1947).
[203] S. M. Neale and P. T. Standring, *Proc. Roy. Soc., Ser. A* **213**, 530 (1952).
[204] S. M. Neale and P. K. Saha, *J. Soc. Dyers Colour.* **73**, 381 (1957).
[205] F. L. Usher and A. K. Wahbi, *J. Soc. Dyers Colour.* **58**, 221 (1942).
[206] S. M. Neale and J. Farrar, *J. Colloid Sci.* **7**, 186 (1952).

The Donnan distribution of mobile ions leads to the following equation:

$$\frac{[Na_\sigma]}{[Na_i]} = \frac{[Cl_i]}{[Cl_\sigma]} = \left(\frac{[D_i]}{[D_\sigma]}\right)^{1/z} = \lambda \tag{71}$$

where the subscripts i and σ refer to the internal cellulose phase and external bulk equilibrium solution, λ is the Donnan distribution coefficient, and z is the basicity of the dye having a general formula Na_zD. The terms $[D_i]$ and $[D_\sigma]$ refer to the equilibrium concentration of the free dye anions in each phase. Combining the above equation with the conditions for electroneutrality in each phase, the following equation due to Peters and Vickerstaff[200] can be obtained

$$\frac{[D_\sigma]}{[D_i]} = \left\{\frac{z[D_\phi]}{2[Na_\sigma]V} + \left[\left(\frac{z[D_\phi]}{2[Na_\sigma]V}\right)^2 + \frac{[Cl_\sigma]}{[Na_\sigma]}\right]^{1/2}\right\}^z \tag{72}$$

At higher salt concentrations (NaCl $\geqslant$ 4 g/liter), the more simplified equation

$$\frac{1}{[D_i]} = \frac{1}{[D_\sigma]} \exp\left[z \sinh^{-1}\left(\frac{z[D_\phi]}{2[Na_\sigma]V}\right)\right] \tag{73}$$

can be derived, assuming $[Na_\sigma] \simeq [Cl_\sigma]$. It is therefore possible to calculate the value of $[D_i]$ for a given set of conditions from the experimental measurements of $[D_\sigma]$, $[D_\phi]$, and the electrolyte concentration used in the dyebath, where $[D_\phi]$ refers to the concentration of dye adsorbed at equilibrium in gram-ions per kilogram of dried fiber. All the other concentration terms are expressed in gram-ions per liter. The concentration of adsorbed dye in the same units will therefore be $([D_\phi]/V)$ gram-ions per liter. The thermodynamic affinity, i.e., the standard free-energy change $-\Delta\mu^\circ$ for the adsorption process can then be calculated from Eq. (74).

$$-\Delta\mu^\circ = RT \ln\left(\frac{[D_\phi]}{V}\right)/[D_i] \tag{74}$$

Marshall and Peters[207] employed Eq. (74) for calculating the affinities of 14 direct dyes on various fibers. The physical chemistry of dyeing cellulose with direct dyes on the basis of these ideas has been very extensively reviewed by Vickerstaff.[18] The electrochemical theory of the dyeing process has been found to give a fairly reasonable explanation of the experimentally measured isotherms, but there are several serious

[207] W. J. Marshall and R. H. Peters, *J. Soc. Dyers Colour.* **63**, 446 (1947).

drawbacks in the theory. For example, although assumptions (1) and (2) can very reasonably be made to describe the distribution of dye ions and counterions between the internal cellulose phase and external solution phase, the other assumptions are not valid in many respects. Deviations of the experimental data from the theory have been chiefly attributed by most workers to a lack of knowledge of the activity of dye ions and the aggregating behavior of the dye.

The volume term V, as defined in assumption (5), was first used by Hanson *et al.*[1] and later by various other workers[191,200,207–210] and is regarded as a constant for a particular type of cellulose independent of temperature, salt, and dye concentrations. However, it was realized that it is a very arbitrary value which can be varied to get agreement between experimental results and postulated theory.[191,207,211]

The second important drawback is the derivation of a suitable expression for the activity of the adsorbed dye ions. In the thermodynamic reasoning of Peters and Vickerstaff,[200] an assumption is made that the activity of the dye may be represented as the product of the ionic activities of the dye and that these are proportional to the ionic concentrations, the proportionality constant having the dimensions of a reciprocal volume ($1/V$). However, simple calculations show that the molar concentrations of the dye in the fiber phase, using this argument, in many cases considerably exceed the solubility limit of the dye in aqueous solutions containing added electrolytes. Therefore, it is apparent that the definition of the activity of the dye in the fiber phase in terms of the concentration $[D_\phi]/V$ is not satisfactory. Further, the exact nature of the adsorption isotherm, whether it is Freundlich, Langmuir, or a direct partition ratio, is not clear. For the purpose of calculation, a linear partition between $[D_1]$ and $[D_\phi]$ is used in assumption (3).

Recently, Sivaraja Iyer *et al.*[159] and Sivaraja Iyer and Baddi,[160] as a result of extensive investigations of the equilibrium adsorption isotherms for CI Direct Blue 1 and CI Direct Yellow 12 on viscose, cotton, and cuprammonium rayon fibers, have proposed the following modification of the electrochemical theory of dyeing. The volume term V is not regarded as constant, but variable with electrolyte concentration, and is defined by the expression

$$V = \frac{A}{\tau} \times 10^{-3} \tag{75}$$

[208] F. Müller, *Melliand Textilber.* **44**, 378 and 488 (1963).

[209] E. H. Daruwalla and G. G. Kulkarni, *Bull. Chem. Soc. Jap.* **37**, 1250 (1964).

[210] P. V. Moryganov and B. M. Melnikov, *Kolloid.-Zh.* **18**, 49 (1956); *CA* **50**, 9022 (1956).

[211] F. H. Holmes, *Shirley Inst., Mem.* **31**, 135 (1958).

liters per kilogram of dried fiber, where A (cm^2) is the available specific surface area and τ (cm^{-1}) is the reciprocal thickness of the electrical double layer[212] and is given by the equation

$$\tau = 2\epsilon\left(\frac{2\pi[Na_\sigma]N \times 10^{-3}}{DkT}\right)^{1/2} \tag{76}$$

where D is the dielectric constant; N, Avogadro's number; k, the Boltzman constant; T, the absolute temperature; and ϵ, the electronic charge in esu. Since $T \propto [Na_\sigma]^{1/2}$ it is apparent that $V \propto 1/[Na_\sigma]^{1/2}$ at any given temperature. For the area A, it is assumed that the surface which is available for the adsorption of a dye molecule is the nitrogen surface area of the water-swollen uncollapsed fiber. For cotton and viscose fibers, for example, the measured areas are, respectively, 116 m^2/gram and 205 m^2/gram. Confirmation of these values has been obtained recently from measurements of heats of wetting of cellulose fibers,[213] streaming potentials,[214] and negative sorption of chloride ions[215] in cellulose–aqueous solution systems. Large values of the surface area have also been reported by Suzawa and Mukai[216] for cellulose fibers from the measurement of ζ potentials. In the variable volume term, the volume of the diffuse double layer is replaced by an equivalent volume called "the inner Donnan phase" which is an equipotential volume having a uniform concentration for all the ions.

The concept of a variable volume term, as defined above, has been used successfully by Schofield and Talibuddin[217] for studies on jute fiber–aqueous electrolyte solution systems in order to obtain values for the true internal surface area of jute fibers. Alexander and Kitchener[218] have applied this concept to explain the sorption of acids and dyes by wool, and Ikeda and Isemura[219] have used the concept of an ionic distribution of the Donnan type between a surface layer of solution and the bulk phase to explain the surface pressure of ionized monolayers. Davies and Rideal[220] have also discussed this aspect. Support for such

[212] E. J. W. Verwey and J. T. G. Overbeek, "Theory of the Stability of Lyophobic Colloids." Elsevier, Amsterdam, 1948.

[213] S. R. Sivaraja Iyer and N. T. Baddi, *Cell. Chem. Technol.* **3**, 561 (1969).

[214] S. R. Sivaraja Iyer and R. Jayaram, *J. Soc. Dyers Colour.* **87** 338 (1971).

[215] S. R. Sivaraja Iyer, R. Jayaram, and B. I. Nemade, *Text. Res. J.* **40**, 1050 (1970).

[216] T. Suzawa and T. Mukai, *Kogyo Kagaku Zasshi* **73**, 2451 (1970); *CA* **74**, 65382 (1971).

[217] R. K. Schofield and O. Talibuddin, *Discuss. Faraday Soc.* **3**, 51 (1948).

[218] P. Alexander and J. A. Kitchener, *Text. Res. J.* **20**, 203 (1950).

[219] S. Ikeda and T. Isemura, *Bull. Chem. Soc. Jap.* **33**, 131 (1960).

[220] J. T. Davies and E. K. Rideal, "Interfacial Phenomena," 2nd ed. Academic Press, New York, 1963.

a variable volume term has been obtained from the results of Balmforth and Bird,[221] who have shown that their data for the adsorption of Solacet dyes on cellulose acetate yarn can be explained on the basis of the current theory of dyeing, if the volume term V is assumed to vary with $1/[Na_\sigma]^{1/2}$. They have, however, not given any theoretical explanation for the use of such a variable volume term. Some aspects of the use of a variable volume term in relation to Warwicker's theory for the diffusion of CI Direct Yellow 12 in cellophane sheet[110] have been discussed in Section I.

Sivaraja Iyer and Jayaram,[161] from recent studies on the measurement of Donnan potentials using Neale and Saha's method,[204] have obtained experimental evidence for a variable volume term in dyed and undyed cellophane–potassium chloride solution systems as follows. The ideal equation for the Donnan potential is given by the expression

$$E_D = \frac{RT}{F} \sinh^{-1} \frac{W}{2K_\sigma V} \tag{77}$$

where W is the surface charge density due to adsorbed dye ions plus the intrinsic ionized carboxyl groups in the cellophane film. At low values of the potential this equation can be approximated to

$$E_D = \frac{RT}{F} \frac{W}{2K_\sigma V} \tag{78}$$

If V is constant (V = 0.44 liter/kg of dried cellophane), a plot of E_D vs. $1/K_\sigma$ or a plot of E_D vs. $\sinh^{-1}(W/2K_\sigma V)$ for a constant W, should be linear. If, on the other hand, $V \propto 1/K_\sigma^{1/2}$, a plot of E_D vs. $\sinh^{-1}(W/2K_\sigma V)$ where V is now a variable quantity calculated from Eq. (75) or a plot of E_D vs. $1/K_\sigma^{1/2}$ should be linear.

Figures 32 and 33 clearly show that linear plots are obtained over a wide range of electrolyte concentration and charge densities W, only if the volume term is variable. These results also indicate that for all the eight dyes studied, the measured Donnan potentials depend solely on the value of the total surface charge and are independent of dye structure. The calculated values of W from the measurement of Donnan potentials indicate that approximately 25% of the total ionizable surface groups (adsorbed dye + carboxyl groups) are dissociated. In the interpretation of the present results, it has been assumed, as Neale and co-workers[203,204] did, that the diffusion potential in the cellophane sheet is very small and is practically cancelled out by the liquid junction potential. This is based on the assumption that the ionic mobility ratio

[221] D. Balmforth and C. L. Bird, *J. Soc. Dyers Colour.* **80**, 534 (1964).

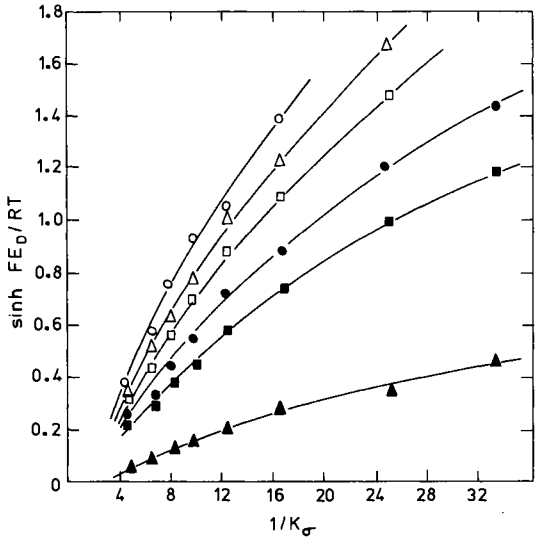


FIG. 32. Sinh FE_D/RT as a function of $1/K_\sigma$ at 26° and with $W \times 10^3$ (equiv/kg) of, ○, 301.1; △, 254.0; □, 227.8; ●, 183.3; ■, 147.3; ▲, 54.4. Reproduced from S. R. Sivaraja Iyer and R. Jayaram.[161]

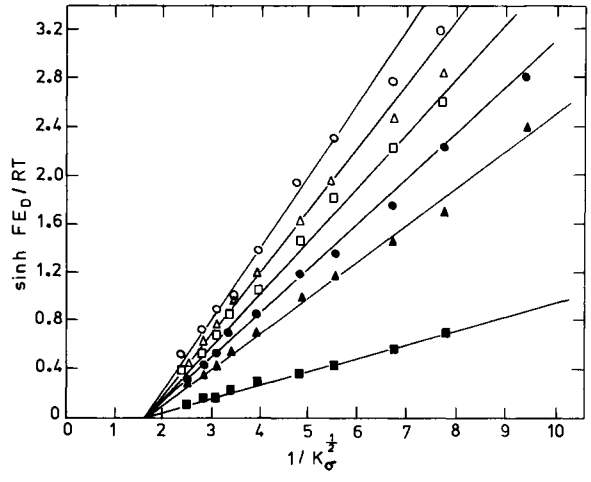


FIG. 33. Sinh FE_D/RT as a function of $1/K_\sigma^{1/2}$ at 26° and with $W \times 10^3$ (equiv/kg) of ○, 301.1; △, 254.0; □, 227.8; ●, 183.3; ▲, 147.3; ■, 54.4. Reproduced from S. R. Sivaraja Iyer and R. Jayaram.[161]

for potassium and chloride ions is unity even in the membrane. Wright[222] has shown that this ionic mobility ratio for the diffusion of potassium chloride in regenerated cellulose is approximately unity, although the absolute value for each ion is less than in a free solution of the electrolyte. Results of recent detailed experimental investigations[223] on the membrane conductivity, membrane potential, and the concentration of sorbed potassium and chloride ions in regenerated cellophane membranes agree with Wright's observations.[222] The measured potentials thus represent the true Donnan potentials in the system studied, provided it is assumed that the adsorbed dye and the intrinsic carboxyl groups are dissociated to about 25%.

Overbeek[224] has discussed the influence of the reduced mobility of the counterions in the charged membranes on the measured Donnan potentials and has come to the conclusion that the first and largest effect on the Donnan potentials is given by the nonideal behavior of mobilities rather than of the ionic activities. He explains the discrepancies between the theoretical and calculated charge densities in terms of the reduced mobility of the counterions in the membrane. It is also possible to calculate the volume term V from a measurement of the ionic distribution of counterions and co-ions in the cellophane membrane for different electrolyte concentrations. Sivaraja Iyer and Kalbag[162] measured such distributions for the cellophane–potassium chloride solution system and used the equation

$$V = \frac{wM_0([K_e^+] - [Cl_e^-] - M_0) - w[K_e^+][Cl_e^-]}{M_0([K_e^+] - [Cl_e^-] - 2M_0)} \tag{79}$$

which can be derived from the material balance equations for the ionic concentrations in the internal solution phase i and external bulk equilibrium solution phase σ, applying the principle of electroneutrality in each phase. In this equation, w is the weight of imbibed solution, M_0 is the bulk electrolyte concentration, and $[K_e^+]$ and $[Cl_e^-]$ refer to the experimentally measured values of the total electrolyte concentration in the cellophane phase containing at equilibrium w grams of imbibed electrolyte solution per gram of dried cellophane. These results are given in Table VI.

At lower electrolyte concentrations, the deviation between theoretical and experimental values of V may be attributed to a decreasing mobility of counterions leading to a decrease in the degree of dissociation of ionizable carboxyl groups.

[222] J. Wright, *J. Phys. Chem.* **58**, 50 (1954).

[223] R. Jayaram, Ph.D. Thesis, Gujarat University, India (1969).

[224] J. T. G. Overbeek, *Progr. Biophys. Biophys. Chem.* **6**, 57 (1956).

TABLE VI

COMPARISON OF THE THEORETICAL VOLUME TERM $V = (A/\tau) \times 10^{-3}$ LITER/KG OF DRY CELLOPHANE AND THE EXPERIMENTAL VALUES OF THE VOLUME, FOR BLANK CELLOPHANE, WHERE[a,b]

$$V = \frac{wM_0([K_e^+] + [Cl_e^-] - M_0) - w[K_e^+][Cl_e^-]}{M_0([K_e^+] + [Cl_e^-] - 2M_0)}$$

M_0 Molarity of KCl (M)	Experimental values of V $\left(\frac{ml}{g \text{ of dry cellophane}}\right)$	Theoretical values of V $\left(\frac{ml}{g \text{ of dry cellophane}}\right)$
0.20	0.162	0.139
0.15	0.174	0.160
0.10	0.216	0.196
0.08	0.225	0.219
0.07	0.259	0.234
0.06	0.327	0.253
0.05	0.377	0.277
0.04	0.445	0.310

[a] S. R. Sivaraja Iyer and V. N. Kalbag.[162]

[b] In the equation for V; w = 1 ml/g of dry cellophane.

McGregor[225] has also recently discussed the importance of experimentally determining the distribution of both counterions and co-ions in textile fiber–inorganic electrolyte solution systems for the proper evaluation of the Donnan distribution coefficient λ and the volume term V, which are very important parameters in the electrochemical theories of dyeing.

The second important assumption in the proposed model is that the Langmuir-type relation given in Eq. (80), holds for the adsorption of dye on the fiber from the dye solution present in the internal volume V.

$$\frac{1}{[D_\phi]} = \frac{\exp(-\Delta\mu^\circ/RT)}{[D_s][D_i]} + \frac{1}{[D_s]} \tag{80}$$

Here $[D_s]$ refers to the saturation value for a monolayer of adsorbed dye under a given set of experimental conditions. This equation can be derived from simple thermodynamic reasoning.[18] The concentration $[D_i]$ of the dye anion in the surface phase i can be calculated from Eq. (81), which is similar to Eq. (73) except for the introduction of the concentration of ionized carboxyl groups in the fiber, since it is found to markedly influence the Donnan distribution, particularly where the

[225] R. McGregor, *Text. Res. J.* **42**, 172 (1972).

concentration of adsorbed dye ion is comparable to the carboxyl ion concentration.

$$[D_i] = [D_\sigma]\exp\left\{-z \sinh^{-1} \frac{z[D_\phi] + [COO^-]}{2[Na_\sigma]V}\right\} \quad (81)$$

The importance of the carboxyl ion concentration in direct dye adsorption has also been discussed by Daruwalla *et al.*[226] Equation (80) can be rearranged to get Eq. (82), which expresses the activity of the adsorbed dye ions by the term $\theta/(1 - \theta)$ where $\theta = [D_\phi]/[D_s]$.

$$\log \frac{\theta}{1 - \theta} = \log[D_i] + \exp(-\Delta\mu^\circ/RT) \quad (82)$$

Fowler[227] has shown on the basis of statistical mechanics that the activity of a substance in the adsorbed state may be represented by $\theta/(1 - \theta)$, where θ is the fraction of sites occupied by the adsorbed molecules. Daruwalla and D'Silva[191] have used the expression $\theta/(1 - \theta)$ to express the activity of the adsorbed dye ions and have shown that the adsorbed dye reaches a saturation value corresponding to the formation of a monolayer. These conclusions imply a Langmuir-type adsorption. The experimental evidence for the formation of a monolayer of adsorbed molecules in direct dye–cellulose fiber systems has already been discussed by Daruwalla in the previous chapter. An important test for the validity of Eq. (82) is that a plot of log $\theta/(1 - \theta)$ vs. log $[D_i]$ should give a straight line of unit slope.

Figures 34 and 35, which are typical of all the isotherms, show reciprocal Langmuir plots for CI Direct Blue 1. It is clear that linear plots are obtained only when $1/[D_\phi]$ is plotted against $1/[D_i]$ or $1/a_i$; i.e., the adsorption of dye from the internal solution phase i of volume V follows a Langmuir-type equation whether one uses a combination of concentrations or activities with a constant or variable volume term. Mean ionic activities for the dye have been calculated using an appropriate form of the Debye-Hückel equation as discussed in the previous chapter. Experimental verification for the use of such an equation has been obtained from solubility[228,229] and isopiestic measurements[230] and through the use of a semipermeable membrane.[231] Porter and Perkins[232]

[226] E. H. Daruwalla, P. J. Kangle, and G. M. Nabar, *Text. Res. J.* **31**, 712 (1961).

[227] R. H. Fowler, *Proc. Cambridge Phil. Soc.* **31**, 260 (1935).

[228] N. T. Baddi and S. R. Sivaraja Iyer, *Kolloid-Z.* **210**, 132 (1966).

[229] M. Mitsushi and G. Aida, *J. Soc. Text. Cell. Ind. Jap.* **18**, 288 (1962); *J. Soc. Dyers Colour.* **78**, 426 (1962).

[230] S. Chadwick and S. M. Neale, *J. Polym. Sci.* **28**, 355 (1958).

[231] D. R. Hardisty and S. M. Neale, *J. Polym. Sci.* **46**, 195 (1960).

[232] J. J. Porter and W. S. Perkins, *Text. Res. J.* **40**, 81 (1970).

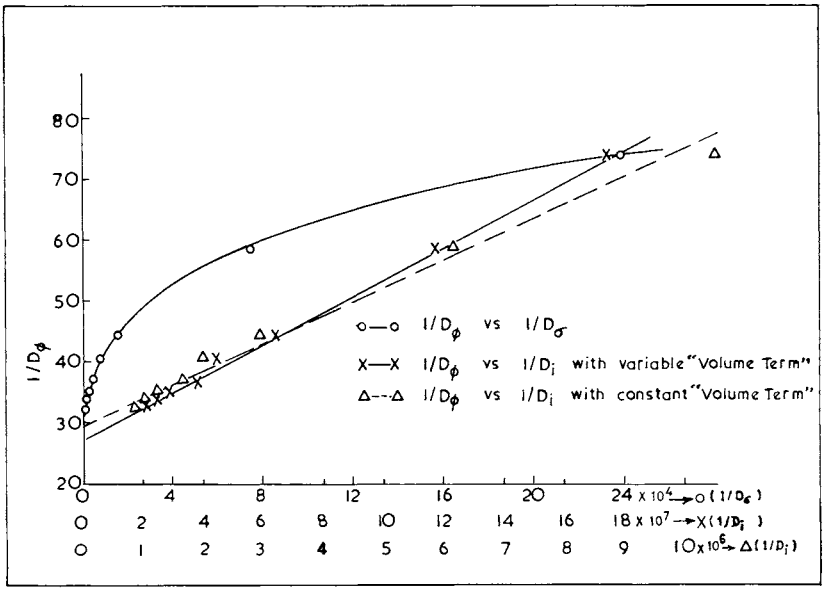


FIG. 34. Langmuir plot using concentrations for adsorption of CI Direct Blue 1 on cotton fibers. NaCl, 7.5 g/liter; temperature, 50°. Reproduced from S. R. Sivaraja Iyer and N. T. Baddi.[160]

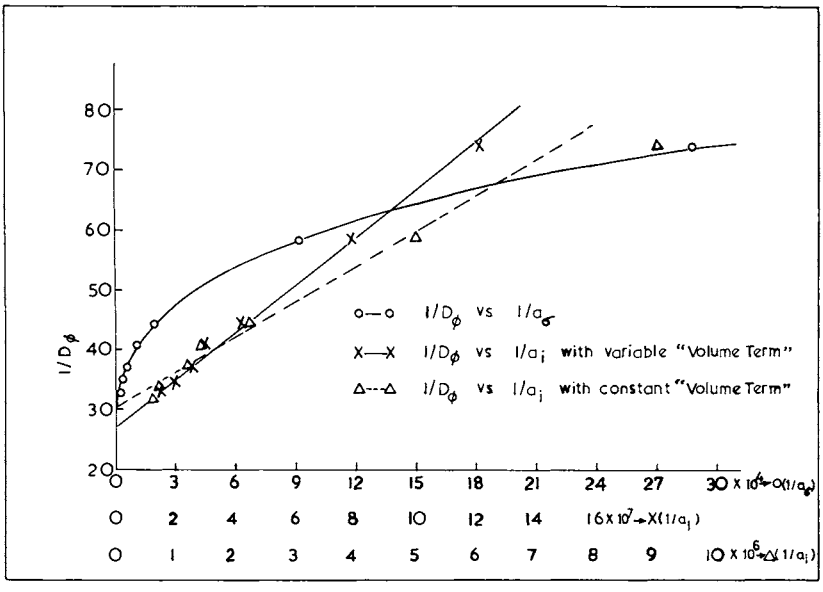


FIG. 35. Langmuir plot using activities for adsorption of CI Direct Blue 1 on cotton fibers. NaCl, 7.5 g/liter; temperature, 50°. Reproduced from S. R. Sivaraja Iyer and N. T. Baddi.[160]

have also shown that the adsorption follows a Langmuir isotherm. The saturation values [D_s], which are obtained from the intercepts of the reciprocal Langmuir plots, increase with increasing electrolyte concentration. An interesting feature of the adsorption isotherms which has been recently studied by Sivaraja Iyer *et al.*[233] is that at equivalent concentrations of the alkali metal chlorides the adsorption of CI Direct Blue 1 on cotton fiber shows a marked increase with increasing size of the alkali metal cations, i.e., in the order of $Li^+ < Na^+ < K^+ < Rb^+ < Cs^+$. Sivaraja Iyer and Ghanekar[119] have also shown that when a series of alkali metal halides are used, the adsorption isotherms are identical for the same equivalent concentrations of the different alkali halides having the same cation, i.e., the nature of the anion has no influence on the adsorption. Comparative studies which were made on the adsorption of CI Direct Blue 1 on a nonpolar hydrophobic surface such as graphon and on a polar hydrophilic surface such as TiO_2 having the same specific surface area ($\simeq 100\ m^2/g$) show that in the case of graphon the nature of the electrolyte cation does not influence the extent of adsorption, whereas for TiO_2 there is a marked influence of the cation on the dye adsorption. For example, in 0.02 *M* NaCl or KCl, the saturation value for KCl is ten times greater than that of NaCl at 35°. All these results taken together suggest that the structure of water at a hydrophilic surface and also around the hydrophobic parts of the adsorbing dye molecules is disturbed by the cations; as discussed in the previous chapter, this water structure-breaking effect will increase with the size of the alkali metal cation, accounting for the increased dye adsorption in the series Li^+ to Cs^+.

The results shown in Figs. 34 and 35 do not help to decide between the use of a variable and a constant volume term. The data given in Table VII, however, clearly show that the closest agreement between theory and experiment using Eq. (82), as discussed earlier, is obtained only when a variable volume term is used, the linear plots for a variable *V* in the case of both CI Direct Blue 1 and CI Direct Yellow 12 having unit slopes.

The above discussion of the electrochemical nature of dyeing and the adherence of the adsorption isotherms to a Langmuir equation for all values of [D_s] suggests that these saturation values are only apparent values depending on the extent to which the concentration of electrolyte cations in the dyebath can suppress the increasing negative potential on the fiber surface due to adsorption of dye anions. The dye anions are adsorbed by virtue of their affinity for the cellulose fiber. Adsorption of the dye anions will repel approaching dye anions due to electrostatic

[233] S. R. Sivaraja Iyer, G. Srinivasan, and N. T. Baddi, *Text. Res. J.* **38**, 693 (1968).

TABLE VII

SLOPES OF THE PLOTS LOG $\frac{\theta}{1-\theta}$ VS. LOG $[D_1]$[a]

Adsorption isotherm	Concentration of NaCl (g/liter)	Temperature (°C)	Slopes: With activities $V = \frac{A}{\tau} \times 10^{-3}$ (liter/kg)	Slopes: With activities V[b]	Slopes: With concentrations $V = \frac{A}{\tau} \times 10^{-3}$ (liter/kg)	Slopes: With concentrations V[b]
Chlorazol Sky Blue FF	10	50	1.00	1.01	1.00	1.10
(CI Direct Blue 1) on	10	60	1.00	1.12	1.00	1.10
cotton fiber	7.5	50	1.00	1.00	1.00	1.00
	7.5	60	1.00	1.12	1.00	1.10
	6.0	50	1.00	0.92	0.92	0.84
	6.0	60	1.00	0.98	0.96	0.90
	4.0	50	1.00	0.78	1.00	0.72
	4.0	60	1.00	0.90	1.00	0.85
Chlorazol Sky Blue FF	10	50	1.00	1.06	1.00	0.92
(CI Direct Blue 1) on	10	60	1.00	1.20	1.00	0.98
viscose fiber	4.0	50	1.00	0.96	1.00	0.95
	4.0	60	1.00	1.06	1.00	1.04
Chlorazol Sky Blue FF	10	50	1.00	0.92	1.00	0.90
(CI Direct Blue 1) on	10	60	1.00	0.72	1.00	0.75
cuprammonium rayon	4.0	50	1.00	0.82	1.02	0.81
fiber	4.0	60	1.00	0.63	0.95	0.60
Chrysophenine G (CI	2.0	35	1.00	1.03	1.00	1.02
Direct Yellow 12) on	2.0	40	1.00	1.01	1.00	1.00
viscose rayon fiber	2.0	45	1.00	1.00	1.00	1.00
Chrysophenine G (CI	2.0	40	1.00	1.04	1.00	1.02
Direct Yellow 12) on	2.0	60	1.00	1.01	1.00	1.00
cellophane sheet (Calculated from results of Willis *et al.*[83])	2.0	97.5	1.00	1.00	1.00	1.00

[a] Reproduced from S. R. Sivaraja Iyer and N. T. Baddi.[160]

[b] V = 0.44 liter/kg for viscose fiber and cellophane sheet; V = 0.37 liter/kg for cuprammonium rayon fiber; V = 0.22 liter/kg for cotton fiber.

repulsion, and the influence of electrolyte counterions in minimizing this surface potential effect due to dye anion adsorption, as mentioned earlier, will be a function of their concentration. Hence, as adsorption proceeds at a given electrolyte concentration and temperature, the equilibrium dye uptake will increase less rapidly with increasing dye concentration at high than at low dye adsorption. Crank[152] also attributes this decreasing gradient in equilibrium adsorption to the

effect of electrolyte counterions and not to the approach of a true saturation value corresponding to the occupation of all the available sites for dye anions. Devanathan[234] has observed apparent Langmuir adsorption isotherms for the adsorption of *n*-amyl alcohol and butanol on a charged mercury surface. This apparent Langmuir relationship has been shown by him to be general, connected with charged interfaces. Further, he has shown in the case of these adsorption isotherms that the free energy of adsorption remains practically independent of coverage, as expected for adsorption processes obeying the Langmuir isotherm. Since $-\Delta\mu^\circ$ (Table VIII) is virtually constant for the dye adsorption process, the adsorption of dye anions on a charged cellulose surface appears to be part of a perfectly general phenomenon associated with charged interfaces.

The affinity or standard free-energy changes $-\Delta\mu^\circ$ calculated from the slopes of the linear plots [Eq. (81)] shown in Figs. (34) and (35) are given in Table VIII. These affinity values are higher than those reported in the literature,[83,200,207,208,210,235] but agree fairly closely with the data of Daruwalla and co-workers.[191,206] The affinity values for a given dye do not vary significantly with $[D_s]$ and are also independent of the type of fiber used. It should, however, be pointed out that the actual numerical values of $-\Delta\mu^\circ$ will depend on the standard states which are chosen for the dye in solution and fiber phases.

The effect of temperature on dyeing is conveniently expressed in terms of the exothermic heat of dyeing $-\Delta H^\circ$, which can be determined with moderate accuracy from isotherm data at two different temperatures, using the standard equation

$$-\Delta H^\circ = \left[\frac{\Delta\mu_1^\circ}{T_1} - \frac{\Delta\mu_2^\circ}{T_2}\right]\left[\frac{1}{T_1} - \frac{1}{T_2}\right] \tag{83}$$

A plot of $-\Delta H^\circ$ vs. the limiting co-areas for the adsorbed dye molecules is shown in Fig. 36. The values of $-\Delta H^\circ$ are a function of the limiting co-area only and are independent of the nature of the cellulose fiber or the alkali metal chlorides added to the dyebath. The increase in heats of adsorption with increasing surface coverage has been attributed by several workers[236–238] to lateral interaction between adsorbed species. The graphical representation of this functional relationship[160] between the values of $-\Delta H^\circ$ and the co-areas (Fig. 36) shows that at large

[234] M. A. V. Devanathan, *Proc. Roy. Soc., Ser. A* **267**, 256 (1962).

[235] S. M. Neale and W. A. Stringfellow, *J. Soc. Dyers Colour.* **56**, 17 (1940).

[236] R. M. Barrer and S. Wasilewski, *Trans. Faraday Soc.* **57**, 1140 (1961).

[237] J. J. Chessick and A. C. Zettlemoyer, *Advan. Catal.* **11**, 290 (1959).

[238] A. V. Kiselev, *Proc. Int. Congr. Surface Activ., 2nd*, p. 168 (1957).

TABLE VIII

AFFINITY VALUES, $-\Delta\mu^\circ$ (KCAL/MOLE)[a]

Adsorption isotherm	Concentration of NaCl (g/liter)	Temperature (°C)	$-\Delta\mu^\circ$ With activities $V = \frac{A}{\tau} \times 10^{-3}$ (liter/kg)	$-\Delta\mu^\circ$ With activities V^b	$-\Delta\mu^\circ$ With concentrations $V = \frac{A}{\tau} \times 10^{-3}$ (liter/kg)	$-\Delta\mu^\circ$ With concentrations V^b
Chlorazol Sky Blue FF	10	50	11.99	9.75	12.02	9.75
(CI Direct Blue 1) on	10	60	11.75	9.41	11.76	9.41
cotton fiber	7.5	50	11.63	9.86	11.85	9.95
	7.5	60	11.46	9.71	11.67	9.74
	6	50	11.49	10.15	11.72	10.15
	6	60	11.37	9.96	11.56	9.89
	4	50	11.18	10.03	11.57	10.38
	4	60	11.08	9.89	11.49	10.22
Chlorazol Sky Blue FF	10	50	12.32	9.89	12.45	9.89
(CI Direct Blue 1) on	10	60	12.04	9.61	12.19	9.61
viscose fiber	4.0	50	11.81	10.30	12.30	10.75
	4.0	60	11.70	10.02	12.13	10.57
Chlorazol Sky Blue FF	10	50	12.32	10.20	12.56	10.22
(CI Direct Blue 1) on	4.0	60	11.81	9.56	11.96	9.56
cuprammonium rayon	4.0	50	11.97	11.85	12.47	11.18
fiber	4.0	60	11.74	11.60	11.90	10.65
Chrysophenine G (CI	2.0	35	6.52	6.33	6.60	6.37
Direct Yellow 12) on	2.0	40	6.48	6.24	6.54	6.32
viscose rayon fiber	2.0	45	6.44	6.14	6.51	6.25
Chrysophenine G (CI	2.0	40	7.05	6.75	7.02	6.72
Direct Yellow 12) on	2.0	60	6.90	6.70	6.92	6.67
cellophane sheet	2.0	97.5	6.67	6.16	6.61	6.02
(Calculated from results of Willis *et al.*[83])						

[a] Reproduced from S. R. Sivaraja Iyer and N. T. Baddi.[160]

[b] V = 0.44 liter/kg for viscose fiber and cellophane sheet; V = 0.22 liter/kg for cotton fiber; V = 0.37 liter/kg for cuprammonium rayon fiber.

limiting co-areas, where dye–dye interaction is negligible, $-\Delta H^\circ$ remains constant at about 14 to 15 kcal/mole. As the co-area decreases to values smaller than about 600 Å^2, $-\Delta H^\circ$ increases with decreasing limiting co-area. There is a sharp increase in $-\Delta H^\circ$ values within the co-area range of 200–400 Å^2. Willis *et al.*[83] report $-\Delta H^\circ$ values ranging from 9.3 to 15.9 kcal/mole for the adsorption of CI Direct Yellow 12 on cellophane sheet in the presence of sodium chloride concentrations up

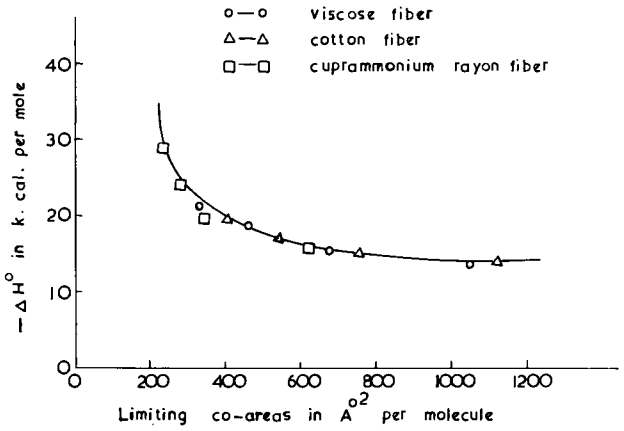


FIG. 36. Plot of $-\Delta H^\circ$ versus limiting co-area for the adsorption of CI Direct Blue 1 on cellulose fibers. Reproduced from S. R. Sivaraja Iyer and N. T. Baddi.[160]

to 4 g/liter. Graham and Fromm,[239] from calculations of $-\Delta H^\circ$ using the data of Willis *et al.*,[83] point out that $-\Delta H^\circ$ increases with increasing salt concentrations for a fixed value of the dye adsorption. However, no attempt was made to explain or discuss this variation of the heat of dyeing with increasing limiting co-area.

Since the standard affinity remains sensibly constant, calculations based on the standard equation, $\Delta\mu^\circ = \Delta H^\circ - T\Delta S^\circ$, where $-\Delta S^\circ$ is the change in entropy, indicate that changes in $-\Delta H^\circ$ values are reflected by a corresponding increase in $-\Delta S^\circ$ values ranging from 10 to 50 cal/deg mole as the co-area decreases. The dye adsorption process can be regarded as one in which the dye undergoes a transition from a state of random distribution of dye ions in solution to a state of restricted movement and a more ordered arrangement of adsorbed dye on the fiber surface. Therefore, as the co-area decreases, the adsorbed dye becomes more closely packed on the surface, resulting in an increased orientation of adsorbed dye ions or molecules and greater restrictions on their freedom of movement. The large increase in values with decrease in co-area is therefore to be expected. It should, however, be pointed out that the enthalpy and entropy changes depend on an interplay of all the energy changes due to molecular interactions in the total system, i.e., in both fiber and solution phases, and this aspect should be borne in mind when interpreting thermodynamic data.[240] Hence, contributions to $-\Delta H^\circ$ and $-\Delta S^\circ$ from, for example, dye aggregation or disaggregation phenomena in the solution and fiber phases, entropy

[239] R. P. Graham and H. J. Fromm, *Can. J. Res., Sect. F* **25**, 303 (1947).

[240] R. McGregor, *J. Soc. Dyers Colour.* **83**, 52 (1967).

changes due to water structure-breaking effects, or changes in the ordering of solute species have also to be considered. Nevertheless, the large values of $-\Delta H^\circ$ and $-\Delta S^\circ$ discussed earlier can be attributed mainly to interactions in the fiber phase since the dyes are not aggregated, and entropy changes due to water structure-breaking effects, for example, should give a positive contribution to $-\Delta S^\circ$. When highly aggregating dyes such as CI Direct Red 28 or CI Direct Red 2 are adsorbed on cellulose or graphon surfaces,[26] or when acid and basic dyes are adsorbed on polar or nonpolar surfaces,[241,242] the adsorption can be endothermic, athermic, or exothermic depending on the relative strengths of dye–substrate, dye–dye, and dye–solvent binding energies. It is likely that the adsorbed dye molecule lies flat on the cellulose surface with the plane of the benzene rings parallel to the surface, until the packing corresponds to a co-area of about 400 Å^2. Further adsorption probably results in a changeover from this orientation to one in which the dye ion lies lengthwise with the plane of the benzene rings perpendicular to the surface. The projected co-area for such a configuration would be of the order of 200 Å^2 for CI Direct Blue 1. Increased lateral dye–dye interaction arising out of such a configuration is responsible for the previously mentioned sharp increase in $-\Delta H^\circ$ values between co-areas of 200–400 Å^2. Such changes in orientation for the adsorption of acid dyes on a polar surface such as alumina have been discussed by Giles *et al.*[241] Giles[242] has also suggested a similar explanation for the adsorption of acid and basic dyes by graphite. At low surface coverages the molecules are thought to lie flat on the surface, but at higher surface coverages they rearrange to a closer packing with the ionic groups away from the solid surface. Such an orientation may give rise to "edge-on" or "end-on" adsorption, depending on the disposition of the charged groups in the molecule.

B. Ionic Dye—Ionic Fiber Systems

1. *Acid Dye–Nylon Fiber System*

Nylon contains carboxyl and amine end groups which can ionize to produce $-COO^-$ ions and alkyl $-NH_3^+$ ions. Since there is usually a greater number of carboxyl end groups than amine end groups, some of the carboxyl end groups remain in the un-ionized form. The fiber also contains weakly basic regularly spaced amide groups along the polymer chain. The zwitterion form NH_3^+—Ny—COO^-, where —Ny— repre-

[241] C. H. Giles, M. M. Allingham, J. M. Cullen, S. K. Jain, and J. S. Woods, *J. Appl. Chem.* **8**, 108 (1958).

[242] C. H. Giles, *in* "Hydrogen Bonding" (D. Hadzi, ed.), p. 449. Pergamon, Oxford, 1959.

sents the aliphatic chain separating the charged groups, is still a matter of considerable speculation, particularly under conditions close to neutral pH. However, this is the structure normally assumed by most workers in explaining the sorption of acid dyes on nylon. Zollinger *et al.*,[243] from a comparative study of the properties of normal nylon 66 and of specially prepared polyamide fibers with double the number of carboxyl groups, conclude that the zwitterion form for nylon is favored. Some recent work by Marshall[244] on the absorption of simple acids by nylon indicates that the process is equivalent to the back-titration of carboxyl groups in the polymer, and that nylon can be regarded as existing in the zwitterion form. Some studies reported by Daruwalla[245] on the electrokinetic potentials of nylon fibers having different amine end group contents under neutral conditions indicate that with increase in amine end group content there is also an increase in the negative ζ-potential value. This increase, which is attributed to an increased ionization of carboxyl groups with increasing amine end group content, thus favors the zwitterion structure.

The equilibrium sorption of acid dyes by nylon fibers as a function of pH generally follows curves of the type shown in Fig. 37,[246] which can be regarded as the titration curves of nylon with anionic dyes. The

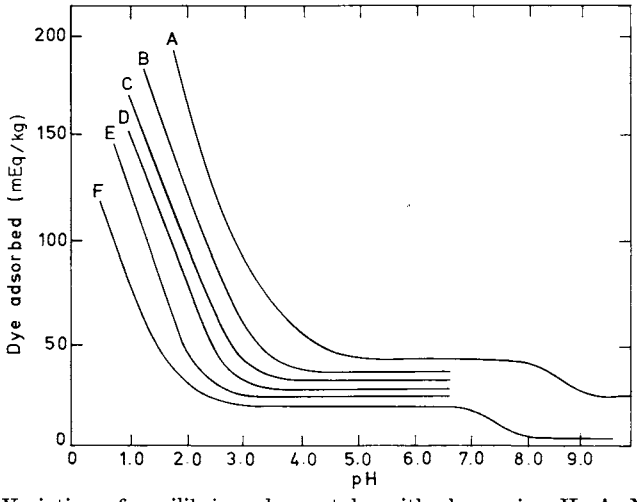


FIG. 37. Variation of equilibrium dye uptake with change in pH: A, Naphthalene Red J; B, Naphthalene Orange G; C, Solway Blue R; D, Lissamine Fast Yellow 2G; E, Azogeranine 2G; F, Solway Blue B. Reproduced by permission from Atherton *et al.*[165]

[243] H. Zollinger, G. Back, B. Milicevic, and A. N. Roseira, *Melliand Textilber.* **42**, 73 (1961).

[244] J. Marshall, *J. Polym. Sci., Part A-1* **6**, 1583 (1968).

[245] E. H. Daruwalla, *Colourage* (*Annu.*) p. 59 (1970).

[246] R. H. Peters, *J. Soc. Dyers Colour.* **61**, 95 (1945).

titration of nylon with hydrochloric acid[247,248] gives a similar sigmoid curve except that the more or less horizontal portion of the curve corresponding to the saturation of amine end groups occurs in a narrow region around pH 3 and is not spread out over a wide range of pH. The curves in Fig. 37 can be divided into three parts. In the first part, at high pH values, the carboxyl groups will be almost completely ionized and amine end groups would begin to lose their protons. Adsorption of anionic dyes would therefore be small. With decrease in pH, the amine end groups will get protonated and the dissociation of the carboxyl groups will be gradually suppressed. The dye anion sorption therefore gradually increases to a maximum value equivalent to the stoichiometric amine end group content, accounting for the more or less horizontal plateau region in the curves in Fig. 37 for the pH region 3–7, the middle zone in the titration curve. Finally, at lower pH values there is a sharp increase in dye uptake that is generally attributed to dye adsorption on protonated imido groups. This phenomenon is usually referred to as overdyeing.

Most of the models[167,246,249,250] for the sorption of acid dye anions by polyamides give a fairly good description of the equilibrium sorption isotherm in the amine dyeing region of pH 3–7 where the dye uptake is relatively insensitive to changes in pH. It is generally assumed that there is a stoichiometric relationship between the amine end group content and the amount of dye adsorbed. The simplified picture that emerges from the results of all these studies in the amine dyeing region is that hydrogen ions are adsorbed by carboxyl groups when nylon fibers are immersed in a dyebath containing an acid and an acid dye, while the dye ion attaches itself to positively charged amino groups, which therefore play a key role in the dyeing process.

It is generally observed that when electrolytes such as NaCl and Na_2SO_4 are added to the dyebath there is a significant desorption of the dye. This is attributed to the exchange of the dye ions by the anions of the inorganic electrolyte. The dyeing of nylon with acid dyes is thus also regarded to some extent as an ion-exchange process similar to that for the dyeing of wool with acid dyes. Myagkov and Pakshver[251] have proposed that a simple ion-exchange equilibrium of the following type exists for the polyamide–acid dye system:

$$—NH_3^+OH(s) + A^-(l) \rightleftharpoons —NH_3^+A^-(s) + OH^-(l) \qquad (84)$$

[247] P. W. Carlene, A. S. Fern, and T. Vickerstaff, *J. Soc. Dyers Colour.* **63**, 388 (1947).

[248] E. Elöd and H. G. Fröhlich, *Melliand Textilber.* **30**, 103 (1949).

[249] R. McGregor and P. W. Harris, *J. Appl. Polym. Sci.* **14**, 510 (1970).

[250] W. R. Remington and E. K. Gladding, *J. Amer. Chem. Soc.* **72**, 2553 (1950).

[251] V. A. Myagkov and A. B. Pakshver, *J. Appl. Chem. USSR* **29**, 774 (1956); *J. Soc. Dyers Colour.* **72**, 456 (1956).

where $—NH_3^+$ is terminal fiber cation; A^-, dye anion; s, solid phase; and l, liquid phase. Ferrini and Zollinger[166] from a study of the adsorption of anionic monoazo dyestuffs on aminopropylene conclude that the dye adsorption follows a Langmuir isotherm with a stoichiometric relationship between the dye adsorbed and amino group concentration, the mechanism of adsorption being a simple ion-exchange process.

However, it has been noted by many workers[248,250,252,253] that the equilibrium sorption as a function of pH in the amine dyeing region deviates from the stoichiometric relationship between amine end group content and concentration of adsorbed dye, and in several cases is either considerably lower or higher than the amine end group content, as can be seen from Fig. 37. Furthermore, for some dyes the stoichiometric relationship between dye adsorption and amine end group content for various values of amine end group content generally exists only at a particular pH. Other dyes do not give a general stoichiometric relationship at any pH value.[253,254] This nonstoichiometric relationship between dye uptake and amine end group content is related to the degree of sulfonation and the affinity of the acid dye. Thus, for example, as can be seen from Fig. 37, dye uptake in excess of the amine end group content is most marked for monosulfonated dyes such as CI Acid Red 88, for CI Acid Orange 7 it is stoichiometric, and for the disulfonated dye CI Acid Blue 45 the adsorption is much less than the amine end group content.

In more recent work, Atherton *et al.*[165] showed that the pH adsorption isotherms at 75° for the dyeing of nylon with various acid dyes had a characteristic shape in which the plateaus were not horizontal, but indicated gradual increases in dye uptake with decrease in pH even in the pH range corresponding to the amine dyeing region (Fig. 38). These results, as well as other data reported in the literature,[169,255] therefore indicate that overdyeing need not occur always at low pH, but can occur to a greater or lower extent even at higher pH once the saturation of amino groups has been achieved. Atherton *et al.*,[165] as well as others,[243,254,256] also showed that the adsorption isotherms at a given pH indicate in some cases a continuous increase in dye adsorption with concentration, whereas in other cases there is a leveling off of the curve to a constant value of dye uptake, the isotherm being similar to a Langmuir sorption isotherm. Some typical results are shown in Fig. 39.

[252] F. C. McGrew and A. K. Schneider, *J. Amer. Chem. Soc.* **72**, 2547 (1950).
[253] H. J. Palmer, *J. Text. Inst.* **49**, T33 (1958).
[254] H. Takasawa, N. Kuroki, and A. Katayama, *Sen-i-Gakkaishi* **24**, 185 (1965).
[255] M. Greenhalgh, A. Johnson, and R. H. Peters, *J. Soc. Dyers Colour.* **78**, 315 (1962).
[256] B. Milicevic, *Textilveredlung* **3**, 607 (1968).

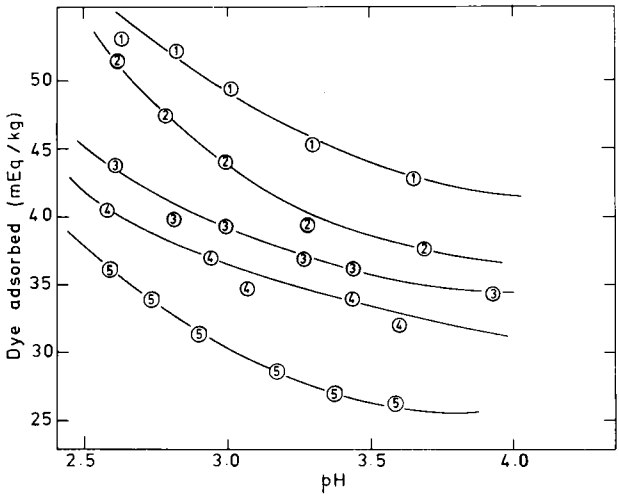


FIG. 38. Variation of equilibrium dye uptake with change in pH. Temperature 75°. 1, Naphthalene Orange G; 2, Naphthalene Red EA; 3, Naphthalene Fast Orange 2G; 4, Solway Blue A; 5, Tartrazine N. Reproduced by permission from Atherton *et al.*[165]

Rattee[257] has recently pointed out that the usual experimental method of immersing polyamide fibers in acid or dye acid solutions and leaving the system to equilibrate is unreliable due to a hydrolytic breakdown of the polymer which results in ions in the dyebath other than those of the acids used. From an analysis of titration liquors from such experiments, Marfell[258] has shown the presence of large quantities of ammonia and smaller but significant quantities of acid compounds in such solutions. Repeated extraction, however, removes these compounds completely. Using this technique to free nylon 6 of such impurities, White[259] obtained titration curves for a series of naphthaleneazo-naphthol dyes ranging from the monosulfonated dye CI Acid Red 88 to the tetrasulfonated dye CI Acid Red 41. The shapes of these acid titration curves are similar to those reported in the literature, but they also clearly indicate that even at high pH in the range 3.5 to 6, the inflection occurs in all cases at adsorbed dye concentrations greater than the amine end group content. These results are shown in Fig. 40 and again indicate that overdyeing can occur even at higher pH.

The considerable overdyeing that takes place at low pH values (pH < 3) in the so-called imido dyeing region of the curves in Figs. 37 and 38 has been established by all the workers referred to earlier. Any

[257] I. D. Rattee, *Colourage* (*Annu.*) p. 23 (1970).

[258] D. J. Marfell, unpublished work referred in Rattee.[257]

[259] M. A. White, Ph.D. Thesis, University of Leeds (1968); see Rattee.[257]

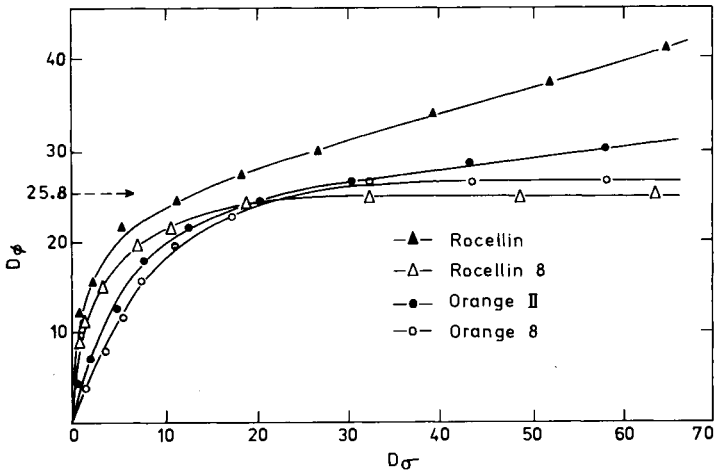


FIG. 39. Adsorption isotherm for nylon 6. Reproduced by permission from Zollinger *et al.*[243]

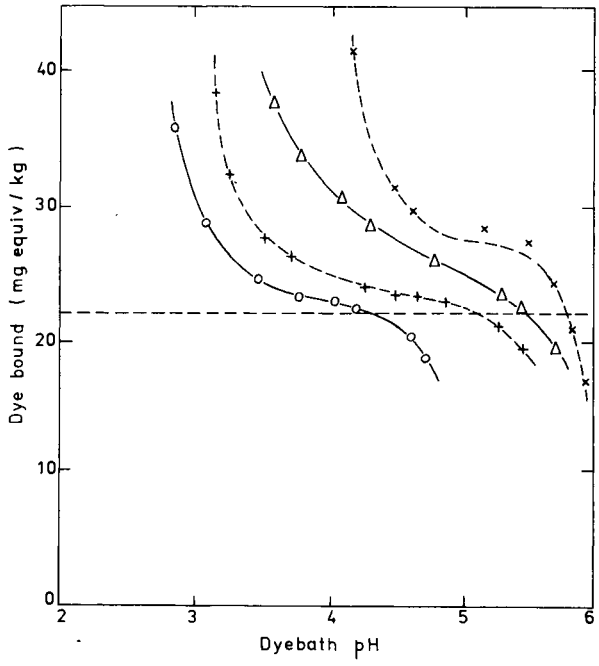


FIG. 40. Dye bound as a function of dyebath pH: ×, CI Acid Red 88 ($Z = 1$); △, CI Acid Red 13 ($Z = 2$); +, CI Acid Red 18 ($Z = 3$); ○, CI Acid Red 41 ($Z = 4$). Reproduced by permission from Rattee.[257]

mechanism of acid dye adsorption should therefore be able to explain overdyeing in both the second and the third region in these curves. Remington and Gladding[250] suggested that overdyeing at low pH values was due to adsorption on new amine end groups formed by acid hydrolysis of amido groups. They observed a rapid adsorption of dye in the initial stages, followed by a slow and steady increase which did not reach a maximum, and therefore considered that the hydrolysis was catalyzed by the presence of the dye. O'Briain and Peters[260] showed that the uptake of dye in this region of low pH is considerably in excess of the number of new amine end groups. They also suggest that overdyeing in this region consists of an initial rapid adsorption of the dye in excess of the amine end groups followed by a gradual hydrolysis. Palmer[253] suggests that dye-catalyzed hydrolysis takes place in the low-pH region. Bhat and Daruwalla[261] have shown that very definite hydrolysis occurs at low pH, but the amount of dye adsorption is considerably in excess of the concentration of the new amine end groups formed at all stages of the dyeing process. White[259] has recently shown that with many dyes the rate of formation of amine end groups is directly proportional to the concentration of the dye adsorbed in excess of the amine end groups. This suggests that dye adsorption is an initiating step in the hydrolysis reaction.

Following the work of O'Briain and Peters,[260] adsorption of dyes in this zone is explained either in terms of protonation of amido groups in the fiber followed by adsorption of the dye ion on the positively charged groups, or adsorption of the dye as undissociated dye acid. The positive charge acquired by nylon at low pH, as shown by electrophoresis, has been advanced as proof for amide protonation.[18] The large number of amide groups in the fiber then explains the considerable overdyeing in excess of the total amine end group content present at any stage of the dyeing process. Bhat and Daruwalla[261] also suggest that amide protonation can explain the overdyeing at low pH. Since considerable overdyeing can occur even at high pH when there is no possibility of amide protonation taking place, the mechanism suggested by Peters,[246] Zollinger *et al.*,[243] and Brody[169] for this region is that of adsorption of undissociated dye acid. Back and Zollinger[262] further suggest that two simultaneous and independent processes occur: (a) adsorption of the dye by a salt binding mechanism to a saturation value corresponding to the amine end group content and (b) a solution mechanism to explain the overdyeing region. Thus, for example, in Fig. 40, the linear in-

[260] C. D. O'Briain and R. H. Peters, *J. Soc. Dyers Colour.* **69**, 435 (1953).

[261] N. J. Bhat and E. H. Daruwalla, *Text. Res. J.* **34**, 435 (1964).

[262] G. Back and H. Zollinger, *Chimia* **13**, 100 (1959).

creasing portion of the curves corresponding to the overdyeing region is regarded as equivalent to the isotherm for a disperse dye type adsorption process. Brody[169] argues that the constancy of the dye diffusion coefficient, irrespective of dye concentration in the overdyeing region, indicates that the dye adsorption mechanism is similar to that for a disperse dye. From comparative studies on the dyeing of acetylated nylon 6 fibers at pH 3 with CI Acid Orange 7 and the disperse dye Solvent Yellow 14 (identical in structure with the acid dye except that it has no sulfonic group), Koshimo and Shimoyama[263] conclude that in the overdyeing region the acid dye behaves like the disperse dye. However, as pointed out by Rattee,[257] the postulated adsorption of dye in the nonionic form in the middle zone of the curves shown in Fig. 40 demands complete back-titration of sulfonic acid groups in this pH region, but titration of the dyes in this pH region shows that no significant back-titration occurs at all pH values. Therefore, it is suggested that the driving force for the dye adsorption process is the dye anion affinity. This hypothesis could explain overdyeing in the middle zone, and since dye adsorption would lead to a decrease in the internal pH as a result of the increased negative surface potential, subsequent hydrolysis of the fiber and protonation of amido groups can also be explained, the effect being greater if the external pH is already low.

When the negatively charged dye anions are adsorbed, the increase in the negative surface potential due to adsorbed dye would lead to electrostatic repulsion of further anions. However, since the dye anions are adsorbed on the fiber surface as a result of their affinity, a situation will thus be reached when there is a balance between the decrease in free energy due to adsorption and the increase in surface free energy due to increase in the surface potential. A saturation value for the adsorption process is then reached. When the dye anion affinity is small, the saturation value will be small and will correspond to the amine end group content, the situation being similar to the adsorption of Cl^- ions, which have a low affinity. For a polysulfonated dye which has a larger negative charge than a monosulfonated dye, the increase in surface potential due to adsorption will be more even at low levels of adsorption, and since these dyes also have a lower affinity for the fiber, the saturation value will approach the amine end group content as can be seen from Figs. 37 and 38. For dyes of high affinity such as the monosulfonated dye CI Acid Red 88, the driving force for adsorption due to a decrease in free energy will be greater than the increase in free energy with

[263] A. Koshimo and T. Shimoyama, *J. Soc. Text. Cell. Ind. Jap.* **19**, 369 (1963).

increase in surface potential. This leads to a much greater adsorption of such dyes before the surface potential effect predominates. Such an approach has already been shown to be quite satisfactory for the adsorption of direct dyes on cellulose fibers.[159,160]

Overdyeing at lower pH regions can be explained in a similar way since protonation of amido groups would decrease the negative surface potential due to adsorbed dye and, hence, the electrostatic repulsion of anions is diminished, leading to more dye adsorption. The addition of electrolytes whose counterions would decrease the negative surface potential due to adsorbed dye should lead to a shift in the titration curves towards a region of high pH. However, the influence of electrolytes on the dye adsorption process in such cases has not been studied in any detail. Its effect would be particularly marked at neutral and higher pH.

If the affinity of the dye anion is the driving force for adsorption, it follows that the concept of amine end group sites can be discarded and that there can be a unified approach to the theory of dye ion adsorption. Recently, McGregor and Harris[167,249] have proposed a simple thermodynamic theory of dyeing, stated to be generally applicable to most dyeing systems, but first applied to polyamide dyeing using the following basic assumptions: (a) At equilibrium both fiber and dyebath behave as homogeneous, electrically neutral phases. (b) The free ions distribute themselves between the dyebath and the fiber according to the laws of thermodynamics. (c) But for the hydrogen ion which determines the degree of dissociation or ionization, there are no specific interactions between any of the ions in the fiber and the ionizable groups in the fiber. (d) The ionizable groups in the fiber influence the distribution of free ions between dyebath and fiber only in so far as they ionize and so must be included in the electrical neutrality conditions, i.e., only in so far as they modify the equilibrium electrical potential difference ($\Delta\phi$) between dyebath and fiber.

A general equation of the type of Eq. (85) can then be derived for the distribution of an ion between two uniformly accessible and homogeneous phases f and s,

$$C_i^{\mathrm{f}} = K_i \lambda^{z_i} C_i^{\mathrm{s}} \tag{85}$$

where C_i^{f} is the equilibrium concentration of ion i in the fiber, C_i^{s} is the equilibrium concentration of ion i in the dyebath or dye solution, and z_i is the charge including sign, on ion i. K_i is given by the equation

$$K_i = \exp\{-\overline{\Delta\mu_i^\circ}/RT\} \tag{86}$$

where $-\overline{\Delta\mu_i^\circ}$ is an apparent standard affinity for the sorption of ion i by

the fiber and corresponds to the standard affinity term generally used in dyeing theory; $-\overline{\Delta\mu_i^\circ}$ however, in general, need not be a constant and can be described by the equation

$$-\overline{\Delta\mu_i^\circ} = -\Delta\mu_i^\circ + v_i\Delta P + RT\ln(\gamma_i^f/\gamma_i^s) + RT\ln\chi_i \qquad (87)$$

Here, only $-\Delta\mu_i^\circ$ is by definition a constant at a given temperature. Substituting this equation and the equation for the Donnan distribution coefficient

$$\lambda = \exp\{-F\Delta\phi/RT\} \qquad (88)$$

in Eq. (85), we get the final equation

$$\frac{C_i^f}{C_i^s} = \exp\left\{\frac{-\Delta\mu_i^\circ + v_i\Delta P + z_iF\Delta\phi + RT\ln(\gamma_i^f/\gamma_i^s) + RT\ln\chi_i}{RT}\right\} \qquad (89)$$

Here, $\Delta\mu_i^\circ$, $v_i\Delta P$, and $z_iF\Delta\phi$ are, respectively, the chemical, mechanical, and electrical works terms, γ represents the activity coefficients, and χ_i refers to "structure factors," for example, the ratio of accessible to nonaccessible portions in the fiber.

The key to the problem is the calculation of the Donnan coefficient λ which enables the values of C_i^f to be determined in any given experimental situation for definite values of C_i^s, z_i, and K_i. The partition coefficient K_i is a measure of the dye ion affinity. The value of the Donnan coefficient λ corresponding to electrical neutrality in the fiber can be solved by a computer for a given set of experimental conditions, such as dye ion affinity, dyebath pH, and ionic end group content in the fiber. Just as in the case of the direct dye–cellulose system discussed earlier, for the calculation of the Donnan coefficient λ it will be necessary to know the volume of the internal solution phase. McGregor and Harris[167] assume that the fiber can be regarded as a homogeneous equipotential volume.

Figure 41 shows computed sorption isotherms for dye anions of different basicities and different affinities. These isotherms are similar to the experimentally observed isotherms obtained by various workers, for example, Fig. 39. Computed values of dye adsorption as a function of pH also give curves similar to those obtained experimentally, as shown in Fig. 42. The marked increase in dye adsorption at high pH as observed in Fig. 42 is attributed to the very high electrolyte concentration, which on the basis of the present model predicts an increase in dye adsorption. The significant influence of carboxyl end group content on dye adsorption at neutral and alkaline pH values can also be explained in terms of the influence of the ionized carboxyl groups on the conditions

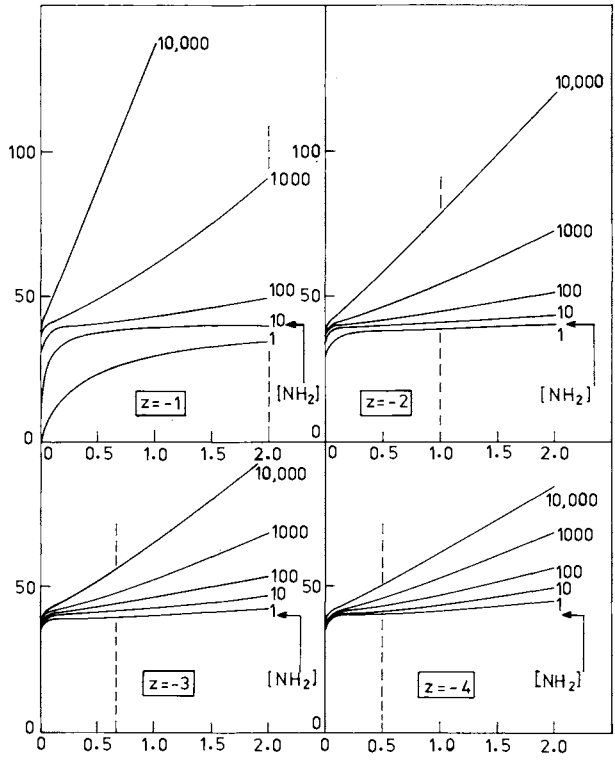


FIG. 41. Computed sorption isotherms for dye anions of different basicity z. *Ordinates*: Dye anion sorption (mEq/kg). *Abscissae*: Dye anion concentration in solution (g-ions/liter) $\times 10^3$. The computations are for a fiber containing 40 mEq/kg of amine end groups ($K_A = 1.0 \times 10^{-9}$) and 40 mEq/kg of carboxyl end groups ($K_B = 0.5 \times 10^{-6}$). The dye sorption takes place from a mixture of the sodium salt of the dye and an acid HA. The solution pH is 3.5. The numbers on the curves are the dye anion K values. Reproduced by permission from McGregor and Harris.[167]

for electroneutrality. This effect is similar to that discussed earlier for the cellulose–direct dye solution system. Overdyeing of the high-affinity dyes having a low basicity can be explained purely in terms of the dye affinity which, if sufficiently high, enables it to be sorbed in excess of the end group content, taking with it sufficient cations to preserve electroneutrality. Dye sorption under such circumstances is similar to the stoichiometric sorption of dye anions, and it is suggested by McGregor and Harris[167] that it is not necessary to explain overdyeing in terms of a second type of "solution mechanism."[169,262]

Calculations of the thermodynamic affinities of dye adsorption using the site adsorption mechanism have been made by Vickerstaff,[18]

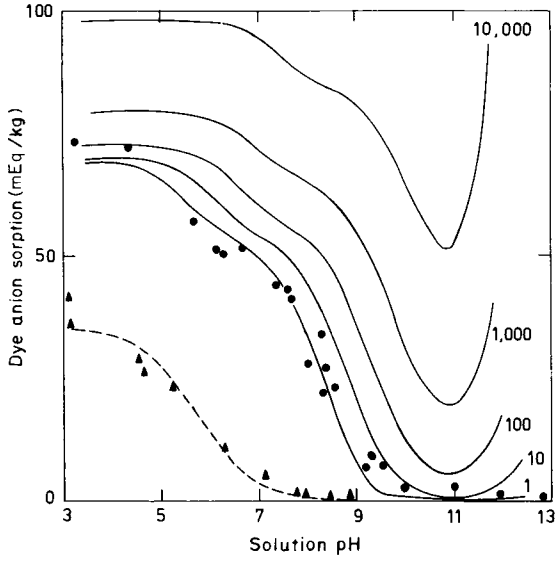


FIG. 42. Computed dye sorptions–pH effects. Theoretical and experimental dye sorptions are shown for some experimental nylon 6 fibers. The unbroken lines represent computations of the equilibrium dye anion sorption as a function of dyebath pH and dye anion K value, for a dibasic dye anion ($z = -2$) at a concentration in solution of 10^{-3} g-ions/liter. The fiber possesses 70 mEq/kg of amine end groups ($K_A = 1.0 \times 10^{-9}$) and 17 mEq/kg of carboxyl end groups ($K_B = 0.5 \times 10^{-6}$). The experimental points for CI Acid Blue 45 (●) correspond approximately to $K = 1$. The broken line is computed for $K = 1$ and for a degraded fiber with 34 mEq/kg of amine end groups ($K_A = 1.0 \times 10^{-9}$) and 35 mEq/kg of carboxyl end groups ($K_B = 0.5 \times 10^{-6}$). This line is in reasonable agreement with direct experimental measurements (▲) of the sorption of CI Acid Blue 45 by the degraded yarn. Reproduced by permission from McGregor and Harris.[167]

Remington and Gladding,[250] McGrew and Schneider,[252] and Atherton *et al.*[165] The basis of the equation developed for the calculation of the affinities is the Gilbert and Rideal model,[171] which was originally proposed for the sorption of hydrochloric acid and dye acids on wool fiber. However, the calculation of affinities will also depend on whether the nylon fiber at the isoelectric point is to be regarded as a zwitterion or as completely un-ionized, on the grounds that the amine and carboxyl end groups in the polymer chain molecule are separated by a long aliphatic chain and hence behave independently of one another. Thus, for example, using Lemin's data, Vickerstaff[18] obtained quite consistent affinity values for CI Acid Blue 45 and CI Acid Red 13 assuming the un-ionized structure for the nylon fiber. Remington and Gladding[250] on the other hand got consistent results with their data assuming the zwitterion structure for nylon. Atherton *et al.*[165] derived the following

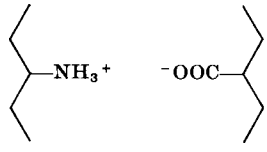
simple thermodynamic formula for the differences in affinity between the dye anion and the anion of a suitable acid present in the dyebath during a desorption process:

$$-(\Delta\mu_D^\circ - \Delta\mu_A^\circ) = RT \log\left(\frac{\theta_D}{(1 - \theta_D)}\right) - RT \log\frac{[D]}{[A]} - RT \log\left(\frac{z}{y}\right) \tag{90}$$

The experimental conditions in the desorption method were maintained such that the ratio of dye concentration [D] to the inorganic ion concentration [A] was kept constant. The factors z and y refer to the basicities of the two anions, respectively. The affinity values for all the dyes are generally small and range from about 4 to 8 kcal/mole. An interesting feature of the results is that the differences in affinity between pairs of dyes that differ from each other by one sulfonic group is on the average about 0.87 kcal/mole, which compares favorably with the average values of 0.95 and 0.75 kcal/mole obtained for the same dyes on wool by Speakman and Clegg,[264] and Lemin and Vickerstaff,[66,265] suggesting thereby a close similarity between the mechanisms for the acid dyeing of nylon and wool fibers.

2. *Acid Dye–Wool Fiber System*

A wool fiber consists of the protein keratin which is built up by the condensation of 18 different α-amino acids to give long polypeptide chains. Some of the side chains terminate in amino groups and others in carboxyl groups, these basic and acidic side chains being present in approximately chemically equivalent quantities, pictured as follows:



When the amphoteric protein fiber is immersed in an aqueous acid solution, the weak carboxyl groups are back-titrated to COOH, resulting in the absorption of H^+ ions. The capacity of a protein fiber for acids should therefore be equivalent to its free amino group content.[18] The complete back-titration of the carboxyl groups leaves the protein with a residual net positive charge due to $—NH_3^+$. Hence, the approach of more hydrogen ions is repelled, whereas anions such as Cl^- are attracted. Absorption of acids occurs in such a way that the net potential effect

[264] J. B. Speakman and G. Clegg, *J. Soc. Dyers Colour.* **50**, 348 (1934).

[265] D. Lemin and T. Vickerstaff, *Fibrous Proteins, Proc. Symp.*, p. 129 (1946).

is zero. The carboxyl and the amino groups, because of their predominant effect on the extent of absorption, are usually regarded as being specific sites for ionic adsorption. Since titration curves for the interaction of proteins with acid dyes are in various respects similar to those for the protein–inorganic acid system, the mechanism of acid dye adsorption is based on the development of two theories which have been proposed to explain the uptake of acids such as HCl by the wool fiber.

One of these theories is based on the application of the principles of a Donnan membrane equilibrium to the system. Peters and Speakman[266] proposed a quantitative analysis of the adsorption of inorganic acids based on a Donnan equilibrium between an internal solution phase in the wool fiber and the external bulk solution. In this model, the swollen wool fiber containing imbibed solution forms an equipotential volume. The potential difference between the fiber and the dyebath is assumed to be located at or very near the surface of the fiber. When an electrolyte is absorbed by such a fiber, although electroneutrality is preserved, the location of the ions in the fiber phase may differ. In the wool fiber –HCl system, for example, the H^+ ions are assumed to be combined with or adsorbed on the fiber surface while the Cl^- ions are unattached, being present as free ions dissolved in the imbibed water within the fiber. This internal solution is generally assumed to have all the properties of a normal aqueous solution. It should be pointed out that the presence of a large electric field due to the high concentration of charged groups in the protein could influence the activity of the dissolved ions. However, in most of the treatments based on this mechanism, the latter effect is generally not considered. The concept of an internal pH which is different from the external pH is one important characteristic of this model. This internal pH is generally higher than the external pH and protects the protein from acid hydrolysis. Experimentally it is observed[266] that with increase in ionic strength of the solution, the rate of hydrolysis increases, and this can be explained on the basis of the Donnan model since with increasing electrolyte concentration the internal pH decreases. The catalytic hydrolysis of wool by acids with high affinity for the fiber can also be explained in a similar manner.[267]

In the theory first put forward by Gilbert and Rideal,[171] the internal solution is not regarded as a normal aqueous solution, and the anions within the fiber are repelled from one another as well as from the negative groups in the fiber. Hence these anions will be associated with the positively charged basic groups in the protein, i.e., there is no separate internal aqueous solution phase, the fiber being regarded as

[266] L. Peters and J. B. Speakman, *J. Soc. Dyers Colour.* **65**, 63 (1949).
[267] J. Steinhardt and C. H. Fugitt, *J. Res. Nat. Bur. Stand.* **29**, 315 (1942).

homogeneous and containing only adsorbed ions. The adsorption of ions follows a Langmuir isotherm. One important criticism of the Gilbert and Rideal theory is that the ions which have little or no affinity for the fiber are also assumed to be adsorbed on specific sites. It is much more likely that such ions are diffusely adsorbed in an internal solution phase which is intimately associated with the fiber. This situation is similar to that assumed for the distribution of electrolyte ions in the internal cellulose phase in the cellulose fiber–aqueous electrolyte solution system discussed in Section II,A. The theory of Peters and Speakman,[266] however, takes this aspect into consideration.

The main difference between these two theories, therefore, is that in the Donnan theory only H^+ ions are specifically adsorbed while the Cl^- ions are present as unbound free ions in the internal solution phase; in the Gilbert and Rideal theory both ions are combined with the protein. A surprising feature is that either theory provides a fairly satisfactory explanation of the data obtained for the titration of hydrochloric acid with wool,[268] although each theory has its own advantages. The suitability of either theory has been a matter of considerable controversy and several arguments have been put forward by various workers for and against these two theories.[18,269–274] The various aspects of both the theories and their application to the adsorption of acids and acid dyes on wool fiber have been extensively reviewed by Vickerstaff.[18]

Current theories which will be discussed here indicate, however, that the Gilbert-Rideal theory can be regarded as a special case of the theory based on a Donnan equilibrium in the system. This has been shown by Peters[275] in a generalized extension of his earlier theory,[266] also extended to include adsorption in the region of the isoelectric point where the earlier theories have not been able to give a satisfactory quantitative explanation. The solid is assumed to possess a large surface on which each sort of ions present can be adsorbed while maintaining the concept of a Donnan distribution of ions between an internal solution phase regarded as an equipotential volume v and a bulk external solution phase. The intrinsic carboxyl and amino groups in the fiber are assumed to possess equilibrium dissociation constants and behave just as if they were dissolved in the internal solution phase. The electrolyte cations

[268] J. Steinhardt and M. Harris, *J. Res. Nat. Bur. Stand.* **24**, 335 (1940).
[269] J. L. Horner, *Trans. Faraday Soc.* **50**, 1000 (1954).
[270] J. A. Kitchener and P. Alexander, *J. Soc. Dyers Colour.* **65**, 284 (1949).
[271] B. Olofsson, *J. Soc. Dyers Colour.* **67**, 57 (1951).
[272] B. Olofsson, *J. Soc. Dyers Colour.* **68**, 506 (1952); **72**, 19 (1956).
[273] B. Olofsson, *J. Polym. Sci.* **12**, 301 (1954).
[274] L. Peters, *J. Soc. Dyers Colour.* **70**, 436 (1954).
[275] L. Peters, *J. Text. Inst.* **51**, T1290 (1960).

such as Na^+ and K^+ are assumed to be present as unbound ions in the internal solution phase. Just as in the case of the cellulose fiber–aqueous electrolyte solution system, the following expression can be derived for the Donnan potential in a wool fiber–inorganic acid (HX) system containing an additional electrolyte such as KCl,

$$\sinh\left[\frac{\psi F}{RT}\right] = \frac{[B^+] - [A^-]}{2vC} \tag{91}$$

where $[B^+]$ and $[A^-]$ are the internal concentrations of $-NH_3^+$ and $-COO^-$ groups, respectively, v is the volume of the internal solution phase, and C is the total ionic strength. Hence the Donnan distribution can be calculated, provided $[B^+]$, $[A^-]$, and v are known under a given set of conditions. By assuming suitable equations based on the law of mass action for the dissociation constants K_A, K_M, K_B, and K_X for the carboxyl group, the carboxylate group, the amino group treated as its conjugate "ammonium acid" ($-NH_3^+$), and the substituted "ammonium chloride" group of the protein ($-NH_3^+ + Cl^- \rightarrow -NH_3Cl$), respectively, Eq. (92) can be derived.

$$([B^+] - [A^-]) = \frac{[B]}{1 + \dfrac{K_B}{\lambda[H]} + \dfrac{[X]}{\lambda K_X}} - \frac{[A]}{1 + \dfrac{\lambda[H]}{K_A} + \dfrac{\lambda[M]}{K_M}} \tag{92}$$

In this equation all the variables are directly measurable except λ. However, λ can be calculated from the amount of acid or alkali combined using the following equations derived on the assumption that H^+ combines with the $-COO^-$ group to form $-COOH$ and OH^- forms hydrated $-NH_2$ groups, i.e., the amount of acid combined $a = [-COOH] - [-NH_2]$. The value of a is given by the equation

$$a = \frac{[A]}{1 + \dfrac{K_A}{\lambda[H]} + \dfrac{[M]}{[H]}\dfrac{K_A}{K_M}} - \frac{[B]}{1 + \dfrac{\lambda[H]}{K_B} + \dfrac{[X]}{K_X}\dfrac{[H]}{K_B}} \tag{93}$$

which on the acid side reduces to the equation

$$\frac{1}{\lambda} = \left(\frac{[A] - a}{a}\right)\frac{[H]}{K_A} - \frac{[M]}{K_M} \tag{94}$$

and on the alkaline side

$$\lambda = \left(\frac{[B] + a}{-a}\right)\frac{K_B}{[H]} - \frac{[X]}{K_X} \tag{95}$$

Near the isoelectric point, the complete equation [Eq. (93)] has to be used. In order to use these equations suitable numerical values must be assigned to the various dissociation constants. Combining these equations for λ with the previous two equations, the value of v can be calculated at any given pH and salt concentration. Using the boundary condition that the midpoint of the wool–HCl titration curve in the absence of salt at 0° has a pH of 2.22[268] and assuming that the Cl^- ion has no affinity and that v equals 30 liters/100 kg, which is the frequently used value for the volume of the internal solution phase, the following equation can be derived:

$$pK_a = 2 \times 2.22 \times \log x \tag{96}$$

Here $x = [A]/2v = 1.5$ molar represents the concentration of the Cl^- ion in the internal volume, and [A] = 90 mole/100 kg represents the total carboxyl content in the fiber. Hence $pK_a = 4.616$ is obtained, which is a reasonable value of the pK of a carboxyl group. Any assumption of v values lower than 30 liters/100 kg gives high values for pK_a which are not reasonable for the weak carboxyl group. If, however, there is finite Cl^- ion adsorption, then a correlation can be established between the value of K_X and v, assuming always that pK_a equals 4.616. Similarly, from the alkali titration curves a correlation can be established between K_M and v. Crewther,[276] however, has more recently pointed out that the value of $pH_{1/2}$ is not the same for all acids as assumed, but varies from acid to acid. Therefore if $pK_a = 4.616$ always, then x in Eq. (96) does not remain constant at 1.5, but should vary.

Peters[275] has shown that if the isoelectric point at pH 6.5 (pH_0)[265,268] is regarded as a suitable zero from which the amount of acid or alkali combined can be measured, then from Eq. (92), using also the approximations that $K_B/[H_0]$ and $[X_0]/K_X$ are negligible, the total number of amino groups [B] is found to be very slightly less than [A]. K_X and K_M therefore have slightly different values. When an electrolyte is present, Eq. (93) becomes

$$\frac{[H_0]}{K_A} = \frac{[A]}{[B]} - 1 + [M]\left(\frac{[A]}{[B]}\frac{1}{K_X} - \frac{1}{K_M}\right) \tag{97}$$

On the Donnan theory therefore, pH_0 should be independent of the electrolyte concentration since $1/K_X = 1/K_M = 0$, whereas the finite values of K_X and K_M on the basis of the Gilbert and Rideal theory leads to a considerable shift in pH_0 according to Eq. (97). However, since it is experimentally found that pH_0 does not significantly change with

[276] W. G. Crewther, *J. Soc. Dyers Colour.* **81**, 156 (1965).

electrolyte concentration, the Gilbert and Rideal theory of negligible internal volume ($v = 0$) does not appear to be valid. From Eqs. (94) and (92), assuming the generalized Gilbert and Rideal theory,

$$pH_{1/2} = pK_A - \log\left(\frac{1}{\lambda} + \frac{[M]}{K_M}\right) \tag{98}$$

whereas the Donnan theory with $v = 30$ gives

$$pH_{1/2} = pK_A + \log \lambda \tag{99}$$

Calculations of the predicted displacement of $pH_{1/2}$ by salt and a comparison of these results with the experimentally observed displacement show that the curve of $\Delta pH_{1/2}$ decreases asymptotically with decreasing electrolyte concentration, tending to a limit $pH_{ext} = pH_{int}$. The Gilbert and Rideal equation, however, shows that at high electrolyte concentration and at $v = 0$, competition between K^+ and H^+ displaces the curve in the opposite direction. This is not observed experimentally; the assumption of strong cation binding is inapplicable and, likewise, there cannot be a strong binding for anions as well. Since dye anions, however, have an affinity for the wool fiber, any explanation of the mechanism of wool dyeing based on a Donnan model should take into account the finite adsorption of dye anions. The affinity of dye anions for the wool fiber is mainly attributed to secondary forces connected with interactions between the hydrophobic portions of the dye molecule and the wool fiber surface.

The above theory also emphasizes the importance of choosing a correct value for the volume v of the internal solution phase. As mentioned earlier, v is usually assigned a value of 30 liter/100 kg and is identified with the volume apparently occupied by the water in the saturated fiber. The existence of an electrical double layer in the wool fiber–aqueous electrolyte solution system suggests that a limiting value for v may be identified with the thickness of the diffuse electrical double layer.[270] As in the case of the cellulose fiber–electrolyte solution system discussed in Section II,A, this volume can be replaced by an equivalent volume called "the inner Donnan phase," in which the potential remains constant throughout and all ions have a uniform concentration. There is consequently a definite boundary between this internal volume and the external bulk solution phase, and the position of this boundary can be displaced by changing the bath conditions.

Delmenico and Peters[277] used the Donnan equilibrium model for the

[277] J. Delmenico and R. H. Peters, *Text. Res. J.* **34**, 207 (1964).

adsorption of acid and alkali by wool to show how the quantities such as λ, v, a^0 ($a^0 = [A] - [B]$), and K_X can be derived from experimentally measurable quantities [M*] and [X*] for the inorganic cation and anion, respectively, given by the equations

$$[M^*] = [M_T] - w[M] = [M_a] + v([m] - [M]) \tag{100}$$

and

$$[X^*] = [X_T] - w[X] = [X_a] + v([x] - [X]) \tag{101}$$

[M*] and [X*], which can be positive or negative, therefore represent the amount of ions in excess of what would be in the wool fiber, if there were no difference between internal and external concentrations. $[M_T]$ and $[X_T]$ refer to the total quantity of ions experimentally determined in the total amount w of water removed with the wool from the equilibrium solution. The sample analyzed contains adsorbed ions M_a and X_a and ions in the internal solution equal to vm and vx. [M] and [X] are the concentrations of the electrolyte cation and anion, respectively, in the bath in which the wool is equilibriated and also [M] = [X]. The parameters λ and v can be calculated from [M*] and [X*] as follows, on the assumption that small inorganic cations are not bound to the wool fiber as shown experimentally by various workers.[268,269,278] On the other hand, it is assumed that a small quantity of anions may be attached to the fiber since anions of inorganic acids have an affinity for the wool fiber, and studies on the interaction of inorganic anions with soluble proteins show that these ions have an affinity for proteins.[279,280] Thus, combining these equations with the equation

$$\lambda = \frac{[h]}{[H]} = \frac{[m]}{[M]} = \frac{[X]}{[x]} \tag{102}$$

the following general equations for λ and v are obtained:

$$\lambda = \frac{[M^*] + v[M]}{v[M]} = \frac{v[X]}{[X^*] - [X_a] + v[X]} \tag{103}$$

$$v = \frac{[M^*]}{[M](\lambda - 1)} \tag{103a}$$

[278] W. S. Barnard, A. Palm, P. B. Starm, D. L. Underwood, and H. J. White, *Text. Res. J.* **24**, 785 (1954).

[279] I. M. Klotz and J. Ayers, *Discuss. Faraday Soc.* **13**, 189 (1953).

[280] G. Scatchard and E. S. Black, *J. Phys. Colloid Chem.* **53**, 88 (1949).

The dissociation constants for the loss of protons from carboxyl groups and for an inorganic anion X^- can be written as

$$K_H = \frac{\lambda[H]([B] - [H_a])}{[H_a] + a^0} \tag{104}$$

and

$$K_X = \frac{[X]([B] - [X_a])}{\lambda[X_a]} \tag{105}$$

At a particular pH value [M*] is zero, i.e., $\lambda = 1$. However, [X*] will have a small but finite value $[X^*] = [X_a] = [X^0]$, i.e., the anion has a small but positive adsorption. Substituting the value $[X_a] = [X^0]$ in Eq. (105)

$$K_X = \frac{[X]([B] - [X^0])}{\lambda[X^0]} \tag{106}$$

and can therefore be calculated from measured quantities. Eliminating v from Eq. (103) and substituting $[X_a]$ by $[X^0]/\lambda$ in Eq. (103) for small values of $[X_a]$ and $[X^0]$,

$$\lambda = \frac{[X^0]}{[X^*]} - \frac{[x]}{[M]}\frac{[M^*]}{[X^0]} \tag{107}$$

If there is no bound anion this equation can be approximated to

$$\lambda = -\frac{[M^*]}{[X^*]} \tag{108}$$

This equation is extensively used for calculating λ and hence the value of v can be obtained from Eq. (103a).

Using suitable experimental methods, the variations of [M*] and [X*] with pH at three electrolyte concentrations and two temperatures, 70° and 90°, were measured. A comparison between the experimental results for wool in sodium chloride solution and the calculated quantities show the expected qualitative trends. Thus for example, when there is positive adsorption of Cl^- ions, i.e., [X*] is positive, then [M*] will be negative as expected and vice versa. Hence, when the salt concentration is increased, λ changes from < 1 to > 1, passing through 1 at a given pH. The calculated v values do not show any significant change with pH or electrolyte concentration on the acid side and have an average value of about 30 liters/100 kg, which is the value usually assigned to v. On the alkaline side, however, v does not remain constant, but increases with

decreasing salt concentration to a value of about 100 liters/100 kg at a NaCl concentration of 0.02 M. This increase in v may perhaps be associated with the increased swelling of wool in alkali solutions, although there is no clear-cut evidence for such a conclusion. The results also show that the calculated values of K_X, a^0, v, and λ are all very sensitive to small experimental errors, and hence the experimental data should be quite accurate if considerable fluctuations in these values are to be avoided. Nevertheless, the results shown in Fig. 43 for the variation of [X*] and [M*] with pH at two values of v, 30 and 40 l/100 kg, indicate that there is reasonably good agreement between the experimental data and the theoretical values. The variation of v does not result in any large separation between the curves for [M*]. Furthermore, for both v values the curve for [X*] remains the same.

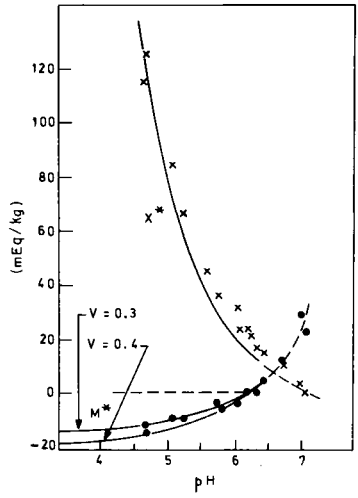


FIG. 43. Calculated curves for variation of [M*] and [X*] with pH. NaCl, 0.05 M; temperature, 40°. (Points experimental.) Reproduced by permission from Delmenico and Peters.[277]

Delmenico and Peters[281,282] extended their studies to include dyeing equilibria for low- as well as high-affinity acid dye anions. In this case the following additional equations are necessary to describe dye binding. The dissociation constant K_D of the bound dye is given by the equation

$$K_D = \frac{[D]}{\lambda} \frac{([B] - [X_a] - [D_a])}{[D_a]} \tag{109}$$

[281] J. Delmenico and R. H. Peters, *Text. Res. J.* **35**, 14 (1965).

[282] J. Delmenico and R. H. Peters, *Proc. Int. Wool. Textile Res. Conf., 3rd*, p. 67 (1965).

The value of λ in the presence of dye is given by the equation

$$\lambda = \frac{[X^0]([B] - [D^*])}{[X^*]([B] - [D^0] - [X^0])} - \frac{[M^*]}{[X^*]} \tag{110}$$

If the quantity of bound dye is very large, then on a negatively charged fiber the concentration of bound inorganic anion can be ignored, i.e., $\lambda \simeq -[M^*]/[X^*]$ [Eq. (108)].

The dissociation constants K_D and K_X can also be written as

$$K_D = [D]([B] - [D^0] - [X^0])/[D^0] \tag{111}$$

and

$$K_X = [X]([B] - [D^0] - [X^0])/[X^0] \tag{112}$$

The product K_{HD} obtained by multiplying Eqs. (104) and (109) gives rise to the following equations in the presence and absence of salt ($[D_a] = [H_a]$), respectively.

$$K_{HD} = \frac{[H][D]([B] - [D_a])([B] - [H_a])}{([H_a] + a^0)[D_a]} \tag{113}$$

and

$$\left\{\frac{[D_a]([D_a] + a^0)}{[H][D]}\right\}^{1/2} = \frac{[B]}{K_{HD}^{1/2}} - \frac{[D_a]}{K_{HD}^{1/2}} \tag{114}$$

These equations are similar to the Gilbert and Rideal equation for the affinity $-\Delta\mu^\circ$ since $\Delta\mu^\circ = -RT \ln K_{HD}$.[283]

The plots of the titration data for CI Acid Red 88 and CI Acid Orange 7 using Eq. (114) are found to be linear and extrapolate to give saturation values 1.13 and 1.04, respectively, which are higher than the actual [B] value (0.9) for the following reasons. While the charge on the wool fiber depends on the relative amounts of ions in solution at low pH, the dyed wool is often negatively charged and the charge remains approximately constant over most of the range of dye adsorption until all the amino groups have been electrically neutralized, or until no more hydrogen ions are available for combination with carboxyl groups. The adsorption of the negatively charged dye anion in spite of the repulsive force due to the net negative charge on the fiber is therefore due to the intrinsic affinity of the dye ion for the fiber. Further adsorption of dye beyond the electrical neutralization point corresponding to the total number of amino groups [B] quickly builds up sufficient repulsion, however, to prevent more dye adsorption. The extent of dye "saturation"

[283] G. A. Gilbert, *Proc. Roy. Soc., Ser. A* **183**, 167 (1944).

beyond total amino group content will be a function of the dye anion affinity. For the above two dyes which have low affinity, the saturation value therefore does not exceed [B] by more than 15–20%. However, for dye anions of high affinity, the saturation value can be considerably in excess of the total amino group content.[282] Under conditions of prolonged dyeing and at low pH, fiber degradation if present can also lead to additional dye uptake. Delmenico and Peters[281] also observed that with increasing dye concentration the calculated values of K_D increase considerably for both CI Acid Red 88 and CI Acid Orange 7. This is attributed to a large decrease in the activity coefficient due to an increase in the aggregation of the dye at higher concentrations. Approximate values of the activity coefficients can be obtained from a comparison of the K_D values for various concentrations of bound dye.

The dye adsorption data as analyzed in terms of the equations discussed above indicate that the extent of dye binding depends on the charge on the fiber. At high pH, the effect of electrolyte, which increases dye adsorption, can be explained in terms of a screening of the charge on the fiber, which reduces the electrical repulsion effect on dye anion adsorption. The desorbing effect of electrolytes can be attributed to competition between dye anions and electrolyte anions; while this effect is present at all pH values, it is more pronounced at lower pH. Another factor which influences desorption of anions in the presence of electrolytes is the formation of dye aggregates. The effect of electrolytes on hydrophobic interactions in the wool fiber–acid dye system, which leads to a decrease in dye uptake, is discussed in Section II,B,2,a. In agreement with the experimental data, the theoretical calculations also show that with increasing amount of bound dye anion, pH^0 shifts to lower values.

One important feature of the work discussed above is that the product $K_H K_D$ of the constants describing hydrogen and dye ion binding is constant over a wide range of dye adsorption, although the individual values vary with the charge on the fiber. It is also observed that the ratio K_D/K_X, obtained from the measured values of [M*], [X*], and [D*] using the appropriate equations, is the same as the ratio K_{HD}/K_{HX}, which can be obtained independently from titration data. This agreement between data obtained by two independent methods indicates the usefulness of these parameters for describing the dyeing equilibria. The values of λ and v calculated from [M*], [X*], and [D*] values show the same qualitative trends as in the case of the adsorption of pure inorganic acids, although considerable fluctuations in these values have been noted. As discussed earlier for the wool fiber–inorganic acid system, these fluctuations can be ascribed to the large effect of

small experimental errors in the measurement of [M*], [X*], and [D*].

From studies on the adsorption of dyes by keratin under near neutral conditions, Marshall[284] showed that the results can be explained using the Donnan model. Variations in the value of K_D due to dye and salt concentration are correlated with the decrease in the activity coefficients with increasing adsorbed dye concentration. This effect is more noticeable with the dibasic dye CI Food Yellow 3 than with the monobasic dye CI Acid Orange 7. Since CI Food Yellow 3 does not aggregate to a greater extent than CI Acid Orange 7, the results cannot be explained on the basis of aggregation. It is suggested that the salt influences the substantivity of the dye anion for the fiber by competing with the dye anions for available sites, this competition being more for the dibasic dye, which at higher dye concentrations has a smaller number of favorable configurations available for adsorption on the fiber surface. As shown by Medley,[72] the increased mobility of the dye anions with increasing salt concentration would also lead to reduction in the substantivity of the dye. This effect is more for the dibasic dye where the mobility increases much more than for the monobasic dye.

The results of the investigations discussed above indicate that the extent of dye adsorption is mainly governed by electrostatic effects due to the charge on the fiber surface. It should therefore be possible to discuss the mechanism of acid dye adsorption on wool fibers as in Section II,B,1 for the nylon–acid dye system, i.e., the driving force for the adsorption process is the thermodynamic affinity of the dye anion for the fiber. Furthermore, there are no specific interactions between any of the ions in the fiber and the ionizable groups in the fiber except for hydrogen ions, which determine the degree of dissociation or ionization of these groups, the distribution of free ions in the system being defined by a Donnan equilibrium.

Rattee[285] has pointed out that in the development of theoretical models for dye adsorption by proteins, a primary source of error in any comparison of theoretical and experimental models is that proteins are only partially stable substrates, soluble products of degradation being formed during a dye adsorption process. This results in a large error in estimated bound dye concentration since part of the dye may be bound to the soluble degradation products.

The thermodynamic affinity or standard free energy change ($-\Delta\mu^\circ$) for the adsorption of an acid or acid dye by wool fiber is usually calculated according to some form of the Gilbert-Rideal equation such as Eqs. (113) and (114), necessarily implying a Langmuir-type isotherm

[284] J. Marshall, *J. Soc. Dyers Colour.* **85**, 251 (1969).
[285] I. D. Rattee, *J. Soc. Leather Trades' Chem.* **50**, 427 (1966).

for the adsorption process which is also the type of isotherm commonly met with in the dyeing of wool with acid dyes. The values of $-\Delta\mu^\circ$ for HCl calculated from the data of Steinhardt and Harris[268] are found to be remarkably constant, with an average value of 5.86 kcal/mole over a very wide range of ionic concentration. Lemin and Vickerstaff[66] obtained affinity values from measured adsorption isotherms for a series of inorganic acids and acid dyes. These affinity values were found to increase with the size of the respective anions and generally range from 4.5 to 8 kcal/mole. This correlation between affinity values and ionic size indicates that the number of charges and coulombic interactions are not the only forces involved, and that contributions to the affinity can also arise from van der Waals forces between the dye and the fiber as well as hydrophobic bonding. An important contribution to the affinity of the dye can arise from short-range forces of the van der Waals type exerted between the hydrophobic parts of the dye anion and the hydrophobic parts of the wool adjacent to $-NH_3^+$ groups. The following facts strongly favor this concept for various dye–fiber systems. For example, small hydrophobic dye molecules have adequate affinity for hydrophobic fibers, while larger dye molecules will be required to dye a hydrophilic fiber. The additive and short-range nonpolar forces require a very close fit of very large areas of the dye and the substrate. This explains the strong correlation between the affinity of the direct dyes and their linear and planar structures, and a more general correlation between affinity and molecular weight for a particular class of dye. The formation of polar bonds may help the dye adsorption process by eliminating hydrated water around polar groups on the dye and the substrate. This would enable the dye molecule to come sufficiently close to the fiber so that the dispersion forces can be effectively operative and bind the dye to the surface.[18]

Meggy[97] suggested that the most important contribution which determines the affinity of a dye anion arises from changes in the structure of water in the system. Water molecules in liquid water are highly aggregated and therefore the introduction of a hydrophobic dye ion results in disaggregation of water molecules. When dye gets adsorbed on the fiber, the water molecules can again reassociate. This energy of reassociation or reordering is measured as the energy of dyeing. This is somewhat akin to the modern concept of hydrophobic bonding which can be pictured as follows. If for example two hydrocarbon molecules or two hydrophobic side chains of a large molecule aggregate, some of the water molecules present in an ordered iceberg structure around each hydrocarbon molecule or the hydrophobic part of each molecule will become free. The positive entropy change resulting from this decreased

degree of order of water molecules due to aggregation is the thermodynamic driving force for hydrophobic bond formation (see also the previous chapter).

Derbyshire and Peters[286] proposed a unified interaction theory for all dye–fiber systems, supported primarily by calorimetric investigations.[287] They postulated that the fundamental contribution to the standard affinity of dyeing arises from nonpolar van der Waals forces. They suggested that electrostatic interactions between charged groups of fibers and dye ions, the dipole interactions, and hydrogen bonding are all of secondary importance, and that hydrophobic parts of the dye molecule have adequate affinity for the hydrophobic parts of the fiber. They found that the heat of dimerization of CI Acid Orange 7, the heat of formation of the solid hydrated dye–acid from infinitely dilute aqueous solution, the directly measured heat of dyeing, and the calculated heat of dyeing are very similar. The heat of formation of the salt between dye–acid and amino acid (glycine) was equal to the heat of neutralization of the amino acid. All these results suggest that the same type of force exists in the adsorption of this dye by wool and also in dye aggregation.

Peters and Lister[288] and Peters[289] studied the adsorption of CI Acid Orange 7 in free acid form by wool and by collagen. They obtained an increase in the entropy, attributed to a greater order in the water originally present on the ionic sites than around the adsorbed dye, suggesting that the entropy is a major contribution to the affinity of the dye for the fiber. Similar positive entropy changes for dye–protein interactions in aqueous homogeneous solutions are well known.[290,291] These positive entropy changes coupled with a small enthalpy change are generally ascribed to the formation of hydrophobic bonds.

Zahn[292] has also emphasized the fact that in a swollen fibrous protein such as wool, salt linkages or ionic pair bonds as well as hydrogen bonds are weak in the presence of water, but it is precisely then that hydrophobic interactions have a stabilizing action. In a wool fiber–aqueous dye solution system hydrophobic reciprocal effects therefore have an important role.

[286] A. N. Derbyshire and R. H. Peters, *J. Soc. Dyers Colour.* **71**, 530 (1955).

[287] A. N. Derbyshire and R. H. Peters, *Trans. Faraday Soc.* **51**, 909 (1955).

[288] L. Peters and G. H. Lister, *J. Soc. Dyers Colour.* **16**, 24 (1954).

[289] L. Peters, *J. Soc. Dyers Colour.* **71**, 725 (1955).

[290] I. M. Klotz, *in* "The Proteins" (H. Neurath and K. Bailey, eds.), 1st ed., Vol. 1, Part B, Chapter 8, p. 727. Academic Press, New York, 1953.

[291] W. Kauzmann, *Advan. Protein Chem.* **14**, 1 (1959).

[292] H. Zahn, *Palette* **21**, 19 (1965).

Ferrini *et al.*[293] and Zollinger,[294] in order to obtain significant information with respect to the potential role of hydrophobic bonding in the dyeing of wool fiber and other solid substrates, experimentally applied the principle known as "the method of minimum structural changes." They studied the adsorption of Orange 8, Butylorange 8, and Rocelline 8 on gabardine wool as a function of temperature, electrolyte concentration, and dielectric constant of the medium. These dyes have the same basic structure, the only difference being in the nature of the side chain, which is benzene, naphthalene, and butylbenzene for Orange 8, Rocelline 8, and Butylorange 8, respectively. The observed decrease in dye adsorption is consistent with hydrophobic bonding as an important factor in wool dyeing. It should, however, be pointed out that the decrease in dye adsorption by the addition of NaCl could also be partially due to competition of Cl^- ions for dye adsorption sites. Calculations of relative thermodynamic quantities from the Langmuir-type isotherm data for these dyes showed that the affinity values are almost identical ($-\Delta\mu^\circ \simeq 5.3$ kcal/mole) for Rocelline 8 and Butylorange 8. However, the contribution of the enthalpy term to the standard affinity is more for Rocelline 8, while for Butylorange 8 the entropy term contributes more to $\Delta\mu^\circ$, indicating a possible contribution to hydrophobic bonding due to the butyl side chain in the latter dye. A comparison of the ΔS° values for Orange 8 and Butylorange 8 showed no significant influence of the side chain, although it did influence $\Delta\mu^\circ$ and ΔH°. These thermodynamic data do not give clear-cut evidence for hydrophobic bonding and some of the difficulties in the interpretation of the data are attributed to aggregation equilibria superimposed on the adsorption equilibria for these dyes.

Sivaraja Iyer *et al.*[295] carried out a comparative study of the adsorption isotherms for three acid dyes on wool fibers at 40°, 50°, and 60° with pH 4.6. The influence of additives such as dioxane and urea on the adsorption isotherms was also studied. These dyes have the same general structure, namely, 1-amino-4-R-aminoanthraquinone-2-sulfonic acid, where the substituent R is a methyl, butyl, or phenyl side chain. The adsorption process could be represented by a Langmuir-type equation. Hence, applying "the principle of minimum structural changes," and since preliminary polarographic measurements showed that these dyes are present only as monomers in solution under the

[293] B. Ferrini, Y. Kimura, and H. Zollinger, *Proc. Int. Wool Text. Res. Conf. 3rd*, p. 291 (1965).

[294] H. Zollinger, *J. Soc. Dyers Colour.* **81**, 345 (1965).

[295] S. R. Sivaraja Iyer, A. S. Ghanekar, and G. S. Singh, *in* "Dye-Polymer Interactions," p. 13. Dept. Chem. Technol., Bombay University, Bombay, 1971.

given experimental conditions, differences in the calculated thermodynamic parameters for the three dyes could be attributed to differences in the nonpolar character of the side chains. Table IX gives thermodynamic parameters calculated from the isotherm data. These results show that in going from the methyl to the butyl or the phenyl dye there is a small but definite increase in the entropy change of the system, while the exothermic enthalpy change ΔH decreases. These results give clear evidence for the presence of hydrophobic bonds in the anionic dye–wool fiber system.

TABLE IX

THERMODYNAMIC PARAMETERS FOR THE INTERACTION OF THE ANIONIC DYES 1-AMINO-4-R-AMINOANTHRAQUINONE-2-SULFONIC ACID WITH WOOL AT 50° AND PH 4.6[a]

Alkyl group	C_s (*mole/kg*)	$\Delta\mu°$ (*kcal/mole*)	$\Delta H°$ (*kcal/mole*)	$\Delta S°$ (*e.u.*)
Methyl	36.4×10^{-2}	−5.3	−7.6	−7.2
Butyl	50.0×10^{-2}	−5.8	−7.1	−4.1
Phenyl	50.0×10^{-2}	−6.0	−5.6	+1.2

[a] Reproduced from S. R. Sivaraja Iyer *et al.*[295]

Salt formation can also lead to an increase in entropy due to a release of water molecules when the salt linkage is formed. However, coulombic interactions (salt linkages) are strengthened by lowering the dielectric constant of the dyebath, but hydrophobic interactions are weakened. In order to distinguish between the influence of these factors, the equilibrium adsorption isotherms for all the three dyes using a 20% (V/V) dioxane–water mixture as the solvent in the dyebath were studied. The dielectric constant of the system is reduced to ~60 and the observed marked decrease in the extent of dye adsorption provides further evidence for the existence of hydrophobic bonding.

The addition of 2 *M* urea also produces a marked decrease in dye adsorption for all the three dyes, giving qualitative evidence for hydrophobic bonding. Urea is known to swell wool fibers in solutions,[74,296] disaggregate dye molecules,[297] rupture hydrophobic bonds,[298] and also increase the dielectric constant of the solution.[299,300] The disaggregating

[296] R. S. Asquith and A. K. Booth, *Text. Res. J.* **40**, 410 (1970).
[297] E. Coates, *J. Soc. Dyers Colour.* **85**, 355 (1969).
[298] G. Némethy, *Angew. Chem., Int. Ed. Engl.* **6**, 195 (1967).
[299] R. Fürth, *Ann. Phys. (Leipzig)* **4**, 70 (1923).
[300] H. Lecher and W. Siefken, *Justus Liebigs Ann. Chem.* **456**, 192 (1927).

effect of urea does not influence dye adsorption since all three dyes are present as monomers in solution. Swelling of the fiber should lead to an increase in adsorption and an increase in the dielectric constant should increase hydrophobic bonding. The observed marked decrease in dye adsorption therefore confirms that the main effect of urea is to rupture dye–fiber hydrophobic bonding.

C. Disperse Dye–Hydrophobic Fiber System

After World War I, a new class of fiber called secondary cellulose acetate was marketed. Owing to its hydrophobic nature, cellulose acetate does not swell very much in aqueous solutions and contains narrow intermicellar canals or pores of the order of 5–10 Å. Therefore only small molecules can diffuse through the fiber, which could not be satisfactorily dyed with most of the dyes (direct, acid, or basic) available at the time. From a study of the dyeing properties, it was concluded that the dye affinity for cellulose acetate is decreased by the presence of sulfonic acid groups while the introduction of strong basic groups has a reverse effect. Since cellulose acetate also has a much higher negative surface potential than cellulose in water, anionic dyes are repelled and cationic dyes are adsorbed. However, basic dyes were found to be unsatisfactory from the point of view of fastness. Hence for dyeing cellulose acetate, a new class of disperse dyes was developed with the essential features of solubility in organic esters, a low aqueous solubility, and a low molecular weight. Such dyes can diffuse rapidly within the fiber and in addition have a fairly high affinity; they are also somewhat volatile due to their relative lack of cohesive energy in the solid state and, in fact, can be used for vapor-phase dyeing.

Subsequently, disperse dyes were found to be the most suitable for dyeing other hydrophobic fibers such as cellulose triacetate and polyester, which do not contain ionic groups. They can also be used to some extent for dyeing polyamide and polyacrylonitrile fibers, which contain ionic groups. All these fibers are strongly hydrophobic and exhibit very slight swelling in water, polyester being regarded as practically anhydrous. Their structures consist of highly ordered crystalline regions with more or less complete restrictions of chain mobility under normal aqueous dyeing conditions, together with regions of random orientation where the polymer segments have a relatively high mobility and behave kinetically as a viscous liquid. The internal structure of these fibers is not static, but can be altered by swelling agents and carriers or by heat treatments such as setting and drawing of the filaments which increase fiber crystallinity. At higher temperatures, the increased mobility of polymer chain segments and

the loosening of interchain bonds by many nonionic compounds results in an increase in accessibility, which is more than what is normally present in the water-swollen regions at ordinary temperatures in the absence of swelling agents. From the point of view of dye adsorption, these fibers therefore cannot in general be regarded as containing a fixed accessibility for dyeing, i.e., there is no fixed specific surface area in such substrates.

Disperse dyes are used in aqueous dyebaths as fine dispersions (particle size of the order of 1–2 μm) containing a dispersing agent, because their solubility in water ranges from 1 to 100 mg/liter even at the high dyeing temperature of 80°–100°. The dyeing temperature is usually around 80° for cellulose acetate; for the other fibers, dyeing can be at the boiling point or between 100° and 130° in pressurized dyeing vessels. The higher temperatures are particularly necessary for polyester fiber since dye uptake is then more rapid owing to a greater loosening up of the fiber structure. The presence of electrolytes or acids generally does not affect dye uptake in disperse dye–hydrophobic fiber systems since the dyes are nonionic. The addition of the dispersing agent prevents precipitation of large dye particles on the fiber, helps in the solubilization of very sparingly soluble dyes, and prevents dye aggregation in the dyebath at high temperatures. Substances called carriers are also added for accelerating the dyeing of hydrophobic fibers, especially polyester, with disperse dyes. The carriers open up and plasticize the fiber structure, enabling more rapid entry of the dye. The mechanism of the action of carriers and dispersing agents on dye uptake has been discussed in Section I,B,1,b. Since disperse dyes are volatile, dyeing can also take place from the vapor phase as in the Thermosol process, mainly used for dyeing the polyester component in fiber blends. In this process, the polyester is dyed by padding the fabric with a suspension of the dye, drying, and then heating for less than 1 minute at about 200°. Under these conditions, dyeing probably consists in the dissolution of the dye from the vapor phase by the fiber which acts as a viscous liquid.

1. *Aqueous Dyeing*

Soon after the introduction of disperse dyes, two theories were put forward to explain the dyeing behavior. Microscopic observations of the dyeing of single acetate rayon fibers from aqueous solutions led Kartaschoff[301] to suggest that positively charged particles of suspended dye were attracted to the negatively charged fiber surface to build up a surface layer of dye particles. Subsequently, the solid dye dissolves in

[301] V. Kartaschoff, *Helv. Chim. Acta* 8, 928 (1925).

the fiber to form a solid solution. This conclusion was also supported by the fact that the fiber was dyed when shaken up with dry aminoanthraquinones. However, Vickerstaff and Waters,[38] Millson and Turl,[302] and Bird *et al.*[303] could not detect any attraction of the dye particles for the fiber when the fibers were dyed from a stable dyebath containing a dispersing agent. A probable reason is that in the presence of dispersing agents, the dye particles are negatively charged and hence are not attracted to the fiber surface. In Kartaschoff's experiments, no dispersing agents were used. However, Kartaschoff's suggestion that secondary cellulose acetate acts as solvent for disperse dyes has some validity even today for all disperse dye–hydrophobic fiber systems.

On the other hand, Clavel[304] suggested without experimental evidence that dyeing takes place from a saturated solution, the suspended particles forming a reservoir of dye which serves to replenish the solution as dye is removed from the dyebath by the fiber. Subsequently, a considerable amount of evidence has been obtained in support of Clavel's theory, which is now generally accepted. The dye which is present in true solution is assumed to be in a monomolecular state, both in the dyebath and in the fiber.[31,54,94,305–307] However, as discussed by Jones,[308] experimental difficulties prevent any definite conclusions being drawn on the monomolecular state of dispersion of the dye in solution and in the substrate. In the previous chapter, Daruwalla has discussed the experimental evidence for state of aggregation of dye in the dyebath and in the substrate. It is probable that in secondary cellulose acetate diffusion consists of transfer from site to site in the fiber, single dye molecules being continually absorbed from and desorbed into the water in the fiber. In the strongly hydrophobic polyester, however, there is practically no swelling in water and hence the dye may be regarded as diffusing from site to site along the fiber molecules without any assistance from water, the fiber behaving as a highly viscous liquid.[309,310] At temperatures above 100° or by adding a swelling agent to the boiling dyebath, the fiber structure can be opened up sufficiently for a rapid diffusion of dye molecules through this more open structure. A characteristic feature of the diffusion process for

[302] H. E. Millson and L. H. Turl, *Text. Res. J.* **21**, 685 (1951).
[303] C. L. Bird, F. Manchester, and P. Harris, *Discuss. Faraday Soc.* **16**, 85 (1954).
[304] R. Clavel, *Rev. Gen. Matier. Color.* **28**, 145 and 167 (1923).
[305] J. Wegmann, *Can. Text. J.* **79**, 37 (1962).
[306] E. H. Daruwalla and H. H. Kundalia, *Text.-Rundsch.* **20**, 345 (1965).
[307] W. McDowell and R. Weingarten, *J. Soc. Dyers Colour.* **85**, 589 (1969).
[308] F. Jones, *Rev. Progr. Coloration Relat. Top.* Vol. I, p. 15 (1969).
[309] C. L. Bird, *J. Soc. Dyers Colour.* **72**, 343 (1956).
[310] C. L. Bird, *Palette* **15**, 25 (1964).

disperse dyes in hydrophobic fibers, discussed in Section I,B,1,b is that the diffusion coefficient is constant, independent of concentration in the fiber, and obeys Fick's law.

A considerable body of evidence now exists which clearly indicates that, in general, for disperse dye–hydrophobic fiber systems the sorption isotherms are linear with a flat plateau corresponding to a saturation value for dye absorption under the given experimental conditions.[18,29,36,38,39,45,126,302,303,311–325] The absorption is also completely reversible. An example of such an isotherm is shown in Fig. 44.

The absorption process has some features which are not met with in other dyeing systems: (a) The absorption increases with increasing aqueous solubility of the dye, whereas in most adsorption systems with ionic dyes, adsorption decreases with increasing dye solubility, both for hydrophilic substrates and cellulose acetate.[324,325] (b) As absorption proceeds, the number of sites available for dye absorption continually increases.[324,325] (c) Fiber saturation values increase with increasing temperature, although the partition coefficient values decrease as the temperature is increased.[319,326,327] Giles[324,325] has shown that the maximum amount of solute absorbed for azobenzene dyes on cellulose acetate increases with the number of hydrogen atoms available for bonding. Each active group increases dye affinity and its ability to penetrate the fiber structure by breaking intersubstrate bonds. The data of Schuler and Remington[319] for the uptake of simple anthraquinone disperse dyes by polyester fiber also show that the amount of dye taken up is more than double the normal water content of the fiber in the air-dried condition, indicating that the dye can penetrate the fiber to a

[311] H. Wahl, Y. Arnould, and M. Simon, *Teintex* **17**, 298 (1952).
[312] C. L. Bird, *J. Soc. Dyers Colour.* **70**, 68 (1954).
[313] C. L. Bird, P. Harris, and F. Manchester, *J. Soc. Dyers Colour.* **71**, 139 (1955).
[314] C. L. Bird and P. Harris, *J. Soc. Dyers Colour.* **73**, 199 (1957).
[315] E. H. Daruwalla and H. A. Turner, *J. Soc. Dyers Colour.* **69**, 240 (1953).
[316] E. H. Daruwalla, S. S. Rao, and B. D. Tilak, *J. Soc. Dyers Colour.* **76**, 418 (1960).
[317] E. H. Daruwalla and V. R. Limaye, *J. Soc. Dyers Colour.* **74**, 464 (1958).
[318] H. C. Olpin and J. Wood, *J. Soc. Dyers Colour.* **73**, 247 (1957).
[319] M. J. Schuler and W. R. Remington, *Discuss. Faraday Soc.* **16**, 201 (1954).
[320] V. S. Salvin, *Amer. Dyest. Rep.* **49**, 600 (1966).
[321] F. Fortess and V. S. Salvin, *Text. Res. J.* **28**, 1009 (1958).
[322] C. H. Giles, T. H. MacEwan, S. N. Nakhwa, and D. Smith, *J. Chem. Soc. London*, p. 3937 (1960).
[323] B. Campbell, *J. Soc. Dyers Colour.* **82**, 303 (1966).
[324] C. H. Giles, *Text. Res. J.* **31**, 141 (1961).
[325] C. H. Giles, *Chem. Ind. (London)* p. 137 (1966).
[326] C. L. Bird, H. K. Partovi, and G. Tabbron, *J. Soc. Dyers Colour.* **75**, 600 (1959).
[327] Ghionis, *Dr. rer. Nat. Thesis*, Technische Hochschule, Stuttgart (1958) (see Bird[329]).

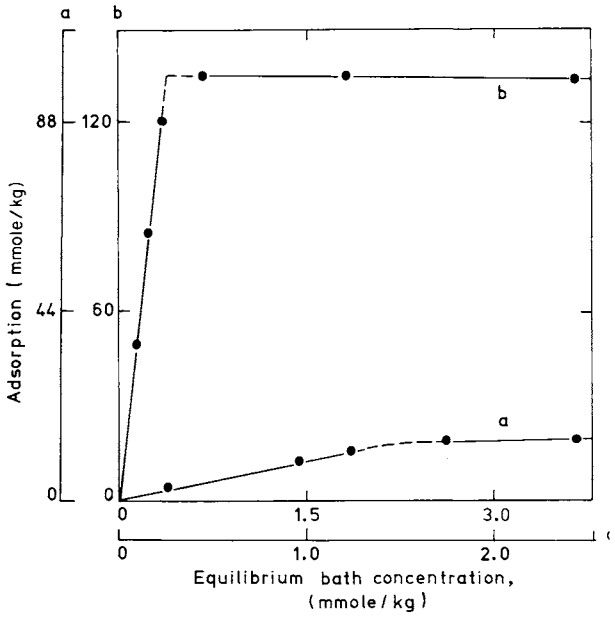


FIG. 44. Adsorption isotherms for nonionic dyes on cellulose acetate at 80°. Typical data from Daruwalla *et al.*[316]

Dye	*Saturation absorption (mmole/kg)*	*Affinity (kcal/mole)*
(a) Nonplanar	3.1	−0.61
(b) Planar	135	−4.77

Reproduced by permission from Giles.[325]

very much greater extent than water. As discussed earlier, such substrates cannot therefore be regarded as possessing a fixed accessibility for dye molecules. Saturation absorption of disperse dyes on hydrophobic fibers is additive in the case of some selected dyes, the partition coefficient of each dye between water and the fiber substrate being the same whether the dyes are used alone or in mixtures. These results also support the conclusion that there is no fixed accessibility in the fiber phase for dye absorption.[18,93,94,317,319,328]

Vickerstaff and Waters[38] obtained Langmuir-type isotherms for the dyeing of four related anthraquinonoid dyes on cellulose acetate. Daruwalla and Turner[315] have shown that under printing conditions a

[328] C. L. Bird and P. Rhyner, *J. Soc. Dyers Colour.* **77**, 12 (1961).

linear isotherm is obtained for 1-amino-4-hydroxyanthraquinone, although the sorption isotherm obtained by Bird and Manchester[39] for the same dye from an aqueous dyebath shows appreciable curvature near the saturation point. However, the latter authors suggested that this curvature was due to the presence of unreactive crystalline dye particles, since a linear isotherm is obtained when a solubilizing dispersing agent is added. Desorption experiments also gave a linear reversible relationship. Other deviations from linear isotherms which have been observed by some workers[305,307,326] can be attributed to the superimposition of a Freundlich type isotherm on the linear isotherm.[54,307,326] This surface-adsorbed dye, however, can be removed by a reduction-clear treatment and does not affect the final linear distribution equilibrium. McDowell and Weingarten[307] have suggested that another reason for deviation from a linear isotherm is the adsorption of dye particles on the walls of the dyeing vessel. These results emphasize the considerable experimental difficulties associated with the measurement of accurate adsorption isotherms in such systems. These authors also point out that a real deviation from linearity in sorption isotherms can occur because of the association of dyes in aqueous solutions as well as changes in the properties of the substrate during a dyeing process.

The linear isotherm which is most commonly obtained for all disperse dye–hydrophobic fiber systems has been explained by most workers in terms of a partition of a solute between two immiscible solvents, the dyebath and the fiber. The partition isotherm is assumed to obey the Nernst equation

$$\frac{[D_\phi]}{[D_\sigma]} = K \tag{115}$$

where K is called the partition coefficient, the concentrations being expressed in mole fractions. This equation is valid for ideal dilute solutions with the further assumption that the solute molecules are present in the same monodisperse state in both solvents. The assumption that the dye is dissolved in the fiber phase arises from analogies with other systems and experimental evidence, such as the fact that cellulose acetate can be dyed with dry disperse dyes. A correlation observed by Bird[329] between the solubility of a disperse dye in secondary cellulose acetate and its solubility in a partially hydrated hydroxy acetate solvent having the same acetyl value also suggests that the hydrophobic dye molecule is effectively extracted from water in a manner similar to

[329] C. L. Bird, *J. Soc. Dyers Colour.* **74**, 688 (1958).

its extraction by an organic solvent. Bird[310] classifies cellulose acetate as a good solvent for the more hydrophilic disperse dyes and polyester as a good solvent for the more hydrophobic disperse dyes. The greater solvent power of polyester for the more hydrophobic dyes is attributed to a considerable van der Waals attraction of the benzene rings in the chain molecule of the fiber for the disperse dyes. Polyacrylonitrile and polypropylene are poor solvents, whereas nylon is a fairly good solvent.[18] The solubility also largely depends on the number of polar groups in the fiber molecule such as ester linkages and the degree of crystallinity of the fiber. However, it should be pointed out that the analogy with a liquid solvent is not very satisfactory since the fibers are not homogeneous in structure, being partially crystalline and partially amorphous. The orientation of the fiber molecules will also cause restrictions in the entropy of the dye molecules dissolved in the substrate.

Another characteristic feature of the linear isotherm described by Eq. (115) is that it is also related to the saturation values S_ϕ and S_σ for the dye in the fiber and in the aqueous phase, respectively, by the equation

$$K = \frac{[D_\phi]}{[D_\sigma]} = \frac{S_\phi}{S_\sigma} \tag{116}$$

Hence the ratios of saturation solubilities should be equal to the partition coefficient K. Bird and Harris[314] used this equation for calculating fiber saturation values for several disperse dyes at 80° on secondary cellulose acetate. Bird *et al.*[326] determined fiber saturation values at 50° and 60° in the presence of a dispersing agent on secondary cellulose acetate. A reasonably good agreement was obtained between these results and those calculated from aqueous solubilities and partition coefficients in the absence of a dispersing agent. Some deviations which were observed for the more hydrophilic disperse dyes are attributed to the adsorption of dispersing agent by the fiber. Bird[310] also points out that the saturation value in a fiber is not an absolute value since, for example, the accessibility of secondary cellulose acetate may vary. Variations in acetyl values for cellulose acetate and cellulose triacetate also influence the fiber saturation value, which decreases with increasing acetyl value. McDowell and Weingarten[307] used a specially designed filter apparatus for the measurement of saturation solubilities of a series of pure disperse dyes at different temperatures in water and in a polyester film. This apparatus avoids most of the experimental difficulties associated with the measurement of an adsorption isotherm. An examination of the solubility ratio with the distribution ratio from measured isotherms shows a reasonably good agreement. Data from

their results are shown in Table X. Some deviations observed in the distribution ratios calculated from partition coefficients and solubility ratios, respectively, for a series of dyes, can be attributed to dye adsorption on the walls of the vessel during the measurements. From studies on the transfer of disperse dyes from thickener films to cellulose acetate fibers by steaming at 100° in the presence and absence of a

TABLE X

SOLUBILITY RATIOS AND DISTRIBUTION COEFFICIENTS FOR SOME DISPERSE DYES[a]

Dye[b]	$-\Delta\mu°$ 100° (*kcal/mole*)	$-\Delta\mu°$ 150° (*kcal/mole*)	$\Delta H°$ (*kcal/mole*)	$\Delta S°$ (*cal/deg mole*)
		Solubility in polyester		
I	−1.43	−0.93	5.16	9.99
II	−1.34	−0.76	5.61	11.47
III	−1.59	−1.33	3.49	5.10
IV	−1.81	−1.67	2.88	2.87
V	−2.37	−2.16	3.95	4.24
VI	−2.31	−1.78	6.31	10.73
		Solubility in water		
I	−6.42	−5.41	14.02	20.37
II	−7.18	−6.13	15.02	21.01
III	−8.21	−7.45	13.89	15.23
IV	−7.66	−7.20	11.07	9.15
V	−9.06	−8.86	10.58	4.08
VI	−9.53	−9.66	8.57	−2.58
		Ratio of solubilities ($C_{p\infty}/C_{w\infty}$)		
I	4.99	4.47	−8.87	−10.38
II	5.85	5.37	−9.40	−9.54
III	6.62	6.11	−10.40	−10.13
IV	5.85	5.53	−8.19	−6.28
V	6.69	6.70	−6.63	0.16
VI	7.22	7.88	−2.26	13.31
		Distribution coefficient		
I	5.07	4.32	−10.72	−15.14
II	5.74	4.85	−12.37	−17.77
III	5.93	5.10	−12.18	−16.75
IV	5.83	5.22	−10.34	−12.09
V	6.85	6.15	−12.08	−14.03
VI	7.04	7.37	−4.63	6.46

[a] Reproduced by permission from McDowell and Weingarten.[307]

[b] I, 1-Amino-4-hydroxyanthraquinone; II, 1,4-dihydroxyanthraquinone; III, CI Disperse Blue 152; IV, *p*-phenylenediamine → phenol; V, CI Disperse Yellow 54; VI, CI Disperse Violet 31.

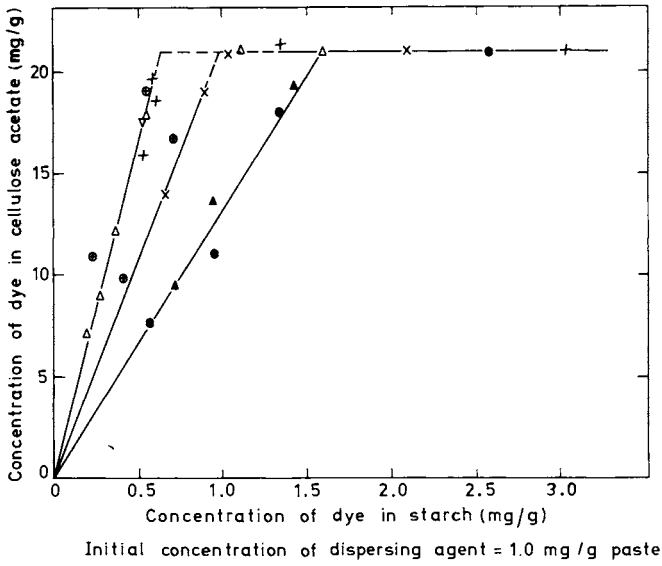


FIG. 45. Effect of dispersing agent on the adsorption isotherms for pure CI Disperse Orange 3.

Adsorption	*Desorption*	*Dispersing agent*
○	△	None
+	▽	Lissapol LS
×	○	Dispersol VL
▲	●	Emulphor EL

Reproduced by permission from Daruwalla and Limaye.[317]

dispersing agent, Daruwalla and Limaye[317] concluded that the presence of the dispersing agent affects the slope of the isotherm but not the fiber saturation value, as shown in Fig. 45. Bird and Manchester[39] obtained similar results for the dyeing of cellulose acetate fiber with disperse dyes from aqueous dyebaths.

It has been pointed out by several workers[18,45,303,305,330-332] that adherence to a linear isotherm does not necessarily indicate a partition of a solid solute between two immiscible solvents, i.e., dyeing proceeds by a mechanism of solid solution of the dye in the fiber phase. Vickerstaff[18] points out that for the formation of a solid solution of the dye in

[330] A. E. Stubbs, *J. Soc. Dyers Colour.* **70**, 120 (1954).
[331] M. Kusunose and K. Odajima, *J. Soc. Text. Cell. Ind. Jap.* **14**, 49 (1958).
[332] H. J. White, Jr., *Text. Res. J.* **30**, 329 (1960).

cellulose acetate it is necessary for the dye to penetrate into the micellar regions of the fiber, and evidence for this is inadequate. It is therefore more probable that the dye diffuses through the water-filled intermicellar canals and becomes fairly evenly adsorbed on sites located on the fiber surfaces. He argues that the distinction between adsorption and a solid solution is largely unreal for the following reasons. In liquid solutions, all the solute molecules are firmly attached to one or more solvent molecules (disperse dye dissolved in ethyl acetate). However, in an analogous system where solvating groups, ester groups in cellulose acetate for example, are attached to a linear cellulose chain, the system can as well be regarded as an adsorption system. Remington and Schroeder[45] point out that, strictly, the concept of a solid solution involves the presence of two or more substances in the same crystal lattice. Since the dye is most probably located only in the amorphous regions of the fiber, it is preferable to describe the mechanism as one of solution, but not solid solution. Independently, Stubbs[330] and Bird *et al.*[303] have suggested that the existence of linear isotherms does not necessarily exclude the possibility that the disperse dye is adsorbed on the fiber surface, the distinction between adsorption and solid solution, for reasons similar to those given by Vickerstaff,[18] being somewhat nebulous.

From a comparative study of the dyeing of cellulose acetate with disperse dyes and cellulose with direct dyes, Kusunose and Odajima[331] conclude that the uptake of disperse dyes by cellulose acetate corresponds to an adsorption process. White[332] considered two models for the dyeing process based on mixing of polymer segments with absorbed molecules or adsorption on sites within the fiber, and concluded that the available experimental results are in better agreement with the site adsorption model involving a linear isotherm. Wegmann[305] studied in detail the dyeing of nylon, cellulose triacetate, and polyester fibers with pure CI Disperse Violet 1 from aqueous solutions under various conditions of temperature, dye concentration, liquor ratio, and additives. The dye concentrations and state of dye in the solution and in the fiber were determined spectrophotometrically. The results are interpreted in terms of a unimolecular distribution of dye when dyeing from a molecular solution, the dye molecules in this unimolecular layer being adsorbed on the fiber surface.

The maximum uptake of dye in the fiber need not necessarily result in the occupation of all the sites in the fiber by dye molecules. The amount of dye uptake will depend on the relative affinities, i.e., whether the chemical potential of the dye in the fiber is higher or lower than that of the solid dye in a dispersion. If, for example, the chemical potential

of the dye in the fiber is higher, the break in the linear isotherm at which "saturation" of the fiber takes place will correspond to a low dyebath concentration. Hence the relationship between equilibrium dye adsorption and dyebath concentration may represent only the initial linear portion of a Langmuir-type isotherm. It is well known that this linear portion corresponds to Henry's law: the amount adsorbed is directly proportional to the equilibrium solute concentration. This can be more clearly seen by making the following assumption in the classical Langmuir equation

$$[D_\phi] = \frac{K[S][D_\sigma]}{1 + K[D_\sigma]} \tag{117}$$

where [S] corresponds to the concentration of adsorption sites and K is the equilibrium constant for the interaction. If $[D_\phi] \ll [S]$, i.e., the experimental conditions are such that the dye solubility is very low leading to a limited dye uptake for a given value of K, then Eq. (117) reverts to Henry's law and is also similar in form to Eq. (115). If the affinity value of the dye for the fiber is also very low, i.e., $K[D_\sigma] \ll 1$, Henry's law is again obtained. Hence a partition isotherm may also result from the solubility characteristics of the dye and the relative weakness of dye–fiber bonding (see also Rattee[168]). Furthermore, if in the usual kinetic derivation of the Langmuir isotherm the assumption is made that the surface molecules are mobile,[333] then,

$$\text{Rate of evaporation} = k_1 S_1 \tag{118}$$

$$\text{Rate of condensation} = k_2 S_2 \tag{119}$$

At equilibrium,

$$S_1/S = b[D_\sigma] \tag{120}$$

where S_1 is the surface area occupied by adsorbed dye molecules and S is the total surface area, i.e., the adsorbed molecule does not occupy any particular binding site on the surface, as usually assumed in the derivation of the Langmuir isotherm.

Since we have inadequate data on the true nature of the disperse dye sorption process, it is desirable at this stage to regard disperse dye uptake as an absorption process without specifying its nature.

Due to increasing interest in the use of nonaqueous solvents in dyeing, dyeing isotherms have been measured for the dyeing of polyester and nylon substrates with disperse dyes from trichloroethylene[334] and

[333] A. W. Adamson, "Physical Chemistry of Surfaces," 2nd ed. Wiley (Interscience) New York, 1967.

[334] L. W. C. Miles, *Shirley Inst., Semin.* **1**, (1969).

perchloroethylene.[335,336] These dyeing isotherms are also linear, although the partition coefficients are much lower than those for dyeing from aqueous dyebaths. Suda *et al.*[337] have recently obtained linear isotherms for the dyeing of cellulose acetate fiber with disperse dyes from *n*-alcoholic solution.

2. *Vapor-Phase Dyeing*

Very few fundamental quantitative studies on the vapor-phase absorption of disperse dyes have been reported, although cellulose acetate can be dyed from the vapor phase.[18,301,303] Majury[338] studied quantitatively the absorption isotherms for the dyeing of cellulose acetate with five model compounds: *p*-nitroaniline, *N*,*N*-dimethyl-*p*-nitroaniline, azobenzene, *p*-aminoazobenzene, and *N*,*N*-dimethyl-*p*-aminoazobenzene. Since it was necessary to know the vapor pressures of the dyes, first, in relation to the absorption isotherm and, second, to give data on the heats of sublimation or vaporization of the dyes, such data were also obtained using manometry and Knudsen's effusion method,[339] the latter being employed for low vapor pressures. Subsequently, accurate vapor pressure data for several disperse dyes and model compounds have been obtained by Bird *et al.*,[340] Jones and Seddon,[178] Jones,[341] Jones and Kraska,[342] and Green and Jones.[343] The saturation vapor pressures of simple disperse dyes are of the order of 10^{-6} to 10^{-4} mm Hg. The absorption isotherms are generally linear, although a slight departure has been noted for some dyes, as for example, *N*,*N*-dimethyl-*p*-aminoazobenzene.

Jones and Seddon[178] made careful measurements of the equilibrium saturation values and absorption isotherms for vapor-phase dyeing of cellulose acetate with three disperse dyes. The dye uptake was measured by a technique in which dyeing from the vapor phase at different controlled temperatures is carried out in an evacuated glass envelope encasing a calibrated silica spring from which is suspended an anhydrous polymer film of known weight. Their results are similar to those obtained by Majury,[338] the isotherms being essentially linear at all temperatures in the range 130°–160°. They also observed that the

[335] B. Milicevic, *Shirley Inst., Semin.* **1**, (1969).
[336] B. Milicevic, *Textilveredlung* **4**, 213 (1969).
[337] Y. Suda, M. Suzuki, and F. Nakajuma, *Bull. Res. Inst. Polym. Text.* **99**, 43 (1972).
[338] T. G. Majury, *J. Soc. Dyers Colour.* **72**, 41 (1956).
[339] M. Knudsen, *Ann. Phys.* (*Leipzig*) **28**, 999 (1909).
[340] C. L. Bird, R. S. Bradley, and F. Jones, *Trans. Faraday Soc.* **56**, 23 (1960).
[341] F. Jones, *Teintex* **34**, 594 (1969).
[342] F. Jones and J. Kraska, *J. Soc. Dyers Colour.* **82**, 332 (1966).
[343] H. S. Green and F. Jones, *Trans. Faraday Soc.* **63**, 1612 (1967).

equilibrium saturation values were quite considerable and suggested that this may be due to the dye acting to some extent as a plasticizer for the fiber and hence loosening up the fiber structure. This conclusion was reached from X-ray diffraction and load extension studies over the temperature range of measurement.

A striking feature of these linear isotherms, therefore, is that although the Nernst-Henry law appears to be obeyed, there is a very substantial amount of dye uptake by the fiber phase, i.e., the concentration in the fiber phase cannot be regarded as sufficiently dilute in dye. In fact, dye uptake in the vapor phase can be as much as ten times the amount of dye absorbed when dyeing from aqueous dyebaths. Majury[338] and Jones and Seddon[178] therefore suggested that the results are best expressed in terms of a solution of the dye in the fiber phase rather than adsorption on a limited number of sites. However, as pointed out by McGregor,[240] a process of mixing of dye molecules and polymer molecules in the fiber, which is implied in a solution process, need not necessarily exclude the possibility that the dye molecules are adsorbed on internal surfaces within the fiber.

It was observed by Thompson[344] that dye sorption at equilibrium decreases with increasing concentration of the water vapor within the substrate, when cellulose acetate is dyed from the vapor phase with model compounds such as *p*-nitroaniline and azobenzene in the presence of unsaturated water vapor. A possible explanation for this decrease is the increased competition for sites between sorbate molecules and water, or structural changes in the substrate due to water, probably the latter since the effect of water is quite considerable.[168] An additional reason for the very high uptake of dye from the vapor phase by anhydrous cellulose acetate may therefore be the absence of water in the substrate under these conditions. The actual values of the partition coefficients obtained in vapor-phase dyeing are about 10^2–10^4 times the values obtained for dyeing from an aqueous dyebath.

Majury,[338] Jones,[178,341] and Jones and Kraska[342] also obtained similar linear absorption isotherms for the vapor-phase dyeing of other hydrophobic fibers such as nylon and Terylene, suggesting that the vapor-phase dyeing of all hydrophobic fibers with disperse dyes follows a similar mechanism.

Studies on the vapor-phase dyeing of polyester fiber with disperse dyes are of considerable importance in relation to the Thermosol process which is widely used to dye the polyester component in polyester–cellulose blends. Datye[345] and Bent *et al.*[346] have independently shown

[344] J. Thompson, Ph.D. Thesis, University of Leeds (1969) (see Rattee[168]).

[345] K. V. Datye, *Textilveredlung* 4, 562 (1969).

that on padding and drying, dye particles in solution are preferentially taken up by the cellulose component, but during the fixation stage the dye is preferentially absorbed by the polyester. Since the fixation stage involves high-temperature anhydrous conditions ($\sim 200°$), the vaporization of the dye plays an important role in the transfer mechanism. It has been shown that in such a transfer mechanism a plot of $\ln[D]_\phi$ vs. $1/T$, where $[D]_\phi$ is the amount of dye absorbed in unit time, should be a line of negative slope independent of the distance x between the polyester substrate and the source of dye for all distances including $x = 0$, i.e., direct contact of dye and polyester. Bent *et al.*[346] found this correlation to be exact, but Datye's data[345] showed slight curvature, and extrapolation to $x = 0$ suggested a small additional contribution by a direct contact mechanism. Dye transfer from solid dye to the substrate is explained by Price[347] as a partial contact mechanism and by Meunier *et al.*[348] as partial dissolution in the substrate.

The standard free-energy changes or thermodynamic affinities $-\Delta\mu°$ are usually calculated from the equation

$$-\Delta\mu° = RT \ln K \tag{121}$$

where K is the partition coefficient given by Eq. (115). Generally, partition coefficients lie within the range 500–3000 for dyeing cellulose acetate from aqueous dyebaths at about 80°, although values of $K \simeq 10^4$ are also known.[314] At temperatures above 100°, the partition coefficients shift to lower values, i.e., in favor of the dyebath. Dyes having a low affinity for the fiber will therefore be absorbed to a lesser extent. Some typical values for the partition coefficients and standard affinities for dyeing from aqueous dyebaths are given in Table XI. Although these values are for a series of model compounds and not commercial disperse dyes, the data are typical of the range of values obtained for disperse dyes. A considerable spread in the partition coefficient values does not result in a large spread in the values of the standard free energy $-\Delta\mu°$ due to the logarithmic relationship between the two quantities. Partition coefficients are thus more informative about the extent of distribution between dyebath and fiber phases. However, the conventional standard affinity of dyeing, $-\Delta\mu°$, is more important since it is a measure of the driving force behind the dyeing process. Moreover, since $-\Delta\mu°$ is practically the same for secondary

[346] C. J. Bent, T. D. Flynn, and H. H. Sumner, *J. Soc. Dyers Colour.* **85**, 606 (1969).

[347] J. Price, *Amer. Dyest. Rep.* **54**, 13 (1965).

[348] P. L. Meunier, J. J. Iannarone, and W. J. Wygand, *Amer. Dyest. Rep.* **52**, 1014 (1963).

TABLE XI

PARTITION COEFFICIENT AND STANDARD AFFINITY VALUES FOR DYEING CELLULOSE ACETATE FIBERS FROM AQUEOUS DYEBATHS[a]

Dye	Temp. (°C)	Dyebath concn. (g/liter)[b]	Secondary acetate		Free energy of dyeing (kcal/mole)	Triacetate		Free energy of dyeing (kcal/mole)
			Uptake (g/liter)	Partition coefficient		Uptake (g/liter)	Partition coefficient	
p-Nitroaniline	60	1.00	55.4	55.4	−2.68	—	—	—
		2.83*	160	56.5		150	53.0	−2.64
	80	0.20	7.0	35.0		—	—	—
		2.00	60.1	30.1		—	—	—
		5.00	151	30.2	−2.40	—	—	—
		6.59*	197	29.9		169	25.6	−2.29
N,*N*-Dimethyl-*p*-nitroaniline	60	0.092*	17.0	185	−3.48	22.0	239	−3.65
	80	0.250*	23.5	94	−3.20	25.4	102	−3.26
Azobenzene	60	0.0293*	59.0	2010	−5.06	—	—	—
	80	0.0760*	56.3	740	−4.66	63.4	833	−4.74
p-Aminoazobenzene	60	0.153*	161	1050	−4.63	134	876	−4.51
	80	0.110	51.1	465	−4.35	—	—	—
		0.370*	185	500		174	470	−4.34
N,*N*-Dimethyl-*p*-aminoazobenzene	60	0.0060*	23.5	3920	−5.50	—	—	—
	80	0.0167*	24.9	1490	−5.15	31.3	1875	−5.31

[a] Reproduced by permission from the Society of Dyers and Colourists.

[b] *, saturated.

cellulose acetate and cellulose triacetate, the origin of this affinity is in the acetyl rather than the hydroxyl groups.[29]

Majury[29] suggested that the strength of interaction between a dye molecule and a substrate may be expressed in terms of an absolute heat of association defined as the difference in heat content of 1 mole of dye in the vapor state and 1 mole of dye absorbed on the substrate. In the vapor state, individual dye molecules do not physically interact with other molecules such as solvent molecules or dye molecules in the solid dye. The vapor state is therefore a useful reference state. However, vapor pressure data for some disperse dyes[342,343] indicate some degree of association greater than unity. The absolute heat of association can be calculated by combining heats of dyeing, solution, sublimation. The heat of dyeing can be calculated directly using Eq. (121) and the following equation:

$$\Delta H_{\text{dyeing}} = \frac{RT_1T_2}{T_1 - T_2} \ln \frac{K_1}{K_2} \tag{122}$$

The heat of solution of the dyes in water can also be calculated similarly from the temperature coefficients of the solubility data using the equation

$$\Delta H_{\text{soln}} = \frac{RT_1T_2}{T_1 - T_2} \ln \frac{S_1}{S_2} \tag{123}$$

The absolute heat of association ΔH_{assn} is given by the equation

$$\Delta H_{\text{assn}} = \Delta H_{\text{sub}} + \Delta H_{\text{soln}} + \Delta H_{\text{dyeing}} \tag{124}$$

where the values of ΔH are used with their proper signs. This equation is based on the assumption that the absorbed dye molecules are not solvated and the heat of wetting of cellulose acetate is not altered as a result of dye absorption. The water in the system is thus assumed to act as a purely inert vehicle for the transfer of dye. Furthermore, this equation shows that the heat of association should be equal to the heat of dyeing from the vapor phase. Some experimental data on the heat of dyeing from the vapor phase were also obtained by Majury.[338] A comparison of these various heat quantities is given in Table XII. An interesting feature of these results is that ΔH_{assn} is practically equal to the heat of absorption from the vapor phase, as suggested by Majury.[338] However, since in aqueous dyebaths water cannot be considered as an inert transfer medium, the fairly close agreement between ΔH_{assn} and $\Delta H_{\text{absorption}}$ in Table XII may be coincidental.

From a comparison of the equilibrium sorption isotherms for dyeing

TABLE XII

ABSOLUTE HEATS OF ASSOCIATION AND HEATS OF ABSORPTION FROM THE VAPOR PHASE ON SECONDARY CELLULOSE ACETATE (KCAL/MOLE)[a]

Dye	*Heat of dyeing + heat of solution*	*Heat of condensation to solid (s) or liquid (l)*	*Absolute heat of association*	*Heat of absorption from vapor phase*
p-Nitroaniline	2.9	−23.3(s)	−20.4	−21.2
N,*N*-Dimethyl-*p*-nitroaniline	3.8	−23.6(s)	−19.8	−21.4
Azobenzene	−1.2	−15.5(l) −17.7(s)	−16.7	−17.8
p-Aminoazobenzene	1.2	−26.5(s)	−25.3	−22.0
N,*N*-Dimethyl-*p*-aminoazobenzene	0.8	−28.9(s)	−28.1	−22.7

[a] Reproduced by permission from the Society of Dyers and Colourists.

from aqueous solution and from the vapor phase, McGregor[240] has shown that there is a fundamental difference between the two systems. In vapor-phase dyeing, not only is the standard affinity of dyeing $-\Delta\mu^\circ$ much greater since the partition coefficient K_{vap} is much greater than K_{soln}, but the values of the standard heats and entropies of dyeing ΔH° and ΔS° are also quite different. Some comparative values from the data of Jones and Seddon[178] and Majury[338] for azobenzene are shown in Table XIII, the difference between the enthalpy changes on vaporization ΔH_v° and the enthalpy change on formation being of the order of −4 kcal/mole. Correspondingly, the molecular interactions in the solution phase cause a decrease in the entropy of the order of 3 cal/degree per mole. Since ΔH_s° is greater than ΔH_v° for dyeing from aqueous solution, molecular interactions within the dyebath must be overcome before dye is taken up by the fiber phase. These results in general emphasize the importance of a proper understanding of the physicochemical properties of aqueous dye solutions, and the role of water and changes in water structure in relation to the dyeing process. Solid-state transitions[349,350] and polymorphic changes[351,352] can also occur through solution and recrystallization mechanisms in the case of nonionic dyes and pigments. The role of water in reducing dye uptake when moist

[349] I. Saito and Y. Arai, *Rep. Govt. Chem. Ind. Res. Inst., Tokyo* **63**, 103 (1968).
[350] L. Flores and F. Jones, *J. Soc. Dyers Colour.* **87**, 304 (1971).
[351] T. W. J. Apperley, *J. Soc. Dyers Colour.* **85**, 562 (1969).
[352] W. Biedermann, *J. Soc. Dyers Colour.* **87**, 105 (1971).

TABLE XIII

THERMODYNAMIC PARAMETERS FOR AZOBENZENE (M.P. 68°) ON CELLULOSE ACETATE[a]

Dyeing conditions	*Fiber saturation uptake (g/liter)*	*Partition coefficient*	$-\Delta\mu_1^\circ$ *(kcal/mole)*	ΔH° *(kcal/mole)*	ΔS° *(cal/mole, °C)*	ΔH_v° *or* ΔH_s°	ΔS_v° *or* ΔS_s°
Aqueous (80°)	56.3	740	4.64	−11.8	−20.3	11.2	16.3
Vapor (105°)	214.7	28,840	7.72	−17.0[b]	−24.4[b]	14.8[b]	19.0[b]
		Difference:	3.08	−5.2	−4.1	3.6	2.7

[a] Reproduced by permission from McGregor.[240]

[b] Based on the vapor concentration in moles per liter.

cellulose acetate fibers are dyed from the vapor phase further emphasizes the fact that water cannot be regarded as an inert vehicle for dye transfer.

The thermodynamic data in Table XIII have been analyzed by McGregor[240] as follows: the standard enthalpy change ΔH_D° ($\Delta H_D^\circ = \Delta H^\circ + \Delta H_v^\circ$) for transporting dye directly from the solid dye into the fiber is rather small, which implies that the strength of dye–fiber interactions is very similar to the strength of dye–dye interactions in the solid dye. Therefore, the heat of dyeing from the vapor phase ΔH° may be reasonably regarded as a direct measure of the strength of dye–fiber bonds, provided there is no interaction between dye molecules in the vapor phase. Since the heat of absorption from the vapor phase is a large exothermic quantity, the sorption of dye molecules from the vapor phase is favored, but since the corresponding entropy change ΔS° is negative, this decrease in entropy will not favor dye absorption. Nevertheless, since dye is absorbed, the driving force is mainly the large ΔH°, which ranges from about -15 to -40 kcal/mole. The decrease is caused by the reduced randomness, i.e., the more ordered packing of the dye molecules when sorbed by the polymer. However, linear correlations between enthalpy changes and entropy changes in a family of measurements, as for example, ΔH_s° and ΔS_s° for a series of disperse dyes, may be spurious when the enthalpy and entropy changes are obtained from the same set of data using an equation of the type

$$\Delta G^\circ = -RT \ln K = \Delta H^\circ - T\Delta S^\circ \tag{125}$$

unless there is a corresponding correlation in the original experimental data, i.e., in the independent K values. Hence, confirmation of any significant relationship between ΔH° and ΔS° should be obtained by a suitable mathematical transformation.[353–355]

The standard heat of dyeing (ΔH_{dye}°) can be calculated from the relationship

$$\Delta H_{dye}^\circ = \Delta H_{fiber}^\circ - \Delta H_{H_2O}^\circ \tag{126}$$

where $\Delta H_{H_2O}^\circ$ and ΔH_{fiber}° are the heat of solution of the dye in water and the heat of absorption of the dye in the fiber, obtained from measurements of saturation solubilities of the dye in water and in the fiber at different temperatures using equations similar to Eq. (123). From the data available for a considerable range of disperse dyes on cellulose acetate, it is observed that $-\Delta H_{dye}^\circ$ is approximately 10 to 11 kcal/mole;

[353] R. McGregor, *J. Soc. Dyers Colour.* **83**, 477 (1967).
[354] R. McGregor and B. Milicevic, *Nature (London)* **211**, 523 (1966).
[355] O. Exner, *Collect. Czech. Chem. Commun.* **29**, 1094 (1964).

$-\Delta H^{\circ}_{H_2O}$ and $-\Delta H^{\circ}_{fiber}$ range from 14 to 18 kcal/mole and 4 to 7 kcal/mole, respectively. Since most disperse dyes are very similar in size and shape, the relatively small spread in the value of the thermodynamic quantities is understandable. However, the data of McDowell and Weingarten[307] (Table X) indicate that enthalpy values obtained using Eq. (126) are much lower than the measured enthalpy of dyeing as calculated from sorption isotherm data using Eq. (122). The considerable differences in the enthalpy values obtained by the two methods may be due to the following reasons:[307] The heat of dyeing calculated from the sorption isotherm corresponds to a differential heat of dyeing, which is related to the absorption of the first few *dn* moles of dye. Heats of dyeing calculated from saturation solubilities are integral heats of dyeing, i.e., they include the total energy changes corresponding not only to the first *dn* moles of dye absorbed by the fiber, but also the heat of dyeing for interaction of dye molecules with absorbed dye molecules already present in the substrate as the saturation limit is approached. Since more heat will be evolved when the first *dn* moles of dye are absorbed, these integral heats of dyeing will be less than the differential heats of dyeing calculated from sorption isotherms. Correspondingly, the entropies of dyeing calculated from isotherm data are higher than those calculated from solubility data.

Arguments based on thermodynamic changes can also be used to explain certain interesting features of the equilibrium sorption isotherms as a function of temperature, when dyeing is carried out from a saturated aqueous dispersion containing excess solid dye. Under such conditions of dyeing, the equilibrium chemical potentials of the dye in solution and the solid dye should be equal to one another. The situation is therefore similar to direct transfer of dye from the solid to the fiber, and hence involves a smaller enthalpy change than in dyeing from an unsaturated dyebath where the standard heat of dyeing is a large negative quantity. The latter involves a marked decrease in partition coefficient with increase in temperature, i.e., a lower equilibrium dye sorption at higher temperatures for the same equilibrium dyebath concentration; in the transfer of dye from the solid to the fiber the variation of saturation dye sorption will not be so significant. Data illustrating such effects have been reported in the literature.[45,326] The situation is also similar when dyeing from the saturated vapor phase, as shown by the results of Majury.[338]

As mentioned earlier, the partition coefficients for dyeing from the vapor phase are higher by 10^2–10^4 than similar data for aqueous dyeing. Consequently, standard free-energy changes for dyeing from the vapor phase are considerably higher. Comparative data of Jones and Seddon[178]

(Table XIV) show that these differences in affinity cannot be explained entirely in terms of the higher temperatures employed in vapor-phase dyeing, since increases in temperature cause a reduction in the partition coefficient, both for dyeing from the aqueous and the vapor phase.

TABLE XIV

THERMODYNAMIC DATA FOR THE ABSORPTION OF NONIONIC DYES[a]

Dye	*Affinity at 80° from water (kcal/mole)*	*Affinity from vapor, kcal/mole (temp., °C)*	*Heat of absorption from vapor phase (kcal/mole)*
Azobenzene	−4.64	−7.72(105.0)	−17.7
p-Aminoazobenzene	−4.36	−9.93(105.1)	−28.6
1-Methylaminoanthraquinone	−5.23	−10.93(150.0)	−21.8
1-Hydroxyethylamineanthraquinone	−4.55	−13.90(150.0)	−37.3

[a] Reproduced by permission from Jones and Seddon.[178]

Hence, if the data for dyeing from the vapor phase at 80° are extrapolated to the higher temperatures corresponding to vapor-phase dyeing, the differences between the two sets of data for $-\Delta\mu^\circ$ become even greater. Since the more recent studies of Jones and Kraska[342] and Green and Jones[343] have shown that some of the dyes are associated in the vapor phase, the partition coefficient and hence the free-energy change will be reduced due to this factor, although the heat of absorption will not be affected. The heats of absorption from the vapor phase are larger than the heats of absorption from aqueous dyebaths since molecular interactions in solution will tend to retain the dye in aqueous dyebaths. This also explains the comparatively lower values of the affinity $-\Delta\mu^\circ$ in the latter case. Correspondingly, as shown in Table XIII, the entropy change ΔS_s° for dyeing from an aqueous dyebath will be more favorable for dyeing, being less negative than the entropy change ΔS_v° for dyeing from the vapor phase. Majury's results[338] reveal that the values of ΔH° are practically the same for dyeing cellulose acetate or nylon fibers with azobenzene from the vapor phase, being, respectively, −17.7 and −18 kcal/mole, and that the sorption process, which has the same energetics for two chemically dissimilar fibers, cannot be explained in terms of specific interactions between the dye molecules and specific chemical groups in the fiber. The constancy of the isosteric heats of absorption over a considerable range of absorbed dye concentration is indicative of a homogeneity of the mixing process. If it

is assumed that the dye is adsorbed on the fiber surface, the adsorbing surface must act homogeneously.

If the dyeing of hydrophobic fibers with disperse dyes is regarded as a process of solution of the dye in the fiber, it is interesting to apply the solubility parameter concept[356] to determine the heat of mixing ΔH_m from a knowledge of the solubility parameters for the polymer and the dye δ_p and δ_d. The solubility parameter is defined as the square root of the cohesive energy density (ced) which can be directly calculated from the molar heat of vaporization ΔH_v° using the equation

$$\text{ced} = \delta^2 = \left[\frac{\Delta H_v^\circ - RT}{nV}\right] \tag{127}$$

where V is the molar volume. The heat of mixing is then given by the equation

$$\Delta H_m = V_d(\delta_d - \delta_p)^2 \tag{128}$$

where V_d is the molar volume of the dye.

If $\delta_d = \delta_p$, then $\Delta H_m = 0$, i.e., both components are acting ideally and will mix in all proportions. Hence a comparison of the values of ΔH_D° (standard enthalpy change on transporting dye directly from the solid state into the fiber) with the values of ΔH_m calculated from Eq. (128) should give a measure of the ideality of mixing. Jones[357] observed that the calculated value of ΔH_m from Eq. (128) and the experimentally measured value of ΔH_D° ($\simeq 0.3$ kcal/mole), obtained from isotherm data for the absorption of saturated azobenzene vapor by cellulose acetate, were both very small quantities. These results suggest that as regards the heat of mixing the system is reasonably ideal. However, later measurements by Jones and Seddon[178] indicate that ΔH_D° is large (~ 2 kcal/mole) and hence this conclusion may not be quite valid. Ibe[358] has carried out an extensive investigation of the solubility parameter concept as applied to the disperse dyeing of hydrophobic fibers. Correlations of the solubility of several disperse dyes in secondary cellulose acetate, cellulose triacetate, and polypropylene with the calculated solubility parameters of both the polymers and the dyes indicate that this concept should be applicable to the dyeing of strongly hydrophobic fibers such as polypropylene from aqueous solution, or to dyeing from the vapor phase. When this concept is applied to the dyeing of more hydrophilic polymers such as cellulose acetate, the presence of water complicates matters, leading to less satisfactory correlations.

[356] J. H. Hildebrand and R. L. Scott, "The Solubility of Nonelectrolytes," 3rd ed. Van Nostrand-Reinhold, Princeton, New Jersey, 1950.

[357] F. Jones, *J. Soc. Dyers Colour.* 77, 57 (1961).

[358] E. C. Ibe, *J. Appl. Polym. Sci.* **14**, 837 (1970).

Milicevic,[187] assuming a model based on simple mixing theory in which the disperse dye molecules occupy mean positions in a quasi-crystalline or liquid (substrate) lattice, obtained an expression for the partial molar enthalpy of mixing $\Delta\bar{H}$ in terms of the site fractions ϕ and ϕ_m of the dye at the dyeing temperature T and the melting point T_m, respectively, assuming $\Delta\bar{H}$ remains constant over the temperature range T to T_m. Equating $\ln \phi/\phi_m$ with $\ln s/s_m$ where s denotes the saturation value, a linear correlation is observed between T/T_m and $\ln s/s_m$ calculated from experimental saturation values in water and cellulose acetate. While this concept appears to be fruitful, there are serious limitations in the model, as pointed out by Milicevic himself.[187] For example, the model can be applied only to very highly dilute systems with the further assumption that water plays no specific role in the mixing. The assumption that the substrate can be treated as a macromolecular liquid is also quite restrictive. Just as in the case of the solubility parameter concept, it is likely that Milicevic's model may be more applicable to the vapor-phase dyeing of anhydrous hydrophobic fibers at high temperatures.

D. Cationic Dye–Acrylic Fiber System

Acrylic fibers contain 85% or more of polyacrylonitrile $(CH_2{=}CH{-}CN)_n$; "modacrylic fibers" contain 35–85% polyacrylonitrile.[359] Copolymerization with about 10% of methyl methacrylate or vinyl acetate opens up the fiber structure. The use of a potassium persulfate–sodium bisulfite redox system for polymerization results in the introduction at the ends of the polymeric chains of sulfate and sulfonate groups, which impart to the fiber its property of dyeability by cationic dyes. Dye uptake is increased by the addition of small amounts of acrylic acid or alkyl hydrogen sulfate during polymerization; another possible source of acid groups in the fiber is the hydrolysis of a few of the nitrile groups. Commercial acrylic fibers vary in acid group content from about 50 to 150 meq/g; strongly and weakly acid groups may be present in roughly equal proportions, or one of the two types may be preponderant. Orlon 42 (Du Pont) contains a higher proportion of strongly acid groups, and Courtelle E (Courtaulds) contains only weakly acid groups.[61] The microstructure of acrylic fibers is sensitive to temperature; the concentration of void spaces which influences dye diffusion and equilibrium adsorption is a function of the spinning procedure used.[360] Hence, acrylic fiber–dye interaction is also sensitive to these parameters.

Acrylic fibers are mainly dyed with cationic dyes, which are classified

[359] D. R. Baer, in *CSD IV*, p. 161.

[360] M. Takahashi, Y. Nikushina and J. Kosugi, *Text. Res. J.* **34**, 87 (1964).

by Baer[359] as follows: (a) dyes with pendant cations (nonresonating charge); (b) dyes with a delocalized positive charge; (c) amine salts. Cationic dyes are taken up by acrylic fiber only above a critical temperature of about 85°, and dyeing is completed at 95°–98°. The dye liquor is maintained at a pH of about 5 by acetic acid, and the addition of electrolytes such as sodium sulfate moderates dye adsorption by the competition of Na^+ ions with dye cations for anionic sites in the fiber. The addition of certain cationic or anionic retarding agents also moderates dye adsorption by competing with cationic dyes for sites in the fiber or by complex formation between dye and the anionic retarder.

Bonche[361] has studied the dyeing of polyacrylonitrile with varying concentrations and compositions of cationic dyes at constant temperature, but variable electrolyte concentration, using 11 different electrolytes. Anions such as Cl^- and SO_4^{2-} were found to have a minor influence, whereas the nature of the cation caused significant changes in dye uptake in the order $K^+ > Na^+ > Li^+ > Al^{3+}$. He suggests that the retarding effect of the cations is independent of the ionic charge, but depends essentially on factors such as mechanical obstruction of the fiber pores, reduction in the ζ potential by inorganic cation adsorption, and ionic interaction with the acid groups present in the fiber substrate, the major factor being mechanical obstruction. Important parameters are therefore ionic volume of the hydrated cation and its electronegativity. Thus for example, K^+ ion has a greater retarding effect than Na^+ ion due to its smaller ionic volume and more electropositive character. Cegarra[61] has shown that the retarding effect of electrolytes is more effective with fibers like Courtelle which contain weak anionic groups.

Several workers[362–364] have suggested that the cationic dyes are bound as in a salt to acid groups in the fiber. Glenz and Beckmann[58] were the first to study systematically the correlation between site content and equilibrium adsorbed dye concentration. They observed that for several cationic dyes the amount of dye adsorbed at equilibrium was more or less the same. Furthermore, from an analysis of the shape of the measured equilibrium isotherms and the limited extent of adsorption, they concluded that the cationic dyes were bound to a definite number of acidic groups, probably at the ends of the polymer chains. Using a special nonaqueous titration method with sodium hydroxide as well as quantitative studies on the adsorption of a free

[361] M. Bonche, *Teintex* **33**, 519 and 585 (1968).
[362] J. Wegmann, *Text.-Rundsch.* **9**, 341 (1954).
[363] J. F. Laucius, R. A. Clarke, and J. A. Brooks, *Amer. Dyest. Rep.* **44**, 362 (1955).
[364] A. Würz, *Melliand Textilber.* **37**, 83 (1956).

carbinol base, they estimated the acidic group content and found a fairly close correlation between the maximum extent of dye sorption and the number of acidic groups in the fiber. Vogel *et al.*[56] and Weltzein and Fester,[365] from studies on the dyeing of Orlon 42 and Dralon with basic dyes, concluded that the mechanism of dye adsorption consists of an interaction between the basic groups in dye molecules and the acidic groups in the fiber, the number of acid groups ($-SO_3H$) being determined from an estimation of the sulfur content. Rosenbaum[366] also observed that at equilibrium basic dyes are sorbed by acrylic fibers to an extent equivalent to the number of acid groups in the fiber.

Acrylic fibers are reported to have a large negative ζ potential of about -44 mV in water.[367] ζ-Potential studies of acrylic fibers by Glenz and Beckmann[58] have shown that even at very low concentrations of dye in the dyebath the fiber gradually loses its negative potential and becomes slightly positive. Any further increase in dye concentration above the amount required to neutralize the ζ potential does not cause a significant change in the potential. These results indicate that the quantity of adsorbed dye remains almost constant and that electrostatic interactions between the negatively charged fiber surface and the positively charged dyes are important factors in the dyeing mechanism. However, other intermolecular forces such as dispersion forces or dipole forces can also be important.[57]

Most of the equilibrium sorption data obtained by various workers[45,56,58,366,368,369] can adequately be described by a Langmuir sorption isotherm. Balmforth *et al.*[369] have carried out detailed studies of the equilibrium sorption isotherms for several commercial acrylic fibers using cationic dyes such as CI Basic Blue 22, CI Basic Orange 21, CI Basic Violet 7, CI Basic Yellow 12, and CI Basic Red 14. Their results confirmed that the adsorption isotherms could adequately be described by the Langmuir-type equation. Some typical isotherms are shown in Fig. 46. Conductometric titrations of the four commercial acrylic fibers used in these experiments, Acrilan 16, Orlon 42, Dralon, and Dralon Neu gave acid group contents which were approximately one-half the Langmuir intercept saturation value. Remington and Schroeder[45] suggested that in Orlon fibers the active sites for basic dye adsorption appear to be acidic groups introduced during polymerization and that these groups are neutralized by colorless cations in the undyed

[365] W. Weltzein and W. Fester, Dissertation, Aachen (1960) (see Beckmann[57]).

[366] S. Rosenbaum, *Text. Res. J.* **33**, 899 (1963).

[367] T. Vickerstaff and G. Weston, unpublished results (see Beckmann[57]).

[368] C. Calin, E. Ionesco, and F. Bril, *Teintex* **30**, 775 (1965).

[369] D. Balmforth, C. A. Bowers, and T. H. Guion, *J. Soc. Dyers Colour.* **80**, 577 (1964).

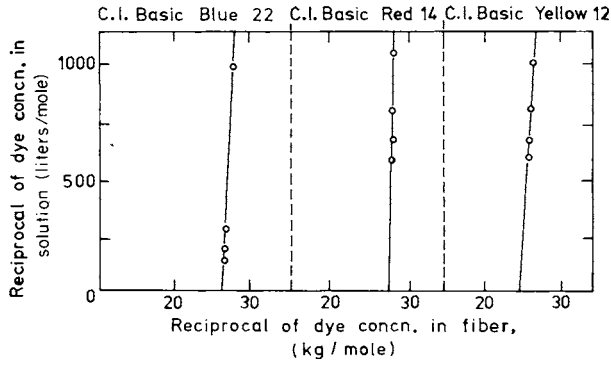


FIG. 46. Langmuir adsorption of cationic dyes on acrylic polymer. Reproduced by permission from Balmforth *et al.*[369]

fibers. Dyeing was supposed to occur through ion exchange with the colorless cations already present in the fiber, these being mainly Na^+ and K^+ ions. Balmforth *et al.*[369] observed that the removal of these colorless cations from the different acrylic fibers using an ion-exchange process gave measured acid group contents equivalent to the Langmuir saturation values (Table XV). This correlation was further confirmed by estimating acidic group content using two other methods—determination of sulfur content and of number-average molecular weights. These data appear to favor an ion-exchange mechanism for the dyeing process.

In keeping with such a mechanism, Balmforth *et al.*[369] also observed that the addition of electrolytes to polymer–dye systems in which the

TABLE XV

ACIDIC DYE SITES OF VARIOUS ACRYLIC FIBERS[a]

Fiber	*Concentration of dye sites (μequiv/g)*	
	From Langmuir intercept	*By conductance titration (after ion exchange)*
Acrilan 16 (Chemstrand)	36	41
Orlon 42 (DuP)	54	54
Dralon (FBy)	44	44
Dralon Neu (FBy)	55	58

[a] Reproduced by permission from Balmforth *et al.*[369]

polymer has been freed from metallic cations by an ion-exchange treatment caused a decrease in dye uptake due to competition with the dye for acid groups in the polymer. With increasing pH beyond 6, there was a significant increase in dye uptake for most of the commercial acrylic fibers examined (Fig. 47). This increase in dye adsorption at high pH was attributed to the presence not only of strong acidic groups in the fibers, but also additional weak acid groups having ionization constants similar to those of carboxylic acids, because the dissociation of strong anionic groups is not very much dependent on changes in pH, whereas with increasing pH weak anionic groups dissociate more easily.

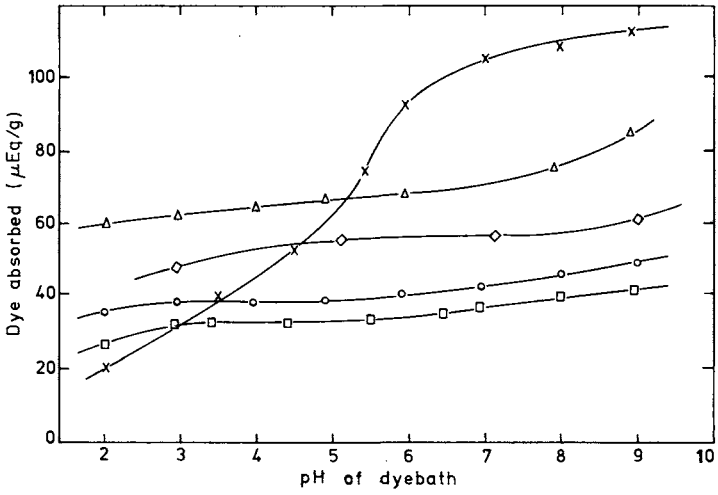


FIG. 47. Variation of the amount of dye adsorbed with pH: ×, Courtelle E; ○, Acrilan 16; △, Beslon; □, Acribel; ◇, Orlon 42. Reproduced by permission from Balmforth *et al.*[369]

The presence of such weak groups was established by a special titration technique.[370] In no case did the dye adsorption exceed the total number of acidic groups. These results are in good agreement with the hypothesis that dyeing takes place by ion exchange, whereby the dye cation displaces H^+, Na^+, or K^+. The continuous increase of dye uptake with pH in Fig. 47 for Courtelle E is attributed to the presence of only weak acid groups in the fiber, the dissociation in such cases being markedly dependent on pH. The correlations shown in Table XV are valid only at the lower pH values, where the interaction appears to take place between sulfate and sulfonate groups in the polymer (strong acid sites)

[370] J. R. Kirby, unpublished results (see Balmforth *et al.*[369]).

and the dye cation. From sorption measurements of basic dyes in polyacrylonitrile, Sand[371] obtained data for the ratios of self-diffusion coefficients of Na^+ and dye cation at 90° and 100° which appear to show satisfactory agreement with the calculated ratios based on an ion-exchange theory.

Rosenbaum[366,372] has discussed the mechanism of dyeing acrylic fibers with cationic dyes in terms of an ion-exchange process. For example, the ion-exchange equilibrium may be written as

$$\text{Fiber—SO}_3\text{H} + \text{D}^+ \rightleftharpoons \text{Fiber—SO}_3\text{D} + \text{H}^+ \tag{129}$$

with an exchange constant given by the equation

$$K_{\text{H}}^{\text{D}} = \frac{[\text{D}_\phi][\text{H}_\sigma]}{[\text{H}_\phi][\text{D}_\sigma]} \tag{130}$$

A general assumption is made that concentrations can be equated to activities both in the solution phase and in the fiber phase in view of the low concentrations of all the ionic and molecular species present in the system. If only H^+ ions are exchanged for the dye ions, Eq. (130) can be written as a Langmuir isotherm:

$$\frac{[\text{D}_\phi]}{S_1 - [\text{D}_\phi]} = \frac{K_{\text{H}}^{\text{D}}}{[\text{H}_\sigma]}[\text{D}_\sigma] = K_{\text{L}}[\text{D}_\sigma]$$

or

$$\frac{[\text{D}_\phi]}{[\text{D}_\sigma]} = \frac{K_{\text{H}}^{\text{D}}}{[\text{H}_\sigma]}(S_1 - [\text{D}_\phi]) = K_{\text{L}}(S_1 - [\text{D}_\phi]) \tag{131}$$

where S_1, the total number of acid groups in the fiber, is given by

$$S_1 = [\text{D}_\phi] + [\text{H}_\phi] \tag{132}$$

If, on the other hand, other competing ions such as Na^+ are also present, an allowance has to be made for an additional exchange coefficient K_{Na}^{D} given by the equation

$$K_{\text{Na}}^{\text{D}} = \frac{[\text{D}_\phi][\text{Na}_\sigma]}{[\text{Na}_\phi][\text{D}_\sigma]} \tag{133}$$

corresponding to the equilibrium

$$\text{Fiber—SO}_3\text{Na} + \text{D}^+ \rightleftharpoons \text{Fiber—SO}_3\text{D} + \text{Na}^+ \tag{134}$$

[371] H. Sand, *Kolloid-Z. Z. Polym.* **218**, 124 (1967).
[372] S. Rosenbaum, *Text. Res. J.* **34**, 159 (1964).

The relevant isotherms obtained are then given by

$$\frac{[D_\phi]}{(S_1 - [H_\phi] - [D_\phi])} = \frac{K_{Na}^{D}}{[Na_\sigma]}[D_\sigma] \tag{135}$$

and

$$\frac{[D_\phi]}{(S_1 - [Na_\phi] - [D_\phi])} = \frac{K_{H}^{D}}{[H_\sigma]}[D_\sigma] \tag{136}$$

where $S_1 = [D_\phi] + [Na_\phi] + [H_\phi]$.

Deviations of the experimental data from Eqs. (135) and (136) have been attributed by Rosenbaum[366] to electrostatic repulsive interactions between the acidic sites, i.e., the sites are not equivalent independent sites as strictly required by the Langmuir theory. As evidence for such interactions, he points out[372] that although the equilibrium dye uptake of polyacrylonitrile fibers increases with sulfonate site content, the increase is less than proportional to the increase in site content, suggesting unfavorable interactions between sites. Furthermore, he assumes[366] that, unlike the situation in aqueous solutions where sulfonic acids behave like strong acids, the ionization of sulfonic acid groups attached to the fiber is suppressed to a large extent by the lower dielectric constant of the fiber phase. However, deviations from Langmuir isotherms can arise from the use of concentrations instead of activities, the presence of more than one type of adsorption site, and uncertainty regarding the actual values of the exchange constants.

In more recent work Harwood *et al.*[373] have studied the sorption isotherms for five basic dyes on acrylic films, and have suggested that the simple ion-exchange mechanism given by Eq. (131) is adequate to explain the results over 80% of the concentration range. The strongly acid groups in the fiber consist of 80% sulfonic groups (S_1) and 20% sulfate groups (S_2). In most cases the apparent saturation values from standard linear Langmuir plots in the form $[D_\phi]/[D_\sigma]$ vs. $[D_\sigma]$ are remarkably consistent for all the dyes and correspond to S_1. These isotherms, however, show "tails" at the higher adsorbed dye concentrations indicating adsorption on more sites than correspond to S_1. This additional sorption value could be correlated with S_2. Some typical results are shown in Fig. 48. Harwood *et al.*[373] further suggest that, alternatively, the results obtained over the whole concentration range ($S_1 + S_2$) can be explained by a simple ion-distribution mechanism over the whole range. The basic aspects of such a mechanism,[167,249,374]

[373] R. J. Harwood, R. McGregor, and R. H. Peters, *J. Soc. Dyers Colour.* **88**, 216 (1972).

[374] R. McGregor, *Colourage* (*Annu.*) p. 1 (1970).

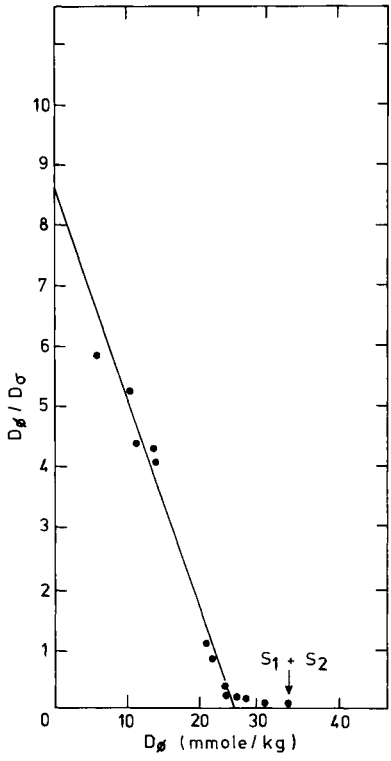


FIG. 48. Typical Langmuir adsorption isotherms for CI Basic Violet 19 on acrylic film. Temperature, 99.3°; pH 3.4. Reproduced by permission from Harwood *et al.*[373]

which give a satisfactory explanation for the dyeing of nylon fiber with acid dyes including overdyeing phenomena, have been discussed in detail in Section II,B,1. Interesting features of this approach are that there is no necessity to assume any particular ion-exchange mechanism, the distribution of all ions present being taken into account by using distribution coefficients based on a Donnan equilibrium for various ionic species present in the system with the corresponding conditions of electroneutrality. On this basis, Harwood *et al.*[373] obtained the following equation for the sorption process:

$$([X_\phi] + [OH_\phi]) = KK_D\left(\frac{[D_\sigma]}{[D_\phi]}\right)([D_\sigma] + [Na_\sigma] + [H_\sigma]) \qquad (137)$$

This equation shows that a significant anion concentration $[X_\phi]$ can provide an explanation for the deviations observed by Rosenbaum[366] using Eq. (135) or Eq. (136) (see also McGregor[374]). Furthermore, these effects would be more apparent for dyes having high K_D values, i.e.,

dyes having a high affinity. The "tails" in the isotherm data shown in Fig. 48 correspond to an overdyeing mechanism, the explanation being similar to that discussed in Section II,B,1 for the dyeing of nylon with acid dyes.

In a more recent publication McGregor *et al.*[374a] have directly measured the distribution of dye ions and inorganic electrolyte cations between acrylic fibers and aqueous solutions. They calculated the K_{Na}^{D} values from these experimental data using Eq. (133). However, a comparison of these values with the value obtained from the Langmuir isotherm described by Eq. (135) showed a considerable discrepancy between theoretical and experimental values. These results also emphasize the importance of the direct measurement of ionic distributions before drawing conclusions from graphical representation of the isotherm data.

Since thermodynamic data have not been reported in the literature for the equilibrium sorption of cationic dyes on acrylic fibers, a discussion of this aspect of the dyeing mechanism is not possible at the moment.

E. Dye–Fiber Bonding

The process of dye uptake in the various dye–fiber systems is influenced by five kinds of binding forces[168]:

(1) Electrostatic or coulombic interactions, which may be of the attractive or repulsive type, are of considerable importance in the adsorption of dye ions on charged textile substrates.

(2) The polar nature of the dye and the substrate gives rise to direct or induced dipolar interactions, which are also electrostatic in origin, but somewhat different from coulombic forces. The energy of interaction depends on the orientation of the dipoles, which is therefore an important parameter in this type of bonding.

(3) Hydrogen bonding is a special type of polar interaction which has been very frequently invoked to explain dye–fiber bonding. The presence of water which can compete with the dye for binding on hydrophilic textile substrates suggests that hydrogen bonding may not be of much importance in such systems; but if there is a high probability of favorable orientation between, for example, negative ions on the fiber surface and groups in the dye molecule containing hydrogen atoms, such bonding can be of importance.

(4) Important binding forces in dye–fiber interactions, which are completely general in occurrence, are dispersion or van der Waals forces. They are always of the attractive type and decrease very rapidly

[374a] R. McGregor, T. H. Guion, T. M. A. Hossain, and J. R. Thagard, *J. Soc. Dyers. Colour.* **89**, 409 (1973).

with distance. The strength of binding requires an effective contact over large areas of the adsorbent surface and adsorbate molecules.

(5) Another important type of binding force, which in recent years has been found to be significant in solute–macromolecule interactions including dye–macromolecule interactions in aqueous solutions, is hydrophobic bonding. Hydrophobic bonding in dye–fiber interactions has been discussed in Section II,B,2, with special reference to the wool fiber–acid dye system, and it may be of general importance in most fiber–dye systems.[233,294]

The relative importance of dye binding forces for various textile fibers is discussed below.

In the case of cellulose fibers, the basic requirements for dye substantivity[375] which have been suggested are (a) a linear configuration of the molecule, (b) ability of the aromatic nuclei to assume a planar configuration,[376] and (c) the presence of hydrogen-bond-forming groups. A linear configuration is not essential for dye substantivity.[375,377,378] The coplanarity hypothesis has been extensively studied experimentally for various dye–cellulose systems.[316,377,379,380] Most of the available evidence indicates that this is an important requirement for dye substantivity. Schirm's[381] suggestion that substantivity is associated with a polynuclear system of conjugated double bonds is another way of expressing the coplanarity hypothesis, since resonance in such a system promotes a coplanar configuration. Several workers[18,286,325,377,382] suggested that the flat molecular configuration resulting from coplanarity allows a close approach and alignment of the dye molecule with respect to the fiber surface so that van der Waals forces become operative. Since these are short-range forces varying inversely as the sixth power of the distance and are additive over all the atoms that are sufficiently close together, maximum substantivity requires a close fit between large areas of the adsorbent surface and the adsorbate molecule. Further evidence is the correlation between affinity and molecular weight.[383] Giles and Hassan[384] have pointed out, using Peters and

[375] *CSD II*, p. 1273.

[376] H. H. Hodgson, *J. Soc. Dyers Colour.* **49**, 213 (1933).

[377] E. H. Daruwalla, *Palette* **25**, 30 (1967).

[378] R. H. Peters and H. H. Sumner, *J. Soc. Dyers Colour.* **71**, 130 (1955).

[379] N. J. Bhat, E. H. Daruwalla, and S. S. Rao, *Text.-Rundsch.* **20**, 133 (1965).

[380] A. Cameron, C. H. Giles, and T. H. MacEwan, *J. Chem. Soc., London* p. 1224 (1958).

[381] E. Schirm, *J. Prakt. Chem.* [N.S.] **144**, 69 (1935).

[382] A. N. Derbyshire and W. J. Marshall, *Discuss. Faraday Soc.* **16**, 140 (1954).

[383] T. Vickerstaff, *J. Soc. Dyers Colour.* **69**, 279 (1953).

[384] C. H. Giles and A. S. A. Hassan, *J. Soc. Dyers Colour.* **74**, 846 (1958).

Sumner's data,[378] that there exists a close correlation between the affinity and molecular weights of vat dyes. The dispersion energy tends to be large for all colored substances since these have low-lying electronically excited states.[385]

Lead[386] argues that, since dispersion forces fall off rapidly with distance, they would be reduced to a negligible value even if the surfaces of dye and cellulose are separated by only a single layer of water molecules. He therefore gives an alternative explanation for dye cellulose substantivity. All dyes which have high substantivities, whether they are direct dyes, vat dyes, naphthols, or natural coloring matters such as bixin, possess an extended conjugate system of π electrons which forms an intermediate layer between cellulose and the regularly arranged water molecules. Under such conditions, substantivity is achieved by interaction between delocalized π electrons and the hydroxyl groups of cellulose, even when they are solvated. Robinson[387] and Bamford[388] have also indicated the possibilities of electron interactions in such systems. Using the refractive index method, studies by Yoshida *et al.*[389] on complex formation between aromatic sulfonic acids and carbohydrates in aqueous solutions indicate the presence of weak complexes. Meybeck,[390] from studies on the dyeing of cellulose with the usual monoazo and bisazo direct dyes, concludes that interaction between π electrons of the conjugated system in the dye and the hydroxyl groups of cellulose is important for dye substantivity.

Sivaraja Iyer *et al.*[233] have shown that the influence of different alkali metal cations on direct dye adsorption can be explained in terms of the water structure-breaking effect of the cation, which enables close contact to be obtained between adsorbent and adsorbate molecules by removal of the intermediate layer of bound water between the hydrophobic parts of the dye molecule and the hydrophobic parts of the cellulose surface (Section II,A). This close contact would assist bonding through dispersion forces. Hence the argument put forward by Lead[386] against the effectiveness of such dispersion forces need not necessarily be valid. In addition, an entropy effect due to the release of bound water molecules can contribute to dye substantivity. Such a possibility has also been discussed by Zollinger.[294]

Substantivity due to hydrogen bond formation has been correlated

[385] H. C. Longuet-Higgins, *Discuss. Faraday Soc.* **40**, 7 (1965).
[386] W. L. Lead, *J. Soc. Dyers Colour.* **73**, 464 (1957); **75**, 195 (1959).
[387] C. Robinson, *Discuss. Faraday Soc.* **16**, 125 (1954).
[388] C. H. Bamford, *Discuss. Faraday Soc.* **16**, 229 (1954).
[389] Z. I. Yoshida, E. Osawa, and R. Oda, *J. Phys. Chem.* **68**, 2895 (1964).
[390] J. Meybeck, *Teintex* **31**, 699 (1966).

with a correspondence between the 10.3–10.4 Å repeat distance of the cellobiose unit and the distance of separation of the hydrogen-bond-forming groups in the dye molecule.[27,391] However, careful studies by Robinson,[387] using atomic models, have shown that the spacing of hydrogen-bond-forming groups is not critical. Venkataraman,[375] Zollinger,[392] and Nursten[393] have also pointed out that for several substantive dyes there is no correlation between the 10.3–10.4 Å spacing and the spacing of hydrogen-bond-forming groups in the dye molecule. A more serious consideration against hydrogen bonding is that the hydroxyl groups of cellulose are too firmly solvated by water to form hydrogen bonds with the dye molecule, as shown by Giles and co-workers[242,394–396] from the results of refractive index measurements and surface-film formation. They suggest that the main source of affinity is van der Waals attraction between dye molecules and the cellulose surface. Comparative studies[384] of dye adsorption on chitin (which adsorbs by hydrogen bonding) and cellulose also do not favor the hydrogen-bonding hypothesis. Gill[397] has argued against hydrogen bonding on the grounds that since water forms hydrogen bonds with both cellulose and dye, the formation of a single hydrogen bond between dye and cellulose involves the rupture of two hydrogen bonds, which he considers improbable on purely energetic grounds. He therefore suggests that dispersion forces are responsible for dye substantivity. The high substantivity of certain carbocyclic vat dyes cannot be explained by a hydrogen-bonding mechanism.[378,375] Wirz and Zollinger[398] have shown that the quaternized benzidine and *m*-tolidine salts have an affinity for cellulose even though they are not able to form hydrogen bonds. They suggest that the affinity is mainly or exclusively due to van der Waals forces. Wegmann[399] suggests that ion–dipole interactions between dipoles on ether groups in the cellulose chain and ionic groups in the dye molecules can contribute to dye substantivity. Coulombic forces do not favor the adsorption of direct dyes on cellulose fibers since the

[391] F. L. Rose, unpublished results (see Vickerstaff,[18] p. 180).

[392] H. Zollinger, *Discuss. Faraday Soc.* **16**, 123 (1954).

[393] H. E. Nursten, *Discuss. Faraday Soc.* **16**, 231 (1954).

[394] F. M. Arshid, C. H. Giles, and S. K. Jain, *J. Chem. Soc., London* p. 559 (1956).

[395] F. M. Arshid, C. H. Giles, E. C. McLure, A. Ogilvie, and T. J. Rose, *J. Chem. Soc., London* p. 67 (1955).

[396] M. M. Allingham, C. H. Giles, and E. L. Neustädter, *Discuss. Faraday Soc.* **16**, 92 (1954).

[397] R. Gill, *J. Soc. Dyers Colour.* **71**, 380 (1955).

[398] R. Wirz and H. Zollinger, *Helv. Chim. Acta* **43**, 1738 (1960).

[399] J. Wegmann, *Amer. Dyest. Rep.* **51**, P276 (1962).

adsorption of dye anions immediately leads to coulombic repulsion effects which prevent adsorption of more dye anions (Section II,A).

Taking into account all the available evidence, it is likely that the main contribution to cellulose–dye substantivity arises from van der Waals forces. The formation of polar bonds and the water structure-breaking effect of electrolyte cations help the dye adsorption process by eliminating hydrated water around polar groups on the dye and the substrate. This would enable a sufficiently close approach of dye and fiber surfaces so that dispersion forces can bind the dye firmly to the cellulose surface.

In cellulose acetate–disperse dye systems, an important requirement for dye substantivity appears to be a coplanar structure for the dye molecule; Daruwalla *et al.*[316] observed that nonionic dyes having a planar structure were adsorbed more readily by cellulose acetate and had much higher affinity than the corresponding nonplanar dyes. Some typical results are shown in Fig. 44. Bhat *et al.*,[379] from a study of adsorption isotherms and affinity values for pairs of quinonoid disperse dyes, found that for each pair of dyes the affinity values were higher for the coplanar dye than for the corresponding non-coplanar analog. They suggest that the higher affinity of the coplanar dye is due to the closer alignment between the dye and polymer chain molecules resulting in a greater possibility of van der Waals forces becoming operative between dye and substrate. Monolayer studies by Giles *et al.*[400] have shown that a strong 1:1 complex is formed by face-to-face packing between planar disperse dyes and the hexacetyl-cellobiose residues in cellulose triacetate. The tendency for the partition coefficient between cellulose acetate and water to increase with increasing molecular weight can be regarded as some evidence for dispersion forces between dye and substrate.[324] As discussed in Section II,C, the strength of dye–fiber interactions in such systems is very similar to the strength of dye–dye interactions in the solid dye. Furthermore, ΔH° values for azobenzene on nylon or on cellulose acetate are practically identical. These results can be explained on the assumption that the same type of forces is involved in dye–dye and dye–fiber interactions and that these forces are most probably dispersion forces.[286] The large affinity of small hydrophobic disperse dye molecules for hydrophobic fibers such as polyester, cellulose acetate, and nylon also indicates that nonpolar forces are of importance (Section II,B,2).

A hydrogen-bonding mechanism was first postulated by Marsden and Urquhart[401] to explain the very high adsorption of simple proton-donor

[400] C. H. Giles, V. G. Agnihotri, and A. S. Trivedi, *J. Soc. Dyers Colour.* **86**, 441 (1970).
[401] R. J. B. Marsden and A. R. Urquhart, *J. Text. Inst.* **33**, T105 (1942).

solutes such as phenol and aniline by cellulose acetate, and Vickerstaff[18] extended this idea to explain disperse dye adsorption. However, it is now known that the polymer can act both as a proton donor and a proton acceptor in binding with polar groups or the π-electron system in a dye molecule.[395,397,402] Evidence for such mechanisms has been obtained from monolayer studies[380,397,403] and refractive index measurements in binary mixtures of polymers or model compounds and dyes.[404] Suda and Ujigawa[405] determined the affinity values for the interaction between anthraquinonoid disperse dyes and aliphatic esters and ketones. The increase in $-\Delta F$ values with decrease of electron density on the α-CH group was explained in terms of hydrogen bonding at the α-CH group. The ability of the polymer to act both as a proton donor and a proton acceptor has also been shown by de Vries and co-workers[406] using gas–liquid chromatography to measure heats of reaction between cellulose acetate and a large number of model compounds. They conclude that phenols and aliphatic alcohols act as proton donors to C=O and OH groups in the secondary cellulose acetate, while aromatic hydrocarbons act as electron donors and the acetate group as an electron acceptor. Adsorption of haloforms is explained by electron transfer from the C=O of the acetate groups to protons in chloroform, bromoform, and iodoform, the heat of adsorption increasing in the indicated order. These results show the importance of both hydrogen-bonding and electron-transfer mechanisms (π-bonding) in disperse dye binding. Other evidence for polar bonding is the correlation between the number of hydrogen atoms available for intermolecular bonding in multifunctional azobenzene dyes and their maximum absorption by cellulose acetate.[324] The influence of polar bonding in disperse dye–hydrophobic fiber systems is shown by the fact that polypropylene, which contains no hydrogen-bonding or ionic groups, can only be dyed to weak fugitive shades; dye uptake here is due to van der Waals forces unless the fiber is modified by incorporation of active groups.[53,407]

Summarizing the available data on bonding in hydrophobic fiber–disperse dye systems, the main types of bonds are hydrogen bonds (with

[402] D. S. Campbell, D. Cathcart, and C. H. Giles, *J. Soc. Dyers Colour.* **73**, 546 (1957).

[403] M. Muramatsu, *Bull. Chem. Soc. Jap.* **32**, 114 and 391 (1959).

[404] Y. Suda and H. Ujigawa, *J. Soc. Fiber Sci. Technol. Jap.* **20**, 617 and 731 (1964); *J. Soc. Dyers Colour.* **81**, 82 and 182 (1965).

[405] Y. Suda and H. Ujigawa, *J. Soc. Text. Cell. Ind. Jap.* **19**, 563 (1963); *J. Soc. Dyers Colour.* **79**, 557 (1963).

[406] M. J. de Vries and J. H. Smit, *J. S. Afr. Chem. Inst.* **20**, 11 (1967); J. H. Smit, J. H. Smith, and M. J. de Vries, *ibid.* p. 144; M. J. de Vries, J. H. Smit, and H. G. Raubenheimer, *ibid.* **21**, 47 (1968) (see Giles[53]).

[407] C. L. Bird and A. M. Patel, *J. Soc. Dyers Colour.* **84**, 560 (1968).

smaller contributions from other polar forces) and van der Waals forces, which come into operation when the dye is attracted near enough to the substrate surface by polar interactions.

The mechanism of acid dye adsorption on wool fiber and the relevant thermodynamic data, which have been discussed in Section II,B,2, indicate that van der Waals forces and hydrophobic bonding are of major importance in dye–fiber bonding. Hydrogen bonding is relatively unimportant, its contributions being masked by ion-exchange and dispersion forces in addition to hydrophobic bonding. Meggy[97] has pointed out that hydrogen bonding can be regarded as relatively unimportant since hydroxyl groups in most dyes are already chelated with another group in the dye molecule. The more recent mechanisms[167,249,373] which have been put forward to explain ionic dye adsorption on nylon and acrylic fibers consider the electrostatic effects purely in terms of their influence on ion distribution between dyebath and substrate, no specific charged sites for binding being considered necessary. Coulombic interactions can therefore be regarded as providing a more favorable distribution for dye anions in the substrate phase, the bonding being mainly due to a close contact between the hydrophobic parts of the dye anions and the hydrophobic parts of the substrate.

The adsorption of cationic dyes by acrylic fibers is usually explained in terms of an ion-exchange process, but, as discussed earlier, the mechanism of adsorption can also be explained without postulating specific interactions between the oppositely charged groups on the fiber and the dye ion. Feitchmayr and Würz,[408] in a discussion of the interaction forces between polyacrylonitrile fibers and disperse dyes or cationic dyes, consider that dipole–dipole and dipole–induced dipole interactions between disperse dye and nitrile groups, as well as dispersion forces, are of major importance. Dyeing polyacrylonitrile fiber not containing anionic groups with cationic dyes involves ion pairs, stabilized to a varying extent as dipoles by interaction with the dipoles of the fiber. In polyacrylonitrile containing anionic groups, ion-pair formation occurs between the substrate anions and dye cations; it is likely, as in other ionic fiber–ionic dye systems, that polar interactions of this type are important in that they enable a closer contact between the dye and the fiber for effective nonpolar bonding between the hydrophobic fiber surface and the hydrophobic parts of the dye molecule. Lack of sufficient experimental data on adsorption isotherms and the associated thermodynamic parameters for such systems makes it

[408] F. Feitchmayr and A. Würz, *J. Soc. Dyers Colour.* **77**, 626 (1961).

difficult to assess more specifically the contributions from the various types of binding forces.

Since dye–fiber bonding and dyeing mechanisms involve the participation of dye, substrate, and dyebath medium, the following factors should also be taken into account: the structure of the fiber, the cohesive forces between the polymer molecules in the fiber substrate, and the influence of water, swelling agents, etc., which open up the fiber structure and enable a more ready diffusion of the dye molecules. Dye–substrate interactions can, in addition, lead to configurational changes in either or both of the components.[168] Most dye molecules which exhibit substantivity for the fiber also associate in solution. The dyeing of hydrophobic fibers with the low molecular weight, weakly polar, sparingly soluble disperse dyes (Section II,C) provides a good example of the interplay of the various effects mentioned above. Another example, discussed in Section II,A, is the effect of dye–dye binding in aqueous solutions on the energy of dyeing of different fiber systems. Stacking phenomena[409] in protein–dye interactions is an example of dye–dye binding on the adsorbent surface, which in turn helps dye adsorption by the protein molecules. Giles[325] suggests that in some cases dye–dye affinity on adsorption may be a major binding force rather than dye–fiber affinity.

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[409] D. F. Bradley and M. K. Wolf, *Proc. Nat. Acad. Sci. U.S.* **45**, 944 (1959).

Chapter V

APPLICATIONS OF SYNTHETIC DYES TO BIOLOGICAL PROBLEMS

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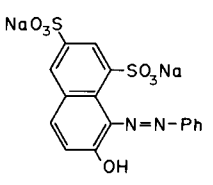
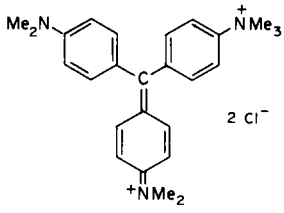
I. Introduction

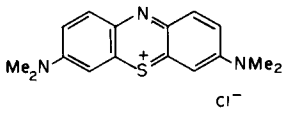
The first application of a colorant (saffron in alcohol) to differentiate between the various parts of cellular and intercellular tissue dates back to van Leuwenhoek[1] in his study of mammalian muscle. The first synthetic dye to be used for this purpose was "Lilac Purple,"[1a] probably identical with Perkin's Mauve. The advantages of using a mixture of contrasting colors to produce differential staining were noticed by Schwartz.[1b] Schwartz's procedure of using ammonium carminate followed by picric acid was modified in the following year by Ranvier,[1c] who combined the two solutions into a single "picrocarmine" stain which is still in use today. Ehrlich[1d,e] made the first attempt to relate staining to the chemical character of dyes and of tissue constituents: acid and basic dyes, respectively, colored basic and acidic constituents, and the neutral product obtained by mixing an acid dye (such as



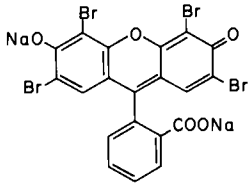
Orange G
CI Acid Orange 10
CI 16230



Methyl Green
CI Basic Blue 20
CI 42585



Methylene Blue
CI Basic Blue 9
CI 52015



Eosine
CI Acid Red 87
CI 45380

[1] A. van Leuwenhoek, "Epistolae Physiologicae super Compluribus Naturae Arcanis." Delphis, Berman, 1719.

[1a] B. Beneke, *Corresp. Ver. Gemeinsch. Arbeit.* **59**, 980 (1862).

[1b] E. Schwarz, *Akad. Wiss. Lit., Mainz Abh., Math.-Naturwiss. Kl.* **55**, *Abt. 1*, 671–691 (1867).

[1c] L. Ranvier, *Arch. Physiol.* **1**, 319–321 (1868).

[1d] P. Ehrlich, *Arch. Anat. Physiol., Physiol. Abt.* 571–579 (1879a).

[1e] P. Ehrlich, *Z. Klin. Med.* **1**, 553–560 (1879b).

Orange G) and a basic dye (such as Methyl Green) colored "neutrophils." The differential coloring of blood smears was thus achieved. Ehrlich was also the first to use staining techniques for diagnosis by distinguishing between normal and disease cells, and he laid the foundations of antibacterial chemotherapy by a search for dyes which could selectively combine with and destroy pathogenic organisms without damage to the host. In 1884 Gram[1f] developed a method for the differentiation of bacteria (gram positive and gram negative) by treatment with Gentian Violet and iodine, and a few years later Romanowsky[1g] discovered the nucleus of the malarial parasite by the differential dyeing of a blood smear with a mixture of Methylene Blue and Eosine.

At the present time synthetic dyes are employed in biology for many purposes. One use, of course, is as pH and redox indicators. A major use is in the staining of live and fixed tissues prior to microscopic examination.[1h–6] Classical microscopic morphology was mainly concerned with fixed and stained specimens, and the introduction of the phase-contrast microscope made possible the observation of living cells. The recent commercial availability of fluorescence microscopes has led to the rapidly increasing application of fluorescent dyes and colorless fluorescent compounds.

The numerous references to *CSD I* and *II* in a book on enzyme histochemistry[7] demonstrate the applicability of chromogenic reactions, especially azo dye formation, for following enzyme activity.

Synthetic dyes and color-formers find use in the elucidation of structural features of proteins and nucleic acids through a study of dye-binding isotherms and the site specificity of some interactions,

[1f] C. Gram, *Fortschr. Med.* **2**, 185–189 (1884).

[1g] D. L. Romanowsky, *St. Petersb. Med. Woch.* **16**, 297–302 307–315 (1891).

[1h] J. R. Baker, "Principles of Biological Microtechnique." Wiley, New York, 1958.

[2] R. D. Lillie, "H. J. Conn's Biological Stains," 8th ed. Williams & Wilkins, Baltimore, Maryland, 1969.

[2a] The Commission also publishes a journal, *Stain Technology* (Vol. 1, 1926), and a laboratory manual "Staining Procedures."

[3] H. Harms, "Handbuch der Farbstoffe für die Mikroskopie." Staufen Verlag, Kamp-Lintfort, 1965.

[4] A. G. E. Pearse, "Histochemistry, Theoretical & Applied," 3rd ed. Little, Brown, Boston, Massachusetts, 1968.

[5] R. D. Lillie, "Histopathologic Technic and Practical Histochemistry," 3rd ed. McGraw-Hill, Blakiston, New York, 1965.

[6] E. Gurr, "Rational Use of Dyes in Biology," Leonard Hill, London, 1965. See also the extensive bibliography in Part 4 of this book; E. Gurr, "Staining Animal Tissues." Leonard Hill, London, 1962; "Encyclopaedia of Microscopic Stains." Leonard Hill, London, 1960; "Synthetic Dyes in Biology, Medicine, and Chemistry." Academic Press, New York, 1971.

[7] M. S. Burstone, "Enzyme Histochemistry and its Application in the Study of Neoplasms." Academic Press, New York, 1962.

covalent as well as noncovalent. Because of the high reactivity of diazonium salts toward amines and phenols under mild conditions, the coupling of diazonium salts with proteins has been extensively used for the estimation of histidine and tyrosine, for probing active sites of enzymes, and for the modification of proteins in immunological studies.

Limitations of space and the complexity of biological materials have restricted this chapter to a bare outline of the present status of synthetic dyes and dye intermediates in the study of biological problems. During the last few years, many books and reviews in this area, some of which we have drawn on heavily, have appeared, and the relevant references are cited to enable the reader to make a detailed study. The objectives of including a chapter on the use of dyes in biology in this series of volumes are to give synthetic dye chemists an insight into applications with which they will not normally be familiar, and to indicate to biologists the wealth of dyes and dye-forming reactions which are available to them.

II. Biological Stains

A. Commercial Stains and Their Standardization

The need for the standardization of stains led to the establishment in the United States in 1922 of the Commission on Standardization of Biological Stains, an independent nonprofit corporation having two main objectives: (a) to collect information on the nature of stains and staining procedures in cooperation with chemists and biologists, and (b) to cooperate with manufacturers and sellers of stains in evolving specifications and test methods so that only stains of approved quality reach the user. The presence of minor quantities of related dyes or other impurities present in a dyestuff sample is believed to account sometimes for its success as a useful biological stain.[2] Performance tests on each batch of a product are therefore carried out in addition to optical and chemical tests. (See Appendix II in reference 2 for details of test methods.) In the United States the Commission Certified Stains are marked "C.C."[2a] The Stain Commission's approach to the standardization of dyes for biological staining is not satisfactory, at least so far as the research worker is concerned. The "certified" label may give the erroneous impression that the certified dyes can be used for purposes other than staining by standard procedures.[8,8a] The presence of unknown impurities in a dye will be a limiting factor in the understanding and quantitative interpretation of dye–substrate interaction. The number of

[8] R. D. Lillie and P. Pizzolato, *Histochemie* **17**, 138 (1969).

[8a] See also R. W. Horobin, *Histochem. J.* **1**, 231 (1969).

dyes on certification basis is less than 50. Until recently, biologists have been somewhat conservative in their choice of stains; but as biological staining is becoming less of an art and more of a science, the variety of dyes used in biology has increased very considerably.[6]

Chromatographic analysis of dyes was discussed in *CSD II* (pp. 1307–1332), and there is now an extensive literature on the subject, which will be reviewed in Vol. VIII. The very brief treatment (pp. 56–57) of this topic in a standard book on biological stains[2] indicates the need for biologists to submit the dyes used by them to chromatographic analysis and to seek the cooperation of color chemists for the isolation of analytically pure dyes from commercial products. A less time-consuming, but more expensive alternative will be to purchase dyes and made-up stains from reputable manufacturers who supply analytical data.

B. Examples of Biological Stains and Staining Methods

1. *Fixatives*

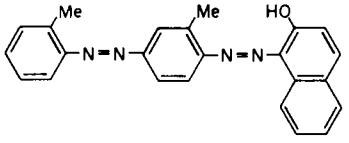
Except in vital coloring, tissue sections are treated with a fixative, which stabilizes the tissue constituents against destruction during embedding, sectioning, and mounting, and also acts as a preservative. Baker[1] has discussed in great detail the action of the coagulant fixatives (mercuric chloride, chromium trioxide, picric acid) and the noncoagulant fixatives (formaldehyde, osmium tetroxide) on proteins, tissues, and cells, and the overall effects of fixation and the subsequent procedures on dyeing. Fixation should be designed to interfere as little as possible with penetration and adsorption of the stain. The interaction of the basic amino acids with formaldehyde and with osmium tetroxide and any alterations in the isoelectric points of proteins by the blocking of carboxyl or amino groups by fixatives can alter the dyeing properties of tissue constituents. Since the object of routine staining is to color the chromatin with a basic dye and the cytoplasm with an acid dye, those fixative mixtures are most suitable which leave the isoelectric points of tissue constituents within favorable limits for dyeing without the use of buffers.

2. *Coloration of Lipids*

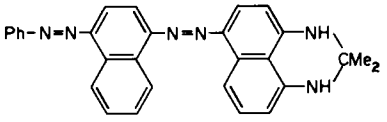
Water-insoluble dyes, soluble in the common organic solvents, are used ("fat stains," "lysochromes"). The technique, in principle, is to treat a tissue section or an emulsion of bacteria from a slant culture or tubercular sputum with a near-saturated solution of the dye in alcohol, alcohol–ether, or ethylene glycol, which do not dissolve the lipids. The specimen is then rinsed with aqueous alcohol and water. The partition

coefficient of the dye between the lipids and the solvent should favor the former.

Sudan IV and Sudan III (the analog from aniline), which exist in neutral solvents in the quinone-hydrazone form, can tautomerize to the azophenol form in the presence of a base, and can therefore slightly color cytoplasm under certain conditions, leading to anomalous results.



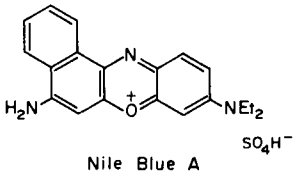
Sudan IV



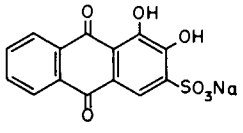
Sudan Black B

Sudan Black is retained strongly by phospholipids and by fatty acids, because of the two secondary amino groups; but if these are acetylated, preferential staining of neutral lipids becomes possible. Sudan Black under prescribed conditions is a specific nonfading stain for tubercle bacilli.

Nile Blue A,[8b] a basic dye for acidic tissue constituents, in aqueous acid solution usually contains enough of the red phenoxazone by spontaneous hydrolysis to function as a lysochrome for neutral fat. Thin-layer chromatography (TLC) and spectrophotometric analysis of Nile Blue sulfate have been studied.[9]



Nile Blue A
Basic Blue 12
CI 51180



Alizarin Red S
CI Mordant Red 3
CI 58005

3. *Mordant Dyeing*

Mordant dyeing methods are important in biological staining, but they are not relevant to this chapter because the dyes mainly used for this purpose are hematoxylin and carminic acid. Among the few examples of synthetic dyes is Alizarin Red S used in the complicated Benda method (1901) for mitochondria, in which the tissue is fixed with solutions containing chromic acid, osmic acid, potassium dichromate,

[8b] *CSD II*, p. 782.

[9] M. G. Dunnigan, *Stain Technol.* **43**, 243 (1968).

and iron alum, and dyed with Alizarin Red S and Crystal Violet, the latter for staining chromatin. A second example is Gallocyanine[9a]; in conjunction with chrome alum it has been recommended for obtaining an approximate estimate of the amount of DNA in a nucleus or of RNA in a nucleolus or in the cytoplasm.[1h] Other mordant dyes which find some use are Alizarin, Purpurin, Anthracene Navy Blue SWR (CI Mordant Blue 32; CI 58605), Celestine Blue B (CI Mordant Blue 14; CI 51050), and Gallamine Blue (CI Mordant Blue 45; CI 51045). Baker, who regards the use of mordants as of great advantage in biological microtechnique, has made a careful study of the action of mordants on tissue constituents.[1h,10] One point in contrast with modern wool dyeing is that the premordanting procedure is generally employed; the metachrome and after-mordanting methods and the preformed metal–azo dye complexes (including the neutral-dyeing 1:2 complexes) have not so far found a place in biological staining.

For the demonstration of certain metals such as calcium and aluminum in tissues, acid-mordant dyes such as Solochrome Cyanine R (CI Mordant Blue 3; CI 43820) and Solochrome Black F (CI Mordant Black 5; CI 26695) are used.[4]

4. *Staining with Acid and Basic Dyes*

The great majority of dyes used for biological staining are acidic and basic dyes, because cell constituents carry both cationic and anionic charges. Brachet[11] put together a composite cell "for the purpose of charting the anatomical features that are shared in varying degrees by all cells," qualified by the statement that there is no typical cell.

Although the innumerable variations of the amino acid composition and sequence in proteins, the presence of inorganic ions and other nonprotein constituents, and physical factors such as membrane permeability influence the behavior of cell constituents toward dyes, many cells and cell structures can be characterized by staining techniques because the presence of acidic groups (carboxyl, phenolic hydroxyl, phosphate) and basic groups (amino, guanidino, and heterocyclic nitrogen) permit the selective attack of basic and acid dyes, respectively, under defined experimental conditions. Broadly, the intensity of staining with a given acid dye will depend on the content of the basic amino acids (lysine, arginine, and to a much smaller extent histidine) in the protein. Conversely, the content of glutamic and aspartic acid (and to a minor extent, of tyrosine) in the protein will determine the intensity

[9a] *CSD II*, p. 783.

[10] J. R. Baker, *Quart. J. Microsc. Sci.* [N.S.] **101**, 255 (1960); **103**, 493 (1962).

[11] J. Brachet, *Sci. Amer.* **205**, 55 (1961).

of staining with a given basic dye. It is convenient to consider separately the interaction of basic dyes with the nucleoproteins and nucleic acids (see Section V). With a particular cell constituent and under specific experimental conditions, the molecular characteristics, the acid or base strength, solubility and other properties of a dye will influence its quantitative uptake.

From the brief reference to the physical chemistry of dyeing in Volume III of this series and the detailed treatment in Chapter III and IV of this volume, it will be clear that theories of dyeing textile fibers are increasingly based on mathematical treatments of the kinetic and the thermodynamic aspects. As recently as 1969 Friedberg and Goldstein stated that "thermodynamic concepts and methods . . . have seldom been applied to problems of histochemical interest." However, kinetic and equilibrium factors have been at least implicitly distinguished, quantitative aspects of dye uptake have been reported, and thermodynamic aspects of histological dyeing have been considered.[11a]

There is a vast literature on the theory of wool dyeing with acid dyes (see Chapters III and IV). The physicochemical aspects are too complex to be summarized here, and they are partly irrelevant for biological coloration processes, because wool is usually dyed at about 90° under conditions which result in maximum exhaustion of the dyebath; but the essential facts from the present point of view are that wool dyeing involves (a) ionic interaction between SO_3^- and NH_3^+, (b) hydrogen bonding, (c) van der Waals attraction, and (d) "hydrophobic bonds" between the hydrophobic parts of the protein and of the dye molecules. The concept of bonding between two hydrophobic side chains of proteins in aqueous solution was suggested by Kauzmann and developed by Némethy and Scheraga.[12] The possible role of the hydrophobic bond in dyeing wool with acid dyes has been discussed by Zollinger.[13] All the four types of interaction are undoubtedly involved in the behavior of tissue proteins towards both acid and basic dyes, but we have meager experimental data; even in the case of wool dyeing, quantitative data are available only in support of a stoichiometric relation between cationic groups in wool and the adsorption of anionic dyes, and a definite assessment of the part played by other forces of attraction has not been made.

[11a] See, e.g., D. J. Goldstein, *in* "Cell Structure and its Interpretation" (S. M. McGee-Russell and K. F. A. Ross, eds.), pp. 67–77. Arnold, London, 1968; *Quart. J. Microsc. Sci.* [N.S.] **104**, 413 (1963); **106**, 299 (1965); S. H. Friedberg and D. J. Goldstein, *Histochem. J.* **1**, 361 (1969).

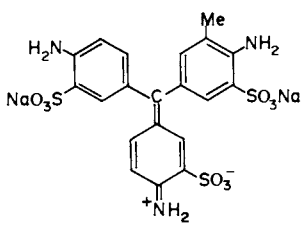
[12] W. Kauzmann, *Advan. Protein Chem.* **14**, 1 (1959); G. Némethy and H. A. Scheraga, *J. Chem. Phys.* **36**, 3382 and 3401 (1962); *J. Phys. Chem.* **66**, 1773 (1962).

[13] H. Zollinger, *J. Soc. Dyers Colour.* **81**, 345 (1965).

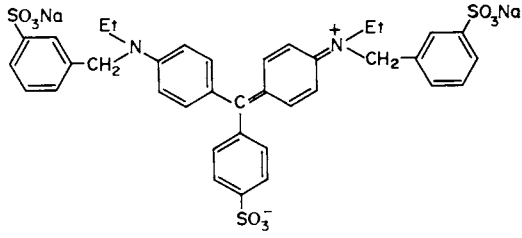
Acid dyes for wool may be broadly classified as (a) low molecular weight dyes, which have a high rate of dyeing and level well, because they dye by an acid–base mechanism and have low affinity for the fiber; (b) milling dyes of higher molecular weight, lower rate of dyeing, and higher affinity; (c) dyes fast to severe milling and potting, which have a low rate of dyeing, aggregate readily in solution and have high affinity for the fiber. Dyes of the three classes can be applied to wool at progressively higher pH (2.5–3, 4–5.5, and 6–8.5, respectively). For biological staining, dyes of group (a) are generally the most suitable.

The differential staining possible with different protein constituents of a tissue, using the same acid dye, is shown by the sharp differences in the behavior of wool and silk towards acids and acid dyes.[13a] Wool has nearly four times the acid-combining power of silk under the usual equilibrium-dyeing conditions; but at temperatures below 50° silk adsorbs more dye than wool because of the horny epidermis of the wool fiber; at higher temperatures wool takes up more dye and in a wool–silk union fabric the dye will migrate from silk to wool.

MacConaill and Gurr have developed the Falg stain (Acid Fuchsine and Light Green SF) as a simple polychrome stain for basic tissue elements. The Falg technique, suitably modified, has been found to be useful for detecting protein malnutrition; when a buccal smear is stained, young cells are colored blue and the old, most cornified cells red. The high or low percentage (80–90% or 2–5%) of the former indicates high protein intake or severe malnutrition.[14]



Acid Fuchsine
CI Acid Violet 19
CI 42685



Light Green SF Yellowish
CI Acid Green 5
CI 42095

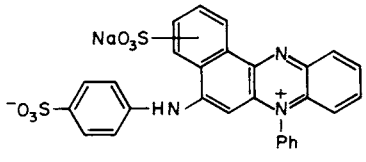
Variations in the side groups of the polypeptide chains are responsible for differences in the amphoteric character and the isoelectric points of the proteins. Utilizing the properties which differentiate one protein from another, the histologist effects differential staining by treatment

[13a] *CSD I*, p. 257; *CSD II*, p. 1296.

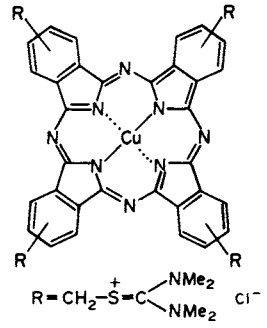
[14] M. A. MacConaill, B. T. Squires, and E. Gurr, *New Sci.* Nov. 10, 290 (1966); B. T. Squires *Proc. Nutr. Soc.* **23**, 16 (1964).

with cationic and/or anionic dyes. Apart from the amino acid residues, cell constituents contain several other charged sites. The phosphoric acid groups of nucleic acids and phospholipids, and the uronic and sulfuric acid groups of mucopolysaccharides, account for many anionic sites, which interact with basic dyes. Uptake of dyes is possible outside the pH ranges predicted on the basis of oversimplified acid–base considerations.

Certain tissues appear more or less "dense" under the microscope, usually because of shrinkage or swelling following fixation.[15] The chromatin of the nucleus is usually much more dense than the surrounding nuclear sap, and this property has been used[15a] to produce differential staining by Acid Fuchsine or Azocarmine.[15b] A correlation of the size of dye particle and the density of substrates with special reference to mucin staining has been made.[16] The selectivity of Alcian Blue (a copper phthalocyanine derivative containing thiuronium groups, which are split off under textile dyeing or printing conditions[16a]) depends partly on its large particle size (as shown by dialysis experiments); it penetrates and stains basophilic structures of low density, but not denser areas.



Azocarmine G
CI Acid Red 101
CI 50085



Alcian Blue
CI Ingrain Blue 1
CI 74240

There are other examples of differential coloration being achieved by taking advantage of the diffusion characteristics of dyes. Acid dyes of the leveling type (e.g., Eosine, Orange G, Ponceau 2R) are often used to give background staining of cytoplasm to provide contrast. Such stains

[15] J. R. Baker [*Quart. J. Microsc. Sci.* [N.S.] **104**, 107 (1963)], has discussed the correct use of the word "dense" in microtechnique.

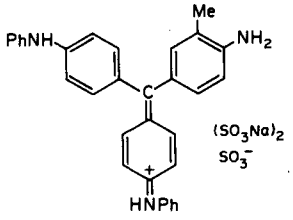
[15a] F. B. Mallory, *J. Exp. Med.* **5**, 15–20 (1900).

[15b] M. Heidenhain, *Z. Wiss. Mikrosk.* **32**, 361–372 (1915).

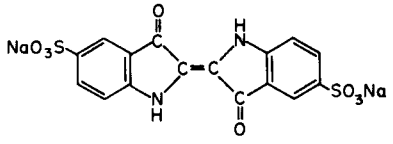
[16] D. J. Goldstein, *Stain Technol.* **37**, 79 (1962); *J. Roy. Microsc. Soc.* [3] **84**, 43 (1965).

[16a] See *CSD II*, p. 1140.

are usually mixtures in which the nonleveling component (e.g., Water Blue I, Indigo Carmine) produces darker areas on collagen; the dyes that diffuse rapidly color cytoplasm, and the slowly penetrating types color collagen.[16b] The rate of diffusion of a dye is related to its molecular weight and state of aggregation, and typical stains for collagen are of the fast-to-milling type of dyes that diffuse slowly and tend to form aggregates.[16c]

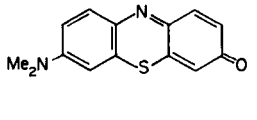


Water Blue I
CI Acid Blue 22
CI 42755

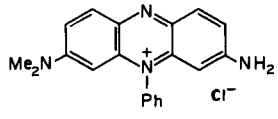


Indigo Carmine
CI Acid Blue 74
CI 73015

The basic dyes are as important for the biological colorist as the acid dyes, although he has fewer of the former to choose from. Methylene Blue and the Azures are outstanding, and figure repeatedly in staining techniques. Azures A, B, and C, prepared from Methylene Blue by several mild oxidation procedures, are stated to be products in which two (unsymmetrically) or one or three of the *N*-methyl groups are removed. A mixture of such demethylation (and possibly more complex oxidation and hydrolytic) products of Methylene Blue, prepared by alkali treatment[16d] is used as Polychrome Methylene Blue. Bernthsen's Methylene Violet (product of hydrolysis of Methylene Blue in which $=NMe_2^+$ is replaced by $=O$) is only used as a biological stain; Tzung[17] has discussed the history, preparation, and reactions of this dye. The chemistry of these products from Methylene Blue needs to be reinvestigated. Methylene Violet (biological stain of NAC) is assigned the structure of a safranine in the *Colour Index* (CI Basic Violet 5; CI 50205).



Methylene Violet (Bernthsen)



Methylene Violet
CI Basic Violet 5
CI 50205

[16b] M. Seki, *Folia Anat. Jap.* **10**, 621 and 635 (1932).
[16c] M. Seki, *Z. Zellforsch. Mikrosk. Anat.* **18**, 1 (1933).
[16d] P. G. Unna, *Z. Wiss. Mikrosk.* **8**, 475–487 (1891).
[17] C. Tzung, *Stain Technol.* **39**, 351 (1964).

In order to color the various tissue elements in contrasting colors, two or more dyes are frequently used in succession, but there are also methods in which mixtures of dyes are used. A widely employed technique is to use a mixture of a basic dye and an acid dye. The discovery of the nucleus of the malarial parasite was due to the development of such neutral stains by Romanowsky,[1g] who mixed Methylene Blue with a slight excess of Eosine in water. This unique combination "Methylene Blue Eosinate" proved so popular that several modifications were subsequently introduced by Nocht,[17a], L. Jenner,[17b] W. B. Leishmann,[17c] J. H. Wright,[17d] G. Giemsa,[17e] W. J. MacNeal,[17f] and others (1898–1954). The following color effects can thus be produced in a blood smear: chromatin of leukocytes, purple; nucleus (in full or part) of parasitic protozoa, red; basophilic cytoplasm of lymphocytes, monocytes, and parasitic protozoa, blue; eosinophilic granules, pink; neutraphilic granules, purple; and red blood corpuscles, pink.

Baker,[1h] who has discussed the "blood dyes" in detail, has stated that Romanowsky dyeing has not yet been fully explained, although we now have sufficient knowledge for the preparation from pure dyes of solutions which give the desired effect. Pure Methylene Blue or an Azure does not produce the polychrome effect; they must be used in conjunction with Eosine. The exact role of Eosine is uncertain; it colors the acidophilic granules in eosinophilic leukocytes, but the extent to which Eosine contributes to the red staining of certain parasite nuclei is in doubt. Replacement of Eosine by other acid dyes or by colorless tribromophenolate anion[17g] produces the Romanowsky effect.[18]

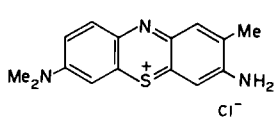
Experiments with Giemsa stain have indicated that ionization of the Azure–Eosine complex is necessary for developing the red nuclear color of Romanowsky-type staining; the addition of Chromotrope 2R (CI Acid Red 29; CI 16570) decreases nuclear staining and accentuates nucleolar staining,[18a] but the precise reason is not clear.

Baker concluded (in 1958) that there was plenty of room for research on the Romanowsky stains.[1h]

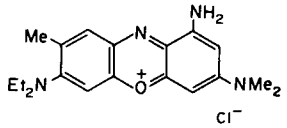
[17a] Nocht, *Centralb. Bakt. Parasit., 1 Abt.* **24**, 839–843 (1898).
[17b] L. L. Jenner, *Lancet* **1**, 370 (1899).
[17c] W. B. Leishman, *Brit. Med. J., Part 2*, 757–758 (1901).
[17d] J. H. Wright, *J. Med. Res.* **7**, 138–144 (1902).
[17e] G. Giemsa, *Centralb. Bakt. Parasit., 1 Abt.* **37**, 308–311 (1904).
[17f] W. J. MacNeal, *J. Amer. Med. Ass.* **78**, 1122 (1922).
[17g] P. G. Unna, *Centralb. Bakt. Parasit., 1 Abt.* **88**, 159 (1922).
[18] See also E. Gurr, *Nature (London)* **202**, 1022 (1964).
[18a] D. W. Menezies, *Stain Technol.* **37**, 45 (1962).

5. *Metachromasy*

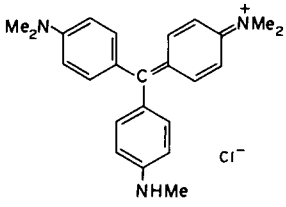
The phenomenon of "metachromasy" or "metachromasia" plays an important part in biological staining.[1h,19–22] Metachromasy has been defined as "the coloring of different tissue constituents in different colors by a single dye." A substrate that causes a metachromatic change is called a "chromotrope" and it carries a charge opposite to that of the



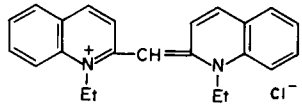
Toluidine Blue O
CI Basic Blue 17
CI 52040



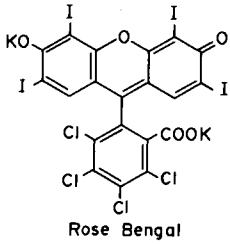
Brilliant Cresyl Blue
CI 51010*



Methyl Violet 2B
CI Basic Violet 1
CI 42535



Pseudoisocyanine



Rose Bengal
CI Acid Red 94
CI 45440

dye. A dye which does not exhibit metachromasy is termed "orthochromatic." The differential staining resulting from impurities formed by chemical changes in a dye in solution (e.g., Nile Red in Nile Blue A) is not metachromasy. A well-known metachromatic dye is Toluidine Blue O, a thiazine. 1,9-Dimethyl Methylene Blue, the analog of

[19] L. Lison, *Arch. Biol.* **46**, 599 (1935).

[20] B. Sylven, *Quart. J. Microsc. Sci.* [N.S.] **95**, 327 (1954); see also L. Jozsa, *Acta Histochem.* **32**, 305 (1969).

[21] An experimental and theoretical re-evaluation of metachromasy was made by J. A. Bergeron and M. Singer [*J. Biophys. Biochem. Cytol.* **4**, 433 (1958)]; see also M. Schubert and D. Hammerman, *J. Histochem. Cytochem.* **4**, 159 (1956).

[22] For a study of the metachromasy of vital-stained cells, see O. Haertel and I. Thaler, *Protoplasma* **62**, 356 (1966).

Methylene Blue from 4-amino-3-methyl-*N*,*N*-dimethylaniline, is a metachromatic stain which gives more consistent results than Methylene Blue, Toluidine Blue, and Azure B.[23] The thiazines provide the most useful metachromatic dyes, but they are also available in the oxazine (e.g., Brilliant Cresyl Blue) and triphenylmethane (e.g., Methyl Violet) class. Taylor has discussed the influence of molecular structure of thiazine and oxazine dyes on their metachromatic properties.[23a] Maxilon Blue RL,[23b] a cationic dye for polyacrylonitrile fiber, is metachromatic.[24]

Metachromatic dyes are mainly polycyclic planar molecules in which an ionic charge is part of the resonance system. Too few dyes have been examined, however, for any generalization concerning structures capable of exhibiting metachromasy; Alcian Blue, in which the cationic charge is on a pendant group attached to copper phthalocyanine, is metachromatic.[25]

Examples of chromotropic tissue constituents are the matrix of cartilage, sections of mucous glands, granules of the basophilic cells of connective tissue, and the corpuscles of amyloid degeneration.[1h] Most chromotropes are acidic (basophil), and often exist in a polymerized form. Glucuronic and mannuronic acids are not chromotropic; the high polymers of mannuronic acid (alginates) are, and the effect increases with the degree of polymerization.[20] Large metachromatic color changes are produced by acid mucopolysaccharides. The metachromasy of Toluidine Blue has been used for the localization of lysosomelike particles, the effect being produced by an acid mucopolysaccharide component.[26] Metachromasy has helped in the diagnosis of genetic defects (such as gargoylism and infantile leukodystrophy) associated with abnormal metabolism of mucopolysaccharides and sulfatides.[26a]

High polymers such as glycogen, starch, and cellulose are by themselves nonchromotropic; the introduction of carboxymethyl groups in cellulose makes it a chromotrope, and the effect increases with increasing number of carboxymethyl groups. Cysteine-rich secretion products exhibit metachromasy after oxidation by peracids.[27]

The metachromatic effect is generally hypsochromic, and the useful metachromatic dyes are blue or violet. The metachromatic change is

[23] K. B. Taylor and G. M. Jeffree, *Histochem. J.* **1**, 199 (1969).

[23a] K. B. Taylor, *Stain Technol.* **36**, 73 (1961).

[23b] See *CSD IV*, p. 191.

[24] D. F. de Almeida, *Stain Technol.* **35**, 129 (1960).

[25] R. W. Mowry and V. M. Emmel, *J. Histochem. Cytochem.* **14**, 799 (1966).

[26] A. M. Dalcq, *Lysosomes, Ciba Found. Symp.*, p. 227 (1963).

[26a] J. H. Menkes and B. R. Migeon, *Annu. Rev. Med.* **17**, 420 (1966).

[27] M. Gabe, *C. R. Acad. Sci., Ser. D* **267**, 668 (1968).

reversible and is dependent on the solvent, the concentration, and the temperature, strongly suggesting that the phenomenon is related to the state of aggregation of the dye. Beer's law is inapplicable to solutions of metachromatic dyes, but there are numerous other dyes which in aqueous solution do not obey Beer's law. The metachromatic effect is produced when the tissues take up dye aggregates as such, or the monodisperse dye ions aggregate on the tissue substrate in a definite manner affecting the light absorption characteristics of the tissue–aggregate complex.[1h,3]

The metachromasy of Toluidine Blue, examined on cells, tissues, and models, has been found to be an inverse, linear, and reversible function of temperature in the range 5°–95°. All the available evidence thus suggests that metachromasy is not a fundamentally different phenomenon in solution and in solid systems, and the temperature studies emphasized the role of structural water and solvent dielectric constant in relation to dye–dye interaction.[28] The stoichiometry of the metachromatic complex of inositol hexaphosphate and Toluidine Blue has been studied. The tetra and lower phosphates are not effective and can thus be distinguished from the hexaphosphate.[29] Recently the stoichiometry of metachromatic interaction has been studied on filter paper, and the simple technique is claimed to be reliable.[29a] With Toluidine Blue and inorganic polyphosphates on filter paper, prominent metachromasy appears at the hexamer stage, reaching maximum intensity at a chain length of 16, followed by a plateau up to 200.[29b] In solution, however, the plateau appears at a chain length of 35.[29c] Toluidine Blue in water has λ_{max} at 620 nm, and in aqueous agar at 540 nm. Comparison with agarose and agaropectin showed that the higher order structure of agar in aqueous solution plays an important part in the appearance of metachromasy.[30] The ultracentrifugal separation of the metachromatic compound of Methylene Blue and chondroitin sulfate has been effected.[31] Direct analysis showed that it contained 1 equivalent of chondroitin sulfate per mole of dye, even when as many as 20 equivalents of the former were used. Mixed polymers between pairs such as Methylene

[28] J. W. Kelly and L. Chang, *J. Histochem. Cytochem.* **17**, 658 (1969); J. W. Kelly, L. Chang, and F. Rothstein, *ibid.* p. 651.

[29] N. C. Mandal *et al.*, *Histochemie* **18**, 202 (1969).

[29a] C. Singh, *Anal. Biochem.* **38**, 564 (1970).

[29b] C. Singh, *J. Sci. Ind. Res., Sect. B* **18**, 249 (1959).

[29c] K. K. Tewari and P. S. Krishnan, *Arch. Biochem. Biophys.* **82**, 99 (1959).

[30] S. Suzuki *et al.*, *Nippon Kagaku Zasshi* **90**, 940 (1969).

[31] M. K. Pal and M. Schubert, *J. Phys. Chem.* **65**, 872 (1961); **67**, 1821 (1963); see also *J. Histochem. Cytochem.* **9**, 673 (1961).

Blue and Acridine Orange have been observed in the presence of polyanions such as chondroitin sulfate.

Pseudoisocyanine in the presence of λ-carrageenin gives a very sharp absorption band at a longer wavelength than the normal absorption band; a large excess of the carrageenin facilitates the disappearance of the band.[32] This reversal of the common metachromasia is attributed to the binding of the dye cations to the polyanion without dye–dye interaction. Heparins occur in the mast cells of many mammals, and they are mucopolysaccharides built from hexuronic acid, hexosamine, and sulfuric acid. The common commercial heparin, prepared from beef lungs or liver, consists of glucuronic acid and glucosamine units and contains both O—SO_3H and NH—SO_3H groups. Stone[33] has studied the secondary structure of heparin by means of the induced anomalous optical rotatory dispersion in the metachromatic absorption band when four planar symmetric dyes were bound to the polyanion. The induced Cotton effect was dependent on the metachromatic reaction of the dyes and on the integrity of the heparin molecule. The data showed that the dyes must be bound to nearby sites and supported the view that the induced asymmetry was largely dependent on dye–dye rather than dye–polymer interactions. The interpretation led to a molecular model of heparin.

Balazs *et al.* have described a new method for studying the binding between the anionic sites of polyanions and Methylene Blue cation (MB^+). The hydrated electron (e_{aq}^-) reacts rapidly with MB^+ according to the reaction $MB^+ + e_{aq}^- \rightarrow MB\cdot$, where $MB\cdot$ represents the Methylene Blue semiquinone. On addition of polyanion, the rate of the e_{aq}^- reaction is markedly decreased, due to neutralization of the positive charge on MB^+ by ion-binding. The changes in rates of e_{aq}^- disappearance can, therefore, be used to study ion-binding. Such ion-binding with cationic dyes is frequently accompanied by spectral shifts in the dye (metachromasia). Measurements of changes in e_{aq}^- disappearance rate demonstrate that the agents which destroy metachromasia in solution (addition of sodium chloride, methanol, and an increase in temperature) lead also to loss of ion-binding. Although loss of ion-binding leads to removal of metachromasia, it does not follow that it is the ion-binding process which is responsible for metachromasia. However, using the pulse radiolysis method, ion-binding can be examined independently of any other process.[34]

[32] M. K. Pal, *J. Indian Chem. Soc.* **47**, 423 (1970); see also von W. Appel and G. Scheibe, *Z. Naturforsch. B* **13**, 359 (1958).

[33] A. L. Stone, *Biopolymers* **2**, 315 (1964); A. L. Stone and H. Moss, *Biochim. Biophys. Acta* **136**, 56 (1967).

[34] E. A. Balazs *et al.*, *Biochem. Biophys. Res. Commun.* **30**, 386 (1968).

Michaelis[35] suggested that metachromatic dyes aggregate on the surfaces of polyanions as they do in solution, although he later doubted the aggregation theory as a general explanation of metachromasy.[21] Bradley and Wolf[36] examined the spectrum of Acridine Orange mixed with seven different polymers over a wide range of polymer–dye ratios. They interpreted all their findings in terms of the aggregation theory with the introduction of a single new concept based on the observation that, although all the polymers exhibited monomer, dimer, and/or higher aggregate bands (α, β, and/or γ bands), the number of sites per dye required to develop the (monomer) band differed from polymer to polymer. All the polymers thus appeared to stack the dye molecules, but differed in the extent to which dilution by excess binding sites tended to unstack them. A numerical parameter, the dye-stacking coefficient, was introduced to express the characteristic tendency of a polymer to promote the reversible association, or stacking, of dye molecules bound to its surface.

The reversible metachromatic spectral shifts of Acridine Orange in presence of polyacrylic acid and a synthetic polyampholyte of the γ-amino acid type have been studied and explained in terms of stacking and unstacking of the dye molecules on binding with the polyelectrolyte.[37]

Hypsochromic color changes resulting from the reversible aggregation of dye molecules when the concentration of the aqueous solution is increased, or the temperature is decreased, or salt or a solvent of low dielectric constant is added, are well known.[38] Rabinowitch and Epstein[39] demonstrated the existence in aqueous solution of dimers and oligomers of Methylene Blue, Rhodamine B, and several other basic dyes and suggested that the planar molecules were attracted by London dispersion forces. By measuring the absorption and fluorescence spectra of Acridine Orange in aqueous solution, Zanker demonstrated its reversible polymerization.[40,41] The metachromatic behavior of dyes

[35] L. Michaelis, *Cold Spring Harbor Symp. Quant. Biol.* **12**, 131 (1947); L. Michaelis and S. Granick, *J. Amer. Chem. Soc.* **67**, 1212 (1945); see also earlier references cited in this important paper on "Metachromasy of basic dyestuffs."

[36] D. F. Bradley and M. K. Wolf, *Proc. Nat. Acad. Sci. U.S.* **45**, 944 (1959). The metachromasy of the nucleic acids is discussed in Section V.

[37] A. N. Dey and S. R. Palit, *Indian J. Chem.* **6**, 260 (1968).

[38] For a discussion of electronic interactions between dye molecules, see S. F. Mason *CSD III*, p. 213.

[39] E. Rabinowitch and L. Epstein, *J. Amer. Chem. Soc.* **63**, 69 (1941); see also other references cited in Scheibe and Zanker.[40]

[40] Cf. G. Scheibe and V. Zanker, *Acta Histochem., Supp.* **1**, 6 (1958), and the discussion on this paper.

[41] V. Zanker, *Z. Phys. Chem.* **199**, 225 (1952).

of many types in aqueous solution has been reviewed by McKay and Hillson.[42] They suggested an interpretation in terms of interaction between dye ions and counterions; but Padday[43] found that electrometric measurements of the halide counterion activity of several dyes at concentrations where marked metachromasy took place showed no indication of ion–counterion association. His work supports the view of Scheibe[44] and others that dimers are probably formed. Trimers could be consistent with his data, but not higher aggregates. He concludes that two ions come together by hydrophobic bonding and the dimer is held together against electrostatic forces by dispersion forces. McKay and Hillson subsequently examined the visible absorption spectra of 1,1′-diethyl-2,2′-carbocyanine bromide in a variety of solvents of high dielectric constant and found that metachromasy was exhibited only in aqueous solution. The abnormally high tendency of water to associate is an important factor responsible for metachromasy. The addition of large amounts of sucrose to aqueous solutions of Methylene Blue tended to destroy its metachromasy.

There appears to be no clear line of demarcation between the spectral shifts produced by polymerization of dyes in solution and by the interaction of dyes with "chromotropes" (such as tissue polyanions).[40] McKay[45] has reviewed the relations between tissue binding of dyes and their ionic states in solution and in tissues, and he has discussed dye metachromasy induced by changes in dye concentration in solution and by "chromotropes." In a paper entitled "On Metachromasis (Have Dyes a Definite Color?)," Scheibe has discussed the variability of the absorption spectra of dyes in solvents and through binding to a matrix.[46]

A few acid dyes (e.g., Orange G; Indigo Carmine; Bordeaux Red, CI Acid Red 17, CI 16180, 1-Naphthylamine → R-Acid) and direct cotton dyes (e.g., Congo Rubine; CI Direct Red 17; CI 22150, but not Congo Red) exhibit metachromasy towards specific basic proteins and bases such as quinine and strychnine.[1h,3,47] A known property of Congo Rubine is its salt sensitivity: the red collidal solution changes to lilac on the addition of sodium chloride.[47a] The effect is usually bathochromic; the λ_{max} of Rose Bengal, for instance, is altered from 545 to 562 nm with

[42] R. B. McKay and P. J. Hillson, *Trans. Faraday Soc.* **61**, 1800 (1965); *Nature (London)* **210**, 297 (1966); *Trans. Faraday Soc.* **63**, 777 (1967).

[43] J. J. Padday, *J. Phys. Chem.* **71**, 3488 (1967).

[44] G. Scheibe, *Kolloid-Z.* **82**, 1 (1938).

[45] R. B. McKay, *in* "Cell Structure and its Interpretation" (S. M. McGee-Russell and K. F. A. Ross, eds.), p. 59. Arnold, London, 1968.

[46] G. Scheibe, *Palette* **35**, 28 (1970).

[47] See also J. W. Kelly, *Stain Technol.* **31**, 283 (1956); **33**, 79 and 89 (1958).

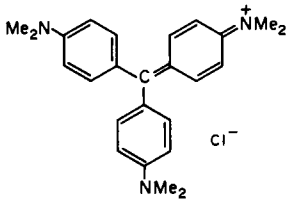
[47a] *CSD I*, p. 511.

bovine albumin,[48] but hypsochromic shifts have also been observed.[49] Singh[29a] (personal communication) has found several simple non-polymeric chromotropes and metachromatic behavior in an old anionic dye, Benzo Azurine G (CI Direct Blue 8; CI 24140; Dianisidine $\rightrightarrows$ 2 moles NW-Acid). No correlation between the structures of anionic dyes and their metachromasy has yet been established.

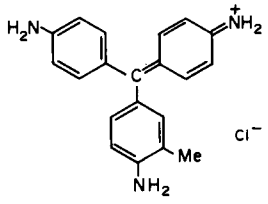
Recent work on the binding of dyes, and in particular Biebrich Scarlet, to polycations is discussed in Section IV,A.

6. *Staining of Bacteria*

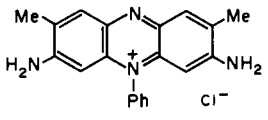
Staining of bacteria, protozoa, and other microorganisms provides an invaluable method for studying their morphology and diagnosis of diseases such as tuberculosis, diphtheria, typhoid, and malaria. According to Lillie[2] the bacteriologist is the chief customer for biological stains, ordering them by the kilo while the zoologist needs only 10-g bottles. The most commonly used dyes are Crystal Violet, Methylene



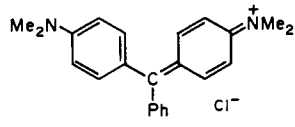
Crystal Violet
CI Basic Violet 3
CI 42555



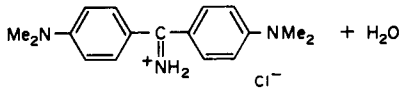
Fuchsine Basic
CI Basic Violet 14
CI 42510
} and related dyes



Safranine O
CI Basic Red 2
CI 50240



Malachite Green
CI Basic Green 4
CI 42000



Auramine O
CI Basic Yellow 2
CI 41000

[48] H. Aizawa, *Shokuhin Eiseigaku Zasshi* **8**, 387 (1968).
[49] C. Singh, *Stain Technol.* **38**, 103 (1963).

Blue, and Fuchsine Basic; among other useful dyes are Toluidine Blue O, Safranine, Malachite Green, and Auramine O.[50]

Ionic interaction between basic dyes and nucleic acids of the protoplasm leads to insoluble products which do not diffuse out of the cell. Bacterial cells have a high content of purine and pyrimidine nitrogen, RNA and DNA constituting about 30% of the dry weight. Unlike mammalian tissue cells, which exhibit structural differentiation on staining, bacteria generally are dyed a uniform shade. From the point of view of response to staining, three broad types of bacteria have been recognized: Gram-positive, gram-negative, and acid-fast.

There are several modifications of Gram's differential stain.[1f] A common procedure is to stain with Crystal Violet, "mordant" with iodine in potassium iodide solution, wash off unfixed Crystal Violet with 95% ethanol, and counterstain with Safranine. Crystal Violet and iodine are specific, but almost any basic dye can be used as counterstain. Bacteria are thus separated into Gram-positive and Gram-negative groups according to their retention of Crystal Violet or the counterstain. Bartholomew[51] has examined the steps in Gram staining for the standardization of the results. The distinction, not always sharp, is of considerable practical value because there is a broad correlation between the Gram classification and sensitivity to certain antibiotics. In general, Gram-positive bacteria are sensitive to the sulfonamides and penicillin, and Gram-negative bacteria to streptomycin. The positive response to the Gram stain reaction has been variously ascribed to a difference in permeability to the alcohol-soluble dye–iodine complex; a difference in the character of the outer layers of the cell, the internal matter being Gram-negative in both bacterial types; the presence of an Mg–RNA–protein complex in Gram-positive bacteria; and differences in other cell constituents. Basu, Biswas, and Pal[52] have recently produced chemical evidence using thiosulfate to explain the role of iodine, which forms a charge-transfer complex with the dye ions bound to the cell component. The stability of the cell component–dye–iodine complex determines the Gram character.

"Acid-fast" bacilli, which include the tuberculosis and leprosy organisms, have a high lipid content and require heat or prolonged treatment for penetration of a dye into the cells; subsequently the stains are fast to alcoholic acid. In the Ziehl-Neelsen method the smear

[50] J. W. Moulder, R. M. Lewert, and J. W. Rippon, "Textbook of Microbiology," 19th ed., pp. 18 and 47. Saunders, Philadelphia, Pennsylvania, 1968.

[51] J. W. Bartholomew and T. Mittwer, *Bacteriol. Rev.* **16**, 1 (1952); J. W. Bartholomew, *Stain Technol.* **37**, 139 (1962); F. L. Tucker and J. W. Bartholomew, *ibid.* p. 157.

[52] P. S. Basu, B. B. Biswas, and M. K. Pal, *Histochemie* **14**, 221 (1968).

is stained with carbol-fuchsine (Fuchsine Basic + phenol) at about 100° for 3–5 minutes or at room temperature for 24 hours, rinsed in water, decolorized by alcoholic hydrochloric acid until the solution is only a faint pink, and counterstained with Methylene Blue. The result is to leave the acid-fast bacteria red and other organisms blue. The role of phenol, which undergoes no chemical reaction with Fuchsine, is not to denature the proteins or disrupt the capsules, but to increase the solubility of the dye in the lipids of the bacilli.[53]

Dyes such as Brilliant Green (CI Basic Green 1; CI 42040; the tetraethyl homolog of Malachite Green) are useful for the selective suppression of bacteria. Thus, Brilliant Green has been used to suppress the growth of *Proteus* in culturing *Salmonella* for the detection of enteric infection; Gentian Violet (Crystal Violet) and Malachite Green are used to suppress contaminating organisms in the demonstration of *Brucella abortus* in milk.[54]

7. *Vital Staining*[1h,55]

a. General Considerations. The coloration of unfixed tissues in their living state by dyes ("vital dyes") is becoming less frequent with the introduction of phase-contrast and interference microscopy; however, differentiations based on minor differences in structure, especially where the refractive indices of tissue components are nearly identical, are sometimes possible with vital dyes.[56]

There are two ways in which vital stains may be employed. One depends on the uptake of dyes (usually anionic azo dyes with a strong tendency to flocculate into particles of colloidal dimensions) by the specialized cells of the excretory mechanism of the organism; the fact that they are foreign bodies is more relevant than their color. The second and more important purpose of vital staining involves the use of specific dyes which penetrate all cells and color certain constituents. The membrane permeability of dyes has been related to the molecular weight, lipid solubility, and the number of methyl groups.[57]

[53] D. J. Lartigue and G. L. Fite, *J. Histochem. Cytochem.* **10**, 611 (1962).

[54] G. S. Wilson and A. A. Miles, "Topley and Wilson's Principles of Bacteriology and Immunity," 5th ed., pp. 1840 and 2061. Arnold, London, 1964.

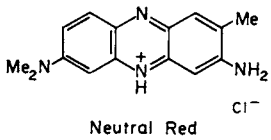
[55] For reviews of vital staining, see L. Stockinger, *Protoplasmologia* **2** (**D1**), 1 (1964); H. Drawert, "Protoplasmology, Handbook of Protoplasmic Research," Vol. 2. Spinger-Verlag, Berlin and New York, 1968; J. M. Gregory, *in* "Cell Structure and its Interpretation" (S. M. McGee-Russell, ed.), p. 87. St. Martin's Press, New York, 1968.

[56] S. A. Shafiq, *Quart. J. Microsc. Sci.* [N.S.] **94**, 319 (1953). The intracellular disposition of vital dyes has been investigated by light and electron microscopy [W. Schmidt, *Z. Zellforsch. Mikrosk. Anat.* **58**, 573 (1962)].

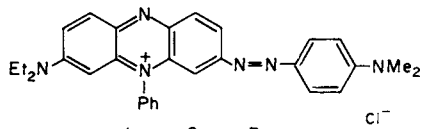
[57] J. F. Danielli, "Cell Physiology and Pharmacology." Elsevier, Amsterdam, 1950.

To be useful as a vital stain, the dye should be able to penetrate and diffuse through the protoplasm of cells, should be nontoxic in the dosages used, and should produce dye localization. The following basic dyes are useful for vital staining: Neutral Red, Methyl Violet 2B, Brilliant Cresyl Blue, Nile Blue A, Azures A & B, Methylene Blue, Bismarck Brown (CI Basic Brown 1; CI 21000; disazo dye from 3 moles of *m*-Phenylenediamine and 2 moles of HNO_2), and Janus Green B. Cells of the reticuloendothelial system and renal tubuli take up and store acid dyes preferentially. The direct cotton dye, Trypan Blue (CI Direct Blue 14; CI 23850; *o*-tolidine $\rightrightarrows$ 2 moles H-acid) is an important vital stain, which has a tendency to aggregate in aqueous solution like many other direct cotton dyes.[57a]

Similar azo dyes are used as anticoagulants in *in vivo* studies because they interfere with the action of thrombin on fibrinogen; examples are Diamine Sky Blue FF (CI Direct Blue 1), Niagara Sky Blue (Benzo Sky Blue; CI Direct Blue 15; CI 24400), and Chlorazol Fast Pink BKS (Sirius Pink BB; CI Direct Red 75; CI 25380).[57b] Alizarin Red S has been used for *in vivo* staining of bone. Other examples are cited later.



Neutral Red
CI Basic Red 5
CI 50040



Janus Green B
CI 11050*

Vital dyes may be used simply by placing the organism in a surrounding medium of the stain, by immersing loose cells or fragments of living tissues in an isotonic solution of the dye ("supravital staining"), by injecting subcutaneously or into a blood vessel of a larger animal, or by oral feeding.[1h]

In vital staining, in contrast with the dyeing of fixed tissues, the nucleus or the ground cytoplasm of cells is generally unaffected except for an occasional tinge, and the regions differentiated are vacuoles, lipid globules, and mitochondria. The resistance to coloration of chromatin of the nucleus may be because of the impermeability of the nuclear membrane or the occurrence of the nucleic acids in combination with proteins, the phosphoric acid residues thereby being unavailable for interaction with basic dyes unless the tissue is fixed. The staining of cytoplasmic inclusions such as vacuoles is not specific for a particular stain, and the term "Neutral Red vacuoles" does not attribute any

[57a] Cf. *CSD II*, p. 1264.
[57b] See *CSD I*, pp. 513 and 515.

special properties for such inclusions; in fact, most lipid globules are also colorable by Neutral Red. Metachromatic coloration by basic vital dyes has also been reported.[58] In fixed tissue dyeing the dye penetrates almost everywhere and nearly the same concentration as in the surrounding medium is reached inside the cells; but in vital staining the dye is present in the cell fluids only at the concentrations permitted by living tissues. This fact, together with the changes that take place on fixation of tissues, makes vital-stained preparations very different in appearance from stained dead tissues.

The coloration of lipid cell inclusions occurs by a process of solution of the stain in the lipid medium.[59] The observed pink coloration of neutral fat globules when Nile Blue is used as a vital stain is due to the nonionic Nile Red present as an impurity in the stain, as mentioned earlier. Supravital staining with basic dyes is a useful method for identification of lipid material in cytology, provided definite conditions and stages of staining (granular or vacuolar stain distribution) are observed.[60]

Ehrlich, who introduced Methylene Blue as a vital stain for nerve dendrites, noted that the dye was reduced by the cells to its leuco base.[1h] The diffusion of the leuco bases through cell membranes was investigated later by other workers. The enzymes present in living cells are capable of effecting such reductions; in supravital staining, tissues are aerated to prevent such decolorization. The ease of reduction of vital dyes varies from dye to dye, and Neutral Red is particularly difficult to convert into the leuco base. The reduction products are nonionic, unlike the parent dyes, and are lipid-soluble.

The staining of mitochondria by Janus Green B is remarkable for its specificity, especially in dilute solutions. Lazarow and Cooperstein[61] consider that the dye is initially taken up throughout the cell, and then gets reduced, except in the mitochondria where the oxidases convert it into the oxidized blue form. The specific coloration is thus due to the vital activities of the cell. Pinacyanol[61a] is superior to Janus Green for supravital staining of mitochondria in blood, because the former does not fade and does not inhibit the effect of Neutral Red.[2,62] Tetrazolium

[58] H. Kinzel, *Protoplasma* **50**, 1 (1958).

[59] Absorption of vital stains (such as Neutral Red & Erythrosine) by the cytoplasm of *Penicillium chrysogenum* is more intense when grown in the presence of fat [O. D. Yudina *et al.*, *Antibiotiki* (*Moscow*) **15**, 145 (1970)].

[60] E. Morgenstern, R. Mueller, and E. Weber, *Histochemie* **10**, 309 (1967).

[61] A. Lazarow and S. J. Cooperstein, *J. Histochem. Cytochem.* **1**, 234 (1953).

[61a] *CSD II*, p. 1150.

[62] See also H. Drawert, *Z. Pflanzenphysiol.* **54**, 145 (1966).

salts, used to follow dehydrogenase activity, are discussed in Section III.

Straus[63] has discussed the concentration of vital stains in intracellular granules or vacuoles after administration to living animals. Like proteins and colloidal materials, the dyes are concentrated in granules of the same type of cells: the cells of the reticuloendothelium system and the epithelial cells of the kidney and intestine. Anionic dyes (such as Trypan Blue) of low degree of dispersion are bound to serum proteins and the protein–dye complexes are then taken up by pinocytosis. The mechanism of ingestion of highly dispersed basic dyes (such as Toluidine Blue, Fuchsine, Neutral Red, and Acridine Orange) is not yet fully understood. They are adsorbed at the surface of amoebae, and they induce pinocytosis. Some basic dyes stain the cytoplasm in a diffuse manner before they are aggregated in vacuoles or granules. An electron-microscopic study of Neutral Red granules of mouse pancreas demonstrated the reaction product of acid phosphatase. The toxicity of certain dyes may cause cell injury; the injured cytoplasmic areas may be sequestered together with lysosomes and dye-containing phagosomes, and may be extruded.

Basic dyes are taken up by lysosomes.[64] Using Acridine Orange, Neutral Red, quinacrine, and Toluidine Blue for vital and supravital staining of actively growing cells, Bastos *et al.* verified that these dyes had the same capacity to stain lysosomes and lysosome-derived bodies. They also found that a sample of Toluidine Blue contains a purple chromatographic fraction (λ_{max} 622 nm) which induced immediate bright yellow fluorescence in cytoplasmic granules of tumor cells, which were unfixed and supravital-stained.[65]

Neutral Red has been used intravitally to demonstrate granulated cells of the juxtaglomerular system in mouse kidney.[66]

b. Examples of Dyes Used as Diagnostic Agents.[67] An important use of dyes is in clinical diagnosis. Azovan Blue (Trypan Blue) (*o*-tolidine $\rightrightarrows$ 2 moles H-acid)[68] is a dye of low toxicity which is used for the determination of blood and plasma volumes. When it is injected intraveneously, it combines with plasma albumin. Sodium anoxynaphthonate

[63] W. Straus, *in* "Enzyme Cytology" (D. B. Roodyn, ed.), p. 275. Academic Press, New York, 1967.

[64] A. M. Dalcq, *Lysosomes, Ciba Found. Symp.*, pp. 226–263 (1963).

[65] A. L. Bastos *et al.*, *Z. Naturforsch. B* **23**, 969 (1968).

[66] S. Sugiyama, *Stain Technol.* **31**, 206 (1956); see also V. Szokoly, Sz. Gomba, and M. B. Soltész, *Nature (London)* **208**, 1331 (1965).

[67] R. G. Todd, ed., "Extra Pharmacopaeia Martindale," 25th ed., p. 553. Pharmaceutical Press, London, 1967.

[68] Evans Blue (CI Direct Blue 53; CI 23860; *o*-tolidine $\rightrightarrows$ 2 moles Chicago acid) is also used for this purpose.

(CI Acid Blue 92; CI 13390; H-acid → *N*-phenyl peri acid) is used for the determination of blood and plasma volume, the measurement of cardiac output, and the definition of the type and position of intracardiac shunts. It is of low toxicity. Indocyanine Green[69] is used as an indicator for determining cardiac output by the dilution method; it has λ_{max} 800 nm, at which oxyhemoglobin and reduced hemoglobin transmit light equally. Its use for testing liver function is mentioned later. Congo Red (CI Direct Red 28; CI 22120; benzidine ⇉ 2 moles naphthionic acid) is used for the diagnosis of amyloid disease.

As a diagnostic agent fluorescein (CI Acid Yellow 73; CI 45350[69a]) has many uses, such as the detection of corneal lesions and foreign bodies in the eye, circulatory disorders and the differentiation of normal and malignant tissues after intravenous injection, localization of brain tumor, early diagnosis of tuberculous meningitis in children, and measurement of circulation rate. Kiton Green V (Naphthalene Green V[69b]) (CI Acid Green 16; CI 44025) is administered intravenously for the detection of retinal lesions and as a diagnostic agent in brain surgery. It has also been used intravenously, like Sulphon Blue, to assess tissue viability in burns and soft tissue trauma. Sulphon Blue (Patent Blue V New[69c]) (CI Food Blue 3; CI 42045) is used by subcutaneous injection to outline lymph trunks in X-ray radiography and by intravenous injection as a direct visual test of the state of the circulation in normal and damaged tissues, particularly for assessing tissue viability in burns and soft tissue trauma. Injection of a buffered aqueous solution of Chlorazol Sky Blue FF (CI Direct Blue 1; CI 24410; Dianisidine ⇉ 2 moles Chicago acid) enables the visualization of lymph nodes during surgery in carcinoma. Indigo Carmine is used to test the efficiency of the kidneys; after an intravenous injection the normal functioning of the kidneys is followed by estimation of the blue color in urine. Phenol Red,[69d] a sulfonphthalein, is used in a renal function test. The incorporation of Phenol Red in a drug provides a method of checking drug ingestion.

Certain dyes, when injected into the circulatory system, are preferentially removed by the liver and are concentrated in the bile. These "cholephilic dyes" are important clinically to assess liver function in

[69] I. J. Fox and E. H. Wood, *Proc. Staff Meet. Mayo Clin.* **35**, 732 (1960). See *ibid.*, pp. 729–782, for a report of a symposium on Indocyanine Green and its clinical applications.

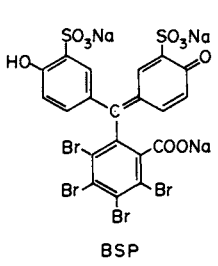
[69a] *CSD II*, p. 747.

[69b] *CSD II*, p. 716.

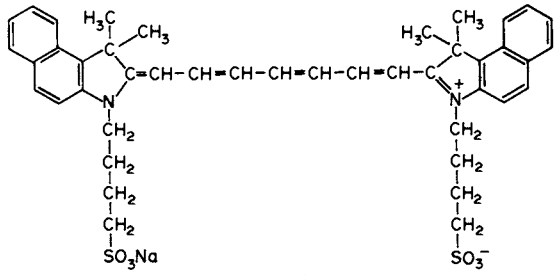
[69c] *CSD II*, p. 715.

[69d] *CSD II*, pp. 736–737.

hepatic disorders. A very detailed review, on which this brief account is based, of the clinical chemistry of cholephilic dyes has recently appeared.[70] At least 200 dyes have been examined, and the most important cholephilic dye is "bromosulfophthalein" ("BSP"); Indocyanine Green and Rose Bengal[70a] have also been used in clinical studies. Indocyanine Green is less irritating than BSP when injected into tissues, but it is much less stable chemically than BSP and less soluble in aqueous media. It is noteworthy that BSP and Indocyanine Green, so frequently used in clinical studies, are far from ideal, and a search for better alternatives is desirable.



BSP



Indocyanine Green

In addition to being nontoxic, cholephilic dyes should be (a) excreted mainly or exclusively by the liver, (b) crystalloid so that they may not be taken up by reticuloendothelial cells, and (c) retained in the circulation long enough after injection to allow blood samples to be taken at convenient intervals. Anionic dyes regardless of their structure are excreted by the liver more readily than cationic dyes.

The transfer of BSP from plasma to bile involves three distinct phases: (a) hepatic uptake of the dye from plasma by a membrane transport system, (b) conjugation of BSP with proteins, and (c) biliary excretion of conjugated and free BSP.

In the standard BSP retention test BSP (usually 5 mg/kg) is given by intravenous injection, and the percentage remaining in the plasma at a given time after injection is determined colorimetrically at one or two wavelengths (580 nm and/or 620 nm). In normal persons only 0–10% of the original dose of BSP is retained 30 to 45 minutes after injection; but the interpretation of the results in terms of liver function has to be made cautiously.

If radioactive dyes are used, external counting at various sites in the

[70] P. Jablonski and J. A. Owen, *Advan. Clin. Chem.* **12**, 309 (1969).

[70a] For a study of the metabolism of Rose Bengal, see M. Jirsa and P. Raban, *Nature (London)* **195**, 1100 (1962).

patient becomes possible, and the minute quantities of radioactive dyes which are adequate would reduce toxic hazards. However, toxic hazards have rarely been reported with unlabeled BSP or Indocyanine Green.

c. Reactive Dyes.[71] Procion Navy Blue M-3R (CI Reactive Blue 9), a reactive dye for cotton, has been suggested as a vital stain for laboratory mammals.[72] With intravenous injection, the animals became bright blue almost instantly. Dense staining of connective tissues, which occurred within minutes, persisted to variable degrees for several months. All connective tissue structures and mucin-containing structures were stained, but no cellular or intracellular staining was seen. The dye has instantaneous anticoagulant activity which can be reversed. Although many of the animals had nonclottable blood, they showed no hemorrhagic manifestations, and most of the animals showed no other evidence of acute or chronic intoxication of consequence. Procion Navy Blue M-3R may be useful in the study of collagen and other connective tissues; since it contains copper, it should be sufficiently electron-dense to make it a useful *in vivo* stain for electron microscopy.

Stretton and Kravitz[73] have developed a technique for determining the geometry and positions of single neurons and their dendritic fields. A single nerve cell is injected with the fluorescent dye Procion Yellow M-4R (CI Reactive Yellow 4; CI 13190), which becomes distributed throughout the cell and remains confined within it during subsequent histological procedures. When viewed with a fluorescence microscope, the dye-filled branches of the neuron appear brilliant yellow against a deep green background of uninjected tissue. This technique has been used, for example, in the study of the morphology and spatial relationships of the giant fibers and fast flexor motor neurons in the crayfish abdomen.[74] Davis[75] has recently applied the dye injection technique to motor neurons supplying relatively complex locomotory appendages, the abdominal swimmerets of the lobster *Homarus americanus*. The general significance of his work is that it demonstrates the usefulness of the dye injection technique for determining the pattern of synaptic connections in a relatively complex network.

The outer segments of cones of living amphibian eye are selectively stained by Procion Yellow M-4RS (a dichlorotriazinyl dye) injected into the vitreous humor. Since the dye does not cross nerve cell membranes,

[71] See *CSD VI* for the chemistry of reactive dyes (Procions, Remazols, etc.). See also W. F. Beech, "Fibre-reactive Dyes." Logos Press, London, 1970.

[72] J. B. Henry, Jr., *Stain Technol.* **43**, 297 (1968).

[73] A. O. W. Stretton and E. A. Kravitz, *Science* **162**, 132 (1968).

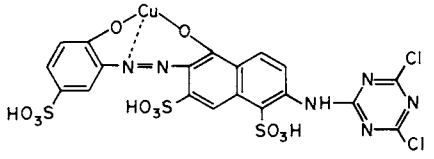
[74] D. Kennedy, A. Selverston, and M. Remler, *Science* **164**, 1488 (1969).

[75] W. J. Davis, *Science* **168**, 1358 (1970).

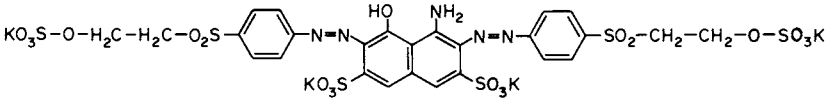
this provides further evidence for structural differences between rod and cone outer segments.[76]

Several reactive dyes of the Procion and Remazol types (particularly Procion Red M-8BS, Orange M-GS, Olive Green M-3GS and Gray M-3GS, and Remazol Black) have proved to be useful as vital indicators of bone growth. They formed covalent bonds with the protein matrices and were preserved *in situ* after fixing, decalcification, and embedding. Upon injection the blood concentrations of the dyes fell rapidly during the first three days, then more slowly, and were almost completely cleared in 14–21 days.[77]

As textile dyes, Procion M and Remazol dyes are primarily used on cellulose fibers, but they can also react chemically with proteins.[71] Procion M dyes are dichloro-1,3,5-triazine derivatives, in which the chlorine atoms are reactive. Remazol dyes contain $-SO_2CH_2CH_2—O—SO_3Na$ as the reactive group. The following are two examples.



Procion Rubine M-B



Remazol Black B

The reactive dyes specifically designed for wool (such as the Procilan, Remalan, Remazolan, and Lanasol dyes) do not appear to have been examined for their use in biological staining.

8. *Fluorescence Microscopy and Fluorochromes*

Many natural products are fluorescent (primary fluorescence),[78,79] but in biological microtechnique the usual practice is to impart fluorescence

[76] A. A. Laties and P. A. Liebman, *Science* **168**, 1475 (1970).

[77] E. C. Seiton and M. B. Engel, *Amer. J. Anat.* **126**, 373 (1969).

[78] A recent book (S. Udenfriend, "Fluorescence Assay in Biology and Medicine," Vol. II. Academic Press, New York, 1969) makes a comprehensive survey of the principles of fluorescence, instrumentation and the application of fluorescence to the assay of proteins, vitamins and other natural products, and as a probe into mechanisms in intact cells.

[79] S. V. Konev ["Fluorescence and Phosphorescence of Proteins and Nucleic Acids" (S. Udenfriend, transl. ed.). Plenum, New York, 1969] has reviewed his own and other work on the electronically excited states of tryptophan, tyrosine, phenylalanine and proteins, the luminescence of biopolymers, and fluorescence as a means of probing the structure and conformation of macromolecules in intact cells.

by suitable stains ("fluorochromes"). The most important dyes useful as fluorochromes in biological staining[2] are (a) the phthaleins: fluorescein, Eosine Y and B, Erythrosine B, Rose Bengal, Rhodamine B[79a]; and (b) the acridine derivative: Acridine Orange.[79b] The benzothiazoles Primuline, Thioflavine S, and Thiazole Yellow G[79c] find some use as fluorescent vital stains.[2] Fluorescence microscopy increases the sensitivity of histochemical methods by several orders of magnitude and has stimulated the commercial development of fluorescence microscopes.[4,78] With the instruments now available, it is possible, for instance, to detect as few as 100 molecules of Acriflavine in 1 square micron.

The use of fluorochromes in antigen–antibody methods and in structural studies of proteins and nucleic acids is discussed in Sections IVC and D, and V.

III. Enzyme Activity and Histochemistry

A. Spectrophotometric Analysis of Enzyme Reactions

Spectrophotometric methods are complementary to manometric, chemical, and other methods in the study of enzyme action.[80] We are not concerned here with the natural substrates and products of enzyme action which absorb light in the UV or visible region and are therefore amenable to spectrophotometric analysis. In estimations such as the hydrolysis of *p*-nitrophenyl phosphate to *p*-nitrophenol by acid or alkaline phosphatase, the formation of *p*-nitrophenol is followed by the absorption at 420 nm in a buffered solution at pH 8. By using the phosphate of a 1-arylazo-2-naphthol the product of enzymic hydrolysis will be insoluble in water and soluble in organic solvents; it can thus be removed continuously and estimated colorimetrically or spectrophotometrically. In the determination of acetyl-CoA, *p*-nitroaniline is preferred to *p*-aminoazobenzene as the acceptor for the acetyl group; λ_{max} for *p*-nitroaniline and *p*-nitroacetanilide are 388 nm and 318 nm, respectively.

The first suggestion to use Methylene Blue as a hydrogen acceptor in enzyme studies was probably made by H. Wieland in 1912. Methylene Blue subsequently found extensive application in biochemistry as an electron acceptor, especially for flavoprotein dehydrogenases. The decolorization of Methylene Blue in evacuated tubes in the presence of an enzyme and its substrate was first demonstrated in 1920 by the

[79a] *CSD II*, pp. 747–751.

[79b] *CSD II*, p. 758.

[79c] *CSD I*, pp. 622–627.

[80] H. U. Bergmeyer, ed., "Methods of Enzymatic Analysis." Academic Press, New York, 1965.

Thunberg process, used for the detection or estimation of the enzyme (e.g., xanthine oxidase, glucose oxidase, liver aldehyde oxidase). Since Methylene Blue is autoxidizable, enzyme activity can be determined even more readily by measuring oxygen uptake manometrically in the presence of enzyme, substrate, and dye (and catalase to destroy H_2O_2 formed by the oxidation of leucomethylene blue). Dehydrogenases requiring di- or triphosphopyridine nucleotides (NAD, NADP) can also be linked to Methylene Blue in the presence of "diaphorases" (flavin enzymes which catalyze the oxidation of reduced pyridine nucleotide by Methylene Blue). It is of historical interest that the observation of Barrón and Harrop[81] on the stimulation of the respiration of erythrocytes in the presence of glucose by Methylene Blue led to the discovery of triphosphopyridine nucleotide by Warburg.[82]

$$\text{Glucose-6-phosphate} + \text{NADP} \xrightarrow{\text{glucose-6-phosphate dehydrogenase}} \text{6-phosphogluconate} + \text{NADPH}_2$$

$$\text{NADPH}_2 + \text{Enz-FMN} \longrightarrow \text{NADP} + \text{Enz}\cdot\text{FMNH}_2$$

$$\text{Enz}\cdot\text{FMN}\cdot\text{H}_2 + \text{Methylene Blue} \longrightarrow \text{Enz}\cdot\text{FMN} + \text{leuco-Methylene Blue}$$

$$\text{Leuco-Methylene blue} + \text{O}_2 \longrightarrow \text{Methylene Blue} + \text{H}_2\text{O}_2$$

(Enz·FMN is Warburg's "old yellow enzyme," the prosthetic group of which is riboflavin monophosphate)

The dyes commonly used in the study of biological oxidations, and also for measuring the O—R potential of enzyme extracts, are listed in Table I. The reduced dyes can also be used as electron donors for the reverse reaction in some cases. The tetrazolium salts and formazans are considered later.

Clark,[83] the source of most of the data in Table I, surprisingly uses the numbers and structures of dyes in *Colour Index*, 1st ed., 1924. The situation becomes more perplexing when it is noted, for instance, that there are 20 Safranines with four constitution numbers mentioned in CI; they are all prepared by the oxidation of various mixtures of aniline, *o*- and *p*-toluidine, *p*-phenylenediamine, 2,5-diaminotoluene, and *p*-aminodimethylaniline. Mahler and Cordes[84] quote $E'_0 = -0.25$ for "Safranine" to which they assign the CI 50240 structure; they name the

[81] E. S. G. Barron and G. A. Harrop, Jr., *J. Biol. Chem.* **79**, 65 (1928).

[82] O. Warburg, "Wasserstoffubertragende Fermente," p. 9. Verlag Dr. Werner Saenger, Berlin, 1948.

[83] W. M. Clark, "Oxidation-Reduction Potentials of Organic Systems." Williams & Wilkins, Baltimore, Maryland, 1960.

[84] H. R. Mahler and E. H. Cordes, "Biological Chemistry," pp. 559–560. Harper, New York, 1966.

TABLE I

DYES USEFUL AS O—R INDICATORS AND ELECTRON ACCEPTORS IN THE STUDY OF DEHYDROGENASES

Dye	E'_0 (pH 7.0)
2,6-Dichlorophenol-indophenol (Tillmans reagent)	0.22
Brilliant Cresyl Blue	0.045
Gallocyanine	0.021
Methylene Blue	0.011
Trisodium indigo trisulfonate	−0.081
Disodium indigo disulfonate	−0.125
Sodium indigo monosulfonate	−0.157
Janus Green B	−0.188
Safranine B	−0.252
Safranine T	−0.289
Neutral Red	−0.325
Benzyl Viologen	−0.359
Methyl Viologen	−0.440
N-Methylphenazinium methosulfate	0.080

"monomethyl compound" (not mentioned in *CI*) Safranine T, for which they quote $E'_0 = -0.289$. Since the O—R potential of a dye can be a critical factor in its choice as an electron acceptor in the study of an oxidoreductase, the collection of new data on dyes of authentic structure and purity is desirable. In this connection Methylene Blue, 2,6-dichlorophenol-indophenol ("DCIP"[84a]), the tetrazolium salts, and the Viologens have the advantage that they are readily obtained as pure homogeneous compounds; further, except Methylene Blue, they are made specifically for biological uses.

Clark[83] has remarked that Janus Green B has been used frequently in biological studies for some strange reason, although it has two sites of reduction; the first step is irreversible, and the second is reversible. Cooperstein *et al.*[85] found the potentials as −0.001 (irreversible) and −0.188 (reversible) at pH 7.48. They also observed that the analog from dimethylsafranine showed only one reduction step at −0.214. After confirming this result, a satisfactory explanation should be found for the marked difference in the effect of the NMe_2 and NEt_2 groups.

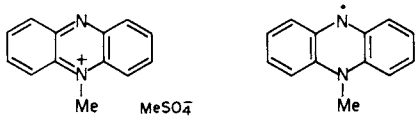
DCIP is a suitable redox dye for the study of dehydrogenases (e.g., in the reaction RCH_2CH_2—CO—S—CoA → RCH=CH—CO—S—CoA).[80]

[84a] *CSD II*, p. 763.

[85] S. J. Cooperstein, A. Lazarow, and J. W. Patterson, *Exp. Cell Res.* **5**, 69 (1953).

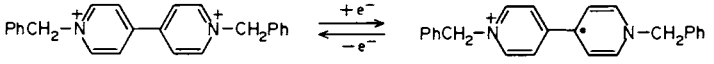
Unlike Methylene Blue, it is relatively nonautoxidizable and its reduction can be measured spectrophotometrically under aerobic conditions.

N-Methylphenazinium methosulfate (I; "phenazine methosulfate"; "PMS") and the viologens are one-electron acceptors.[86] PMS has been found to be the electron carrier of choice in the assay of succinic dehydrogenase[87]; for the assay of the enzyme in higher organisms and aerobic yeast mitochondria, the reduction of DCIP is followed spectrophotometrically, using PMS as catalyst, because DCIP does not react directly with the enzyme.[88] Phenazine derivatives such as (I) resemble the flavins in their ready formation of free radicals (II).[89] Points to be remembered in the storage and use of (I) are that it undergoes photochemical oxidation to the deep blue pyocyanine,[89a] and that the phenazyl (II) forms at low temperature a dimer which exhibits a new band at 800 nm in the absorption spectrum.[90]



(I) (II)

The low potential of the viologens enables them to be used for studying low E'_0 systems such as xanthine dehydrogenase and hydrogenases.[91] Benzyl viologen has also been used for the study of nitrite and nitrate reductases.[92] Colorless benzyl viologen takes up an electron to form the blue ion-radical (III).



Benzyl viologen
Colorless

(III) Blue

B. Solid Substrates for Enzyme Reactions

Dyes have also been used in "solid-substrate" assay for enzyme reactions. The principle involved in this method is to use a substrate

[86] For a discussion of these compounds in connection with the formation of stable free radicals, see A. R. Forrester, J. M. Hay, and R. H. Thomson, "Organic Chemistry of Stable Free Radicals." Academic Press, New York, 1968.

[87] V. Massey and T. P. Singer, *J. Biol. Chem.* **229**, 755 (1957).

[88] O. Arrigoni and T. P. Singer, *Nature (London)* **193**, 1256 (1962).

[89] F. Dickens and H. McIlwain, *Biochem. J.* **32**, 1615 (1938).

[89a] *CSD II*, p. 779.

[90] H. McIlwain, *J. Chem. Soc., London* p. 1704 (1937).

[91] L. Michaelis, *Chem. Rev.* **16**, 243 (1935); L. Michaelis and E. S. Hill, *J. Amer. Chem. Soc.* **55**, 1481 (1933); *J. Gen. Physiol.* **16**, 859 (1933).

[92] O. Prakash and J. C. Sadana, *Arch. Biochem. Biophys.* **148**, 614 (1972); J. C. Sadana and W. D. McElroy, *ibid.* **67**, 16 (1957).

which is not soluble in the hydrolyzing buffer, but will release a soluble, highly colored product. Thus, a suitable amount of such a solid substrate is suspended in an enzyme reaction mixture for a fixed period of time, and the enzyme reaction is then stopped by removing the substrate through filtration or centrifugation, by changing the pH, adding trichloroacetic acid, or heating. The color intensity gives a measure of the enzyme activity in unit time. Originally the dye–substrate complexes depended on the physical adsorption of the dye and were used for the assay of collagenase[93] (azo coll, hide powder with a deep red azo dye), proteases[94] (Congo coll, Congo red elastin,[94a] orcein elastin[94b]), pepsin and acid proteases (fibrin blue), and peptic and tryptic activity (azo albumin).[94c] In this method migration of dye from substrate protein to other soluble protein could occur to give a false positive result. More recently, therefore, substrates having covalently bound dye have been used, such as amylose-azure (Remazol Brilliant Blue bound to amylose)[95] for α-amylase, Remazol Brilliant Blue attached to cellulose powder or filter paper for cellulase,[96] and hide powder azure (the same dye bound to collagen) for neutral proteases.[96] These methods are less specific and accurate than those with specific substrates for proteolytic enzymes which vary widely in specificity, but dye-bound substrate techniques are rapid and simple, and they are suitable for rapid screening for protease producing organisms. Filter paper with bound Remazol Brilliant Blue has been suggested as suitable for the estimation of cellulase activity as well as rapid screening for cellulolytic microorganisms.[96a]

C. Histochemistry[4,7,80]

Enzymatic histochemical staining reactions proceed in general as follows[97]:

$$\text{Substrate} \xrightarrow{\text{enzymatic reaction}} \text{stain precursor} \xrightarrow[\text{with developer}]{\text{capture reaction}} \text{stain}$$

[93] C. L. Oakley, G. H. Warrack, and W. E. Van Heyningen, *J. Pathol. Bacteriol.* **58**, 229 (1946).

[94] W. L. Nelson, E. I. Ciaccio, and G. P. Hess, *Anal. Biochem.* **2**, 39 (1961).

[94a] M. A. Naughton and F. Sanger, *Biochem. J.* **78**, 156 (1961).

[94b] L. A. Sachar, K. Wintek, N. Sicher, and S. Frankel, *Proc. Soc. Exp. Biol. Med.* **90**, 323 (1955).

[94c] R. M. Tomarelli, J. Charney, and M. L. Harding, *J. Lab. Clin. Med.* **34**, 428 (1949).

[95] H. Rinderknecht, P. Wilding, and B. J. Haverback, *Experientia* **23**, 805 (1967).

[96] H. Rinderknecht, M. C. Geokas, P. Silverman and B. J. Haverback, *Clin. Chim. Acta* **21**, 197 (1968).

[96a] R. P. Poincelot and P. R. Day, *Appl. Microbiol.* **23**, 875 (1972).

[97] S. J. Holt *et al.*, *Proc. Roy. Soc., Ser. B* **148**, 465, 481, 495, 506, and 520 (1958).

Thus dye formation takes place from a colorless precursor (e.g., an azoic coupling component). Holt[97] has studied the theoretical basis of the staining methods classified under this scheme. A localization factor was defined which gave an indication of the intrinsic localization possibilities of any cytochemical method. A prospective substrate should not associate with protein, except possibly the specific enzyme species, but it should yield a stain precursor and a stain, both of which should be highly substantive towards protein. Considering the term "substantivity" in the light of structural factors governing the substantivity of synthetic dyes for cellulosic and protein fibers,[98] Holt has discussed the bearing of lipid solubility and substantivity of dyes on cytochemical staining.

Staining by the action of acid or basic dyes with amphoteric tissue proteins is minimal or does not take place at all under histochemical conditions. The concept of substantivity of dyes for fibers, in terms, for instance, of the heat of dyeing, does not apply to the histochemical connotation of substantivity according to Burstone,[7] who relates it to

> ... affinity or insoluble nature of the reaction products formed by substrates *in situ*. Three types of dyes may result from histochemical reactions: (1) dyes which are amorphous in appearance even at the highest optical level (e.g., dyes derived from Naphthol AS derivatives and 4,4'-dichloroindigo and nitro-blue tetrazolium); (2) dyes which are initially amorphous or of fine crystalline pattern, but which subsequently crystallize (e.g., derivatives of 6-benzoyl-2-naphthol and 6-bromo-2-naphthol, and indoaniline dyes from α-naphthol and phenylenediamines); (3) dyes which initially exhibit a grossly crystalline pattern (e.g., unsubstituted indigo and simple formazan dyes).

According to O. Sullivan (cited by Burstone[7]) the following conditions are necessary for obtaining successful cytochemical localization[99]: (1) The preparatory processing and subsequent treatment of tissues should not affect the distribution of enzyme activity. (2) Rapid penetration of the substrate and developing agent should occur with reference to all cellular components. (3) The substrate should specifically interact with one enzyme or group of enzymes. (4) The developing agent should not affect the enzymatic process or the penetration of the substrate. (5) The product of the enzyme-catalyzed reaction should combine very rapidly with the developing agent and the velocity constant should not be affected by different cellular environments. (6) The colored product should be immediately precipitated, i.e., it should have a very low solubility, should not supersaturate, should

[98] *CSD II*, p. 1253; T. Vickerstaff, "The Physical Chemistry of Dyeing," 2nd ed., p. 172. Oliver & Boyd, Edinburgh. 1954.

[99] See also M. M. Nachlas, A. C. Young, and A. M. Seligman, *J. Histochem. Cytochem.* **5**, 565 (1957).

deposit in an amorphous or submicrocrystalline state, and should be stable. (7) No preferential adsorption of any of the substances involved should occur in special sites which are unrelated to enzyme distribution.

Table II lists examples of substrates for a series of enzymes. Such materials and the relevant reactions can also be used for the localization of enzyme activity in enzyme separation methods, e.g., by electrophoresis on acrylamide gels or other supports.[99a]

The pigments produced in tissue sections by the enzymatic processes of Table II are of the following types: (1) azoic; (2) indigoids; (3) indophenols; (4) azines; (5) miscellaneous oxidation products; and (6) formazans.

TABLE II

ENZYME SUBSTRATES[7]

Enzyme	*Examples of substrates*
Phosphatase	Phosphates of Naphthol AS and its analogs
Esterase	Naphthol AS acetate; indoxyl acetate
Sulfatase	Naphthol AS sulfate Phenolphthalein disulfate
Glycosidase (hydrolases attacking glycosidic linkages; most important histochemically are β-glucuronidases and β-glucosaminidases)	Glycosides of 6-bromo-2-naphthol, Naphthol AS, 8-hydroxyquinoline, umbelliferone, phenolphthalein
Proteolytic enzymes (mainly aminopeptidase)	Naphthol AS chloroacetate; L-cysteine-β-naphthylamide; DL-alanyl-3-aminocarbazole
Oxidases:	
(a) Cytochrome *c*	A mixture of (a) *p*-aminodiphenylamine and amides of 1-hydroxy-2-naphthoic acid; (b) α-naphthol and 1-amino-4-dimethylamino-naphthalene
(b) Peroxidases	Wide variety of aromatic amines, phenols, phenolic acids; leuco derivative of Malachite Green
(c) Monoamine oxidase (MAO; tyraminase)	Tryptamine + a tetrazolium salt, tryptamine + 2-hydroxy-3-naphthoylhydrazide (condensation product of the aldehyde from tryptamine and the hydrazide developed with a diazonium salt)
(d) Tyrosinase and Dopa oxidase	Tyrosine (→ Dopa → *o*-quinone → red hallochrome → black melanin)
Dehydrogenases	Tetrazolium salts

[99a] For references, see A. H. Gordon, "Electrophoresis of Proteins in Polyacrylamide and Starch Gels," p. 62. Elsevier, Amsterdam, 1969.

Very few preformed azo dyes themselves have been used as reagents in enzyme histochemistry; two examples are 4-*p*-nitrophenylazo-1-naphthol phosphate and a hydroxyphenylazonaphthol glucuronide.

The action of hydrolytic enzymes on esters and glycosides of Naphthol AS and its analogs liberates the naphthol, which is then coupled with suitable diazonium salts to form azoic pigments. Burstone[7] prepared the phosphates of many known and new arylamides of naphthoic acid as substrates for the histochemical demonstration of alkaline phosphatase. The β-anthraquinonylamide of 2-hydroxy-3-naphthoic acid was found to produce exceedingly sharp localizations. The condensation product of 2-hydroxy-3-naphthoic acid with *p*-amino-azobenzene is useful for a noncoupling technique.

The data on coupling rates available for azoic dyes produced on textiles or prepared as pigments are not directly applicable to histochemical conditions, and relevant data have to be collected by specific experiments. With the object of producing highly insoluble and deeply colored products to facilitate histochemical localizations and increasing the permanance of stained preparations, Burstone and Weisburger[100] studied the diazotization and coupling behavior of many amines (such as 1-amino-2-bromo-4-toluidinoanthraquinone) other than those used normally for the preparation of azo and azoic dyes.

Numerous compounds yielding colored products by oxidation have been used for the study of oxidases and peroxidases. They include amines and phenols which give by oxidation dyes of more or less complex and indefinite structures, indoxyls and thioindoxyls which give characterizable indigoid and thioindigoid dyes, and leuco bases of basic dyes such as Malachite Green.

Ehrlich observed in 1885 the formation of a blue dye when α-naphthol and *p*-aminodimethylaniline were injected into living tissues. This formation of indophenol blue[100a] is often referred to as the "nadi reaction" (naphthol + diamine), and the role of cytochrome oxidase in this reaction was demonstrated by Keilin and Hartree.[101] However, the very easy formation of indophenol blue by atmospheric oxygen limits its value,[102,103] and new substrates have been investigated. The best results are obtained with amides of 1-hydroxy-2-naphthoic acid in

[100] M. S. Burstone and E. K. Weisburger, *J. Histochem. Cytochem.* **9**, 301 and 349 (1961).

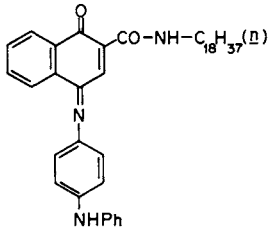
[100a] *CSD II*, p. 763.

[101] D. Keilin and E. F. Hartree, *Proc. Roy. Soc., Ser. B* **127**, 167 (1939).

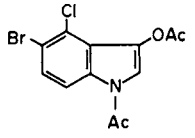
[102] G. Gomori "Microscopic Histochemistry," p. 153. Univ. of Chicago Press, Chicago, Illinois, 1952.

[103] L. Lison, "Histochemie et cytochemie animales," 3rd ed., Vol. 2, p. 597. Gauthier Villars, Paris, 1960.

conjunction with *p*-aminodiphenylamine. Thus the indoaniline (IV) is produced by the action of cytochrome oxidase in tissue sections, and a more precise and permanent localization is effected by post-mordanting with divalent cobalt. Other useful amines for the reaction are 3-amino-9-ethylcarbazole and 8-amino-1,2,3,4-tetrahydroquinoline, which are resistant to autoxidation, making long incubation periods feasible.[7] The formation of azamethine and indoaniline dyes[103a] has been discussed by J. Bailey and L. A. Williams in Vol. IV of this series in connection with the photographic color development process. Many examples of developers and couplers are cited, and some may prove to be useful in the study of oxidases.



(IV)



(V)

Azines can be produced under mild conditions of oxidation, for instance, by post-incubation treatment of stained sections with an arylamine in the presence of an oxidizing agent. Because of their stability in comparison with the indoanilines and their intense color, they offer better permanence and greater contrast for photomicrography.[7] The chemistry of the azine dyes, discussed by Bailey and Williams in Vol. IV as photographic image dyes, is relevant for a consideration of their use in histochemistry and cytochemistry.

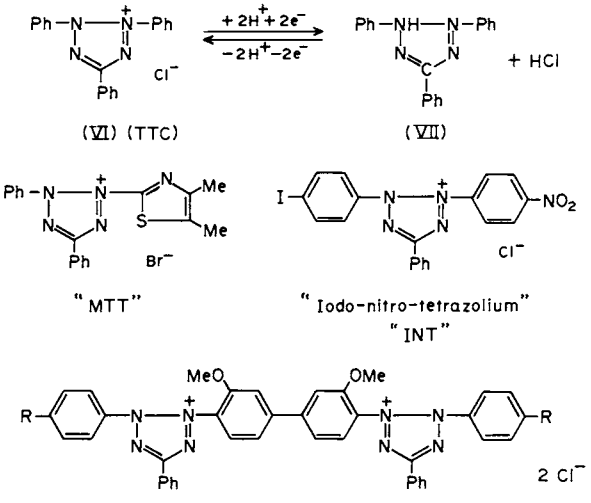
The oxidation of indoxyl and substituted indoxyls to indigoid dyes is a chromogenic reaction suitable for cytochemical staining to localize cellular enzymes. Holt[97] synthesized a series of substituted indoxyl acetates and examined them as substrates for esterases. The relationships between solubility, molecular association, and structure in indigoid dyes were investigated. Aerial oxidation of indoxyls to indigoids was followed kinetically by a spectrophotometric method, and a free-radical mechanism involving intermediate formation of leucoindigoids was postulated. 5-Bromo-4-chloro-diacetylindoxyl (V) was found to be the most effective reagent for accurate cytochemical localizations.

Formazans and tetrazolium salts have been known since 1892, and

[103a] *CSD II*, p. 1201.

Wizinger has carried out extensive work on metal complexes of formazans, particularly those with hydroxyl and carboxyl groups *ortho* to the formazan chain.[104] No formazan appears to have attained commercial success as a dye; but the tetrazolium salts and the formazans have proved to be of great interest in biology. Hooper[105] has recently reviewed the synthesis and properties of formazans and tetrazolium salts, including the uses of tetrazolium salts in biochemistry, microbiology, histochemistry, and cytochemistry. Formazans (e.g., VII) are conveniently prepared by coupling aryldiazonium salts with benzaldehyde phenylhydrazone or appropriate derivatives. Tetrazolium salts (e.g., VI) are then obtained by the oxidative cyclization of the formazans. Colorless tetrazolium salts (VI) are reduced to colored formazans (VII). Kuhn and Jerchel[105a] demonstrated the reduction of tetrazolium salts by plant tissues at pH 7.2, and Lakon[105b] used triphenyl tetrazolium chloride (TTC; VI) for testing the viability of seeds by the formation of the red formazan (VII). Formazans are not reoxidized to tetrazoles in biological systems.

A simple formazan, such as (VI), is orange-red in color, and by the introduction of nitro groups or iodine atoms or by dimerization, violet,



R = H; Blue tetrazolium chloride (BT)

R = NO_2 (Nitro-BT; Nitro blue tetrazolium)

[104] For references, see R. Price, *CSD III*, p. 373.

[105] W. D. Hooper, *Rev. Pure Appl. Chem.* **19**, 231 (1969); see also A. W. Nineham *Chem. Rev.* **55**, 355 (1955).

[105a] R. Kuhn and D. Jerchel, *Ber. Deut. Chem. Ges. A* **74**, 941 (1941).

[105b] G. Lakon, *Ber. Deut. Bot. Ges.* **60**, 299, 434 (1942).

blue, and green hues become available. Four examples of commonly used tetrazolium salts are MTT, INT, BT, and nitro-BT.[106]

Pearse[4] has given a detailed account of the properties of the tetrazolium salts and their role as electron acceptors from the oxidized substrate in dehydrogenase histochemistry. Above pH 9 the tetrazolium salts are reduced by thiols and can be used for the study of enzymatic processes involving thiol (sulfhydryl) groups. The reduction potentials of some common tetrazolium salts are given in Table III.[107]

TABLE III

REDUCTION POTENTIALS OF TETRAZOLIUM SALTS

Compound	E'_0 (22°, pH 7.2)
TTC	−0.49
Neo-tetrazolium (NT)	−0.17
BT	−0.16
MTT	−0.11
INT	−0.09
Nitro-BT	−0.05

The wide variety of applications of tetrazolium salts, all of which involve reduction to the corresponding formazans, has led to searching investigations of the reaction by polarography, ESR spectroscopy, and classical chemical and biochemical experiments.[105]

IV. Chemical Modification of Proteins

The "primary structure" of a protein refers to the sequence of amino acids linked covalently; the "secondary structure" to the conformational aspects of the polypeptide backbone resulting from hydrogen bonding between CO and NH groups in neighboring chains or in the same chain (sheet or helical structures); and the "tertiary structure" to the further folding and refolding of the polypeptide chains resulting from the interactions between the side chains to form a compact molecule of highly organized and specific structure.[108] Perutz has stated that the nature of this anatomy constitutes the central problem of protein chemistry and that it can be solved only by X-ray analysis.[108] Starting

[106] S. S. Karmarkar, R. J. Barrnett, M. M. Nachlas, and A. M. Seligman, *J. Amer. Chem. Soc.* **81**, 3771 (1959); **82**, 575 (1960); and other papers by the Seligman school.

[107] See D. Jerchel, H. Geyer, and H. Holtkamp, *Justus Liebigs Ann. Chem.* **613**, 137 (1958), for more extensive data.

[108] M. F. Perutz, "Proteins and Nucleic Acids, Structure and Function." Elsevier, Amsterdam, 1962.

with the classic work of Sanger,[108a] who determined the complete primary structure of insulin, the amino acid sequence of several other proteins has been unravelled by chemical methods. Sanger used 2,4-dinitrofluorobenzene for attacking the *N*-terminal amino acid; the corresponding chloro compound is a very old and well-known dye intermediate. Other reagents for this purpose and for the fission of protein chains at specific sites are now available. The interaction of proteins with reagents which do not degrade, but modify them, has provided extensive knowledge of their chemical and biological properties. The specific chemical modification of proteins is the subject of several recent reviews,[109,110] two of which are devoted to group-specific reagents[111] and to active sites of enzymes.[112] Habeeb has discussed the changes in protein conformation which are associated with chemical modification.[112a]

A brief account is given in this section of some of the dyes, color-formers, and fluorescent compounds which have proved useful for investigating protein structures and properties.

A. Dye Binding

In the separation of proteins by electrophoresis on supporting media the usual method for making the protein zones visible is to stain them with dyes.[113] Among the dyes commonly used are Amido Black 10B and Light Green SF. On cellulose acetate strips Coomassie Brilliant Blue R was found to be much more sensitive than Amido Black. Procion Blue RS (Procion Brilliant Blue M-R) has the advantage of the best reproducibility and accuracy; the possibility of this reactive dye combining chemically with the protein has also been considered.[114] In the important technique of disk electrophoresis in polyacrylamide gel, which combines the principles of electrophoresis and gel filtration,[114a] a protein sample of a few micrograms is concentrated into an extremely

[108a] F. Sanger, *Biochem. J.* **39**, 507 (1945).

[109] G. E. Means and R. E. Feeney, "Chemical Modification of Proteins." Holden-Day, 1971; see also A. N. Glazer, *Annu. Rev. Biochem.* **39**, 101 (1970); R. B. Freedman, *Quart. Rev., Chem. Soc.* **25**, 431 (1971).

[110] C. H. W. Hirs, ed., "Methods in Enzymology," Vol. 11. Academic Press, New York, 1967.

[111] L. A. Cohen, *Annu. Rev. Biochem.* **37**, 695 (1968).

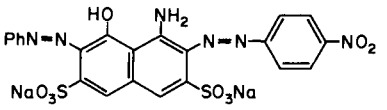
[112] B. L. Vallee and J. F. Riordan, *Annu. Rev. Biochem.* **38**, 733 (1969).

[112a] A. F. S. A. Habeeb, *in* "Chemistry of the Cell Interface" (H. D. Brown, ed.), Part B, p. 259. Academic Press, New York, 1971.

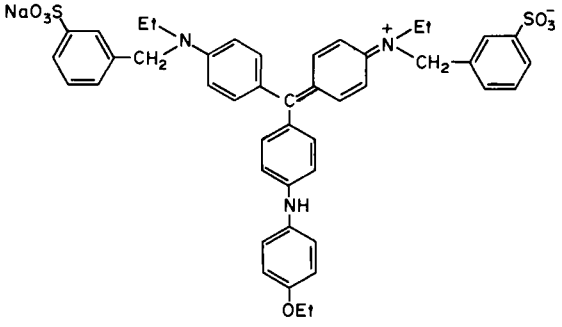
[113] W. Grassman, K. Hannig, and M. Knedl, *Deut. Med. Wochenschr.* **76**, 333 (1951).

[114] S. F. de St. Groth, R. G. Webster, and A. Datyner, *Biochim. Biophys. Acta* **71**, 377 (1963).

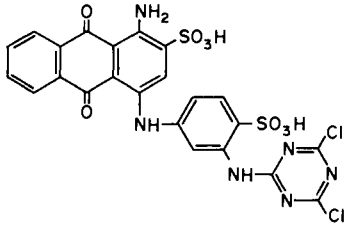
[114a] L. Ornstein, *Ann. N. Y. Acad. Sci.* **121**, 321 (1964).



Amido Black 10 B
CI Acid Black 1
CI 20470



Coomassie Brilliant Blue R
CI Acid Blue 83
CI 42660



Procion Brilliant Blue M-R

sharp layer at the origin by proper selection of the gel components and buffer ions.[114b] The separated proteins are located in the transparent gel matrix by staining with dyes, and enzyme activity can be localized by histochemical procedures. (See Section III.)

Proteins such as gelatin, casein, fibrin, and edestin were found by titration to combine with acid dyes, such as Biebrich Scarlet, Naphthylamine Brown, Tropeolin O, and Metanil Yellow in stoichiometric proportions,[115] which could be correlated with the proportion of free basic groups of arginine, histidine, and lysine residues. Titration curves were used for the determination of basic groups of proteins by means of

[114b] O. Gabriel, *in* "Methods in Enzymology" (W. B. Jakoby, ed.), Vol. 22, p. 578. Academic Press, New York, 1971.

[115] L. M. Chapman, D. M. Greenberg, and C. L. A. Schmidt, *J. Biol. Chem.* **72**, 707 (1927).

Orange G and of carboxyl, phenolic, and thiol groups by Safranine O.[116]

Using the equilibrium dialysis method Fredericq[117] studied the interaction of bovine plasma albumin and insulin with anions derived from benzene and naphthalene derivatives, and with a large number of anionic dyes of the azo, phthalein, indigoid, and anthraquinone classes. He found that the affinity between the anions and proteins was proportional to the number of aromatic rings of the anions, and that van der Waals forces were greater than electrostatic attraction. Substituents such as hydroxyl, nitro, and halogens on the aromatic rings increased the binding affinity, whereas amino substituents depressed it.

Klotz has carried out extensive work on dye–protein interaction. In a review of dye-binding methods, which must be consulted for references to earlier work, Rosenberg and Klotz[118] have given a detailed account of the basic principles; experimental procedures for equilibrium dialysis, precipitation, and spectrophotometry; and the information about proteins which can be obtained from dye-binding methods. The results of equilibrium dialysis were used to deduce the number and nature of the binding sites on proteins and the energy of binding. The presence of sites of widely different affinities for dyes was established. Most of the studies were on serum albumins because of their almost unique ability to bind a variety of small molecules. Specific structural features (such as differences between human and bovine serum albumin) were shown more clearly by spectral studies. The azomercurial (VIII) reacts with the single SH group in BSA to form an R—Hg—S– protein conjugate, and the acid–base equilibrium of the NMe_2 group was studied by measuring absorption spectra as a function of pH. Denaturation of a protein can destroy or enhance dye-binding ability, and the kinetics of denaturation can be followed spectrophotometrically.

A comparison of the binding properties of BSA with 4-hydroxyazobenzene, azobenzene-4-sulfonic acid, and 4-hydroxyazobenzene-4′ sulfonic acid revealed that 4-hydroxyazobenzene was strongly bound at pH 2.9 although it contained no anionic substituent.[119] At 28°, 4-hydroxyazobenzene-4′-sulfonic acid was more strongly bound than azobenzene-4-sulfonic acid, which in turn was bound more strongly than 4-hydroxyazobenzene, both at 2° and at 28°. Forbes *et al.*[119] have rightly stated that spectral changes must be used cautiously as a

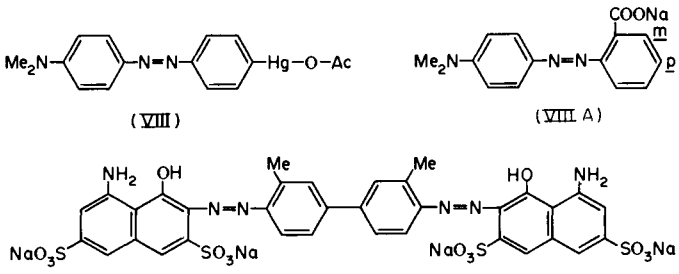
[116] H. Fraenkel-Conrat and M. Cooper, *J. Biol. Chem.* **154**, 239 (1944).

[117] E. Fredericq, *Bull. Soc. Chim. Belg.* **63**, 158 (1954); **64**, 639 (1955); **65**, 644 (1956).

[118] R. M. Rosenberg and I. M. Klotz, *in* "A Laboratory Manual of Analytical Methods of Protein Chemistry" (P. Alexander and R. J. Block, eds.), Vol. 2, p. 133. Pergamon, Oxford, 1960.

[119] W. F. Forbes, B. S. Harrap, and B. Milligan, *Aust. J. Chem.* **15**, 82 and 841 (1962).

measure of the interactions between proteins and organic molecules; but there is no evidence for their improbable suggestion that one type of spectral change in the binding of Methyl Orange by protein is the result of out-of-plane twisting of the dye's conjugated system.



Trypan Blue
CI Direct Blue 14
CI 23850

From the spectral shifts produced by the interaction of BSA with the three dyes [(VIII A) and the *m*- and *p*-isomers], it was found that under the experimental conditions they did not compete for binding sites. It was concluded that the three dyes were bound at different sites and that the *para*-isomer caused unfolding of the protein.[120]

Trypan Blue has the structure of a typical direct cotton dye: a symmetrical and elongated planar molecule which can attach itself to cellulose and to proteins by van der Waals forces as well as hydrogen bonding, and to proteins also as an anionic compound with four sulfonate groups. It has a tendency to aggregate in aqueous solution and its absorption spectrum is also sensitive to pH changes. Lang and Lasser[121] found that it is not amenable to equilibrium dialysis because of its strong binding to dialysis tubing. The effect of BSA on its spectrum disclosed unusual properties of the system, but the qualitative spectral changes which were measured at pH 7.4 and 3.3 over a wide range of dye–protein ratios were interpreted in terms of sets of binding sites. A set was defined as a number of occupied sites which were spectroscopically indistinguishable.

Glazer[122] studied by spectrophotometry and equilibrium dialysis the interaction of trypsin, α-chymotrypsin, their zymogens, and a few other proteins with 50 dyes of the azo, acridine, phenothiazine, and other

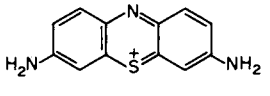
[120] R. K. Burkhard, F. A. Moore, and S. J. Louloudes, *Arch. Biochem. Biophys.* **94**, 291 (1961).

[121] J. H. Lang and E. C. Lasser, *Biochemistry* **6**, 2403 (1967).

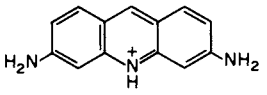
[122] A. N. Glazer, *Proc. Nat. Acad. Sci. U.S.* **54**, 171 (1965); **65**, 1057 (1970); *J. Biol. Chem.* **242**, 3326 and 4528 (1967).

classes. Dyes with a net charge of more than 2 at pH 7 were avoided, and the buffer composition was so chosen that electrostatic interaction was minimized. Dye concentrations were below 5×10^{-4} *M*, and a molar excess of protein was used. The protein–dye interactions were highly specific.

Thionine did not bind to chymotrypsin, but in the structurally very similar trypsin it had one binding site, overlapping with the site involved in substrate binding. Proflavine bound to both chymotrypsin and trypsin, and the difference in behavior between the phenothiazine and acridine dyes was attributed to the size of the sulfur atom; however, the difference in color is sufficient to show that other factors are involved.

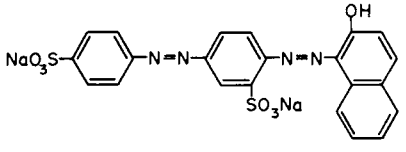


Thionine
CI 52000*

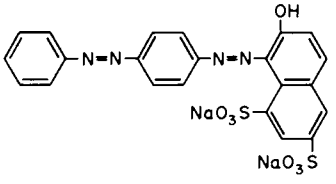


Proflavine

α-Chymotrypsin had a strong binding site for Biebrich Scarlet, overlapping with the active site region, and complex formation was accompanied by a red shift in the visible spectrum. The dye did not bind strongly to chymotrypsinogen, nor to trypsin. The isomeric dye Croceine Scarlet MOO was totally unaffected in its spectrum by chymotrypsin, and it was a much poorer inhibitor of the enzyme than Biebrich Scarlet. It was suggested that a major contribution to the binding of Biebrich Scarlet was the naphthalene ring unsubstituted by hydrophilic sulfonic



Biebrich Scarlet WS
CI Acid Red 66
CI 26905



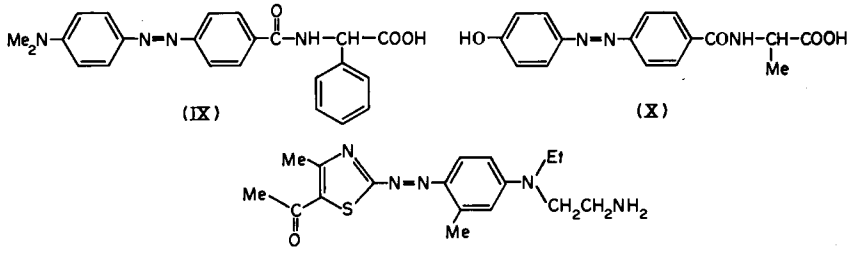
Croceine Scarlet MOO
CI Acid Red 73
CI 27290

groups. Summarizing his results, Glazer found that strong dye-binding to simple globular proteins took place predominantly in areas overlapping the active sites. The dyes had no structural relationship to those of the normal ligands. Glazer has suggested that this phenomenon is a reflection of the special stereochemical features of such sites, their hydrophobicity relative to other portions of the protein surface, and possibly greater flexibility in these regions of the protein molecule.

Biebrich Scarlet binds and stacks to polycations, such as histone, like cationic dyes on polyanions.[123]

Meier[123a] has discussed dye-sensitized photooxidation, which is of interest in connection with the use of dyes as sensitizing agents for the photochemical oxidation of proteins; one object is to make a specific attack on the histidine residue.[124]

The configurational adaptability of the binding regions of proteins was indicated by equilibrium dialysis studies and spectral investigations of the binding of BSA with optical isomers of the azo dye (IX).[125] It was observed that for low values of moles of optically active dye per mole of protein, only one or two sites were involved, and the protein had preference for the *l*-isomer, indicating that the binding site possessed configurational asymmetry. When 9 or more moles of dye were used per mole of protein both isomers were found to bind almost equally well. In the case of the azo dye (X), derived from optically active alanine, the binding capability of BSA was more for the *d*-isomer than for the *l*-isomer. However, the selectivity completely disappeared when BSA was denatured by heating to 90°.[126]



Stryer and Blout[127] studied the optical rotatory dispersion (ORD) of complexes of cationic dyes such as (XI), Acridine Orange, and pseudoisocyanine with L- and D-poly-α-glutamic acids (PGA). At pH above 5.5, L- or D-PGA had random conformation. The ORD of the dye–polypeptide complex was identical with that of random PGA, showing that the ORD was due to PGA alone. At pH below 5.1, PGA had a helical conformation and the dye–PGA complexes showed anomalous

[123] J. W. Winkelman and D. F. Bradley, *Biochim. Biophys. Acta* **126**, 536 (1966).

[123a] *CSD IV*, p. 498.

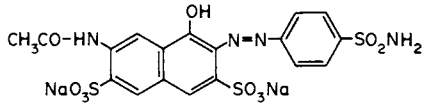
[124] W. J. Ray, Jr., *in* "Methods in Enzymology" (C. W. Hirs, ed.), Vol. 11, p. 490. Academic Press, New York, 1967.

[125] F. Karush, *J. Phys. Chem.* **56**, 700 (1952).

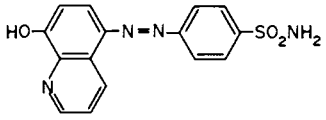
[126] J. Koga, N. Morita, and N. Kuroki, *Bull. Univ. Osaka Prefect., Ser. A* **17**, 347 (1968); *Nippon Kagaku Zasshi* **90**, 707 (1969).

[127] L. Stryer and E. R. Blout, *J. Amer. Chem. Soc.* **83**, 1411 (1961).

rotatory dispersion (Cotton effect) in the dye absorption band. The observed Cotton effect was very high for the dye–PGA complexes. Thus the dye molecules, although they had no asymmetric center, exhibited a Cotton effect in their absorption bands if bound to helical polypeptides, but not if bound to random coil structures. The inflection points of the induced Cotton effects were in good agreement with the absorption maxima of the dyes, thus suggesting that the dye chromophores acquired optical rotatory power. The sign of the induced Cotton effect was opposite for the L- and D-amino acid polymers, which had α-helical conformations of opposite screw sense. ORD studies of complexes of dyes with biopolymers are therefore useful in the elucidation of their conformations.



(XII)



(XIII)

The optically inactive sulfonamide dyes (XII) and (XIII) showed characteristic circular dichroism spectra when bound to carbonic anhydrase, and they were sensitive probes for the active center of the enzyme.[128,129] The dyes bind at or near the metal atoms at the active center. The hypsochromic and bathochromic shifts of the principal absorption bands of (XII) induced by the binding to carbonic anhydrase were similar to those observed in less polar solvents, suggesting a hydrophobic cleft at the active center of the enzyme.

When proteins are denatured, more sites become available for dye-binding and the binding constants are smaller; but no systematic work on the binding of a variety of dyes by proteins submitted to different denaturing treatments has been carried out so far.[130]

Dye binding by native proteins makes them less amenable to denaturation. Thus Bromophenol Blue protects BSA from denaturation by acetamide, except at very high concentrations, but the same dye makes horse carbonylhemoglobin more susceptible to denaturation at low pH.[130] Binding of HSA with Methyl Orange and other anionic dyes decreased its susceptibility to hydrolytic attack by proteases. Occupation of two strong binding sites in HSA was sufficient for this effect. It

[128] I. E. Coleman, *J. Biol. Chem.* **243**, 4574 (1968).

[129] R. Einarsson and M. Zeppezauer, *Acta Chem. Scand.* **24**, 1098 (1970).

[130] Cf. J. Steinhardt and J. A. Reynolds, "Multiple Equilibria in Proteins," p. 314. Academic Press, New York, 1970.

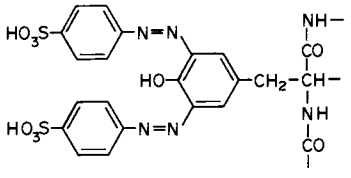
was concluded that the protein conformations stabilized by different ligands were characteristic of the ligands.[131]

B. Coupling with Diazonium Salts

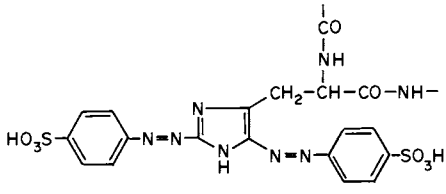
For the characterization of the "active sites" in proteins, which determine their enzymatic activity or antibody–antigen specificity, covalent labeling is regarded as the most valuable method, next only to X-ray crystallography.[132] Among numerous reagents which are being developed for these purposes, diazonium salts find some use because they react to form products whose absorption bands extend into the visible region and do not overlap with protein absorption bands, and different products can be spectrally distinguished.

1. *Action of Diazonium Salts on Amino Acids*

It was long ago recognized that diazonium salts couple with proteins,[133] but the reaction was first systematically studied by Pauly,[134] who showed that diazotized sulfanilic or arsanilic acid ("Pauly reagents") reacted with proteins to give colored derivatives (azo proteins). He considered that the color formation was mainly due to the coupling of four diazonium groups with one of a tyrosine and one of a histidine residue in a protein to form the bis-coupled products (XIV) and (XV).



(XIV)



(XV)

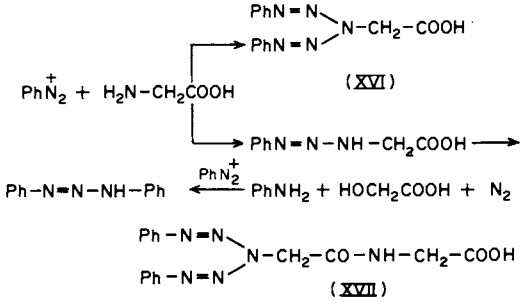
The reaction became a useful tool in protein chemistry and came to be recognized as Pauly's diazo reaction. The Pauly reagent (diazotized sulfanilic acid) has to be kept moist and at low temperature because it is explosive when dry. It was later shown that a very large excess of diazonium salt was essential to obtain the brownish red bisazo-tyrosine; a moderate excess gave monoazo tyrosine as the main product.[135] The

[131] G. Markus, D. K. McClintock, and B. A. Castellani, *J. Biol. Chem.* **242**, 4402 (1967).
[132] S. J. Singer, *Advan. Protein Chem.* **22**, 1 (1967).
[133] BASF, *DRP* 82,446 (1894).
[134] H. Pauly, *Hoppe-Seyler's Z. Physiol. Chem.* **42**, 508 (1904); **94**, 284 (1915).
[135] M. Tabachnick and H. Sobotka, *J. Biol. Chem.* **234**, 1726 (1959).

UV absorption maximum of mono(*p*-sulfophenylazo)tyrosine is at 325 nm, identical with that of mono(phenylazo)-*p*-cresol.[136] The bisazo derivative (XIV) has λ_{max} 480 nm.[136] Histidine couples with diazonium salts first at the 2-position, and then immediately at the 5-position, giving the bisazo dye (XV).[137] Higgins *et al.*[137] have carried out a kinetic analysis of the mono- and bis-coupling of histidine and tyrosine with diazotized sulfanilic acid in the indicated positions. Unlike carbocyclic compounds such as tyrosine, resorcinol, and *m*-phenylenediamine in which the second coupling is slower, the second coupling in histidine proceeds 1.4 times faster than the first. The mono- and bis-coupled products of histidine with diazotized sulfanilic acid have λ_{max} 380 and 490 nm, respectively. In the Pauly reaction care must be taken to see that no free nitrous acid remains in the diazonium solution, because it may react with the free amino groups in the protein and convert them into hydroxy derivatives as in the Van Slyke estimation.

While aryldiazonium coupling to *N*-benzoylhistidine esters leads almost entirely to 2,4-bisarylazo derivatives, coupling in the *N*-acetyl series leads predominantly to the 2-arylazo derivative. The results recorded earlier on azo coupling to histidine therefore do not apply to side-chain-derived histidine or to histidine in peptide sequence.[137a]

Glycine reacts with diazotized aniline in neutral or weakly alkaline solution to give 3-carboxymethyl-1,5-diphenylpentaza-1,4-diene (XVI).[137,138] Diazoaminobenzene (1,3-diphenyltriazene) was also isolated from the reaction mixture as expected, and glycine was partially converted into glycolic acid as indicated.[139] Glycylglycine under the



[136] R. J. Morris and W. R. Brode, *J. Amer. Chem. Soc.* **70**, 2485 (1948).

[137] H. G. Higgins *et al.*, *Aust. J. Chem.* **6**, 195 (1953); **10**, 99 (1957); *Arch. Biochem. Biophys.* **85**, 409 (1959).

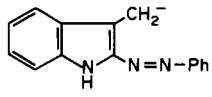
[137a] W. Nagai, K. L. Kirk, and L. A. Cohen, *J. Org. Chem.* **38**, 1971 (1973); *J. Amer. Chem. Soc.* **95**, 8389 (1973).

[138] A. N. Howard and F. Wild, *Biochem. J.* **65**, 651 (1957).

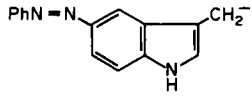
[139] H. Zahn, B. Wollemann, and O. Waschka, *Hoppe-Seyler's Z. Physiol. Chem.* **294** 100 (1954).

same conditions yielded the bis-coupled product (XVII) exclusively. It was shown that only compounds such as glycine, β-alanine, and lysine, which have a $-CH_2NH_2$ group, could react with two molecules of a diazonium salt to give products such as (XVI) and (XVII). Amino acids such as α-alanine, phenylalanine, valine, and leucine containing $RCH(NH_2)-$ yield the diazoaminobenzene derivative as the main product, together with the corresponding hydroxycarboxylic acid.

Howard and Wild[138] suggested that the tryptophan residue in a protein probably reacts first at the imino group, and the resulting triazene derivative rearranges to the *C*-azo derivative (XVIII) or (XIX). They used indole-3-acetic acid as the model, but no *C*-azo derivative was isolated or characterized, although some color changes were observed. Indole itself gives 3-phenylazoindole (λ_{max} 368 nm).[140]

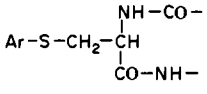


(XVIII)

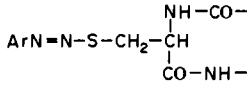


(XIX)

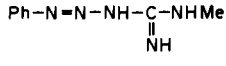
Diazonium salts can react with sulfhydryl groups of cysteine, forming an aryl alkyl sulfide (XX), presumably through the extremely unstable *S*-azo derivative (XXI).



(XX)



(XXI)



(XXII)

By analogy with the reaction of diazonium salts with compounds such as methylguanidine and cyanoguanidine (dicyandiamide),[141] it has been suggested that at least one diazonium group reacts with the guanidino group of arginine.[138] Methyl guanidine reacted with diazotized aniline to form (XXII) in 80% yield. Use of a large excess of the diazonium salt failed to show any evidence of further reaction. Nevertheless, it was suggested that further reaction involving a total of three diazonium groups and one guanidino group of arginine was possible, since the latter contained three active hydrogen atoms. As/N values in the products of the reaction between various proteins and diazotized *p*-arsanilic acid supported this contention.[138]

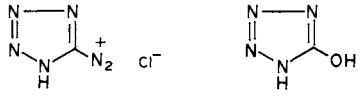
[140] W. Madelung and O. Wilhelmi, *Ber. Deut. Chem. Ges.* **57**, 234 (1924); J. H. Binks and J. H. Ridd, *J. Chem. Soc., London* p. 2398 (1957).

[141] F. H. S. Curd and F. L. Rose, *J. Chem. Soc., London* p. 729 (1946).

The aliphatic hydroxyl groups in serine, threonine, and hydroxyproline may react slowly with diazonium salts to give an aldehyde or ketone with the evolution of nitrogen.

2. *Diazo-1H-tetrazole (DHT)*

The visible absorption bands of the bisazo dyes from histidine and tyrosine (e.g., by coupling with diazotized sulfanilic acid) are located very close to each other (490 nm for histidine and 480 nm for tyrosine), and their resolution into component bands often becomes difficult. The intensities of these absorption bands are also of similar order. The formation of the bisazo dyes requires a high concentration and excess of the diazonium reagent. The usual diazonium salts partially decompose to phenols, which subsequently couple with further diazonium reagent under the reaction conditions and give colored products. These impurities greatly interfere with the spectrophotometric analysis. To overcome these difficulties, "diazo-1*H*-tetrazole" (DHT; XXIII) was introduced.[142] The new reagent (XXIII), prepared by the diazotization of 5-aminotetrazole, reacts with proteins smoothly at the optimum pH of 8.8, although it is highly explosive, and it has two major advantages. First, the decomposition product, 5-hydroxytetrazole (XXIV) is itself colorless and does not couple further with undecomposed reagent to give colored products. Second, by using an appropriately large concentration of DHT for the reaction with alkali-denatured proteins, all histidine and tyrosine residues can be converted into the bisazo dyes. High concentration of the reagent and the denaturation of the protein prior to coupling



(XXIII) (XXIV)

are two important factors for successful protein analysis by this method. The bisazohistidine derivative has λ_{max} 480 nm (ϵ 20500) and the tyrosine derivative 550 nm (ϵ 13800). The relatively wide difference in the two absorption maxima makes the accurate spectral determination of histidine and tyrosine residues in a protein molecule possible.[143] As mentioned earlier, the monoazo derivative of histidine is formed first, and it reacts with DHT faster than the unsubstituted histidyl residue. If a sufficient concentration of DHT is not used, a considerable propor-

[142] H. Horinishi, Y. Hachimori, K. Kurihara, and K. Shibata, *Biochim. Biophys. Acta* **86**, 477 (1964).

[143] M. Sokolovsky and B. L. Vallee, *Biochemistry* **5**, 3574 (1966); T. F. Spande, B. Witkop, Y. Degani, and A. Patchornik, *Advan. Protein Chem.* **24**, 97 (1970).

tion of the monoazotyrosine may be formed, since the second coupling of tyrosine is much slower. Monoazotyrosine has λ_{max} 478 nm (ϵ 5200), which is quite close to that of the bisazohistidine, and the overlapping of the two bands may present difficulties in the spectrophotometric analysis of histidine.

Coupling of DHT with cellulase from *Penicillium notatum* resulted in the complete loss of activity of the enzyme, even when a very small excess of the reagent was used. This happened before the formation of any bisazo derivatives of histidine and tyrosine as shown by the spectral studies at 480 and 550 nm. It has been suggested that the histidyl residues are more reactive, and the inactivation may be due to the formation of monoazo derivatives of histidine residues.[144] Sixty percent of the antithrombin activity of hirudin was reduced by treatment with DHT, which probably blocked the imidazole and part of the phenol residues.[145]

Coupling of carboxypeptidase A with an 8-fold molar excess of DHT at pH 8.8 doubled the esterase activity, while the peptidase activity remained steady at about 90%. With gradually increasing DHT concentration, the esterase activity was not increased further, but the peptidase activity progressively decreased to nearly zero at a 45-fold molar excess of the reagent. The dependence of the quantitative variations of the two activities on the concentration of the reagent may be due to the modification of different amino acid residues in the enzyme. A comparison of these changes with the increase of absorption at 480 nm, corresponding to the formation of monoazotyrosyl and bisazohistidyl derivatives, suggested that the histidine and tyrosine residues were involved in the mechanism of the action of carboxypeptidase.[146] From studies of the action of DHT on several enzymes, Shibata[147] concluded that DHT serves two useful purposes: (1) the spectrophotometric estimation of total histidine content in denatured proteins can be carried out without separating the individual amino acids, and (2) free and bound histidine residues in native protein can be distinguished. An exception to this generalization is rabbit muscle myokinase.[148] It was found that the "histidine-specific" reagent DHT

[144] G. Patterson, *Arch. Biochem. Biophys.* **126**, 776 (1968).

[145] C. Tertrin, P. de la Llosa, and M. Jutisz, *Bull. Soc. Chim. Biol.* **49**, 1837 (1967).

[146] M. Sokolovsky and B. L. Vallee, *Biochemistry* **6**, 700 (1967).

[147] K. Shibata *et al.*, *Arch. Biochem. Biophys.* **111**, 520 (1965); **113**, 371 (1966); *Biochim. Biophys. Acta* **105**, 167 (1965); see also T. G. Bak and R. Sato, *ibid.* **146**, 328 (1967); M. Marumatu and S. Fujii, *J. Biochem.* (*Tokyo*) **66**, 455 (1969); R. F. Steiner, *Arch. Biochem. Biophys.* **115**, 257 (1966).

[148] R. H. Schirmer, I. Schirmer, and L. Noda, *Biochim. Biophys. Acta* **207**, 165 (1970), see also Patterson.[144]

did not show any advantage over other Pauly reagents. When this protein was reacted with increasing concentrations of DHT, cysteine reacted first, followed by lysine and tyrosine. The histidine residues were the last to couple. However, at high DHT concentrations the bisazo derivatives of histidine and tyrosine were formed; they could be analyzed by the usual spectrophotometric method.

Shibata has studied in great detail the action of DHT on insulin before and after oxidative cleavage of the disulfide bonds, and he has demonstrated its value in understanding some of its structural features, such as the influence of neighboring amino acid units on the reactivity of monocoupled histidine and tyrosine residues.[149]

Reaction of bovine serum albumin with diazotized arsanilic acid and other diazonium salts showed that the coupling of tyrosine preceded the coupling of histidine residues, probably due to the differences in the environment at the various sites of attachment.[150] Since no absorption peaks near 480 nm were observed, it was inferred that only monoazo derivatives of tyrosine and histidine resulted, together with colorless derivatives of other amino acid residues.

3. *Immunological Activity of Azoproteins*

The study of the antigen-antibody reaction, the basis of immunology, was at one stage facilitated by the use of azoproteins as antigens.[150a] A great variety of azoproteins can be obtained, and as coupling with diazonium salts is carried out under very mild conditions (0°–20° and pH 7–9), denaturation of the proteins is considerably minimized. The serological specificity of determinant chemical groups or "haptens" has been studied extensively with nonprotein chemical substituents such as the arylazo groups of azoproteins. The classical work of Landsteiner[151] clearly demonstrated that immunization or sensitization with the protein–hapten conjugates usually elicits the production of antibodies, some of which are specifically directed towards the haptens. Rabbit antisera were obtained in separate experiments by injection of sulfophenylazo derivatives of horse-serum proteins on the one hand and similar azo derivatives of chick-serum proteins on the other. The *o*-, *m*-, and *p*-sulfophenylazo derivatives were serologically shown to be distinct.

[149] T. Suzuki, O. Takenaka, and K. Shibata, *J. Biochem.* (*Tokyo*) **66**, 815 (1966); see also W. L. Koltum, *J. Amer. Chem. Soc.* **79**, 5681 (1957).

[150] E. W. Gelewitz, W. L. Riedman, and I. M. Klotz, *Arch. Biochem. Biophys.* **53**, 411 (1954).

[150a] E. A. Kabat and M. M. Mayer, "Experimental Immunochemistry," 2nd ed., pp. 446, 457, and 798. Thomas, Springfield, Illinois, 1961.

[151] K. Landsteiner, "The Specificity of Serological Reactions." Harvard Univ. Press, Cambridge, Massachusetts, 1946.

Thus antiserum to horse-serum proteins containing *p*-sulfophenylazo groups was shown to react with chick-serum proteins containing only *p*-sulfophenylazo groups, and not *m*- or *o*-sulfophenylazo groups in precipitation tests. In similar experiments it was shown that the antibodies can differentiate the sulfophenyl from the corresponding derivatives of arsonic and carboxylic acids. Kinetic studies have revealed that the combination of antibody with hapten takes place extremely rapidly. The polarity, shape, and rigidity of the haptens of the azoproteins determined the serological specificity.[151,152] To cite one example, the mono-*p*-nitroanilides of both *d*- and *l*-tartaric acids were prepared. The nitro groups were reduced, and the resulting amines diazotized and coupled with proteins. Tests with these azoproteins showed that the antibodies against the *d*-tartaric acid derivatives were different from those against the *l* derivatives.

A recent patent describes the preparation of azoproteins by the coupling of mammalian (human, horse, and rabbit) blood serum albumins with diazotized *p*-aminophenylazohistamine. The purified homogeneous azoproteins were found to be antigenic to histamine and were of use in the treatment of allergic conditions.[153]

In "affinity labeling" a reagent has the steric requirements for first combining specifically and reversibly with a particular active site in a protein; it also has a small reactive group which then attacks one or more amino acid residues in the site to form irreversible covalent bonds.[154] Even a diazonium salt so reactive as 2,4-dinitrobenzenediazonium fluoroborate can be used effectively to label the active sites of anti-DNP antibodies, because its specific reaction at concentrations around 10^{-5} *M* in an aqueous buffer at pH 5.0 and about 4° is complete within 1 second, whereas its reaction with normal γ-globulin is much slower.[154]

Antibodies directed to the *p*-azobenzenearsonate hapten have been treated with *p*-arsonophenyldiazonium fluoroborate in the absence or presence of the specific active site protector, *p*-nitrobenzenearsonate. In the unprotected antibody azo linkages to tyrosyl residues were formed predominantly, whereas a variety of azo linkages were produced in the protected antibody and normal γ-globulin.[155] The results of several such studies have been discussed by Singer and Doolittle.[156]

[152] F. Haurowitz, "Immunochemistry and the Biosynthesis of Antibodies." Wiley (Interscience), New York, 1968.

[153] Human Oltoanyagtermelo es Kutato, *BP* 1,017,479; *CA* **64**, 12470g (1966).

[154] S. J. Singer, *Advan. Protein Chem.* **22**, 25 (1967).

[155] L. Wofsy, H. Metzger, and S. J. Singer, *Biochemistry* **1**, 1031 (1962).

[156] S. J. Singer and R. F. Doolittle, *Science* **153**, 13 (1966); see also N. O. Thorpe and S. J. Singer, *Biochemistry* **8**, 4523 (1969).

C. Treatment with Compounds Containing Reactive Halogen

Dyes containing reactive chlorine were the first representatives of reactive dyes for wool and cotton, and they continue to be the most numerous and technically the most important. Because of the simplicity and variety of the methods by which reactive halogen can be attached to a dye molecule, great flexibility in the design of reactive dyes becomes possible. Similarly, compounds containing reactive halogen have proved to be useful as group-specific reagents[111] for proteins and for their chemical modification.

2,4-Dinitrofluorobenzene (DNFB) has found more specialized application than *N*-terminal sequence determination.[111] Dinitrophenyl groups at all the reactive sites other than the amino groups can be displaced by 2-mercaptoethanol.[157] The selective adsorption of DNFB at lipophilic sites prior to its chemical reaction with BSA has been demonstrated.[158] Ribonuclease reacts with DNFB at lysine-1 (α-amino group) and lysine-41 (ϵ-amino group); the blocking of the latter leads to inactivation.[159] Chymotrypsin reacts with DNFB at the histidine residue, whereas chymotrypsinogen reacts at some other site, since the resulting DNP–chymotrypsinogen gives enzymatically active DNP–chymotrypsin on activation with trypsin.[160,161] The bifunctional reagent, 1,5-difluoro-2,4-dinitrobenzene, originally used for the study of the tertiary structure of insulin,[162] has been applied to establish by intramolecular cross-linkage the spatial proximity of ϵ-amino groups of two lysine residues at positions 7 and 41 in the polypeptide chain of bovine pancreatic ribonuclease A.[163]

2,4,6-Trinitrobenzenesulfonic acid (TNBS) has advantages over picryl chloride; it combines preferentially with primary amino groups in proteins.[111,164] Proline, histidine, and tyrosine residues do not react with TNBS, which has been used to characterize the reactivities of amino groups in several enzymes.[165] The selective reactivity of this reagent for ϵ-amino groups is shown by its reaction with cytochrome *c*.[166] The nitroimidazole reagents (XXVA) and (XXVB) are specific for

[157] S. Shaltiel, *Biochem. Biophys. Res. Commun.* **29**, 178 (1967).

[158] N. M. Green, *Biochim. Biophys. Acta* **74**, 542 (1963).

[159] C. H. W. Hirs, M. Halmann, and J. H. Kycia, *Arch. Biochem. Biophys.* **111**, 209 (1965); A. L. Murdock, K. L. Crist, and C. H. W. Hirs, *ibid.* **114**, 375 (1966).

[160] V. Massey and B. S. Hartley, *Biochim. Biophys. Acta* **27**, 361 (1956).

[161] J. R. Whitaker and B. J. Jandorff, *J. Biol. Chem.* **223**, 751 (1956).

[162] H. Zahn and J. Meienhofer, *J. Makromol. Chem.* **26**, 126 and 153 (1958).

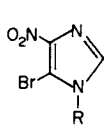
[163] P. S. Marfey *et al.*, *J. Biol. Chem.* **240**, 3264 and 3270 (1965).

[164] K. Satake *et al.*, *J. Biochem.* (*Tokyo*) **47**, 454 (1960), *et sequa.*

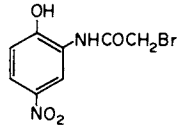
[165] R. B. Freeman and G. K. Radda, *Biochem. J.* **114**, 611 (1969).

[166] J. Ozols and P. Strittmatter, *J. Biol. Chem.* **241**, 4793 (1966).

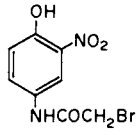
sulfhydryl groups in proteins.[167] Reagents (XXVI) and (XXVII) react specifically with the methionine residue near the active serine unit of chymotrypsin.[168,169] Burr and Koshland[168] use (XXVI) as a "reporter group," defined as an environmentally sensitive group which has been covalently bound to a protein at a specific location in which a physical signal can be studied as a measure of local environmental conditions. The compound (XXVIII) reacts with cysteine and tryotophan residues in addition to methionine to give colored derivatives, but it can be used for the quantitative estimation of tryptophan in proteins.[170] Cyanuric fluoride has also been mentioned as a group-specific reagent,[171,172] but its advantage over the readily available cyanuric chloride is not clear. Cyanuric chloride and its derivatives containing two or one reactive chlorine atom merit consideration as reagents whose structures can be progressively varied.



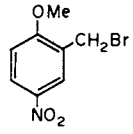
(XXV A) R=H
(XXV B) R=Me



(XXVI)



(XXVII)



(XXVIII)

An interesting reagent which reacts selectively with cysteinyl residues is azobenzene-2-sulfenyl bromide (XXIX),[173] which exists as 2-phenylbenzo-1-thia-2,3-diazolium ion (XXX), since it has unusual solubility and stability in water.[174] Moreover, unlike 2,4-dinitrophenylsulfenyl

[167] L. A. Cohen and W. Schreiber, cited in Cohen.[111]

[168] M. Burr and D. E. Koshland, Jr., *Proc. Nat. Acad. Sci. U.S.* **52**, 1017 (1964).

[169] A. Conway and D. E. Koshland, Jr., *Biochim. Biophys. Acta* **133**, 593 (1967).

[170] H. R. Horton, H. Kelly, and D. E. Koshland, Jr., *J. Biol. Chem.* **240**, 722 (1965); T. F. Spande, M. Wilchek, and B. Witkop, *J. Amer. Chem. Soc.* **90**, 3256 (1968).

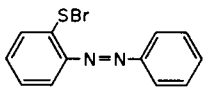
[171] K. Kurihara, H. Horinishi, and K. Shibata, *Biochim. Biophys. Acta* **74**, 678 (1963).

[172] M. J. Gorbunoff, *Biochemistry* **6**, 1606 (1967).

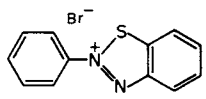
[173] A. Fontana, F. M. Veronese, and E. Scoffone, *Biochemistry* 7, 3901 (1968).

[174] A. Burawoy, F. Liversedge, and C. E. Vellins, *J. Chem. Soc., London* p. 4481 (1954).

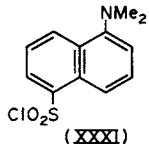
chloride, which reacts with cysteine and tryptophan residues, (XXIX) lacks reactivity towards tryptophan.[175]



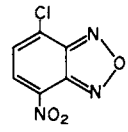
(XXIX)



(XXX)



(XXXI)



(XXXII)

1-Dimethylaminonaphthalene-5-sulfonyl chloride (dansyl chloride; XXXI) was first described in a German patent in 1894[176] and first used by Weber[177] for the preparation of fluorescent conjugates of albumin. Dansyl chloride is about 100 times more sensitive than DNFB as an end group reagent; the dansyl derivatives of amino acids have an intense yellow fluorescence, are more stable to acid hydrolysis than the corresponding DNP–amino acids, and quantitative separation can be effected chromatographically or electrophoretically. *N*-terminal amino acid in a protein can be determined with milligram quantities using dansyl chloride.[178] 7-Chloro-4-nitrobenzo-2-oxa-1,3-diazole (XXXII), more stable and more soluble in aqueous solutions than dansyl chloride, reacts with amino acids to give fluorescent derivatives.[179]

Edelman and McClure[180] have defined fluorescent probes as "small molecules which undergo changes in one or more of their fluorescent properties as a result of noncovalent interaction with a protein or other macromolecule." They have described experiments in which the probe TNS (2-toluidinylnaphthalene-6-sulfonate; *N*-*p*-tolyl Brönner acid) was used to detect protein denaturation, zymogen activation, and subtle changes in conformation which accompany enzyme–ligand interaction. TNS is nonfluorescent in water, but becomes strongly fluorescent when bound to proteins. The fact that TNS fluoresces more strongly in non-

[175] E. Scoffone, A. Fontana, and R. Rocchi, *Biochemistry* **7**, 971 (1968); A. Fontana, E. Scoffone, and C. A. Benassi, *ibid.* p. 980.

[176] K, *DRP* 90,274 (*Frdl.* 4, 603); see also G. D. Glebova, *CA* **57**, 16510g (1962).

[177] G. Weber, *Biochem. J.* **51**, 155 (1952).

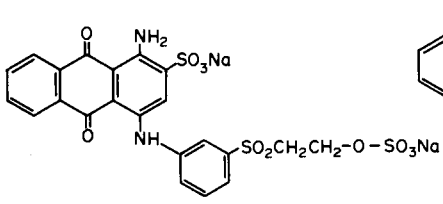
[178] W. R. Gray and B. S. Hartley, *Biochem. J.* **89**, 59P (1963); N. Seiler, *Methods Biochem. Anal.* **18**, 259 (1970).

[179] P. B. Ghosh and M. W. Whitehouse, *Biochem. J.* **108**, 155 (1968).

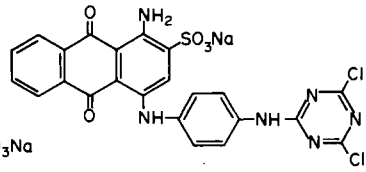
[180] G. M. Edelman and W. O. McClure, *Accounts Chem. Res.* **1**, 65 (1968).

polar than in polar solvents suggested that it is a hydrophobic probe. By using the sulfonyl chloride, a covalent bond is formed and the region of attachment is labeled after noncovalent association with a protein. Examples of similar fluorescent probes are *N*-phenylnaphthionic acid and *N*-phenyl Peri-acid (1-anilinonaphthalene-4- and 8-sulfonic acids).[181] Edelman and McClure have remarked that "a search for probes capable of binding to regions of proteins other than hydrophobic sites should be extremely rewarding."

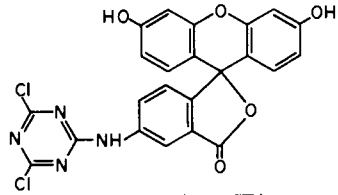
The numerous reactive groups discovered in connection with reactive dyes for cotton, wool, and the synthetic polyamide fibers offer a wide choice of reagents (including bifunctional compounds such as Remazol Black B) for the study of proteins.[182]



Remazol Brilliant Blue R



(XXXIII)



(XXXIV)

Zahn and Rouett[183] found that vinyl sulfone dyes (such as Remazol Brilliant Blue R) and acrylamides (Dye—NH—COCH=CH_2) reacted almost exclusively with the ϵ-amino group of the lysine residues in wool. Shore[184] has reviewed the mechanism of the reaction of proteins with reactive dyes. Although both he and Zahn were primarily concerned with wool, their work is relevant to the problem of the use of dyes and compounds containing reactive groups for studying protein structure. Shore studied dichlorotriazinyl and monochlorotriazinyl dyes (Procion M and H) and found that there are several degrees of reactivity among

[181] E. Daniel and G. Weber, *Biochemistry* **5**, 1893 (1966); see Edelman and McClure[180] for further references to the literature.

[182] See *CSD VI*.

[183] H. Zahn and F. P. Rouett, *Textilveredlung* **3**, 241 (1968); G. Reinert, Dissertation, Technische Hochschule, Aachen (1967).

[184] J. Shore, *J. Soc. Dyers Colour.* **84**, 408, 413, and 545 (1968); **85**, 14 (1969).

the primary amino and imidazole groups in proteins, depending on the effects of neighboring groups. A dichlorotriazine dye such as (XXXIII) can react with polypeptides at the functional groups of lysine, hydroxylysine, cysteine, and tyrosine in addition to the terminal amino groups under mild conditions. Monochlorotriazine dyes also probably react at the cysteine thiol groups, the primary amino groups of lysine, the imidazole group of histidine, and the N-terminal amino acids.[184] Several factors, including the pH and the amino acid composition and sequence, influence the reactivity of these dyes with specific sites in proteins.[184]

The dichlorotriazinylaminofluorescein (XXXIV) was found to react specifically with the ϵ-amino group of lysine and the imidazole group of histidine at slightly acid pH.[185]

D. Fluorescent Antibody Methods

Fluorescent antibody methods have now become so important that a book on the subject has appeared recently.[186] The basis of the technique[187] is the ability of antigens to precipitate antibodies. Serum proteins are treated with a fluorescent dye containing a reactive group capable of entering into covalent combination with vulnerable sites such as amino groups in the protein molecules. An important condition is that the fluorescent protein derivative thus obtained should be unaltered in immunological reactivity. When a few drops of the conjugated serum containing antibodies are placed on a smear or section containing homologous antigen, the antigen–antibody complex is precipitated and thus localized. Unreacted and nonantibody proteins can be rinsed off, and the preparation examined under a fluorescence microscope. Sites of the fluorescent antibody deposition can be observed against the dark background of non-antigen-containing material.

Coons, Creech, and Jones[188] laid the foundation of the fluorescent antibody method in 1941, when they used β-anthryl isocyanate for labeling antipneumococcus Type III serum. The important advance they made was twofold. Unlike earlier workers, who studied the action of diazonium salts and other reagents on the immunological properties of antibodies, they sought to produce labeled, but immunologically

[185] V. E. Barkii, V. B. Ivanov, Yu. E. Sklyar, and G. I. Mikhailor, *Izv. Akad. Nauk SSSR, Ser. Biol.* **5**, 744 (1968); *CA* **70**, 79127 (1969).

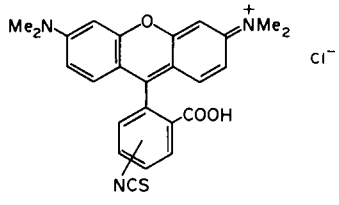
[186] M. Goldman, "Fluorescent Antibody Methods." Academic Press, New York, 1968. See also "Fluorescent Protein Tracing" (R. C. Nairn, ed.), 2nd ed. Livingstone, Edinburgh, 1964.

[187] See also V. J. Lewis and J. B. Brooks, *J. Bacteriol.* **88**, 1520 (1964).

[188] A. H. Coons, H. J. Creech, and R. N. Jones, *Proc. Soc. Exp. Biol. Med.* **47**, 200 (1941).

unaltered antibodies; by using fluorescent dyes they vastly increased the sensitivity of the technique. Their interest was in immunology, but the technique is now used for numerous other purposes, such as the localization of infectious agents or specific proteins in tissues and the identification of microorganisms in cultures or pathogenic exudates. Goldman[186] has made an exhaustive review of both the immunological and nonimmunological applications of this fluorescence technique.

The most widely used labeling agents at the present time are the isocyanate[189] and isothiocyanate[190] of fluorescein and Rhodamine B. The isothiocyanates are preferred and, in fact, have largely replaced the isocyanates, because the former have greater storage stability than the isocyanates. The isothiocyanate (XXXV) of the methyl analog ("Tetramethyl Rhodamine B") is more suitable than the isothiocyanate of Rhodamine B.[191] The isocyanates and isothiocyanates are prepared by carrying out the phthalein condensation[191a] with 4-nitrophthalic anhydride, catalytic reduction to the amine, and subsequent reaction with phosgene or thiophosgene.



(XXXV)

Corey and Churchill[192] have determined by an NMR study the orientation of the two nitrofluoresceins obtained by the condensation of 4-nitrophthalic anhydride with resorcinol. The positions of the nitro group in the two isomers were the reverse of those determined by Borek from IR data.[193] It is not clear if the two isomeric isocyanates or isothiocyanates exhibit any qualitative or quantitative difference when they are used for labeling in antigen–antibody studies, because Corey and Churchill have stated that "despite increasing use of antibodies labeled with fluorescein in immunochemical studies, the exact structures of the labeling substances have remained unknown."

[189] A. H. Coons *et al.*, *J. Immunol.* **45**, 159 (1942).

[190] J. L. Riggs *et al.*, *Amer. J. Pathol.* **34**, 1081 (1958).

[191] M. L. Smith, T. R. Carski, and C. W. Griffin, *J. Bacteriol.* **83**, 1358 (1962).

[191a] *CSD II*, pp. 747 and 750.

[192] H. S. Corey and F. C. Churchill, *Nature (London)* **212**, 1040 (1966). See H. S. Corey and R. M. McKinney [*Anal. Biochem.* **4**, 57 (1962)] for the chromatography of nitro, amino and isothiocyanate derivatives of fluorescein.

[193] F. Borek, *J. Org. Chem.* **26**, 1292 (1961).

Two other compounds which have found limited application in antibody work are 1-dimethylaminonaphthalene-5-sulfonyl chloride ("dansyl chloride")[178] and the chloride of Lissamine Rhodamine B (CI Acid Red 52; CI 45100; Sulforhodamine B).[193a,194] Among several other sulfonyl chlorides and cyanuric chloride derivatives listed by Goldman[186] (p. 114), the chloride of 3-hydroxypyrene-5,8,10-trisulfonic acid (CI Solvent Green 7; CI 59040) is stated to have good possibilities.

V. Dye-Binding by Nucleic Acids and Nucleoproteins

Some of the most important applications of dyes in biology are in the field of nucleic acids: for the localization and estimation of DNA, RNA, and nucleoproteins in histochemistry, for studying the conformation and differentiation of double-stranded and less highly organized or single-stranded DNA, for the protection and stabilization of DNA, and as highly selective mutagens. Dye–nucleic acid interactions are dependent on the three components of the nucleic acids: purine and pyrimidine bases, deoxyribose and ribose, and orthophosphoric acid. The strong UV absorption of the purine and pyrimidine bases at about 260 nm can be used for the cytochemical detection and estimation of the nucleic acids,[194a] but it does not distinguish between DNA and RNA.

A. Histochemistry and Cytochemistry

1. *Feulgen Reaction*

The Feulgen reaction, discovered in 1924 by Feulgen and Rossenbeck[195] as a specific test for thymonucleic acid (DNA) and later used by Feulgen and Voit for lipid aldehydes (plasmal reaction),[196] has become the most important technique for localization and estimation of DNA. Of all the methods employed for the staining of chromosomes, the Feulgen reaction is considered to be the most effective.[197] The Feulgen reaction involves two distinct steps: (1) hydrolysis of the fixed tissue with 1 *N* HCl at about 60° for a few minutes and (2) treatment with Schiff's reagent for 1–3 hours, when nuclei are selectively stained a magenta or purple color against a clear cytoplasmic background.

[193a] *CSD II*, p. 746.

[194] C. S. Chadwick *et al.*, *Immunology* **1**, 315 (1958).

[194a] T. Caspersson, *Arch. Physiol. Scand., Suppl. 8* (1936).

[195] R. Feulgen and H. Rossenbeck, *Hoppe-Seyler's Z. Physiol. Chem.* **135**, 203 (1924).

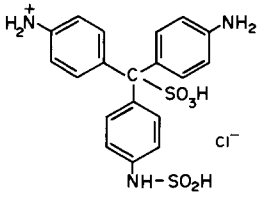
[196] R. Feulgen and K. Voit, *Pflueger's Arch. Gesamte Physiol.* **206**, 389 (1924).

[197] A. K. Sharma and A. Sharma, "Chromosome Techniques," pp. 110–152. Butterworth, London, 1965.

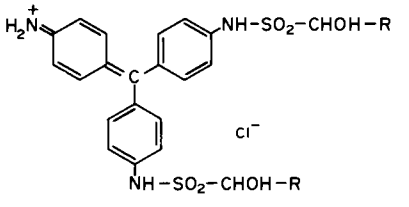
Kasten[198] has reviewed in great detail the history, chemistry, and applications of Schiff's reagent to cytochemistry. Although it is convenient to deal with the reagent in this section because its major use is in the Feulgen reaction, the characteristic behavior towards aldehydes is the basis of several other applications.[199] In the periodic acid–Schiff (PAS) reaction, which was first used on tissue sections to demonstrate mucins, 1,2-glycols (or other compounds amenable to the Malaprade oxidation) are treated with periodic acid to form dialdehydes and then with Schiff's reagent.

Schiff's reagent, as he originally made it in 1866 by saturating an aqueous solution of Fuchsine with sulfur dioxide, continues to be used in organic laboratories for the detection of aldehydes, but numerous other sources of sulfur dioxide (e.g., sodium thiosulfate + HCl)[200] and optimum conditions for the use of the reagent in biological microtechnique have been suggested. Simple aldehydes give a deep red to purple color almost immediately; more complex aldehydes respond more slowly; many aromatic aldehydes give a negative test. Kasten has provided tables of compounds, including those involved in histochemistry, which give a color reaction with Schiff's reagent, and of compounds which fail to give a color reaction.

Complex and partly reversible reactions are involved, both in the formation of Schiff's reagent and its interaction with aldehydes. Wieland and Scheuing[201] suggested structure (XXXVI) for Schiff's reagent from Pararosaniline and (XXXVII) for the dye finally obtained. However, there is considerable evidence in favor of the corresponding *N*-alkyl ω-sulfonic acid structure (–NH—CHR—SO_3H) for the dye.[202] More than one dye can be finally formed, depending on the aldehyde and the conditions of the reaction; their precise structures have not yet



(XXXVI)



(XXXVII)

[198] F. H. Kasten, *Int. Rev. Cytol.* **10**, 1 (1960).

[199] For references, see Gurr.[6]

[200] D. W. Menzies, *Stain Technol.* **36**, 341 (1961).

[201] H. Wieland and G. Scheuing, *Ber. Deut. Chem. Ges.* **54**, 2527 (1921).

[202] Cf. H. Hormann, W. Grassmann, and G. Fries, *Justus Liebigs Ann. Chem.* **616**, 125 (1958).

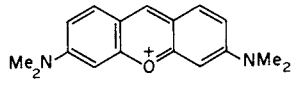
been established.[203] It has been demonstrated by paper chromatography that in the reaction between Schiff's reagent and formaldehyde 3–7 dyes with λ_{max} ranging from 535 to 574 nm are formed.[204]

Kasten surveyed over 400 dyes and found that several dozen, all of which had the common feature of an amino group, could be used as Schiff-type reagents.

The chemical basis of the Feulgen test has been the subject of controversy, although its specificity for DNA is beyond doubt.[4] Overend and Stacey[205] have shown that mild acid hydrolysis breaks the glycosidic linkages between the sugars and the purine bases. The "apurinic acids" thus formed are firmly held through phosphate linkages in the main nucleic acid chain. Deoxyribose of DNA reacts in its aldehyde form with Schiff's reagent. Even if the sugar–base glycosidic linkages in RNA are cleaved during the Feulgen test, ribose does not form a dye with Schiff's reagent under the conditions of the test. Since the Schiff reaction is applicable to aldehydes in general, interfering compounds must be eliminated and appropriate controls must be run for the correct interpretation of the results.

B. Staining with Cationic Dyes

In view of the strongly acidic character of the nucleic acids (p$K \approx 2$), basic dyes, such as Methyl Green, Pyronine G, Toluidine Blue, and Acridine Orange, are used for their characterization, localization, and estimation. Pyronine is a selective stain for RNA and Methyl Green for DNA; Toluidine Blue and Acridine Orange stain both RNA and DNA metachromatically.



Pyronine G
CI 45005*

There are other acidic substances in tissues besides nucleic acids capable of binding basic dyes, such as proteins with free carboxyl groups, phosphoproteins, and acid polysaccharides. Since the pK of protein carboxyl groups is around 5, at lower pH levels carboxyl binding by most basic dyes is negligible. Thus in the pH range 3–5 basophilia in tissues is due to nucleic acids or *O*-sulfates of polysaccharides. The latter stain a different color from RNA with many basic dyes and can thus be distinguished. They can also be distinguished from RNA by running a control on which the periodic acid–Schiff (PAS) reaction has been

[203] See also P. J. Stoward, *J. Histochem. Cytochem.* **14**, 681 (1966); R. V. Nauman, P. W. West, and F. Tron, *Anal. Chem.* **32**, 1307 (1960).

[204] T. Barka and L. Ornstein, *J. Histochem. Cytochem.* **8**, 208 (1960).

[205] W. G. Overend and M. Stacey, *Advan. Carbohyd. Chem.* **8**, 61 (1953).

performed. The phosphoprotein binding of basic dyes can be distinguished by running a control section with ribonuclease. Thus basic dye staining of tissue sections, if carried out near pH 3–5 in conjunction with controls treated with RNase, DNase, and PAS reagents, can be used to specifically localize nucleic acids.

Protein amino groups have a marked effect upon dye binding by nucleic acids. Part of the effect of pH on nucleic acid binding in sections is certainly due to the presence of protein amino groups. This effect of proteins can be eliminated in sections by treatment with acetic anhydride, chloramine-T, nitrous acid, or proteolytic enzymes, which may result in a severalfold increase in dye binding by nucleic acids.

The Pappenheim-Unna stain,[2,3] a mixture of Methyl Green and Pyronine, was used by Brachet[206] for demonstrating RNA in both nucleus and cytoplasm by treating a control section with RNase. Material staining red with Pyronine and removable by treatment with RNase is considered to be RNA. This is the method of choice for investigating the RNA content of the majority of tissues.

Kurnick[207] considered that the apparent distinction between nucleic acids in tissues stained with Pyronine was a function of their relative states of polymerization. He studied the stoichiometry of Methyl Green–DNA interaction and suggested the use of the stable complex as a substrate for determining DNase activity. Rosenkranz and Bendich[208] suggested that the specificity of Methyl Green for DNA depended upon its double-stranded structure. Differences in behavior of cationic dyes towards nucleic acids and polyphosphates, demonstrated by a critical electrolyte concentration method, were considered by Scott[209] to be a consequence of interactions between the dyes and the purines and pyrimidines of the polynucleotides. Dye binding with the bases was modified by the secondary structure of the polynucleotides. Single-stranded molecules had more sites available for interaction than molecules with considerable double-strandedness or other secondary structures. Planar dyes like Pyronine reacted best with nucleic acids having freely accessible purines and pyrimidines, as in RNA and denatured DNA. Nonplanar Methyl Green was unique as a poor precipitant for polyanions, which displayed a very marked and specific interaction with double-stranded DNA, but not heat-denatured DNA.

[206] J. Brachet, *C.R. Soc. Biol.* **133**, 88 (1940); *Arch. Biol.* **53**, 207 (1942).

[207] N. B. Kurnick, *Cold Spring Harbor Symp. Quant. Biol.* **12**, 141 (1947); *Arch. Biochem.* **29**, 41 (1950); N. B. Kurnick and A. E. Mirsky, *J. Gen. Physiol.* **33**, 265 (1950).

[208] H. S. Rosenkranz and A. Bendich, *J. Biophys. Biochem. Cytol.* **4**, 663 (1958).

[209] J. E. Scott and I. H. Willett, *Nature (London)* **209**, 985 (1966); J. E. Scott, *Biochem. J.* **99**, 3P (1966); *Histochemie* **9**, 30 (1967).

Alcian Blue behaved as if it was bound only by electrostatic forces, except with rat liver RNA(I), whose structure is abnormally open. Alcian Blue differed from other cationic dyes because of its bulky planar structure, its polyvalence, and the cationic charges' not being part of the chromophore. Scott's reference to Alcian Yellow as a copper phthalocyanine derivative is erroneous.

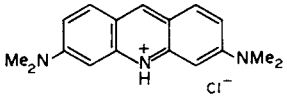
Gallocyanine (CI Mordant Blue 10, CI 51030[209a]), in conjunction with chrome alum, is useful for the quantitative estimation of the nucleic acids, but it does not differentiate between DNA and RNA.[210]

Toluidine Blue has also been used to differentiate DNA and RNA in fixed specimens. Feder and Wolf[211] showed that in acrolein-fixed tissues DNA stains deep blue (orthochromatic) and RNA pale purple (metachromatic). Love[212] used Toluidine Blue for differentiating ribonucleoproteins. Several basic dyes differentiate between native and denatured DNA in solution, the former forming a cohesive clot, and the latter a precipitate.[212a]

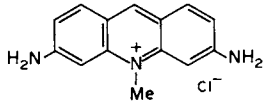
C. Acridine Dyes

Introduced as a fluorochrome around 1940, Acridine Orange (AO) has now attained great importance in biological studies and is the subject of several reviews and numerous papers. Bertalanffy[213] has reviewed its applications as a fluorochrome in cell physiology, cytochemistry, and medicine. Kasten[214] has discussed in great detail its properties and its role in the cytochemistry of nucleic acids. It stains tumor cells selectively and has been used for the cytodiagnosis of cancer. There was some interest at one time in its ability to retard tumor growth.[215]

The absorption spectra of AO and proflavine show that they aggregate



Acridine Orange NO
CI Basic Orange 14
CI 46005



Acriflavine
CI 46000

[209a] *CSD II*, pp. 783–785.

[210] H. Mayersbach, *Acta Histochem.* **3**, 128 (1956).

[211] N. Feder and M. K. Wolf, *J. Cell Biol.* **27**, 327 (1965).

[212] L. Love, *Nature (London)* **180**, 161 (1957); *J. Histochem. Cytochem.* **10**, 227 (1962); L. Love, A. M. Clark, and G. P. Studzinski, *Nature (London)* **203**, 1384 (1964); L. Love and G. Rabotti, *J. Cell Biol.* **25**, 164 (1965).

[212a] C. Singh and J. Misra, *Experientia* **22**, 599 (1966).

[213] L. von Bertalanffy, *Protoplasma* **57**, 51 (1963).

[214] F. H. Kasten, *Int. Rev. Cytol.* **21**, 141 (1967).

[215] M. R. Lewis and P. G. Goland, *Amer. J. Med. Sci.* **215**, 282 (1948).

in aqueous solution and their metachromatic behavior can be interpreted in terms of monomer–dimer equilibria.[41,216]

Staining with AO of fresh blood samples from chicks infected with *Plasmodium gallinaceum* results in differential fluorescence of the nucleic acids of the *Plasmodia*: DNA, bright green, and cytoplasmic RNA, orange-red.[217]

A quantitative estimation of the DNA content of living squamous cells has been made by taking advantage of the specific binding affinity of acriflavine for nucleic acids under controlled conditions.[218]

The stem end of the human Y chromosome exhibits a brilliant fluorescence when it is stained with quinacrine (Atebrin; Atabrine[218a]) or quinacrine mustard.[219] The technique shows up the number of Y chromosomes in a human interphase (nondividing) cell,[220] and it has also been used to show that the short arm and not the stem of the Y chromosome is associated with the X chromosome in meiosis.[220] On human spermatozoa the stain has made it possible to distinguish the male-determining from the female-determining spermatozoa.[221] As stated in a note in *Nature*,[222] "The acridine dyes certainly hold a lot of interesting secrets in store, and in the coming months laboratories throughout the world will doubtless be fluorescing with excitement to find out just what these may be."

In 1956 Peacocke and Skerrett[223] studied DNA–proflavine complexes by the equilibrium dialysis method and noticed the heterogeneity of binding sites on DNA. They distinguished two main stages in this binding: a strong binding (complex I) which was saturated at a dye to DNA phosphate ratio (D/P) of 0.22; and a weaker binding (complex II) which was saturated at a D/P of 1. Bradley and Wolf[36] suggested that the secondary weak binding (complex II) represented dye binding by ionic groups of polyanions by stacking or aggregation, and they introduced a numerical parameter, the "stacking coefficient" (see Section II,B,5). Polymers whose binding sites are free to assume optimal positions for the stacking of dye molecules bound to them will have high

[216] M. E. Lamm and D. M. Neville, *J. Phys. Chem.* **69**, 3872 (1965); G. R. Haugen and W. H. Melhuis, *Trans. Faraday Soc.* **60**, 386 (1964).

[217] G. P. Dutta, *Stain Technol.* **44**, 223 (1969).

[218] D. Roth, M. London, and M. Manjon, *Stain Technol.* **42**, 125 (1967).

[218a] *CSD II*, pp. 139, 757, and 1327.

[219] L. Zech, *Exp. Cell Res.* **58**, 463 (1969).

[220] P. L. Pearson, M. Bobrow, and C. G. Vosa, *Nature (London)* **226**, 78 (1970); P. L. Pearson and M. Bobrow, *ibid.* p. 959.

[221] P. Barlow and C. G. Vosa, *Nature (London)* **226**, 961 (1970).

[222] Anonymous, *Nature (London)* **226**, 897 (1970).

[223] A. R. Peacocke and J. H. N. Skerrett, *Trans. Faraday Soc.* **52**, 261 (1956).

stacking coefficients, while those polymers ose binding sites are rigidly held in positions less than optimal fc acking will have lower stacking coefficients. Thus a double-stranded DNA has a lower stacking coefficient for AO than RNA and denatured DNA.[224]

From a study of the course of dissociation of DNA complexes with proflavine and AO by heating, Kleinwachter *et al.*[225] found that dyes bound more weakly to the surface (complex II) dissociated in a wide temperature interval, whereas dissociation of the dye bound more strongly (complex I) occurred in the temperature region of the helix-coil transition of the complex and was of a cooperative character. In his classical paper on "The Nature of the Interaction of Nucleic Acids and Nuclei with Basic Dyestuffs" Michaelis[35] suggested that "each dye cation combined with one phosphate group must lie in the space between the planes of the pyridine [pyrimidine] or purine rings, and so they are prevented from approaching each other in such a way as to interfere optically with each other and from exhibiting the spectrum of a higher dyestuff aggregate."

Lerman[226] interpreted the strong binding (complex I) of the amino-acridines to DNA in terms of intercalation, a process in which dye molecules slide between adjacent hydrogen-bonded base pairs in the DNA double helix in a plane perpendicular to the helical axis. A local unwinding and extension of the helix takes place to accommodate the intercalated dye molecule, thus forcing adjacent base pairs further apart (up to 6.8 Å) than normal (3.4 Å). Support for the intercalation model was found by Lerman in the greatly reduced reactivity of the acridine amino groups of the DNA–proflavine complex towards diazotisation. In the proposed model the proflavine molecule is sandwiched firmly between base pairs above and below, so that it is largely shielded from direct contact with the surrounding medium, and the amino groups of proflavine are positioned next to the oxygen of the desoxyribose residue from above and below, and thus protected from diazotization.

Support for the intercalating model has been given by X-ray diffraction,[226] small-angle X-ray scattering,[227] flow-polarized fluorescence, and flow dichroism.[228] The electrooptical properties of the deoxyribonucleohistone–proflavine complexes showed that they are consistent with an intercalation of the proflavine cations between adjacent

[224] A. L. Stone and D. F. Bradley, *J. Amer. Chem. Soc.* **83**, 3627 (1961).

[225] V. Kleinwachter, Z. Balcharova, and J. Bohacek, *Biochim. Biophys. Acta* **174**, 188 (1969).

[226] L. S. Lerman, *J. Mol. Biol.* **3**, 18 (1961); **10**, 367 (1964); *Proc. Nat. Acad. Sci. U.S.* **49**, 94 (1963).

[227] V. Luzzati, F. Masson, and L. S. Lerman, *J. Mol. Biol.* **3**, 634 (1961).

[228] L. S. Lerman, *J. Cell. Comp. Physiol., Suppl.* **1**, 64 (1964).

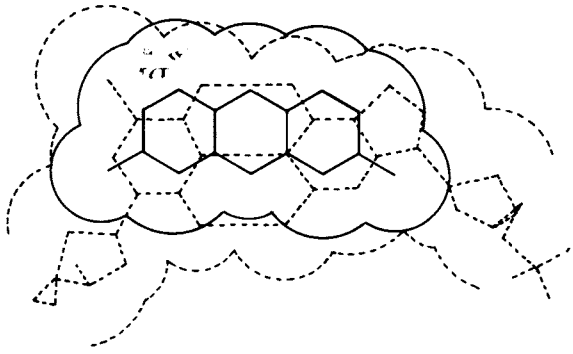


FIG. 1. An intercalated dye molecule in a DNA double helix.[226]

nucleotide pairs (at most one proflavine molecule per five nucleotide pairs) in the gel-forming deoxyribonucleohistone; for higher proflavine content the excess dye molecules would be externally attached to the helix.[229] Electron micrographic measurement of the lengthening of DNA as the result of dye binding showed that only every second space between base pairs is available for intercalation.[230]

Additional evidence for the intercalation mechanism of dye binding by nucleic acids has been provided by an NMR study of the interaction of purine and pyrimidine derivatives with Acridine Orange.[231] An up-field shift of the proton signals of the bases was observed in solution containing AO, consistent with the formation of mixed aggregates of dye and base. The data further indicated that the interaction was more favored in purine-rich regions of polynucleotides and deoxyribopolynucleotides, presumably because of the presence of a large mobile π-electron system in the purine ring.

Acridine dyes are mutagenic, and the intercalation model[232,233] for their binding has helped to explain their mutagenic action at the molecular level. As discussed above, the unwinding and extension of the helix, which take place as a result of the binding of acridine dyes by intercalation, force the base pairs further apart than normal, and if this happened between bases of one chain of DNA, it might lead to the deletion (or addition) of a base in replication, and in turn to substitution of one amino acid for another, or a break in a polypeptide chain, or

[229] C. Houssier and E. Fredericq, *Biochim. Biophys. Acta* **120**, 434 (1966).

[230] J. Cairns, *Cold Spring Harbor Symp. Quant. Biol.* **27**, 311 (1962).

[231] F. E. Hruska and S. S. Danyluk, *Biochim. Biophys. Acta* **161**, 250 (1968).

[232] S. Brenner, L. Barnett, F. H. C. Crick, and A. Orgel, *J. Mol. Biol.* **3**, 121 (1961); A. Orgel and S. Brenner, *ibid.* p. 762.

[233] G. Streisinger *et al.*, *Cold Spring Harbor Symp. Quant. Biol.* **31**, 77 (1966).

production of no protein at all. Thus mutation can be pictured as an error introduced in replication by a local unwinding of the nucleic acid chain.

The intercalation concept of the interaction of DNA with flat planar molecules is also being examined as a possible explanation of the biological activity of some antibiotics and chemotherapeutic agents.[233a]

In a modified intercalation model elaborated by Peacocke[234] the acridine molecule is located between two adjacent bases on the same polynucleotide chain. The main basis for the modification was that the strong binding with an aminoacridine was not reduced by denaturation. In a recent review Blake and Peacocke[235] have discussed the nature of the interaction between aminoacridines and nucleic acids and the structures of the complexes.

Semmel and Daune[236] studied the binding of proflavine, Acridine Orange, Pyronine, and Toluidine Blue by ribosomal RNA and synthetic polyribonucleotides, which causes a red shift in the absorption spectra of the dyes. The first dye molecules were intercalated between the base pairs of the organized regions of the polyribonucleotides. The resultant partial disorganization of the macromolecules permitted subsequent binding of other dye molecules with smaller binding constant, but with the same spectral effect. At high temperatures the secondary structure of the polyribonucleotides disappeared, and the dyes were then bound to the phosphates, as shown by the blue shift of the absorption spectra of the dyes.

The interaction of AO and proflavine with DNA at pH 6.5 has recently been studied at several ionic strengths by equilibrium dialysis, absorption spectrophotometry, and low-shear viscosimetry.[237] The results led to an intercalation model with the following novel features: (1) Every slot between two successive base pairs of the DNA helix constitutes a possible binding site for one intercalated dye molecule. Intercalated dye is distributed randomly over all possible binding sites with one restriction — intercalation does not occur at sites immediately adjacent to one already occupied. (2) Every intercalated dye molecule is a possible binding site for an additional, nonintercalated dye molecule. The pair of bound dye molecules so formed constitutes a spectroscopically distinct dimer species. This binding of nonintercalated dye mole-

[233a] M. Waring, *Prog. Mol. Subcel. Biol.* **2**, 216 (1971).

[234] N. J. Pritchard, A. Blake, and A. R. Peacocke, *Nature (London)* **212**, 1360 (1966).

[235] A. Blake and A. R. Peacocke, *Biopolymers* **6**, 1225 (1968).

[236] M. Semmel and M. Daune, *Biochim. Biophys. Acta* **145**, 561 (1967).

[237] R. W. Armstrong, T. Kurucsev, and U. P. Strauss, *J. Amer. Chem. Soc.* **92**, 3174 (1970).

cules proceeds in a random fashion and does not affect the contour length of the DNA.

Dye–nucleic acid interactions have been widely used for studying the secondary structures of nucleic acids, to distinguish between double-stranded native DNA from single-stranded and denatured DNA. The fluorescence maxima of complexes of AO with double-stranded DNA at 530 nm (yellow-green) and with single-stranded DNA at 640 nm (red) have been used to study the conformation of DNA, both in solution state and in cytochemistry. Thus its interaction with AO showed polyoma virus to contain double-stranded DNA.[238] Double-stranded DNAs of a number of phages have single-stranded regions inside phage particles; after isolation, transformation from a partially single-stranded to a completely double-stranded form takes place.[238a]

Intracellular nucleic acids and nucleoproteins can be characterized by binding with AO and determining the ratio between the fluorescence intensities at 530 and 650 nm.[239] The former emission corresponds to intercalated AO in DNA and the latter to the aggregated dye bound to RNA. Rigler used AO binding to determine the degree of ordered structure of DNA in deoxyribonucleoprotein in different stages of cell activity. He found that the binding of AO to nucleic acids in nucleoproteins was influenced by the amino groups of the proteins; but Lerman[240] has questioned the direct effect of the protein charge. Optically inactive proflavine and AO exhibit a large positive Cotton effect in the presence of DNA. Only a small number of dye molecules in close proximity are required for optical activity to occur.[241] Denaturation of DNA does not destroy the optical activity. In the ORD curves of AO bound to DNA two Cotton effects of opposite sense can be distinguished. There are apparent differences in the behavior of the two dyes because of the tendency of AO to aggregate. The circular dichroism of an isolated intercalated proflavine molecule depends on salt concentration.[241a]

Strong interaction of nucleic acids with dyes stabilizes their structures against heat denaturation and enzymatic degradation.[225,242,243]

[238] H. D. Mayor, *Proc. Soc. Exp. Biol. Med.* **108**, 103 (1960).

[238a] M. Gabrilovich and L. N. Romanovskaja, *Biochim. Biophys. Acta* **213**, 231 (1970).

[239] R. Rigler, Jr., *Acta Physiol. Scand., Suppl.* **267**, 1–122 (1966).

[240] L. S. Lerman, *Prog. Mol. Subcel. Biol.* **2**, 382 (1971).

[241] A. Blake and A. R. Peacocke, *Biopolymers* **4**, 1091 (1966); **5**, 383 and 871 (1967).

[241a] H. J. Li and D. M. Crothers, *Biopolymers* **8**, 217 (1969); *J. Mol. Biol.* **39**, 461 (1969).

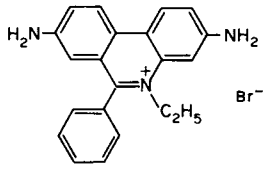
[242] N. F. Gersch and D. O. Jordan, *J. Mol. Biol.* **13**, 138 (1965).

[243] M. D. Faddeeva, *Tsitologiya* **11**, 225 (1969).

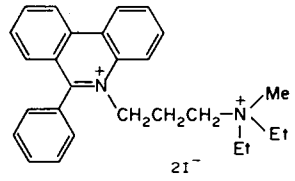
Kurnick and Radcliffe[244] have shown that AO, acriflavine, and quinacrine inhibit DNAs by binding to DNA.

D. Ethidium Bromide

Ethidium (also known as homidium bromide), useful as a trypanocide, contains the phenanthridine ring system, isomeric with acridine. It has recently proved to be of great interest as an intercalating dye, particularly for the study of closed circular DNA.



Ethidium bromide



Propidium iodide

Bauer and Vinograd[245] have recently reviewed the occurrence and properties of closed circular DNAs with reference to intercalating dyes as probes for their structure. Double-helix DNAs have been found to have two additional characteristics of closed circularity and superhelicity in many DNAs occurring in animal and bacterial viruses, animal mitochondria and extramitochondrial cytoplasm, protozoa, and bacteria. A serious problem in the study of circular DNA is its separation in pure form from an overwhelming preponderance of linear DNAs, and it was found by Radloff, Bauer, and Vinograd that the detection and isolation of the closed circular DNA from HeLa cells could be achieved by a dye-buoyant density method. The special properties of closed circular DNA are explained by the hypothesis that the total number of rotations of one strand about the other is invariant. The closed molecule is therefore subject to a topological restraint, one consequence of which is the restricted binding of intercalating dyes, which leads to a smaller buoyant density decrement when the sedimentation velocity of the DNA is determined in alkaline CsCl by band centrifugation. One of the requirements for a suitable dye is strong binding at high CsCl concentrations, which is fulfilled by ethidium bromide and not by the aminoacridines. Propidium diiodide was subsequently found to be an improvement over ethidium bromide. Some 46 different closed circular DNAs have been extracted and studied by this method; nearly half involved the use of ethidium bromide.

[244] N. B. Kurnick and I. E. Radcliffe, *J. Lab. Clin. Med.* **60**, 669 (1962).

[245] W. Bauer and J. Vinograd, *Progr. Mol. Submol. Biol.* **2**, 180 (see this paper for earlier references); see also D. R. Helinski and D. B. Clewell, *Amer. Rev. Biochem.* **40**, 899 (1971).

Crawford and Waring measured the supercoiling of polyoma virus DNA by its interaction with ethidium bromide.[246,247]

Lerman[240] studied intercalability into DNA which had undergone polymer-and-salt-induced transition (ψ transition), using ethidium because its tendency to bind to DNA in an aggregated state, in addition to the intercalated binding, was smaller than that of AO. He observed that affinity for the dye was stronger in non-ψ than in ψDNA, and he considered the out-of-plane benzene ring in the 9-position to be involved, because the benzene ring was unlikely to be accommodated in the space between nucleotide pairs occupied by the phenanthridine ring system. The ψ conformation, which appeared to correspond to DNA *in vivo*, required close association between parts of the DNA molecule not covalently adjacent.

Canter *et al.*[248] have used covalently and noncovalently bound fluorescent dyes for studying the tertiary structure of transfer RNA in solution. Covalent labeling was achieved by periodate oxidation of the 3′ end of RNA and condensing the resultant bis-aldehydes with a suitable compound, such as acriflavine, to form a Schiff's base, or other acridine derivatives which formed hydrazones. Ethidium bromide was used for noncovalent binding (intercalation), and a variety of fluorescence techniques provided information on the tertiary structure.

VI. Dyes as Antibacterial and Therapeutic Agents

A section of "Extra Pharmacopoeia Martindale" is devoted to dyes in medicine and pharmacy,[249] to which reference must be made for the original literature. The dyes used at the present time for medicinal purposes are limited in number, although it has been stated that they are "important bacteriostatic agents in medicine, surgery and veterinary practice."[250] In addition to their utility in diagnostic procedures (see Section II,B,6,7) they are employed as antiseptics, chemotherapeutic agents against protozoa, and wound-healing agents.[251] Further, many

[246] L. V. Crawford and M. J. Waring, *J. Mol. Biol.* **25**, 23 (1967).

[247] See also M. J. Waring, *Progr. Mol. Submol. Biol.* **2**, 216.

[248] C. R. Canter *et al.*, *Progr. Mol. Submol. Biol.* **2**, 297.

[249] R. G. Todd, ed., "Extra Pharmacopoeia Martindale," 25th ed., pp. 553–570. Pharmaceutical Press, London, 1967. In the 26th ed. (N. W. Blacow, ed.), 1972, dyes are discussed in the sections on "flavouring and colouring agents," "diagnostic agents," "antiseptics and disinfectants," and miscellaneous topics. See also C. H. Browning *in* "Experimental Chemotherapy" (R. J. Schnitzer and F. Hawking, eds.), Vol. 2, p. 1, Academic Press, 1964.

[250] J. W. Baker, I. Schumacher, and D. P. Roman, *in* "Medicinal Chemistry" (A. Burger, ed.), 3rd ed., Part I, p. 627. Wiley (Interscience), New York, 1970.

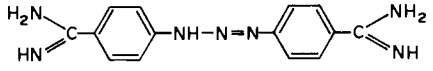
[251] L. S. Goodman and A. Gilman, eds., "The Pharmacological Basis of Therapeutics," 3rd ed., p. 1044. Macmillan, New York, 1965.

of the reactions and products first developed in connection with synthetic dyes have provided useful leads for the synthesis of drugs.

Drugs are often colored for distinguishing them during pharmaceutical operations and subsequent use. Approved food colors are obviously suitable; in the United States there is an additional list of D and C colors which are approved for use only in drugs and cosmetics.

A. Diazo and Azo Compounds

4,4′-Diamidinodiazoaminobenzene (XXXVIII) (Berenil, Hoechst) is a veterinary trypanocide; limited clinical trials in human trypanosomiasis have been favorable.[252] *N*,*N*-Diacetyl-4-aminoazotoluene (Dimazon) and Sudan IV are used for promoting epithelial growth. The azo dye pyridium (aniline → 2,6-diaminopyridine hydrochloride) is an inhibitor of *E. coli* and some cocci and finds use as a dental, surgical, and urinary antiseptic,[250] but is more frequently employed as a urinary analgesic.[251] Congo Red has some use as a hemostatic agent.



(XXXVIII)

B. Phthaleins

Phenolphthalein continues to be widely employed as a cathartic in candy and other forms, one advantage it has being its relative nontoxicity. Phenolsulfophthalein (Phenol Red) and fluorescein are valuable as diagnostic agents. Merbromnin (Mercurochrome[252a]), because of its nonirritant character and probably also its attractive red color, is a popular antiseptic, although it is only a weak bacteriostatic agent.[251]

C. Basic Dyes

Many basic dyes of the triphenylmethane, acridine, and thiazine classes have bacteriostatic properties, and a few continue to be used, but mostly for external treatment. Basic dyes are able to stain bacteria (see Section II,B,6) by the cations forming complexes with nucleic acids and nucleoproteins, and this is also the broad basis of the antibacterial activity of the dyes. The activity is usually inhibited or diminished by serum, and concentrations of 0.05% to 0.5% have to be used in the treatment of fungal skin affections, wounds, and burns.

Cyanine dyes useful as medicinals are mentioned by G. E. Ficken.[252b]

[252] N. W. Blacow, ed., "Extra Pharmacopaeia Martindale," 26th ed., p. 1859. Pharmaceutical Press, London, 1972.

[252a] *CSD II*, p. 749.

[252b] See *CSD IV*, p. 337.

1. *Acridines*

Albert has carried out extensive work on structure–activity relationships among the acridines; his results and conclusions concerning the dependence of antibacterial activity on ionization, the competition of drug ions with hydrogen ions of groups in the bacterial cell, and the shape and size of drug molecules are of interest in wider fields of chemotherapy.[253]

Acriflavine and proflavine as external antiseptics, and quinacrine[253a] as an anthelmintic continue to be used. Acriflavine is actively bacteriostatic in dilutions of 1:3,000,000, and the relatively fragile gonococcus is sensitive to dilutions as high as 1:50,000,000. The gonococcus is killed in 2–3 minutes by the dye in dilutions of about 1:400,000.[50] Quinacrine, formerly one of the official antimalarials, has now been superseded by less toxic and more effective drugs.

Acrisorcin, the 4-hexylresorcinol salt of 9-aminoacridine, is useful as a topical antifungal agent.

2. *Triphenylmethanes*

Triphenylmethane dyes in high dilutions exhibit both bacteriostatic and bactericidal activity; in general, the Gram-positive organisms are more sensitive to dyes.[50] Malachite Green inhibits the growth of *B. subtilis* in a dilution of 1:4,000,000 and staphylococci in 1:1,000,000; concentrations of about 1:30,000 are required to inhibit typhoid bacilli. Brilliant Green is active in even higher dilutions. In the triaminotriphenylmethane dyes antibacterial activity increases markedly with *N*-methylation. The parent Pararosaniline and Rosaniline are active only in 1:500,000; but Methyl Violet and Crystal Violet are active (e.g., against the diphtheria bacillus) in dilutions of 1:5,000,000.[50]

Malachite Green has antiseptic properties and can be applied to wounds as a spray. It has also been used in the treatment of mycotic skin infections. Brilliant Green (the ethyl homolog of Malachite Green) in combination with Crystal Violet and proflavine is used as an antiseptic. Brilliant Green also has the valuable property of stimulating tissue proliferation and is therefore used in the treatment of indolent ulcers.

Condensation of 2-hydroxy-3-naphthoic acid with formaldehyde gives the 1,1′-dinaphthylmethane derivative, pamoic acid. The pamoic acid derivative of Pararosaniline is claimed to have antischistosomal activity.[254]

[253] A. Albert, "The Acridines," 2nd ed. Arnold, London, 1966; "Selective Toxicity," 4th ed. Barnes & Noble, New York, 1968.

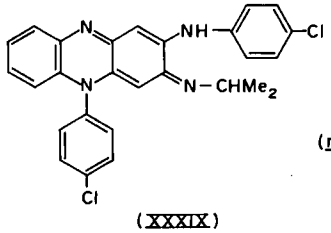
[253a] *CSD II*, p. 757.

[254] Parke, Davis & Co., *BeP* 588,814.

Magenta (CI Basic Violet 14, CI 42510) has antifungal and antibacterial action, especially against Gram-positive bacteria. Crystal Violet is a potent nonirritant antiseptic with a selective action on Gram-positive organisms. It also has anthelmintic properties. For threadworms in children it has been superseded by piperazine. In the United States "Methylrosanilin chloride" ("Gentian Violet"; mainly Crystal Violet mixed with Methyl Violet, pentamethyl pararosaniline) is official as a 1% solution in 10% alcohol for topical use as a "local antiinfective."

3. *Azines, Oxazines, Thiazines*

Barry synthesized numerous phenazines as potential antimycobacterial agents and found that many were highly active.[255] One of the most potent was (XXXIX). None has proved useful in human tuberculosis, but some may find a place in the chemotherapy of leprosy.[256] Benzophenoxazines were being investigated for selective tumor-staining and growth-retarding action when screening disclosed exclusive activity against mycobacteria. Several, such as (XL), were ten times as effective as streptomycin, but the production of hemolytic anemia in experimental animals precluded clinical trials.



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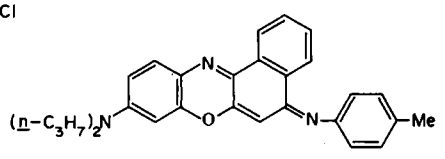


(XL)

The weak antimalarial activity of Methylene Blue, discovered by Ehrlich, led to synthetic work on new antimalarials. The phenothiazine ring system has acquired great importance in connection with antipsychotic agents.

Methylene Blue has mild antiseptic properties; because it is excreted mainly by the kidneys, it has been used in the treatment of urinary tract infections by oral administration, but more effective drugs are now available. It is an antidote to cyanide poisoning and is useful in the treatment of idiopathic methemoglobinemia by oral administration with large doses of ascorbic acid and in the treatment of drug-induced methemoglobinemia by intravenous injection. Toluidine Blue is admini-

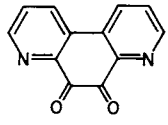
[255] V. C. Barry *et al.*, *Bull. Int. Union Tuberc.* **29**, 582 (1959).

[256] For references, see A. Lewis and R. G. Shepherd, *in* "Medicinal Chemistry" (A. Burger, ed.), 3rd ed., Part I, p. 443. Wiley (Interscience), New York, 1970.

stered intravenously to inhibit the anticoagulant effect of heparin; it acts by forming an inactive complex. It has also some use in the treatment of idiopathic functional uterine bleeding, but for these purposes it is being superseded by more effective drugs.

4. *Quinones*

4,7-Phenanthroline-5,6-quinone (XLI) (Phanquone, Ciba) represents a novel chemical type among amebicides. It is a widely used amebicide whose efficacy is improved by combination with halogenated 8-hydroxyquinoline. It is also active against both Gram-positive and Gram-negative bacteria. Many benzoquinones and hydroquinones exhibit antiamebic properties.[257]



(XLI)

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[257] E. F. Elslager, *in* "Medicinal Chemistry" (A. Burger, ed.), 3rd ed., Part I, p. 545. Wiley (Interscience), New York, 1970.

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