



**ISAO NODA  
YUKIHIRO OZAKI**

# **TWO-DIMENSIONAL CORRELATION SPECTROSCOPY**

Applications in Vibrational and Optical Spectroscopy

 **WILEY**

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# Two-dimensional Correlation Spectroscopy – Applications in Vibrational and Optical Spectroscopy

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**Isao Noda**

*Procter and Gamble, West Chester, OH, USA*

and

**Yukihiro Ozaki**

*Kwansei-Gakuin University, Sanda, Japan*



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# Preface

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In the last decade or so, perturbation-based *generalized two-dimensional (2D) correlation spectroscopy* has become a surprisingly powerful and versatile tool for the detailed analysis of various spectroscopic data. This seemingly straightforward idea of spreading the spectral information onto the second dimension by applying the well-established classical correlation analysis methodology, primarily for attaining clarity and simplicity in sorting out the convoluted information content of highly complex chemical systems, has turned out to be very fertile ground for the development of a new generation of modern spectral analysis techniques. Today there are more than several hundred high-quality scientific publications based on the concept of generalized 2D correlation spectroscopy. The trend is further promoted by the rapid evolution of this very unique concept, sometimes extending well beyond the spectroscopic applications. Thus, in addition to the widespread use in IR, X-ray, fluorescence, etc., we now see successful applications of 2D correlation techniques in chromatography, microscopy, and even molecular dynamics and computational chemistry. We expect the generalized 2D correlation approach to be applied to many more different forms of analytical data.

This book is a compilation of work reflecting the current state of generalized 2D correlation spectroscopy. It can serve as an introductory text for newcomers to the field, as well as a survey of specific interest areas for experienced practitioners. The book is organized as follows. The concept of two-dimensional spectroscopy, where the spectral intensity is obtained as a function of two independent spectral variables, is introduced. In Chapter 1, some historical perspective and an overview of the field of perturbation-based 2D correlation spectroscopy are provided. The versatility and flexibility of the generalized 2D correlation approach are discussed with the emphasis on how different spectroscopic probes, perturbation methods, and their combinations can be exploited. The rest of this book is organized to provide a comprehensive coverage of the theory of perturbation-based two-dimensional correlation spectroscopy techniques, which is generally applicable to a very broad range of spectroscopic techniques, and numerous examples of their application are given for further demonstration of the utility of this versatile tool.

Chapter 2 covers the central theoretical background of the two-dimensional correlation method, including heterospectral correlation, pertinent properties and interpretation of features appearing in 2D correlation spectra, model 2D spectra generated from known analytical functions, and the fundamental relationship between classical cross correlation analysis and 2D correlation spectroscopy. Chapter 3 provides a rapid and simple computational method for obtaining 2D

correlation spectra from an experimentally obtained spectral data set, which is followed by the practical considerations to be taken into account for the 2D correlation analysis of real-world spectral data in Chapter 4. These three chapters should fully prepare the reader to be able to construct and interpret 2D correlation spectra from various experimental data.

The next three chapters deal with more advanced topics. Chapter 5 introduces the concept of sample–sample correlation and hybrid correlation, and Chapter 6 explores the relationship between 2D correlation spectroscopy and classical statistical and chemometrical treatments of data. Matrix algebra notations are used in these chapters. Chapter 7 examines other types of 2D spectroscopy not covered by the rest of this book, such as nonlinear optical 2D spectroscopy based on ultrafast laser pulses, 2D mapping of correlation coefficients, and newly emerging variant forms of 2D correlation analyses, such as moving-window correlation and model-based correlation methods.

The remaining chapters of the book are devoted to specific examples of the application of 2D correlation spectroscopy to show how the technique can be utilized in various aspects of spectroscopic studies. Chapter 8 is focused on the so-called dynamic 2D spectroscopy techniques based on a simple periodic perturbation. Although it represents the most primitive form of 2D correlation methods, this chapter demonstrates that surprisingly rich information can be extracted from such studies. Generalized 2D correlation studies of basic molecules are discussed in Chapter 9, followed by applications to polymers and liquid crystals in Chapter 10 and reaction kinetics in Chapter 11. Chapter 12 covers the application of 2D correlation in the field of protein research, and Chapter 13 deals with other biological and biomedical science applications. Chapter 14 examines the intriguing potential of heterospectral correlation, where data from more than one measurement technique are now combined by 2D correlation. Finally, Chapter 15 explores the possibility of extending the 2D correlation method beyond the boundary of optical spectroscopy techniques.

We hope this book will be not only useful but also enjoyable to read. In spite of its powerful utility, generalized 2D correlation is fundamentally a simple and relatively easy technique to implement. We will be most gratified if the book can inspire readers to try out some of the specific 2D techniques discussed here in their own research area or even to attempt the development of a new form of 2D correlation not yet explored by us.

Isao Noda and Yukihiro Ozaki  
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# 1 Introduction

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## 1.1 TWO-DIMENSIONAL SPECTROSCOPY

An intriguing idea was put forward in the field of NMR spectroscopy about 30 years ago that, by spreading spectral peaks over the second dimension, one can simplify the visualization of complex spectra consisting of many overlapped peaks.<sup>1–4</sup> It became possible for the spectral intensity to be obtained as a function of two independent spectral variables. Following this conceptual breakthrough, an impressive amount of progress has been made in the branch of science now known as *two-dimensional (2D) spectroscopy*. While traditional field of 2D spectroscopy is still dominated by NMR and other resonance spectroscopy methods, lately a very different form of 2D spectroscopy applicable to many other types of spectroscopic techniques is also emerging. This book's focus is on this latter type of 2D spectroscopy.

The introduction of the concept of 2D spectroscopy to optical spectroscopy, such as IR and Raman, occurred much later than NMR in a very different form. The basic concept of *perturbation-based* two-dimensional spectroscopy applicable to infrared (2D IR) was proposed first by Noda in 1986.<sup>5–7</sup> This new form of 2D spectroscopy has evolved to become a very versatile and broadly applicable technique,<sup>8,9</sup> which gained considerable popularity among scientists in many different areas of research activities.<sup>10–14</sup> So far, over several hundred scientific papers related to this topic have been published, and the technique is establishing itself as a powerful general tool for the analysis of spectroscopic data. 2D spectra appearing in this book are all based on the analysis of perturbation-induced spectral variations.

So, what does a 2D correlation spectrum look like? And what kind of information does it provide us with? Figure 1.1 shows an example of a *stacked-trace* or *fishnet* plot of a 2D IR correlation spectrum in the CH stretching region of an atactic polystyrene film under a mechanical (acoustic) perturbation.<sup>15</sup> The IR correlation intensity is plotted as a function of two independent wavenumber axes. Figure 1.2 is the same spectrum plotted in the form of a counter map. The stacked-trace or pseudo three-dimensional representation provides the best overall view of the intensity profile of a correlation spectrum, while the contour map representation is better to observe the detailed peak shapes and positions. It should be immediately apparent that the 2D IR spectrum consists of much sharper and better resolved peaks than the corresponding 1D spectrum. This enhancement

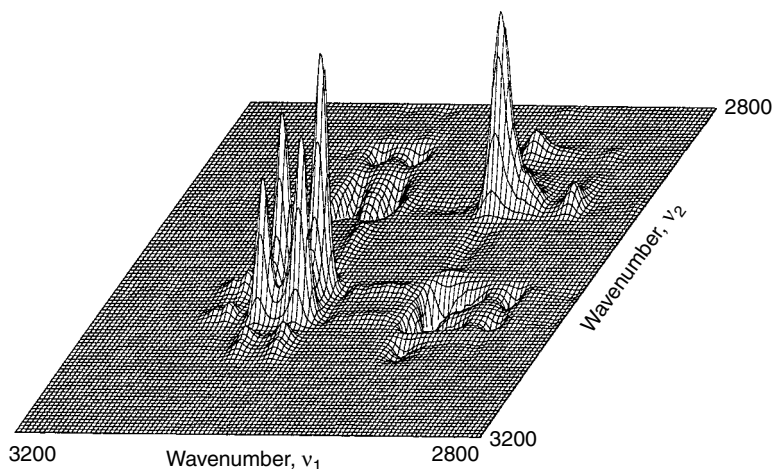


**Figure 1.1** Fishnet representation of a 2D IR correlation spectrum of an atactic polystyrene film under a mechanical perturbation. (Copyright © 1990 by Chemtracts, originally published in *ChemTracts: Macromolecular Chemistry*, **1**(2): 89–105.)

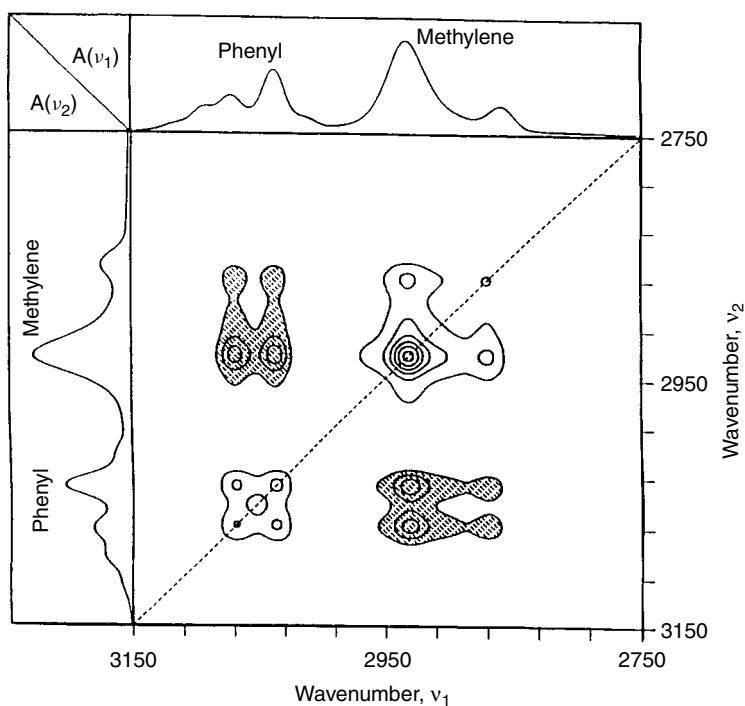


**Figure 1.2** Contour map representation of a 2D IR correlation spectrum of polystyrene film. (Reproduced with permission from Ref. No. 121. Copyright (1999) Wiley-VCH.)

of the resolution is a direct consequence of spreading highly overlapped IR peaks along the second dimension. The appearance of positive and negative *cross peaks* located at the off-diagonal positions of a 2D spectrum indicates various forms of correlational features among IR bands. Correlations among bands that belong to, for example, the same chemical group, or groups interacting strongly, can be effectively investigated by 2D spectra. Basic properties of 2D spectra and a procedure to interpret their features are described in Chapter 2.

2D IR spectra, such as those shown in Figures 1.1 and 1.2, may look very different from conventional IR spectra, but in fact they are measured with a spectrometer not that different from an ordinary commercial instrument. Sometimes the spectrometer is equipped with an additional peripheral attachment designed to stimulate or perturb a sample, but quite often 2D correlation spectroscopy does not require any special attachment at all. When a certain perturbation is applied to a sample, various chemical constituents of the system are selectively excited or transformed. The perturbation-induced changes, such as excitation and subsequent relaxation toward the equilibrium, can be monitored with electromagnetic probes such as an IR beam to generate so-called *dynamic spectra*. The intensity changes, band shifts, and changes in band shapes are typical spectral variations observed under external perturbation. The monitored fluctuations of spectral signals are then transformed into 2D spectra by using a correlation method described in Chapters 2 and 3. The experimental approach, therefore, is relatively simple and broadly applicable to many aspects of spectroscopic studies. One of the important characteristic points of Noda's 2D spectroscopy lies in the fact that 2D correlation spectra consist of two orthogonal components, the synchronous and asynchronous correlation spectrum, which individually carry very distinct and useful information for the subsequent analysis.

The main advantages of the 2D correlation spectroscopy discussed in this book lie in the following points: (i) simplification of complex spectra consisting of many overlapped peaks, and enhancement of spectral resolution by spreading peaks over the second dimension; (ii) establishment of unambiguous assignments through correlation of bands; (iii) probing the specific sequential order of spectral intensity changes taking place during the measurement or the value of controlling variable affecting the spectrum through asynchronous analysis; (iv) so-called *heterospectral* correlation, i.e., the investigation of correlation among bands in two different types of spectroscopy, for example, the correlation between IR and Raman bands; and (v) truly universal applicability of the technique, which is not limited to any type of spectroscopy, or even any form of analytical technique (e.g., chromatography, microscopy, and so on).

## 1.2 OVERVIEW OF THE FIELD

Some historical perspective and overview of the field of 2D correlation spectroscopy should be useful for the reader. It is difficult to describe the development of optical 2D correlation spectroscopy without mentioning the significant

influence of 2D NMR on the field of multi-dimensional spectroscopy.<sup>1-4</sup> The direct and indirect influence of 2D NMR on the earlier development of 2D IR correlation spectroscopy was profound. The whole idea of obtaining 2D spectra had previously been totally alien to the field of IR and other vibrational spectroscopy. The success of 2D NMR motivated the desire to extend this powerful concept into general optical spectroscopy applications. A conceptual breakthrough in the development of practical optical 2D spectroscopy was realized for IR studies around 1986.<sup>5-7</sup> It was developed separately from 2D NMR spectroscopy with a significantly different experimental approach, not limited by the manipulation of pulse-based signals. Most importantly, this new approach turned out to be adaptable to a vast number of conventional spectroscopic techniques.

Today, it may seem almost surprising to us that this powerful yet simple idea of obtaining a spectrum as a function of two independent spectral axes had not been practiced in vibrational spectroscopy until only several decades ago. The 2D technique had been virtually ignored in the optical spectroscopy community for a long period, due to the apparent difficulty in implementing the elegant experimental approach based on multiple pulses, which has been so successfully employed in 2D NMR using radio frequency (rf) excitations. Common optical spectroscopy techniques, such as IR, Raman, and ultraviolet-visible (UV-vis) are governed by physical phenomena having time scales which are very different from those of NMR. The characteristic time scale of molecular vibrations observed in IR absorption spectroscopy is on the order of picosecond, compared to the micro- to millisecond ranges usually encountered in NMR. In NMR, the double Fourier transformation (FT) of a set of time-domain data collected under multiple-pulse excitations generates 2D spectra.<sup>1-4</sup> Direct adaptations of such a procedure based on pulsed excitations to conventional vibrational spectroscopy was rather difficult several decades ago. Nowadays, it has become possible to conduct certain experiments based on ultrafast femtosecond optical pulses in a fashion analogous to pulse-based 2D NMR experiments.<sup>16-21</sup> Chapter 7 of this book briefly discusses such ultrafast optical measurements. However, such measurements are still in their infancy and typically carried out in specialized laboratories with the access to highly sophisticated equipments. Ordinary commercial IR spectrometers cannot adequately provide rapid excitation and detection of vibrational relaxation responses to carry out such measurements. Thus, the specific experimental procedure developed adequately for 2D NMR had to be fundamentally modified before being applied to practical optical spectroscopy.

The first generation of optical 2D correlation spectra were obtained from IR experiments based on the detection of various relaxation processes, which are much slower than vibrational relaxations but closely associated with molecular-scale phenomena.<sup>5-7</sup> These slow relaxation processes can be studied with a conventional IR spectrometer using a standard time-resolved technique. A simple cross-correlation analysis was applied to sinusoidally varying dynamic IR signals to obtain a set of 2D IR correlation spectra. This type of 2D IR correlation spectroscopy has been especially successful in the study of samples stimulated

by a small-amplitude mechanical or electrical perturbation. The technique was first applied to the analysis of a rheo-optical dynamic IR dichroism measurement of a polymer film perturbed with a small-amplitude oscillatory strain. Dynamic fluctuations of IR dichroism signals due to the submolecular level reorientation of polymer chain segments were analyzed by a 2D correlation scheme. In addition to such mechanically stimulated experiments, similar 2D IR investigations based on time-dependent IR signals induced by sinusoidally varying electrical or photo-acoustic perturbations have also been tried. One can find many examples of the applications of 2D IR correlation spectroscopy in the studies of polymers and liquid crystals. Chapter 8 of this book presents some of the useful applications of 2D spectra based on sinusoidal perturbations.

One of the major shortcomings of the above 2D correlation approach, however, was that the time-dependent behavior (i.e., waveform) of dynamic spectral intensity variations must be a simple sinusoid to effectively employ the original data analysis scheme. To overcome this limitation, Noda in 1993 expanded the concept of 2D vibrational correlation spectroscopy to include a much more general form of spectroscopic analysis, now known as the *generalized* 2D correlation spectroscopy.<sup>8</sup> The mathematical procedure to yield 2D correlation spectra was modified to handle an arbitrary form of variable dependence much more complex than simple sinusoidally varying time-dependent spectral signals.<sup>8</sup> The type of spectral signals analyzed by the newly proposed 2D correlation method became virtually limitless, ranging from IR, Raman, X-ray, UV-vis, fluorescence, and many more, even to fields outside of spectroscopy, such as chromatography.<sup>10–14</sup> Most importantly, the generalized 2D correlation scheme lifted the constraint of the perturbations and excitation types. As a result, perturbations with a variety of physical origins, such as temperature, concentration, pH, pressure, or any combination thereof, have been tried successfully for 2D correlation spectroscopy applications.<sup>10–14</sup> Hetero-spectral correlation among different spectroscopic techniques, such as IR-Raman and IR-NIR, has also become straightforward with the generalized 2D scheme. Such a generalized correlation idea truly revolutionized the scope of potential applications for 2D spectroscopy, especially in the field of vibrational spectroscopy.

Parallel to the development of generalized 2D correlation spectroscopy by Noda, some other variants of 2D correlation methods have been proposed. For example, in 1989 Frasinski *et al.*<sup>22</sup> developed the 2D covariance mapping and applied it to time-of-flight mass spectroscopy using a picosecond laser pulse ionization technique. Barton II *et al.*<sup>23,24</sup> proposed a 2D correlation based on statistical correlation coefficient mapping. Chapter 7 of this book discusses more on this approach. 2D correlation maps generated from the idea of Barton II *et al.* display correlation coefficients between two series of spectra, for example, between IR and NIR spectra of a sample, respectively. The main aim of their approach lies in investigating relations between spectral bands in IR and NIR regions. The 2D correlation analysis by Barton II *et al.* set an important direction for the eventual development of the generalized 2D correlation spectroscopy. The

idea by Barton II *et al.* was closely followed by Windig *et al.*,<sup>25</sup> who employed a 2D correlation coefficient map to define the purest available variables in the IR–NIR system of spectra. These variables are subsequently used for chemometric alternating least-squares regression to extract pure IR and NIR spectra of components. In 2001, Šašić and Ozaki<sup>26</sup> expanded statistical 2D correlation spectroscopy originally proposed by Barton II *et al.* to incorporate several improvements concerned with objects and targets of correlation analysis, as well as a relatively simple matrix algebra representation that the methodology utilizes. See Chapter 7 for a further description of their work. Ekgasit and Ishida<sup>27</sup> proposed to refine the 2D correlation method through the normalization of spectral intensities and phase calculation. Their method seems to work for synthetic spectra, but the robust applicability to real-world spectra, especially those with substantial noise, has yet to be determined.

One of the interesting recent developments in generalized 2D correlation spectroscopy was the introduction of sample–sample 2D correlation spectroscopy by Šašić *et al.*<sup>28,29</sup> An in-depth discussion on this subject is found in Chapter 5. Usually 2D maps have spectral variables (wavelengths, wavenumbers) on their axes and depict the correlations between spectral features (variable–variable correlation maps). One can also produce 2D maps that have samples (observed at different time, temperature, concentration, etc.) on their axes and provide information about the correlations among, for example, the concentration vectors of species present (sample–sample correlation maps). Information obtained by variable–variable and sample–sample 2D correlation spectroscopy is often complementary, and general features of variable–variable correlation maps are expected to be equally applicable to the sample–sample correlation maps. Recently, Wu *et al.*<sup>30</sup> proposed hybrid 2D correlation spectroscopy to further expand the concept. Chapter 5 describes the basic concept of this approach.

Meanwhile, studies on ultrafast laser pulse-based optical analogues of 2D NMR have also been getting very active.<sup>16–21</sup> For example, the recent conceptual development of 2D Raman experiments based on pulsed excitations is creating a possible link for vibrational spectroscopy and 2D NMR. The detailed discussion on nonlinear optical 2D spectroscopy, which is rapidly establishing itself as an independent branch of physical science, is beyond the scope of this book. The content of this volume is mainly concerned with 2D correlation spectroscopy proposed by Noda, but in Chapters 5–7 different types of 2D correlation methods will also be discussed.

### 1.3 GENERALIZED TWO-DIMENSIONAL CORRELATION

The concept of *generalized two-dimensional (2D) correlation* is the central theme of this book. It is a formal but very versatile approach to the analysis of a set of spectroscopic data collected for a system under some type of external perturbation.<sup>8</sup> The introduction of the generalized 2D correlation scheme

has opened up the possibility of utilizing a powerful and versatile analytical capability for a wide range of spectroscopic applications. Recognition of the general applicability of the 2D correlation technique to the investigation of a set of ordinary spectra obtained not only for time-dependent phenomena but also from a static or stationary measurement was clearly a major conceptual departure from the previous approach. The unrestricted selection of different spectroscopic probes, perturbation methods and forms, and the combination of multiple analytical methods provided the astonishing breadth and versatility of application areas for generalized 2D correlation spectroscopy.

### 1.3.1 TYPES OF SPECTROSCOPIC PROBES

The basic idea of generalized 2D correlation is so flexible and general that its application is not limited to any particular field of spectroscopy confined to a specific electromagnetic probe. Thus far, generalized 2D correlation spectroscopy has been applied to IR,<sup>15,26,27,29–75</sup> NIR,<sup>23,24,29,76–97</sup> Raman,<sup>98–106</sup> ultraviolet–visible (UV–vis),<sup>107–109</sup> fluorescence,<sup>110–112</sup> circular dichroism (CD),<sup>46,47</sup> and vibrational circular dichroism (VCD)<sup>113</sup> spectroscopy. Furthermore, the application of 2D correlation spectroscopy is not even restricted to optical spectroscopy. It has, for example, been applied to X-ray<sup>15,114</sup> and mass spectrometry.<sup>115</sup> An interesting testimony of the versatility of generalized 2D correlation was demonstrated by Izawa *et al.*,<sup>116–118</sup> where the basic idea of 2D correlation is applied to time-resolved gel permeation chromatography, which is totally outside of conventional spectroscopic applications.

### 1.3.2 EXTERNAL PERTURBATIONS

The generalized 2D correlation scheme enables one to use numerous types of external perturbations and physical stimuli that can induce spectral variations.<sup>10,13,14</sup> The perturbations utilized in the 2D correlation analysis may be classified into two major types. One type yields the spectral data set as a direct function of the perturbation variable itself (e.g., temperature, concentration, or pressure), and the second type gives it as a function of the secondary consequence caused by the perturbation, such as a time-dependent progression of spectral variations caused by the application of a stimulus.

Temperature<sup>29,37,38,76–79,81,82,87,100</sup> and concentration<sup>43,50,56,80,83–86,101,102,104–106</sup> are the most commonly used static perturbations for generalized 2D correlation spectroscopy. Typical examples of temperature-induced spectral variations studied by 2D correlation analysis involve dissociation of hydrogen-bonded systems in alcohols,<sup>29,76,79,81,82</sup> and amides,<sup>77–79,87,100</sup> the denaturation of proteins,<sup>57,59,60</sup> and the melting and premelting behavior of polymers.<sup>61,78</sup> Alcohols such as oleyl alcohol and butanol and *N*-methylacetamide show complex temperature-dependent spectral variations due to the dissociation



of hydrogen bonds, and resulting spectral changes were analyzed by 2D correlation spectroscopy. Šašić *et al.*<sup>29</sup> utilized sample–sample 2D NIR correlation spectroscopy to explore the dissociation of associated oleic acids in the pure liquid state. Thermal denaturation of proteins has long been a matter of keen interest. 2D correlation spectroscopy has provided new insight into the denaturation process of proteins.<sup>57,59,60,80,86</sup> For example, a 2D NIR correlation spectroscopy study of the thermal denaturation of ovalbumin revealed an interesting relationship between the temperature-induced secondary structural changes and changes in the extent of hydration.<sup>80</sup> Although most thermal studies are concerned with the static effect of temperature itself on the spectra, one can also apply 2D correlation analysis to a dynamic experiment where the time dependent response caused by a temperature shift (e.g., T-jump or thermal modulation) induces dynamic spectral variations.

A number of 2D correlation spectroscopy studies have been carried out for concentration- or composition-dependent spectral modifications of simple molecules, proteins,<sup>43,56,80,86,106</sup> polymers,<sup>50,83,84,101,102,104,105</sup> and multicomponent mixtures.<sup>23,24,31,85</sup> For example, systematic studies of polymer blends and copolymers exhibiting specific interactions of components using 2D IR, 2D NIR, 2D Raman, and hetero-correlation analysis have been reported.<sup>50,83,84,101,102,104,105</sup> Concentration changes often induce nonlinear structural perturbations for a variety of molecules. 2D correlation analysis may be uniquely suited for finding such changes, because if the systems yield nonlinear responses of spectral intensities to concentration changes (i.e., apparent deviation from the classical Beer–Lambert law), some new features not readily analyzable by conventional techniques may be extracted from 2D correlation analysis. The first example of a 2D correlation study of multicomponent mixtures was carried out for complex liquid detergent formulations comprising a number of ingredients by use of a simple 2D covariance analysis.<sup>31</sup>

2D correlation spectroscopy of pressure-dependent spectral variations is also becoming popular.<sup>26,48,49,65</sup> Several research groups have reported 2D IR studies of pressure-induced protein denaturation.<sup>48,65</sup> For example, pressure-induced spectral changes of polymer films were also subjected to 2D correlation analysis to investigate the morphologically influenced deformation mechanism of polyethylene under compression.<sup>49</sup> Magtoto *et al.*<sup>53</sup> reported IR reflection–absorption measurement of pressure-induced chemisorption of nitric oxide on Pt (100). Noda *et al.*<sup>49</sup> investigated combined effects of pressure and temperature by means of 2D IR spectroscopy.

Other perturbations that yield a series of sequentially recorded spectral data are, for example, pH, position, angle, and excitation wavelength. Murayama *et al.*<sup>66</sup> reported a 2D IR correlation spectroscopy study of pH-induced structural changes of human serum albumin (HSA). They investigated protonation of carboxylic groups of amino acid residues as well as secondary structural alternations of HSA. Nagasaki *et al.*<sup>55</sup> applied 2D correlation analysis to polarization angle-dependent IR band intensity changes to investigate the molecular orientation and

structure of a ferroelectric liquid crystal with a naphthalene ring in the chiral smectic- $C^*$  phase. Spectroscopic analysis of various chemical reactions is also a popular area. The application of reaction-based 2D IR includes complex reaction kinetics, electrochemistry, and photochemistry.<sup>32–34</sup>

Mechanical deformation,<sup>5–7,15,36,41,67–69</sup> an electric field,<sup>35,45</sup> and chemical reactions<sup>32–34</sup> are representative examples of stimuli that may give rise to spectral changes that we describe as producing secondary effects as a consequence of the perturbation. A simple but interesting example of this type of application is the time-dependent compositional change of a mixture by physical or chemical reactions. Noda *et al.*<sup>11</sup> analyzed transient IR spectra of a polystyrene/methyl ethyl ketone/toluene solution mixture during an evaporation process. An example of chemical reactions studied by 2D correlation analysis is an H to D exchange reaction to probe the secondary structure of a protein.<sup>44,63</sup> Šašić *et al.*<sup>64</sup> applied sample–sample correlation spectroscopy to analyze IR spectra of chemical reactions.

Dynamic 2D IR and 2D NIR spectroscopy based on small-amplitude oscillatory mechanical perturbation is well established in polymer science and engineering. An Electric field is another stimulus frequently used for 2D correlation spectroscopy. It is particularly useful for exploring the mechanism of the reorientation of liquid crystals. Ataka and Osawa<sup>33</sup> first applied 2D IR spectroscopy to electrochemical systems. IR spectra near the electrode surface were collected as a function of applied potential.

## 1.4 HETEROSPECTRAL CORRELATION

One very intriguing possibility of 2D correlation spectroscopy is *2D heterospectral correlation* analysis,<sup>15,23,24,97,100,104–106,114,119,120</sup> where two completely different types of spectra obtained for a system under the same perturbation using multiple spectroscopic probes are compared. Chapter 14 of the book provides actual examples of this approach. 2D hetero-spectral correlation may be divided into two types. The first type is concerned with the comparison between closely related spectroscopies, such as IR/NIR and Raman/NIR spectroscopy. In this case, the correlation between bands in two kinds of spectroscopy can be investigated. Therefore, it becomes possible to make band assignments and resolution enhancements by 2D heterospectral correlation. The second type of heterospectral correlation is heterocorrelation between completely different types of spectroscopy or physical techniques such as IR and X-ray scattering. This type of heterospectral correlation is useful for investigating the structural and physical properties of materials under a particular external perturbation.

Heterospectral correlation analysis provides especially rich insight and clarification into the in-depth study of vibrational spectra. For example, the investigation of the correlation between IR and Raman spectra of a molecule by heterospectral correlation is very attractive from the point of better understanding

of its complementary vibration spectra. Likewise, the correlation between NIR and IR spectroscopy is very interesting, because, by correlating NIR bands with IR bands for which the band assignments are better established, one may be able to investigate the less-understood band assignments in the NIR region.

## 1.5 UNIVERSAL APPLICABILITY

We have already alluded to the fact that the generalized 2D correlation spectroscopy described in this book may utilize a number of different spectroscopic probes, e.g., IR, Raman, NIR, fluorescence, UV, and X-ray, in a surprisingly flexible manner by combining the spectroscopic measurement with various physical perturbations, e.g., mechanical, thermal, chemical, optical, and electrical stimuli, to explore a very broad area of applications, ranging from the study of basic small molecules to characterization of polymers and liquid crystals, as well as complex biomolecules. This technique is truly a generally applicable versatile tool in spectroscopy.

It is also useful to point out that the fundamental concept of generalized 2D correlation analysis may be applied to any analytical problems, not at all limited to spectroscopy. Thus, any branch of analytical sciences, such as chromatography, scattering, spectrometry, and microscopy, as well as any other field of scientific research, including molecular dynamics simulations, life science and biology, medicine and pharmacology, or even topics traditionally dealt in social science, can benefit by adopting this scheme. The possibility of extending the 2D correlation method beyond the boundary of optical spectroscopy techniques will be explored in Chapter 15.

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## 2 Principle of Two-dimensional Correlation Spectroscopy

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This chapter provides a tutorial on the fundamental concept behind perturbation-based 2D correlation spectroscopy. Discussions include a formal mathematical procedure to generate 2D correlation spectra, basic properties of synchronous and asynchronous spectra, and closed form analytical expressions for 2D spectra obtained from representative signals of well-known waveforms. Practical numerical computation methods to generate 2D correlation spectra from spectral data set with arbitrary waveforms will be described in Chapter 3. For those who are interested, more detailed discussions on these topics are found in published literature.<sup>1–7</sup>

### 2.1 TWO-DIMENSIONAL CORRELATION SPECTROSCOPY

#### 2.1.1 GENERAL SCHEME

A schematic description of a 2D correlation spectroscopy experiment based on an external perturbation is depicted in Figure 2.1. In an ordinary spectroscopic measurement, some type of electromagnetic probe, for example an IR beam, is applied to the system of interest. The characteristic interaction between the probe and system constituents, such as different chemical groups, is represented in the form of a spectrum and then analyzed to elucidate the detailed information about the system. In 2D correlation spectroscopy which we discuss in this book, an additional external perturbation is applied to the system during the spectroscopic measurement. This external perturbation stimulates the system to cause some selective changes in the state, order, surroundings, etc. of system constituents. The overall response of the stimulated system to the applied external perturbation leads to distinctive changes in the measured spectrum. This spectral variation induced by an applied perturbation is referred to as a *dynamic spectrum* in 2D correlation.

In the generalized 2D correlation spectroscopy scheme, a series of perturbation-induced dynamic spectra are collected first in a systematic manner, e.g., in a sequential order during the process. Such a set of dynamic spectra is then transformed into a set of 2D correlation spectra by cross-correlation analysis. The



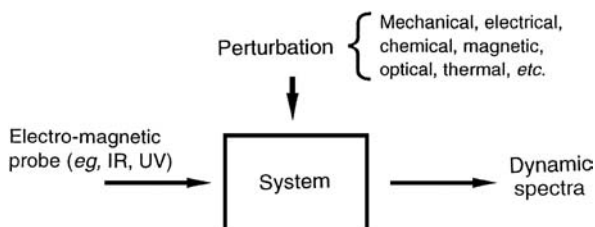


**Figure 2.1** General scheme for obtaining perturbation-based 2D correlation spectra. (Reproduced with permission from I. Noda, *Appl. Spectrosc.*, **47**, 1329 (1993). Copyright (1993) Society for Applied Spectroscopy.)

specific mathematical procedure to construct 2D correlation spectra is relatively straightforward and will be discussed later. One often finds that 2D correlation spectra provide useful information which is not readily available from or at least not apparent in the original set of conventional 1D spectra. This is the prime motivation behind constructing 2D correlation spectra.

### 2.1.2 TYPE OF EXTERNAL PERTURBATIONS

The simple conceptual scheme presented in Figure 2.1 to induce dynamic spectra is a very general one, encompassing a vast number of possible experimental situations. The scheme at this point does not explicitly specify the physical nature or any mechanism by which the applied perturbation affects the system. There are, of course, numerous physical perturbations, which could be used to stimulate the system of interest. For example, various molecular-level excitations may be induced by mechanical, electrical, thermal, magnetic, chemical, or even acoustic excitations.<sup>7</sup> Each perturbation affects the studied system in a unique and selective manner, governed by the specific interaction mechanism coupling the macroscopic stimulus to microscopic or molecular level responses of individual constituents of the system. The physical information contained in a dynamic spectrum, therefore, is dictated by the specific nature of perturbation method.

The *waveform* of the applied perturbation, likewise, can also be selected freely. Thus, a simple sinusoid, or a sequence of pulses can be applied as a possible perturbation, as well as those having much more complex waveforms, such as random noises. The linear response of the system, which leads to the superposition of spectral variations to a sequence of multiple stimuli, is not a prerequisite for 2D correlation analysis. Nonlinear responses actually provide even richer possibilities for 2D correlation spectroscopy. A detailed study on individual interaction mechanisms between the perturbation and various system constituents or the determination of appropriate response functions for system constituents based on dynamic spectra, however, is beyond the scope of this discussion. The main point to be stressed here is that any spectroscopic experiment, which utilizes an external perturbation to generate some form of dynamic spectra, is a good potential candidate for benefiting from 2D correlation analysis.

In many 2D correlation studies, a dynamic spectrum is detected as a straightforward transient function of time under a given perturbation. For example, time-dependent evolution and subsequent relaxation of spectral signals arising from the reorientation of dipole transition moments in a mechanically stretched polymer film may be studied by 2D analysis. Many other transient experiments, such as chemical reactions, may also be analyzed by this method. More importantly, however, spectral variations used in generalized 2D correlation analysis do not even have to be time dependent. It is possible to collect dynamic spectra as a direct function of the quantitative measure of the imposed physical effect itself. Spectral changes as a function of any reasonable physical variables, such as temperature, pressure, concentration, stress, electrical field, etc. may also be studied.<sup>7</sup> As long as the spectral feature changes systematically under some external conditions, it is possible to apply the same correlation method to generate a set of useful 2D spectra. Because 2D correlation analysis historically evolved from statistical time-series analysis,<sup>8,9</sup> certain traditional terminology, such as dynamic spectra, will be retained to describe the perturbation-induced spectral changes, even though the temporal aspect of the measurement may no longer be relevant to some 2D studies.

## 2.2 GENERALIZED TWO-DIMENSIONAL CORRELATION

### 2.2.1 DYNAMIC SPECTRUM

Let us consider a perturbation-induced variation of a spectral intensity  $y(\nu, t)$  observed during a fixed interval of some external variable  $t$  between  $T_{\min}$  and  $T_{\max}$ . While this external variable  $t$  in many cases is the conventional chronological time, it can also be any other reasonable measure of a physical quantity, such as temperature, pressure, concentration, voltage, etc., depending on the type of experiment. The variable  $\nu$  can be any appropriate spectral index used in the field of spectroscopy, including Raman shift, wavenumber or wavelength in IR, NIR and UV-visible studies, scattering angles of X-ray or neutron beam, etc.

The *dynamic spectrum*  $\tilde{y}(\nu, t)$  of a system induced by the application of an external perturbation is formally defined as

$$\tilde{y}(\nu, t) = \begin{cases} y(\nu, t) - \bar{y}(\nu) & \text{for } T_{\min} \leq t \leq T_{\max} \\ 0 & \text{otherwise} \end{cases} \quad (2.1)$$

where  $\bar{y}(\nu)$  is the *reference spectrum* of the system. While selection of a proper reference spectrum is not strictly specified, in most cases, it is customary to set  $\bar{y}(\nu)$  to be the *stationary* or *averaged* spectrum given by

$$\bar{y}(\nu) = \frac{1}{T_{\max} - T_{\min}} \int_{T_{\min}}^{T_{\max}} y(\nu, t) dt \quad (2.2)$$

In some applications, however, it is possible to select a different type of reference spectrum by choosing a spectrum observed at some fixed reference point  $t = T_{\text{ref}}$ , i.e.,  $\tilde{y}(\nu) = y(\nu, T_{\text{ref}})$ . For example, a reference point can be chosen as the original or ground state of the system, sometimes well before the application of the perturbation ( $T_{\text{ref}} \rightarrow -\infty$ ). It can also be picked at the beginning ( $T_{\text{ref}} = T_{\text{min}}$ ) or the end ( $T_{\text{ref}} = T_{\text{max}}$ ) of the course of spectral measurement period, or even well after the full relaxation of the perturbation effect ( $T_{\text{ref}} \rightarrow +\infty$ ). The reference spectrum could also be set simply equal to *zero*; in that case, the dynamic spectrum is identical to the observed variation of the spectral intensity. Each selection of the reference spectrum has its own merit for the specific type of 2D correlation analysis. Without any prior knowledge about the specific physical origin of the dynamic spectrum, the reference spectrum defined by Equation (2.2) probably provides the most robust and preferred form to be used for the correlation analysis.

### 2.2.2 TWO-DIMENSIONAL CORRELATION CONCEPT

The fundamental concept governing 2D correlation spectroscopy is a quantitative comparison of the patterns of spectral intensity variations along the external variable  $t$  observed at two different spectral variables,  $\nu_1$  and  $\nu_2$ , over some finite observation interval between  $T_{\text{min}}$  and  $T_{\text{max}}$ . The 2D correlation spectrum can be expressed as

$$X(\nu_1, \nu_2) = \langle \tilde{y}(\nu_1, t) \cdot \tilde{y}(\nu_2, t') \rangle \quad (2.3)$$

The intensity of 2D correlation spectrum  $X(\nu_1, \nu_2)$  represents the quantitative measure of a comparative similarity or dissimilarity of spectral intensity variations  $\tilde{y}(\nu, t)$  measured at two different spectral variables,  $\nu_1$  and  $\nu_2$ , during a fixed interval. The symbol  $\langle \rangle$  denotes for a cross-correlation function designed to compare the dependence patterns of two chosen quantities on  $t$ . The correlation function generically defined by Equation (2.3) is calculated between the spectral intensity variations measured at two independently chosen spectral variables,  $\nu_1$  and  $\nu_2$ , which gives the basic two-dimensional nature of this particular correlation analysis.

In order to simplify the mathematical manipulation, we treat  $X(\nu_1, \nu_2)$  as a complex number function

$$X(\nu_1, \nu_2) = \Phi(\nu_1, \nu_2) + i\Psi(\nu_1, \nu_2) \quad (2.4)$$

comprising two orthogonal (i.e., real and imaginary) components, known respectively as the *synchronous* and *asynchronous* 2D correlation intensities. The synchronous 2D correlation intensity  $\Phi(\nu_1, \nu_2)$  represents the overall similarity or coincidental trends between two separate intensity variations measured

at different spectral variables, as the value of  $t$  is scanned from  $T_{\min}$  to  $T_{\max}$ . The asynchronous 2D correlation intensity  $\Psi(\nu_1, \nu_2)$ , on the other hand, may be regarded as a measure of dissimilarity or, more strictly speaking, out-of-phase character of the spectral intensity variations.

The terminology, such as the synchronous or asynchronous spectrum, was adopted for purely historical reasons. Because earlier conceptual development of perturbation-based 2D correlation analysis had relied heavily on the framework of statistical time-series analysis, the variable  $t$  associated with the external perturbation was originally assumed to be the chronological time.<sup>5,6</sup> With the generalized scheme of 2D correlation depicted in Figure 2.1, the variable  $t$  can be any reasonable physical quantity, such as temperature, pressure, concentration, and so on. However, in order to avoid the unnecessary coinage of awkward terms, such as the *synthermal* or *asynbaric* spectrum, traditional terms like *synchronous* and *asynchronous* spectrum will be consistently used to refer to the real and imaginary components of the complex 2D correlation spectrum.

It is also necessary to point out that the above separation of the 2D correlation intensity into two orthogonal components may be somewhat arbitrary and simplistic. There are many different ways to represent the 2D correlation intensities, each of which contains a distinct informational content according to the specific functional form chosen to define  $\langle \rangle$ . However, we will focus our attention strictly on the simplest, but surprisingly useful, form of the 2D correlation function, known as the *generalized 2D correlation spectrum*.

### 2.2.3 GENERALIZED TWO-DIMENSIONAL CORRELATION FUNCTION

The generalized 2D correlation function given below

$$\Phi(\nu_1, \nu_2) + i\Psi(\nu_1, \nu_2) = \frac{1}{\pi(T_{\max} - T_{\min})} \int_0^\infty \tilde{Y}_1(\omega) \cdot \tilde{Y}_2^*(\omega) d\omega \quad (2.5)$$

formally defines the synchronous and asynchronous correlation intensity introduced in Equation (2.4). The term  $\tilde{Y}_1(\omega)$  is the forward Fourier transform of the spectral intensity variations  $\tilde{y}(\nu_1, t)$  observed at a given spectral variable  $\nu_1$  with respect to the external variable  $t$ . It is given by

$$\begin{aligned} \tilde{Y}_1(\omega) &= \int_{-\infty}^{\infty} \tilde{y}(\nu_1, t) e^{-i\omega t} dt \\ &= \tilde{Y}_1^{\text{Re}}(\omega) + i\tilde{Y}_1^{\text{Im}}(\omega) \end{aligned} \quad (2.6)$$

where  $\tilde{Y}_1^{\text{Re}}(\omega)$  and  $\tilde{Y}_1^{\text{Im}}(\omega)$  are, respectively, the real and imaginary component of the Fourier transform. It is useful to remember that the real component  $\tilde{Y}_1^{\text{Re}}(\omega)$  is an even function of  $\omega$ , while  $\tilde{Y}_1^{\text{Im}}(\omega)$  is an odd function. The Fourier frequency  $\omega$  represents the individual frequency component of the variation of

$\tilde{y}(v_1, t)$  traced along the external variable  $t$ . According to Equation (2.1), the above Fourier integration of the dynamic spectrum is actually bound by the finite interval between  $T_{\min}$  and  $T_{\max}$ . The conjugate of the Fourier transform  $\tilde{Y}_2^*(\omega)$  of the spectral intensity variation  $\tilde{y}(v_2, t)$  observed at another spectral variable  $v_2$  is given by

$$\begin{aligned}\tilde{Y}_2^*(\omega) &= \int_{-\infty}^{\infty} \tilde{y}(v_2, t) e^{+i\omega t} dt \\ &= \tilde{Y}_2^{\text{Re}}(\omega) - i\tilde{Y}_2^{\text{Im}}(\omega)\end{aligned}\quad (2.7)$$

Once the appropriate Fourier transformation of the dynamic spectrum  $\tilde{y}(v, t)$  defined in the form of Equation (2.1) is carried out with respect to the variable  $t$ , Equation (2.5) will directly yield the synchronous and asynchronous correlation spectrum,  $\Phi(v_1, v_2)$  and  $\Psi(v_1, v_2)$ .

## 2.2.4 HETEROSPECTRAL CORRELATION

A very intriguing possibility found in 2D correlation spectroscopy is the idea of 2D *hetero-spectral correlation analysis*, where two completely different types of spectra obtained for a system using multiple spectroscopic probes under a similar external perturbation are compared. Thus, a dynamic spectrum  $\tilde{y}(v, t)$  measured by one technique (e.g., IR absorption) may be compared to another dynamic spectrum  $\tilde{z}(\mu, t)$  measured with a completely different probe (e.g., Raman scattering). Thus, the general form of the heterospectral 2D correlation will be given by

$$X(v, \mu) = \langle \tilde{y}(v, t) \cdot \tilde{z}(\mu, t') \rangle \quad (2.8)$$

By using the form presented in Equation (2.5), the synchronous and asynchronous heterospectral correlation spectrum can be obtained from

$$\Phi(v, \mu) + i\Psi(v, \mu) = \frac{1}{\pi(T_{\max} - T_{\min})} \int_0^{\infty} \tilde{Y}(\omega) \cdot \tilde{Z}^*(\omega) d\omega \quad (2.9)$$

If there is any commonality between the response patterns of system constituents monitored by two different probes under the same perturbation, one should be able to detect the correlation even between different classes of spectral signals. Heterospectral correlation has become one of the most active areas of research in 2D correlation spectroscopy.

## 2.3 PROPERTIES OF 2D CORRELATION SPECTRA

### 2.3.1 SYNCHRONOUS 2D CORRELATION SPECTRUM

The intensity of a synchronous 2D correlation spectrum  $\Phi(v_1, v_2)$  represents the simultaneous or coincidental changes of two separate spectral intensity variations

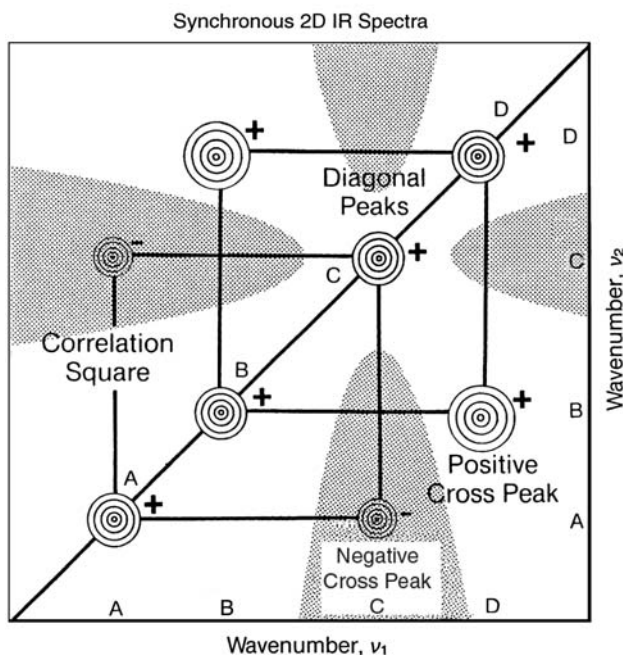


**Figure 2.2** Schematic contour map of a synchronous 2D correlation spectrum. Shaded areas indicate negative correlation intensity. (Reproduced with permission from I. Noda, *Appl. Spectrosc.*, **44**, 550 (1990). Copyright (1990) Society for Applied Spectroscopy.)

measured at  $\nu_1$  and  $\nu_2$  during the interval between  $T_{\min}$  and  $T_{\max}$  of the externally defined variable  $t$ . Figure 2.2 shows a schematic example of a synchronous 2D correlation spectrum plotted as a contour map. A synchronous spectrum is a symmetric spectrum with respect to a diagonal line corresponding to coordinates  $\nu_1 = \nu_2$ . Correlation peaks appear at both diagonal and off-diagonal positions.

The intensity of peaks located at diagonal positions mathematically corresponds to the autocorrelation function of spectral intensity variations observed during an interval between  $T_{\min}$  and  $T_{\max}$ . The diagonal peaks are therefore referred to as *autopeaks*, and the slice trace of a synchronous 2D spectrum along the diagonal is called the *autopower spectrum*. In the example spectrum shown in Figure 2.2, there are four distinct autopeaks located at the spectral coordinates: A, B, C, and D. The magnitude of an autopeak intensity, which is always positive, represents the overall extent of spectral intensity variation observed at the specific spectral variable  $\nu$  during the observation interval between  $T_{\min}$  and  $T_{\max}$ . Thus, any region of a spectrum which changes intensity to a great extent under a given perturbation will show strong autopeaks, while those remaining near constant develop little or no autopeaks. In other words, an autopeak represents the overall susceptibility of the corresponding spectral region to change in spectral intensity as an external perturbation is applied to the system.

*Cross peaks* located at the off-diagonal positions of a synchronous 2D spectrum represent simultaneous or coincidental changes of spectral intensities observed at two different spectral variables  $\nu_1$  and  $\nu_2$ . Such a synchronized change, in turn, suggests the possible existence of a coupled or related origin of the spectral intensity variations. It is often useful to construct a *correlation square* joining the pair of cross peaks located at opposite sides of a diagonal line drawn through the corresponding autopeaks to show the existence of coherent variation of spectral intensities at these spectral variables. In the example spectrum, bands A and C are synchronously correlated, as well as bands B and D. Two separate synchronous correlation squares, therefore, can be drawn.

While the sign of autopeaks is always positive, the sign of cross peaks can be either positive or negative. The sign of a synchronous cross peak becomes positive if the spectral intensities at the two spectral variables corresponding to the coordinates of the cross peak are either increasing or decreasing together as functions of the external variable  $t$  during the observation interval. On the other hand, a negative sign of the cross peak intensity indicates that one of the spectral intensities is increasing while the other is decreasing. In the example spectrum, the sign of the cross peaks at the spectral coordinates A and C is negative, indicating that the intensity of one band is increasing while that of the other is decreasing. The sign of the cross peak at the coordinates B and D, on the other hand, is positive, indicating that both bands apparently decrease (or increase) together.

### 2.3.2 ASYNCHRONOUS 2D CORRELATION SPECTRUM

Figure 2.3 shows an example of an asynchronous 2D correlation spectrum. The intensity of an asynchronous spectrum represents sequential or successive, but not coincidental, changes of spectral intensities measured separately at  $\nu_1$ , and  $\nu_2$ . Unlike a synchronous spectrum, an asynchronous spectrum is antisymmetric with respect to the diagonal line. The asynchronous spectrum has no autopeaks, and consists exclusively of cross peaks located at off-diagonal positions. By extending lines from the spectral coordinates of cross peaks to corresponding diagonal positions, one can construct asynchronous correlation squares. In Figure 2.3, asynchronous correlation is observed for band pairs A and B, A and D, B and C, as well as C and D. From the cross peaks, it is possible to draw four asynchronous correlation squares.

An asynchronous cross peak develops only if the intensities of two spectral features change out of phase with each other (i.e., delayed or accelerated if time is the external variable). This feature is especially useful in differentiating overlapped bands arising from spectral signals of different origins. For example, different spectral intensity contributions from individual components of a complex mixture, chemical functional groups experiencing different effects from some external field, or inhomogeneous materials comprising multiple phases or regions, may

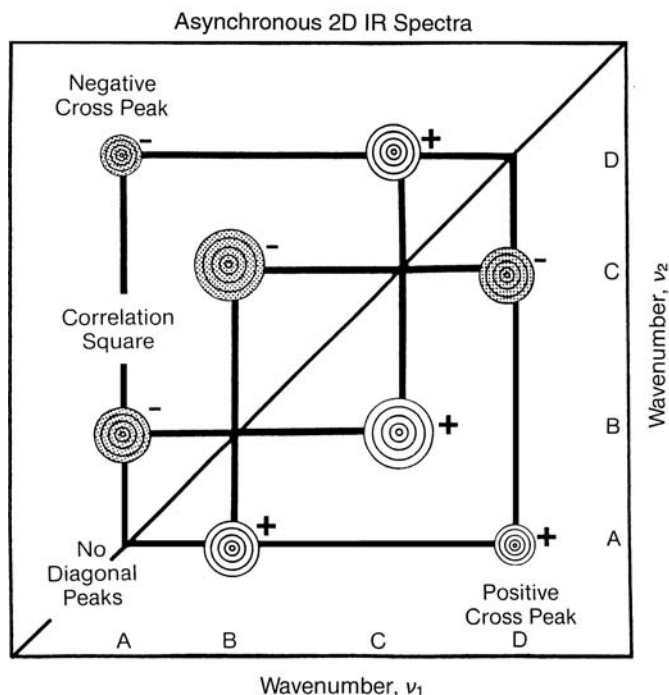


**Figure 2.3** Schematic contour map of an asynchronous 2D correlation spectrum. Shaded areas indicate negative correlation intensity. (Reproduced with permission from I. Noda, *Appl. Spectrosc.*, **44**, 550 (1990). Copyright (1990) Society for Applied Spectroscopy.)

all be effectively discriminated. Even if spectral bands are located close to each other, as long as the characteristic patterns of sequential variations of spectral intensities along the external variable are substantially different, asynchronous cross peaks will develop between their spectral coordinates.

As in the synchronous spectrum case, the sign of an asynchronous cross peak can be either negative or positive. It provides useful information on the *sequential order* of events observed by the spectroscopic technique along the external variable. The sign of an asynchronous cross peak becomes positive if the intensity change at  $\nu_1$  occurs predominantly before that at  $\nu_2$  in the sequential order of  $t$ . On the other hand, the peak sign becomes negative if the change at  $\nu_1$  occurs predominantly after  $\nu_2$ . However, this sign rule is reversed if the synchronous correlation intensity at the same coordinate becomes negative, i.e.,  $\Phi(\nu_1, \nu_2) < 0$ . The example spectrum (Figure 2.3) indicates the intensity changes (either increase or decrease) at bands A and C occur after the changes at B and D. The set of sequential order rules (sometimes referred to as Noda's rule) described above are quite reliable, as long as variation patterns of spectral intensities during the observation period are reasonably monotonic.



### 2.3.3 SPECIAL CASES AND EXCEPTIONS

There are certain exceptions to the interpretation rules of 2D correlation spectra discussed in the previous sections. They apply to the analysis of 2D spectra derived from special kinds of dynamic signals. The first case is the correlation of spectral intensity variations having very different waveforms. For example, we may observe the spectral intensity at  $\nu_1$  increases gradually, while that at  $\nu_2$  changes rapidly in an erratic manner. In this case, the set of spectral intensity changes cannot be meaningfully described in terms of the simple synchronous correlation based on coordinated increase or decrease of signals. More importantly, the sequential order of intensity changes derived from the asynchronous correlation analysis does not make much sense, especially if the changes involve many ups and downs of highly mismatched frequencies. These signals are truly *uncorrelated*, so correlation spectra will not provide much useful information.

Fortunately, one can usually avoid such comparison of virtually uncorrelated signals in normal perturbation-based 2D correlation spectroscopy experiments. The fundamental assumption behind the generalized 2D correlation scheme is that the spectral intensity changes induced by the same external perturbation should carry relatively similar classes of waveforms. Such signal pairs share close ranges of Fourier frequency components and are safely analyzed and interpreted within the context of the generalized 2D correlation scheme.

Interpretation of 2D correlation spectra generated from spectral data involving features other than simple intensity variations, such as band position shifts or line shape changes is another important case where conventional rules of 2D spectroscopy do not apply. In this case, characteristic patterns of a cluster of 2D correlation peaks, instead of the analysis of individual peaks, must be considered. We will cover this subject in full detail in Chapter 4.

## 2.4 ANALYTICAL EXPRESSIONS FOR CERTAIN 2D SPECTRA

If the waveform of a dynamic spectrum can be explicitly expressed as a simple analytical function of time or other physical variables, it is sometime possible to directly derive the expression for the corresponding 2D correlation spectrum in a closed analytical form. In this section, four such examples are provided to further illustrate the relationship described in the previous section and to demonstrate the utility of 2D correlation spectra. The waveforms of examples are chosen to represent the actual behavior of dynamic spectra often encountered in real-world experiments.

### 2.4.1 COMPARISON OF LINEAR FUNCTIONS

A dynamic spectrum which changes linearly with time, or any one of appropriate external variables, is obviously of great interest. A linear function is the simplest

form of variations of spectral intensities. One encounters numerous cases of such variations in spectral intensities. For example, if the concentration of a component in a solution mixture is systematically changed, the spectral intensities of bands associated with this component should vary linearly with respect to the concentration, provided that the Beer–Lambert law applies.

An interesting question arises with respect to the pattern of linearly changing spectral intensities. For a given increase in the concentration of a component, intensities of strongly absorbing bands will obviously increase more rapidly than those for weaker bands. Does this higher rate of intensity change mean the changes in the stronger bands occur ahead of the weaker ones, even though variations in both stronger and weaker bands result from the concentration change of the same component? The answer becomes clear as we obtain the closed form expression for the 2D correlation intensities for linearly varying signals.

A spectral signal with a linearly varying intensity with respect to the external variable  $t$  during the observation interval between  $T_{\min}$  and  $T_{\max}$  has the general form

$$y(\nu, t) = k(\nu)t + c(\nu) \quad (2.10)$$

where  $k(\nu)$  and  $c(\nu)$  are, respectively, the wavenumber-dependent rate of change for the spectral intensity and an arbitrary constant term. One of the most familiar examples of such linear variations is the dependence of an optical absorbance of a solution mixture on concentration  $t$  with finite baseline contributions. From Equation (2.2) the reference spectrum obtained by taking the average over the observation interval between  $T_{\min}$  and  $T_{\max}$  is given by

$$\bar{y}(\nu) = k(\nu)(T_{\max} + T_{\min})/2 + c(\nu) \quad (2.11)$$

The dynamic spectrum according to Equation (2.1) now becomes

$$\tilde{y}(\nu, t) = \begin{cases} k(\nu)[t - (T_{\max} + T_{\min})/2] & \text{for } T_{\min} \leq t \leq T_{\max} \\ 0 & \text{otherwise} \end{cases} \quad (2.12)$$

Note that the constant term  $c(\nu)$  is eliminated from the dynamic spectrum. By determining the analytical expression for the Fourier transform of the dynamic spectrum in Equation (2.12) using Equations (2.6) and (2.7) and substituting the result into Equation (2.5), one can obtain the closed form analytical expressions for the 2D correlation intensities of linearly varying dynamic spectra.

$$\Phi(\nu_1, \nu_2) = \frac{1}{12}k(\nu_1) \cdot k(\nu_2)(T_{\max} - T_{\min})^2 \quad (2.13)$$

$$\Psi(\nu_1, \nu_2) = 0 \quad (2.14)$$

It is important to note that, in generalized 2D correlation analysis, any pair of linearly varying signals are considered to be fully synchronized, i.e., having

the identical pattern of change. This is true even if the apparent rate of change for individual spectral signals measured at different wavenumbers may be vastly different. The asynchronicity detected by the 2D correlation is governed not by the difference in the instantaneous rate of spectral intensity variations but rather by the sequential order of integrated pattern of events occurring during the entire observation interval. Signals changing intensities predominantly in the early stage of the observation period are considered to be ahead of signals changing intensities in the later stage. Asynchronous 2D correlation analysis detects this difference in the distribution of intensity changes during the observation. As linear signals have the uniform distribution of intensity change throughout the observation interval, any two linear signals are considered to possess the same variation pattern and, therefore, to be fully synchronized.

It is also clear from the above result that 2D correlation analysis of a data set consisting exclusively of linearly varying dynamic spectra will be relatively uninteresting, as little useful information will be found beyond what is obtainable directly from 1D spectra. No selective spreading of overlapped peaks or enhanced spectral resolution will be realized. 2D correlation analysis becomes a truly meaningful tool only if the dynamic spectrum behaves nonlinearly with respect to the external variable. In other words, 2D correlation may be regarded as a technique to effectively contrast the different nonlinear behaviors of spectral intensity variations.

#### 2.4.2 2D SPECTRA BASED ON SINUSOIDAL SIGNALS

The construction of 2D correlation spectra based on sinusoidally varying signals, especially IR dichroism signals, has been reported extensively.<sup>5,6</sup> It is demonstrated here that a dynamic spectrum having such a waveform can be readily analyzed in terms of the generalized 2D correlation formalism. More importantly, the analysis of a simple sinusoid provides a useful insight into the understanding of a more general form of 2D correlation intensity defined in Equation (2.5). As the Fourier transform of a dynamic spectrum, which is the fundamental building block of the 2D correlation functions, may be viewed as an alternative expression for a complex waveform in terms of a series of sinusoidal functions, the relationship between a simple sinusoid and 2D correlation spectra should be equally applicable to the individual Fourier components of more complex signals.

A sinusoidally varying dynamic spectrum with a fixed frequency  $\Omega$  has the general form

$$\tilde{y}(v, t) = A'(v) \sin \Omega t + A''(v) \cos \Omega t \quad (2.15)$$

where  $A'(v)$  and  $A''(v)$  are the amplitudes of two orthogonal components of the sinusoidal function. Alternatively, Equation (2.15) can be expressed as

$$\tilde{y}(v, t) = \hat{A}(v) \sin[\Omega t + \beta(v)] \quad (2.16)$$

where  $\hat{A}(\nu)$  and  $\beta(\nu)$  are the wavenumber-dependent magnitude and phase angle of the sinusoidal signal given by

$$\hat{A}(\nu) = \sqrt{A'(\nu)^2 + A''(\nu)^2} \quad (2.17)$$

$$\beta(\nu) = \arctan \frac{A''(\nu)}{A'(\nu)} \quad (2.18)$$

The observation period for the sinusoidal signals is assumed to be much greater than the periodicity of the signal, i.e.,  $T_{\max} - T_{\min} \gg 2\pi/\Omega$ , such that the contribution of the signal to the constant reference spectrum is negligible. Figure 2.4 shows an example of a dynamic spectrum containing four different bands, all with their intensities varying sinusoidally with time under the same periodicity but having distinct phase angles.

Sinusoidal functions are readily Fourier transformed to yield explicit analytical expressions for 2D correlation intensities. By Fourier transforming the expression in Equation (2.15) and substituting it in Equation (2.5), the closed-form analytical expressions for the intensities of corresponding 2D correlation spectra are obtained as

$$\Phi(\nu_1, \nu_2) = \frac{1}{2}[A'(\nu_1)A'(\nu_2) + A''(\nu_1)A''(\nu_2)] \quad (2.19)$$

$$\Psi(\nu_1, \nu_2) = \frac{1}{2}[A''(\nu_1)A'(\nu_2) - A'(\nu_1)A''(\nu_2)] \quad (2.20)$$

In terms of the magnitude and phase angle of the sinusoidal signals, the 2D correlation intensities for sinusoids can also be expressed as

$$\Phi(\nu_1, \nu_2) = \frac{1}{2}\hat{A}(\nu_1)\hat{A}(\nu_2)\cos[\beta(\nu_1) - \beta(\nu_2)] \quad (2.21)$$

$$\Psi(\nu_1, \nu_2) = \frac{1}{2}\hat{A}(\nu_1)\hat{A}(\nu_2)\sin[\beta(\nu_1) - \beta(\nu_2)] \quad (2.22)$$

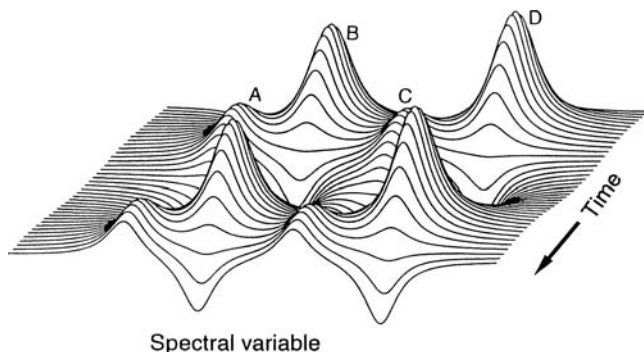


**Figure 2.4** Dynamic spectrum containing four different bands, all with their intensities varying sinusoidally with time under the same periodicity but having distinct phase angles. (Reproduced with permission from I. Noda, *Appl. Spectrosc.*, **47**, 1329 (1993). Copyright (1993) Society for Applied Spectroscopy.)

Equations (2.21) and (2.22) provide the interpretation of the 2D correlation intensities obtained from sinusoidally varying signals. The synchronous and asynchronous correlation intensities represent, respectively, the similarity and dissimilarity of the signal phase angles measured at  $\nu_1$  and  $\nu_2$ . If the phase angles are similar, the synchronous correlation intensity becomes significant. If there is enough discrepancy between phase angles, on the other hand, the asynchronous correlation intensity dominates. In other words, the asynchronicity between sinusoidal signals with a fixed frequency is uniquely determined only by the phase angle difference.

### 2.4.3 EXPONENTIALLY DECAYING INTENSITIES

An exponentially decaying dynamic spectrum is expressed in the general form

$$\tilde{y}(\nu, t) = A(\nu) e^{-k(\nu)t} \quad (2.23)$$

where  $A(\nu)$  is the initial value of the intensity, and  $k(\nu)$  is the characteristic rate constant of the decay process. Figure 2.5 shows an example of a decaying transient spectrum containing four bands, all decreasing their intensities exponentially but with different decay constants. For simplicity, we assume that  $T_{\min} = 0$  and  $T_{\max} \gg 1/k(\nu)$  so that the reference spectrum can be set to zero. The Fourier transform of an exponentially decaying function is obtained in a straightforward manner, as long as the observation period is reasonably long. Thus, an explicit analytical expression for each 2D correlation spectrum can be obtained

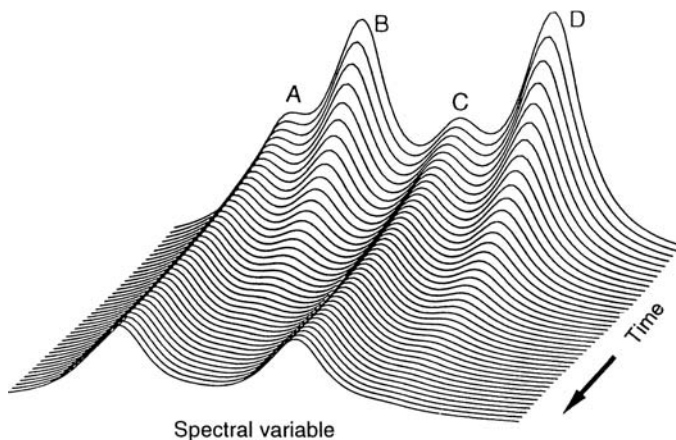


**Figure 2.5** Decaying transient spectrum containing four bands, all decreasing their intensities exponentially but with different decay constants. (Reproduced with permission from I. Noda, *Appl. Spectrosc.*, **47**, 1329 (1993). Copyright (1993) Society for Applied Spectroscopy.)

from the dynamic spectrum. Using Equation (2.5) with the Fourier transform of Equation (2.23), the 2D correlation spectra for exponentially decaying signals become

$$\Phi(v_1, v_2) = \frac{A(v_1)A(v_2)}{T_{\max} - T_{\min}} \frac{1}{k(v_1) + k(v_2)} \quad (2.24)$$

$$\Psi(v_1, v_2) = \frac{A(v_1)A(v_2)}{\pi T_{\max} - T_{\min}} \frac{\ln k(v_1)/k(v_2)}{k(v_1) + k(v_2)} \quad (2.25)$$

For a pair of signals having relatively similar decay constants, i.e.,  $k(v_1) \approx k(v_2)$ , the above result in Equation (2.25) further simplifies to

$$\Psi(v_1, v_2) = \frac{2A(v_1)A(v_2)}{\pi T_{\max} - T_{\min}} \frac{k(v_1) - k(v_2)}{k(v_1) + k(v_2)} \quad (2.26)$$

It is interesting to note that the asynchronicity between two exponentially decaying functions is uniquely determined by the rate constant difference,  $k(v_1) - k(v_2)$ . If two signals have the same decay constant, the asynchronous correlation intensity becomes zero regardless of the initial values of the signals.

#### 2.4.4 DISTRIBUTED LORENTZIAN PEAKS

The last example of a dynamic spectrum analyzed by the 2D correlation method represents responses distributed in the time domain as Lorentzian peak functions. Peak functions, reflecting delayed and distributed responses to a single excitation, are often encountered in spectroscopic measurements coupled with chromatographic separation schemes. Lorentzian and Gaussian peaks are commonly observed peak functions. Fourier transforms of such functions are well known and serve as excellent test functions for studying the results of 2D correlation analyses.

A dynamic spectrum having Lorentzian response function in the time domain is expressed in the general form

$$y(v, t) = \frac{A(v)w^2(v)}{w^2(v) + [\theta(v) - t]^2} \quad (2.27)$$

where  $A(v)$  is the maximum peak height of the spectral intensity, and  $\theta(v)$  is the characteristic delay time (e.g., chromatographic retention time) of the system. The time-domain spread of a Lorentzian peak is determined by the half width  $w(v)$ . Figure 2.6 shows an example of a distributed transient spectrum containing four bands. Each band intensity increases at some distinct time then decreases after a while. With several simplifying assumptions, namely a

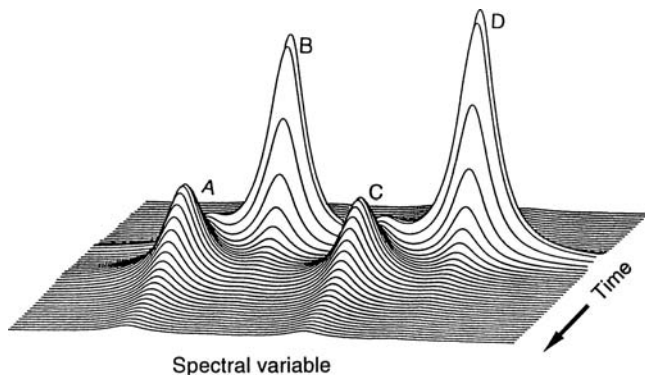


**Figure 2.6** Distributed transient spectrum containing four bands. Each band intensity increases at some distinct time then decreases after a while. (Reproduced with permission from I. Noda, *Appl. Spectrosc.*, **47**, 1329 (1993). Copyright (1993) Society for Applied Spectroscopy.)

reasonably long observation period and similar half width  $w(\nu)$ , the closed-form analytical expressions for corresponding 2D correlation spectra become

$$\Phi(\nu_1, \nu_2) = \frac{\pi A(\nu_1)A(\nu_2)}{T_{\max} - T_{\min}} \frac{w(\nu_2) + w(\nu_1)}{[w(\nu_2) + w(\nu_1)]^2 + [\theta(\nu_2) - \theta(\nu_1)]^2} \quad (2.28)$$

$$\Psi(\nu_1, \nu_2) = \frac{\pi A(\nu_1)A(\nu_2)}{T_{\max} - T_{\min}} \frac{\theta(\nu_2) - \theta(\nu_1)}{[w(\nu_2) + w(\nu_1)]^2 + [\theta(\nu_2) - \theta(\nu_1)]^2} \quad (2.29)$$

In asynchronous 2D correlation, the discrimination of events occurring at different times results from the term  $\theta(\nu_2) - \theta(\nu_1)$  in Equation (2.29), which becomes nonzero only if the time-domain peak positions are different.

#### 2.4.5 SIGNALS WITH MORE COMPLEX WAVEFORMS

Most of the everyday experimental data obtained for dynamic spectra are actually very complex and difficult to be described by a simple analytical expression such as those discussed in the previous sections. In order to obtain 2D correlation spectra from experimentally obtained spectral data, one usually resorts to the use of a numerical computation method to manipulate a series of discrete spectral data points. The computation of the generalized 2D correlation spectra formally defined in Equation (2.5) may be carried out in a relatively straightforward manner even for a discrete set of spectral data using a fast Fourier transform algorithm. However, since the evaluation of 2D correlation intensity must be made at every point of a 2D spectral plane, the total number of computational steps even using a fast Fourier transform algorithm may become rather large. Fortunately, there are several computational shortcuts one can take to obtain an

adequate numerical estimation of 2D correlation intensity. Some of the practical computational methods are described in the following chapter.

## 2.5 CROSS-CORRELATION ANALYSIS AND 2D SPECTROSCOPY

There is substantial overlap between the basic concept of 2D correlation spectroscopy and that of the classical statistical theory of cross-correlation analysis, especially those related to the time-series analysis.<sup>8,9</sup> In this section, some mathematical tools of correlation analysis useful to 2D correlation spectroscopy are examined. The external variable  $t$  will be referred to as *time* throughout this section, even though it is understood that this variable could be any other reasonable physical quantities, such as temperature, concentration, etc.

### 2.5.1 CROSS-CORRELATION FUNCTION AND CROSS SPECTRUM

The classical *cross-correlation function* between distinct dynamic spectral intensity variations observed at  $\nu_1$  and  $\nu_2$  along  $t$  for a fixed period between  $T_{\min}$  and  $T_{\max}$  is given by

$$C(\tau) = \frac{1}{T_{\max} - T_{\min}} \int_{T_{\min}}^{T_{\max}} \tilde{y}(\nu_1, t) \cdot \tilde{y}(\nu_2, t + \tau) dt \quad (2.30)$$

where  $\tau$  is the *correlation time*. The function compares the time dependence of two separate functions (i.e., intensities of dynamic spectra measured at two different wavenumbers) shifted by a fixed constant  $\tau$ . The corresponding cross spectrum  $S(\omega)$ , which is the Fourier transform of the cross-correlation function, is given by

$$\begin{aligned} S(\omega) &= \frac{\tilde{Y}_1^*(\omega) \cdot \tilde{Y}_2(\omega)}{T_{\max} - T_{\min}} \\ &= \phi_{\omega}(\nu_1, \nu_2) - i\psi_{\omega}(\nu_1, \nu_2) \end{aligned} \quad (2.31)$$

The real and negative imaginary component of the cross spectrum,  $\phi_{\omega}(\nu_1, \nu_2)$  and  $\psi_{\omega}(\nu_1, \nu_2)$ , are referred to, respectively, as the *cospectrum* and *quad-spectrum*, which can be expressed in terms of the real and imaginary components of the Fourier transform of the dynamic spectrum intensities as

$$\phi_{\omega}(\nu_1, \nu_2) = \frac{1}{T_{\max} - T_{\min}} [\tilde{Y}_1^{\text{Re}}(\omega) \cdot \tilde{Y}_2^{\text{Re}}(\omega) + \tilde{Y}_1^{\text{Im}}(\omega) \cdot \tilde{Y}_2^{\text{Im}}(\omega)] \quad (2.32)$$

$$\psi_{\omega}(\nu_1, \nu_2) = \frac{1}{T_{\max} - T_{\min}} [\tilde{Y}_1^{\text{Im}}(\omega) \cdot \tilde{Y}_2^{\text{Re}}(\omega) - \tilde{Y}_1^{\text{Re}}(\omega) \cdot \tilde{Y}_2^{\text{Im}}(\omega)] \quad (2.33)$$



It is necessary to point out that the term *spectrum* (e.g., cross spectrum, cospectrum, and quad-spectrum) used here specifically refers to the Fourier domain spectral representation of time-dependent signals. This mathematical spectrum should not be confused with the optical intensity spectrum measured with an electromagnetic probe. From Equation (2.5) the cospectrum and quad-spectrum are related to the synchronous and asynchronous 2D correlation spectra by

$$\Phi(\nu_1, \nu_2) = \frac{1}{\pi} \int_0^\infty \phi_\omega(\nu_1, \nu_2) d\omega \quad (2.34)$$

$$\Psi(\nu_1, \nu_2) = \frac{1}{\pi} \int_0^\infty \psi_\omega(\nu_1, \nu_2) d\omega \quad (2.35)$$

In other words, the cospectrum and quad-spectrum represent the individual Fourier component of the synchronous and asynchronous 2D correlation spectrum. In turn, the synchronous spectrum and asynchronous spectrum may be regarded as the integral sums of the contributions of cospectrum and quad-spectrum over the entire positive Fourier frequency of the external variable.

### 2.5.2 CROSS-CORRELATION FUNCTION AND SYNCHRONOUS SPECTRUM

We now derive an important relationship between the cross-correlation function and synchronous 2D correlation intensity. By applying the well-known Wiener–Khinchine theorem,<sup>10</sup> the cross spectrum can be directly related to the time-domain Fourier transform of dynamic spectral intensity variations (see Appendix).

$$C(\tau) = \frac{1}{2\pi(T_{\max} - T_{\min})} \int_{-\infty}^{\infty} \tilde{Y}_1^*(\omega) \cdot \tilde{Y}_2(\omega) e^{i\omega\tau} d\omega \quad (2.36)$$

By setting the correlation time to  $\tau = 0$ , Equation (2.36) reduces to

$$C(0) = \frac{1}{2\pi(T_{\max} - T_{\min})} \int_{-\infty}^{\infty} \tilde{Y}_1^*(\omega) \cdot \tilde{Y}_2(\omega) d\omega \quad (2.37)$$

Since the imaginary component of a cross spectrum must consist exclusively of an odd function, the integration over the symmetric range of  $\omega$  from  $-\infty$  to  $+\infty$  leaves only the real component

$$C(0) = \frac{1}{\pi(T_{\max} - T_{\min})} \text{Re} \left\{ \int_0^\infty \tilde{Y}_1(\omega) \cdot \tilde{Y}_2^*(\omega) d\omega \right\} \quad (2.38)$$

The notation  $\text{Re}\{\}$  stands for the real part of a complex number. According to Equation (2.5), the above expression is identical to the real part of the generalized 2D correlation function. Thus, the synchronous 2D correlation intensity

$\Phi(\nu_1, \nu_2)$  can be directly calculated from the cross-correlation function  $C(\tau)$  with  $\tau = 0$ . Most importantly, since the determination of the cross-correlation function according to Equation (2.30) does not require the use of the Fourier transformation of dynamic spectra, the synchronous 2D correlation spectrum can be directly computed as

$$\Phi(\nu_1, \nu_2) = \frac{1}{T_{\max} - T_{\min}} \int_{T_{\min}}^{T_{\max}} \tilde{y}(\nu_1, t) \cdot \tilde{y}(\nu_2, t) dt \quad (2.39)$$

This result shows that the synchronous 2D correlation intensity is nothing but the time average of the product of dynamic spectral intensity variations measured at two different spectral variables,  $\nu_1$  and  $\nu_2$ .

The manipulation of a classical cross-correlation function of dynamic spectral intensity variations yields only a synchronous 2D correlation spectrum. An asynchronous 2D correlation spectrum must be calculated by other means if one wishes to circumvent the use of the Fourier transformation of dynamic spectra. Although a heuristic approximation using a disrelation spectrum discussed later, for example, provides a reasonable estimate of an asynchronous 2D correlation spectrum, a more reliable computational method based on a rigorous mathematical derivation is desired. Such a method may be developed by utilizing the time-domain Hilbert transformation of dynamic spectra.<sup>2</sup>

### 2.5.3 HILBERT TRANSFORM

For a given analytic function  $g(t)$ , the *Hilbert transform*  $h(t)$  of the function is given by

$$h(t) \equiv \frac{1}{\pi} \text{pv} \int_{-\infty}^{\infty} \frac{g(t')}{t' - t} dt' \quad (2.40)$$

The integration symbol  $\text{pv} \int$  denotes that the *Cauchy principal value* is taken, such that the singularity at the point where  $t' = t$  is excluded from the integration. It is well known that the Hilbert transform operation is closely associated with the Kramers–Kronig analysis of various spectra which are coupled by the orthogonal dispersion relationship.

It can be easily pointed out by observing Equation (2.40) that the Hilbert transform  $h(t)$  may be regarded as the convolution integral between the two functions,  $g(t)$  and  $1/t$ . From the convolution theorem,<sup>10</sup> the Fourier transform of the Hilbert transform  $h(t)$  becomes proportional to the product of the Fourier transforms of  $g(t)$  and  $1/t$ .

$$\begin{aligned} H(\omega) &= \int_{-\infty}^{\infty} h(t) e^{-i\omega t} dt \\ &= \frac{1}{\pi} \int_{-\infty}^{\infty} \frac{1}{t} e^{-i\omega t} dt \cdot \int_{-\infty}^{\infty} g(t) e^{-i\omega t} dt \end{aligned} \quad (2.41)$$

Thus, the Fourier transforms of functions  $g(t)$  and  $h(t)$  are related by

$$\begin{aligned} H(\omega) &= i \operatorname{sgn}(\omega) \cdot G(\omega) \\ &= \begin{cases} -G^{\operatorname{Im}}(\omega) + i G^{\operatorname{Re}}(\omega) & \omega > 0 \\ 0 & \text{if } \omega = 0 \\ G^{\operatorname{Im}}(\omega) - i G^{\operatorname{Re}}(\omega) & \omega < 0 \end{cases} \end{aligned} \quad (2.42)$$

where  $G^{\operatorname{Re}}(\omega)$  and  $G^{\operatorname{Im}}(\omega)$  are the real and imaginary component of the Fourier transform of the function  $g(t)$ . The value of *signum function*  $\operatorname{sgn}(\omega)$  becomes  $-1$ ,  $0$ , or  $+1$ , respectively, if the variable  $\omega$  is smaller than, equal to, or greater than  $0$ .

The above result provides a simplified view of the Hilbert transform relationship. The Hilbert transformation of a function *shifts* the phase of each Fourier component of a function *forward* by  $\pi/2$  if  $\omega > 0$ , and backward by  $\pi/2$  if  $\omega < 0$ . Thus, the functions  $g(t)$  and  $h(t)$  are *orthogonal* to each other and have the relationship

$$\int_{-\infty}^{\infty} g(t) \cdot h(t) dt = 0 \quad (2.43)$$

#### 2.5.4 ORTHOGONAL CORRELATION FUNCTION AND ASYNCHRONOUS SPECTRUM

Let the orthogonal spectrum  $\tilde{z}(v_2, t)$  be the time-domain Hilbert transform of the dynamic spectrum  $\tilde{y}(v_2, t)$ .

$$\tilde{z}(v_2, t) = \frac{1}{\pi} \operatorname{pv} \int_{-\infty}^{\infty} \frac{\tilde{y}(v_2, t')}{t' - t} dt' \quad (2.44)$$

Thus, the orthogonal spectrum is a function where the *phase* of each Fourier component of the temporal variation of the dynamic spectrum is shifted by  $\pi/2$ . The two-dimensional *orthogonal correlation function* between different spectral intensity variations observed at  $v_1$  and  $v_2$  for a period between  $T_{\min}$  and  $T_{\max}$  is given by

$$D(\tau) = \frac{1}{T_{\max} - T_{\min}} \int_{T_{\min}}^{T_{\max}} \tilde{y}(v_1, t) \cdot \tilde{z}(v_2, t + \tau) dt \quad (2.45)$$

where  $\tau$  is the correlation time. The orthogonal correlation function defined above is nothing but a cross-correlation function between the dynamic spectrum and orthogonal spectrum. By applying the Wiener–Khinchine theorem (see Appendix), the orthogonal correlation function can be directly related to the Fourier transforms of the dynamic spectrum and orthogonal spectrum as

$$D(\tau) = \frac{1}{2\pi(T_{\max} - T_{\min})} \int_{-\infty}^{\infty} \tilde{Y}_1^*(\omega) \cdot \tilde{Z}_2(\omega) e^{i\omega\tau} d\omega \quad (2.46)$$

where  $\tilde{Z}_2(\omega)$  is the Fourier transform of  $\tilde{z}(v_2, t)$ . By setting  $\tau = 0$ , and by using the relationship  $\tilde{Z}_2(\omega) = i \operatorname{sgn}(\omega) \cdot \tilde{Y}_2(\omega)$  based on Equation (2.42) and the definition of the cross spectrum (Equation 2.31), the above equation reduces to

$$\begin{aligned} D(0) &= \frac{i}{2\pi(T_{\max} - T_{\min})} \int_{-\infty}^{\infty} \operatorname{sgn}(\omega) \tilde{Y}_1^*(\omega) \cdot \tilde{Y}_2(\omega) d\omega \\ &= \frac{1}{2\pi} \int_{-\infty}^{\infty} i \operatorname{sgn}(\omega) S(\omega) d\omega \\ &= \frac{1}{\pi} \int_0^{\infty} \psi_{\omega} d\omega = \Psi(v_1, v_2) \end{aligned} \quad (2.47)$$

Thus, the asynchronous 2D correlation spectrum can also be computed directly from the dynamic spectrum and the orthogonal spectrum.

$$\Psi(v_1, v_2) = \frac{1}{T_{\max} - T_{\min}} \int_{T_{\min}}^{T_{\max}} \tilde{y}(v_1, t) \cdot \tilde{z}(v_2, t) dt \quad (2.48)$$

This result shows that the asynchronous correlation intensity is equivalent to the time average of the product of dynamic and orthogonal spectrum measured at two different spectral variables,  $v_1$  and  $v_2$ . Note that the evaluation of Equation (2.48), like that of Equation (2.39), does not require the Fourier transformation of dynamic spectral data.

### 2.5.5 DISRELATION SPECTRUM

Another convenient way to estimate the asynchronous spectrum by circumventing the need to transform spectral data into the Fourier domain is to compute a special type of a heuristic 2D spectrum known as the *disrelation* spectrum.<sup>1</sup> The disrelation spectrum  $\Lambda(v_1, v_2)$  is given by

$$\Phi^2(v_1, v_2) + \Lambda^2(v_1, v_2) = \Phi(v_1, v_1) \cdot \Phi(v_2, v_2) \quad (2.49)$$

The total joint variance of the spectral intensity changes during the observation period, which is represented as the product of the two autopower spectrum intensities at  $v_1$  and  $v_2$  on the right-hand side of the above equation, is separated into two parts: the correlated portion and the *disrelated* portion. It can be easily observed that the absolute value of the disrelation spectrum intensity may be directly calculated from a set of only synchronous correlation intensities. By rearranging Equation (2.49) one obtains

$$\Lambda(v_1, v_2) = \operatorname{sgn}(\kappa) \sqrt{\Phi(v_1, v_1) \cdot \Phi(v_2, v_2) - \Phi^2(v_1, v_2)} \quad (2.50)$$

where  $\kappa$  is some constant to determine the sign of this spectrum, which is tentatively given by the slope of the cross correlation function (Equation 2.30) evaluated at  $\tau = 0$ .

$$\kappa = dC(\tau)/d\tau|_{\tau=0} \quad (2.51)$$

The 2D disrelation spectrum can often be substituted effectively for the asynchronous spectrum, as long as the time dependence of the dynamic spectrum is not very complex. Even though the mathematical form of the disrelation spectrum in Equation (2.50) does not look at all like the form given by Equation (2.48),  $\Lambda(\nu_1, \nu_2)$  can often serve as an excellent substitution for  $\Psi(\nu_1, \nu_2)$  to highlight the basic asynchronous features of different spectral intensity variations. The similarity between the two types of 2D correlation spectra becomes especially noticeable when the dynamic spectrum  $\tilde{y}(\nu, t)$  is changing in a relatively monotonic fashion with respect to  $t$ . It was found in many instances that the only difference between  $\Psi(\nu_1, \nu_2)$  and  $\Lambda(\nu_1, \nu_2)$  is a simple proportionality constant independent of the spectral coordinate. In such cases, the contour map representation of  $\Lambda(\nu_1, \nu_2)$  becomes virtually indistinguishable from that of  $\Psi(\nu_1, \nu_2)$ .

The mathematical justification for using the derivative of the cross-correlation function  $\kappa$  to estimate the sign of the disrelation spectrum is based on the fact that the first derivative of a monotonic function often serves as a reasonable approximation to its orthogonal function, i.e., the Hilbert transform. Equation (2.47) relates the orthogonal correlation function  $D(\tau)$  at  $\tau = 0$  to the asynchronous spectrum  $\Psi(\nu_1, \nu_2)$ . The orthogonal correlation function is the Hilbert transform of the cross-correlation function  $C(\tau)$ . The Fourier transform of a cross-correlation function, i.e., the cross spectrum  $S(\omega)$ , is related to the Fourier transform of the orthogonal correlation function by

$$\int_{-\infty}^{\infty} D(\tau) e^{-i\omega\tau} d\tau = i \operatorname{sgn}(\omega) \cdot S(\omega) \quad (2.52)$$

The Fourier transform of the derivative of the cross-correlation function, on the other hand, is given by

$$\int_{-\infty}^{\infty} \frac{dC(\tau)}{d\tau} e^{-i\omega\tau} d\tau = i\omega \cdot S(\omega) \quad (2.53)$$

Thus, the replacement of the orthogonal correlation function by the first derivative of the cross-correlation function in the correlation-time domain is equivalent to the replacement of a *signum* function  $\operatorname{sgn}(\omega)$  by a linear function  $\omega$  in the Fourier domain. It is straightforward to obtain the expression for the derivative  $\kappa$  of the cross-correlation function at  $\tau = 0$  as

$$\kappa = \frac{1}{\pi} \int_0^{\infty} \omega \cdot \psi_{\omega}(\nu_1, \nu_2) d\omega \quad (2.54)$$

The apparent similarity between the above expression for  $\kappa$  and that for the asynchronous spectrum (Equation 2.35) is obvious, except for the factor  $\omega$  which tends to weigh the higher frequency component of quad-spectrum more heavily. One may even consider the possibility of using this quantity  $\kappa$  as a substitute for the asynchronous correlation intensity  $\Psi(v_1, v_2)$ . Although such a substitution seems to over represent the contributions of dynamic signal components with higher frequency changes, this effect is often small for most simple waveforms. The absolute value of  $\Lambda(v_1, v_2)$  is probably a better match for the magnitude of  $\Psi(v_1, v_2)$  compared to  $\kappa$ . The sign of  $\kappa$ , on the other hand, follows that of  $\Psi(v_1, v_2)$  well. Thus, Equation (2.50) is a reasonable compromise between strong points of the two heuristic estimates to  $\Psi(v_1, v_2)$ .

## APPENDIX 2.1

The Wiener–Khinchine theorem establishes a convenient relationship between a cross-correlation of two functions and their Fourier transforms.<sup>10</sup> The theorem utilizes the fact that the term  $\tilde{y}(v_2, t + \tau)$  appearing in Equation (2.30) can be expressed as an inverse Fourier transform of  $\tilde{Y}_2(\omega)$  as shown in the bracket below.

$$C(\tau) = \frac{1}{T_{\max} - T_{\min}} \int_{T_{\min}}^{T_{\max}} \tilde{y}(v_1, t) \left[ \frac{1}{2\pi} \int_{-\infty}^{\infty} \tilde{Y}_2(\omega) e^{i\omega(t+\tau)} d\omega \right] dt \quad (2.A1)$$

Taking the constant term outside the integration, the equation can be simplified to

$$C(\tau) = \frac{1}{2\pi(T_{\max} - T_{\min})} \int_{T_{\min}}^{T_{\max}} \tilde{y}(v_1, t) \int_{-\infty}^{\infty} \tilde{Y}_2(\omega) e^{i\omega t} e^{i\omega\tau} d\omega dt \quad (2.A2)$$

By rearranging the order of integration, one obtains

$$C(\tau) = \frac{1}{2\pi(T_{\max} - T_{\min})} \int_{-\infty}^{\infty} \left[ \int_{T_{\min}}^{T_{\max}} \tilde{y}(v_1, t) e^{i\omega t} dt \right] \tilde{Y}_2(\omega) e^{i\omega\tau} d\omega \quad (2.A3)$$

The integration boundary ( $T_{\min}, T_{\max}$ ) of the term enclosed in the bracket above may be expanded to  $(-\infty, +\infty)$ , as long as the condition explicitly set by Equation (2.1) holds. The integration then becomes equivalent to the conjugate of the Fourier transform.

$$C(\tau) = \frac{1}{2\pi(T_{\max} - T_{\min})} \int_{-\infty}^{\infty} \tilde{Y}_1^*(\omega) \cdot \tilde{Y}_2(\omega) e^{i\omega\tau} d\omega \quad (2.A4)$$

Thus, the Fourier transform of a cross-correlation function can be directly related to the product of Fourier transforms of dynamic spectra, which is the cross

spectrum given by Equation (2.31).

$$\begin{aligned} \int_{-\infty}^{\infty} C(\tau) e^{-i\omega\tau} d\tau &= \frac{\tilde{Y}_1^*(\omega) \cdot \tilde{Y}_2(\omega)}{T_{\max} - T_{\min}} \\ &= S(\omega) \end{aligned} \quad (2.A5)$$

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## 3 Practical Computation of Two-dimensional Correlation Spectra

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### 3.1 COMPUTATION OF 2D SPECTRA FROM DISCRETE DATA

Spectral data collected in a typical measurement are usually obtained in the form of a digitized discrete data set instead of continuous functions. Efficient manipulation of such discrete data has become an important aspect of 2D correlation analysis. In this chapter, practical numerical computation methods for 2D spectra based on discrete data are examined.<sup>1-3</sup>

#### 3.1.1 SYNCHRONOUS SPECTRUM

A discrete set of dynamic spectra measured at  $m$  equally spaced points in time  $t$  between  $T_{\min}$  and  $T_{\max}$  is represented by

$$\tilde{y}_j(\nu) = \tilde{y}(\nu, t_j) \quad j = 1, 2, \dots, m \quad (3.1)$$

where the  $j$ th point in  $t$  is given by  $t_j = T_{\min} + (T_{\max} - T_{\min})(j - 1)/(m - 1)$ . We assume that the reference spectrum  $\bar{y}(\nu)$ , typically the average spectrum given by  $\bar{y}(\nu) = \sum_{j=1}^m y(\nu, t_j)/m$ , has already been subtracted from raw data as  $\tilde{y}(\nu, t_j) = y(\nu, t_j) - \bar{y}(\nu)$ . By transforming the integral in Equation (2.39) to a discrete summation form, the synchronous 2D correlation intensity may be directly calculated from the dynamic spectra by

$$\Phi(\nu_1, \nu_2) = \frac{1}{m-1} \sum_{j=1}^m \tilde{y}_j(\nu_1) \cdot \tilde{y}_j(\nu_2) \quad (3.2)$$

For a set of data collected at varying intervals not equally spaced in time, it is necessary to modify Equation (3.2). The computation of 2D spectra for unevenly sampled data set will be discussed later.



It is often convenient to represent a discrete set of data in terms of a matrix notation. The column vector depicting the dynamic spectra of Equation (3.1) is given by

$$\tilde{\mathbf{y}}(v) = \begin{bmatrix} \tilde{y}(v, t_1) \\ \tilde{y}(v, t_2) \\ \vdots \\ \tilde{y}(v, t_m) \end{bmatrix} \quad (3.3)$$

By using this notation, the synchronous 2D correlation spectrum is concisely represented by the inner product of two dynamic spectrum vectors:

$$\Phi(v_1, v_2) = \frac{1}{m-1} \tilde{\mathbf{y}}(v_1)^\top \tilde{\mathbf{y}}(v_2) \quad (3.4)$$

It is worth pointing out that the discrete form of a synchronous 2D correlation spectrum (Equations 3.2 and 3.4), derived from data observed at  $v_1$  and  $v_2$  over a period between  $T_{\min}$  and  $T_{\max}$  with an equal increment, results in the expression similar to that of statistical covariance of the spectral intensity with  $m-1$  degree of freedom.

### 3.1.2 ASYNCHRONOUS SPECTRUM

By adopting Equation (2.48) for a discrete set of dynamic spectra, one obtains the computational formula for an asynchronous spectrum.

$$\Psi(v_1, v_2) = \frac{1}{m-1} \sum_{j=1}^m \tilde{y}_j(v_1) \cdot \tilde{z}_j(v_2) \quad (3.5)$$

The discrete orthogonal spectra  $\tilde{z}_j(v_2)$  can be directly obtained from the dynamic spectra  $\tilde{y}_j(v_2)$  by using a simple linear transformation operation

$$\tilde{z}_j(v_2) = \sum_{k=1}^m N_{jk} \cdot \tilde{y}_k(v_2) \quad (3.6)$$

where

$$N_{jk} = \begin{cases} 0 & \text{if } j = k \\ 1/\pi(k-j) & \text{otherwise} \end{cases} \quad (3.7)$$

By using the matrix notation of Equation (3.3), the asynchronous correlation spectrum can be written as

$$\Psi(v_1, v_2) = \frac{1}{m-1} \tilde{\mathbf{y}}(v_1)^\top \mathbf{N} \tilde{\mathbf{y}}(v_2) \quad (3.8)$$

where the *Hilbert–Noda transformation matrix*  $\mathbf{N}$  is given by

$$\mathbf{N} = \frac{1}{\pi} \begin{bmatrix} 0 & 1 & \frac{1}{2} & \frac{1}{3} & \dots \\ -1 & 0 & 1 & \frac{1}{2} & \dots \\ -\frac{1}{2} & -1 & 0 & 1 & \dots \\ -\frac{1}{3} & -\frac{1}{2} & -1 & 0 & \dots \\ \dots & \dots & \dots & \dots & \dots \end{bmatrix} \quad (3.9)$$

The above transformation method for carrying out the discrete Hilbert transform is robust and reliable, as long as the functional form of dynamic spectra is in alignment with Equation (2.1).<sup>2</sup>

### 3.2 UNEVENLY SPACED DATA

Spectra analyzed in the previous sections are assumed to be sampled with a fixed increment along the external variable to create a discrete data set consisting of equally spaced spectral traces. The results in Equations (3.2) and (3.5) are applicable only for such evenly spaced data. There are, however, many occasions in the real world where spectral measurements are taken with unevenly spaced increments of the external variable. The 2D correlation analysis of such unevenly spaced data sets cannot be effectively carried out by the computational method used earlier. One way to circumvent this limitation is to convert the unevenly spaced data into evenly spaced data through interpolation or a curve-fitting procedure. It would be much more convenient, however, if one could directly analyze the original data without such data conversion. Fortunately, only a modest modification to the original computational procedure is required to obtain the 2D correlation spectra reflecting the effect of uneven sampling of spectral data.<sup>4</sup> This section provides a general method to efficiently calculate the synchronous and asynchronous 2D correlation spectra from spectral data collected with arbitrary and irregular increments.

For unevenly spaced data, the reference spectrum  $\bar{y}(\nu)$ , taken as the discrete time-average of the observed spectral data, can be calculated by a simple numerical integration method.

$$\bar{y}(\nu) = \frac{\sum_{j=1}^m y_j(\nu) \cdot (t_{j+1} - t_{j-1})}{\sum_{j=1}^m (t_{j+1} - t_{j-1})} \quad (3.10)$$

We must also define two additional points in time  $t_0$  and  $t_{m+1}$  located outside the observation period as

$$t_0 = 2t_1 - t_2 \quad (3.11)$$

$$t_{m+1} = 2t_m - t_{m-1} \quad (3.12)$$

The denominator of Equation (3.10) then becomes

$$\sum_{j=1}^m (t_{j+1} - t_{j-1}) = 3t_m - t_{m-1} + t_2 - 3t_1 \quad (3.13)$$

If the discrete data are collected at an equal increment  $\Delta t$ , such that

$$\begin{aligned} \Delta t &= t_j - t_{j-1} = t_{j+1} - t_j \\ &= \frac{t_m - t_1}{m - 1} \end{aligned} \quad (3.14)$$

then Equation (3.10) simplifies to the familiar straight average value of all data points

$$\bar{y}(v) = \frac{1}{m} \sum_{j=1}^m y_j(v) \quad (3.15)$$

The synchronous and asynchronous 2D correlation spectrum calculated for unevenly spaced spectral data set are given by

$$\Phi(v_1, v_2) = \frac{1}{2(t_m - t_1)} \sum_{j=1}^m \tilde{y}_j(v_1) \cdot \tilde{y}_j(v_2) \cdot (t_{j+1} - t_{j-1}) \quad (3.16)$$

$$\Psi(v_1, v_2) = \frac{1}{2(t_m - t_1)} \sum_{j=1}^m \tilde{y}_j(v_1) \cdot \tilde{z}_j(v_2) \cdot (t_{j+1} - t_{j-1}) \quad (3.17)$$

The discrete Hilbert transform  $\tilde{z}_j(v_2)$  of unevenly spaced data set  $\tilde{y}_j(v_2)$  can be obtained by the numerical integration of Equation (2.44) to yield

$$\tilde{z}_j(v_2) = \frac{1}{2\pi} \sum_{k=1}^m \frac{\tilde{y}_k(v_2)}{t_k - t_j} \cdot (t_{k+1} - t_{k-1}) \quad (3.18)$$

The above expression can be rewritten in a more compact form

$$\tilde{z}_j(v_2) = \sum_{k=1}^m N_{jk} \cdot \tilde{y}_k(v_2) \quad (3.19)$$

where  $N_{jk}$  is the element of the *Hilbert–Noda transformation matrix* for unevenly spaced data set

$$N_{jk} = \begin{cases} 0 & \text{for } j = k \\ \frac{t_{k+1} - t_{k-1}}{2\pi(t_k - t_j)} & \text{otherwise} \end{cases} \quad (3.20)$$

It can be easily shown that Equations (3.16), (3.17), and (3.20), respectively, reduce to Equations (3.2), (3.5), and (3.7) for an evenly spaced data set, where the condition Equation (3.14) holds.<sup>4</sup>

### 3.3 DISRELATION SPECTRUM

The disrelation spectrum  $\Lambda(v_1, v_2)$  is an attractive substitute for the asynchronous spectrum due to its ease of computation.<sup>1</sup> As already discussed in the previous chapter, the absolute value of the disrelation intensity is obtained directly from the corresponding synchronous spectrum. We derived the expression

$$\Lambda(v_1, v_2) = \text{sgn}(\kappa) \sqrt{\Phi(v_1, v_1) \cdot \Phi(v_2, v_2) - \Phi^2(v_1, v_2)} \quad (2.50)$$

Numerical values of synchronous 2D correlation intensities are readily calculated from a discrete data set using Equation (3.2), as long as the discrete data set is equally spaced. For an unevenly spaced data set, the synchronous spectrum must be obtained by using Equation (3.16) instead of Equation (3.2). The sign of the disrelation spectrum, however, must be calculated separately from the slope of the cross-correlation function. Fortunately, it is actually quite straightforward to estimate the first derivative of a cross-correlation function. The constant  $\kappa$  to determine the sign of disrelation spectrum is given by

$$\kappa = \frac{1}{2(T_{\max} - T_{\min})} \sum_{j=1}^{m-1} [\tilde{y}_j(v_1) \cdot \tilde{y}_{j+1}(v_2) - \tilde{y}_{j+1}(v_1) \cdot \tilde{y}_j(v_2)] \quad (3.21)$$

Note that the term  $1/(T_{\max} - T_{\min})$  is actually irrelevant to the determination of the sign of  $\kappa$ . Furthermore, it turns out that the sign of  $\kappa$  determined by Equation (3.21) holds without further modification even for unevenly spaced data as well. The sign of  $\kappa$  thus obtained, along with the synchronous correlation intensities from Equation (3.2), can be substituted into Equation (2.50) to obtain the desired numerical value of the disrelation spectrum intensity.

### 3.4 COMPUTATIONAL EFFICIENCY

It is important to note that the numerical calculation of 2D correlation spectra using Equations (3.2) and (3.5) is quite efficient in a typical machine computation scheme, even compared to the fast Fourier transform algorithms, if the number of dynamic spectra  $m$  is not too large. First of all, these operations do not involve the evaluation of transcendental functions, i.e., the need for the higher order Taylor series expansion of the exponential function of complex numbers.

**Table 3.1** Computational efficiency<sup>2</sup>

|                                                                    |                     |
|--------------------------------------------------------------------|---------------------|
| Fast Fourier transform                                             |                     |
| Elementary computational steps                                     | $m \log_2 m$        |
| Complex number operations                                          | $\times 2$          |
| Transformation of both $\tilde{y}(v_1, t)$ and $\tilde{y}(v_2, t)$ | $\times 2$          |
| Determination of $e^{i2\pi/m}$                                     | Taylor expansion    |
| <i>Power-of-two</i> algorithms                                     | $m \rightarrow 2^n$ |
| Discrete Hilbert transform                                         |                     |
| Elementary computational steps                                     | $m^2$               |
| Real number operations                                             | $\times 1$          |
| Transformation of only $\tilde{y}(v_2, t)$                         | $\times 1$          |
| Determination of $1/(k - j)$                                       | Simple division     |

More detailed comparative analysis reveals the difference in the computational efficiency between the fast Fourier and direct discrete Hilbert transform approach.

Table 3.1 summarizes the number of computational steps required to carry out the evaluation of generalized 2D correlation spectra from a set of  $m$  discrete dynamic spectra. The number of elementary computational steps required to perform a fast Fourier transform operation using a standard mathematical method, such as Sande–Tuckey or Cooley–Tuckey algorithm,<sup>5,6</sup> is  $m \log_2 m$ . The direct discrete Hilbert transform, on the other hand, will require  $m^2$  elementary computational steps. The advantage of the fast Fourier transform approach becomes significant as  $m$  becomes very large. The fast Fourier transform algorithms operate most effectively if the number of data points  $m$  is equal to  $2^n$ . This requirement will sometimes affect the relative computational efficiency, especially when the number of data points  $m$  is not extremely large. The effective number of data points for the fast Fourier transform operation is often raised by appropriate zero-filling.

In order to calculate generalized 2D correlation spectra by the Fourier transform approach, it is necessary to carry out the time-domain transformation of two separate traces of spectral intensity variations measured at  $v_1$  and  $v_2$ . The Hilbert transform approach, on the other hand, requires the transformation of only one trace of intensity variations measured at  $v_2$ . Furthermore, because the Fourier transformation is a mathematical operation using complex numbers, the computation actually requires twice as many steps as in real number operations such as the Hilbert transform. Combining these two factors, the Fourier transform method should require four times as many computational steps.

Figure 3.1 shows a plot of the expected number of computational steps as a function of the number of spectral data  $m$ . Clearly the number of required computational steps becomes much higher for the Hilbert transform method as the number of dynamic spectra  $m$  becomes large. However, the plot also shows that the direct discrete Hilbert transform method may compete well against the fast Fourier transform algorithm, as long as the number of dynamic spectra is kept below 40. The advantage of the Hilbert transform method may be even greater, as

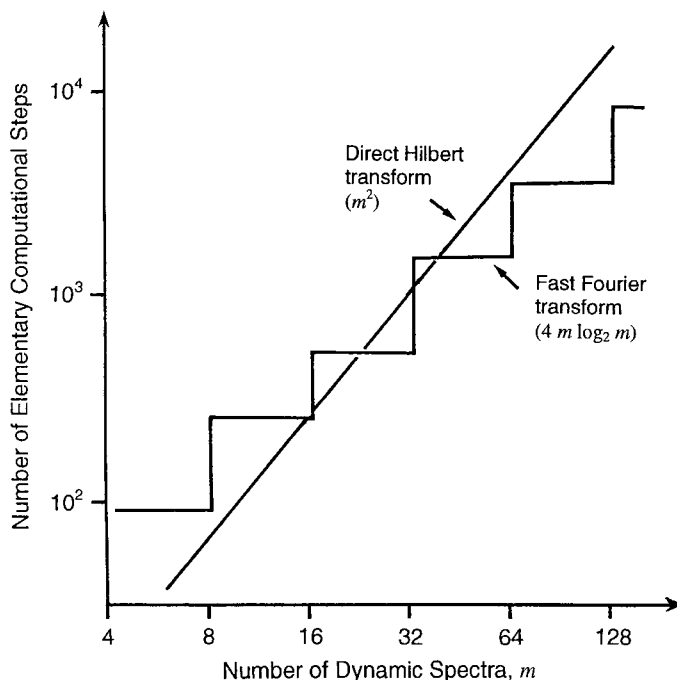


**Figure 3.1** Possible number of elementary computational steps as a function of the number of dynamic spectra  $m$  for the fast Fourier transform and direct discrete Hilbert transform method. An additional penalty ( $\times 2 - \times 8$ ) is possible for the fast Fourier transform route due to the requirement for the Taylor expansion of transcendental functions. (Reproduced with permission from I. Noda, *Appl. Spectrosc.*, **54**, 994 (2000). Copyright (2000) Society for Applied Spectroscopy.)

it involves the evaluation of only simple divisions as opposed to the determination of exponential functions by the Taylor expansion. Noda's experience with various computer programs developed for 2D correlation analysis in the past indicates that the actual cross-over point where the fast Fourier transform method becomes more efficient than the direct discrete Hilbert transform method may well be much higher, somewhere between 64 and 128.<sup>2</sup>

The computation of a disrelation spectrum is even faster than the direct computation of an asynchronous spectrum using either the fast Fourier or the discrete Hilbert transform method, especially if the total number  $m$  of spectral traces for a given dynamic spectrum becomes large. The use of the disrelation spectrum becomes even more attractive if the main purpose of 2D analysis is to simply differentiate overlapped peaks by taking advantage of the high-resolution feature of 2D correlation spectroscopy. In this case, the information concerning temporal relationships among spectral variations is not important. Therefore, only the magnitude part of Equation (2.50), which depends on the synchronous spectral intensities, needs to be evaluated.

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## 4 Generalized Two-dimensional Correlation Spectroscopy in Practice

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In this chapter, an illustrative example of a set of 2D correlation spectra constructed from spectral data obtained in an actual laboratory experiment is presented to show how such 2D correlation maps can be constructed and analyzed to obtain physically meaningful information. A simple model transient system based on the evaporation of volatile components from a solution mixture is probed with time-resolved FT IR measurement. The resulting spectral data set is then subjected to the generalized 2D correlation analysis to demonstrate how 2D correlation spectra can be used to reflect pertinent features observed in a real experimental situation.

Real-world spectral data often contain various imperfections, distortions, and noise interfering with the pertinent information. One of the most serious problems in 2D correlation spectroscopy is the appearance of artifacts in 2D maps. Spectral noises, baseline fluctuations, and band shifts are three major causes for the artifacts and misleading features. In the second part of this chapter, we discuss how we can avoid the artifacts, and how we can distinguish between real peaks and artifacts or misleading features in synchronous and asynchronous correlation spectra. To avoid the artifacts produced by noise some pretreatment methods are useful. Several methods have been proposed that will allow one to discriminate the real peaks from the artifacts in 2D maps. Finally, the proper interpretation of complex features not related to intensity changes, such as band position shift and line broadening, will be discussed.

### 4.1 PRACTICAL EXAMPLE

#### 4.1.1 SOLVENT EVAPORATION STUDY

Let us study a very simple example to demonstrate how straightforward the actual 2D correlation analysis really is. The system described here is a three-component solution mixture of polystyrene (PS) dissolved in a 50:50 blend of methyl ethyl ketone (MEK) and perdeuterated toluene.<sup>1</sup> The initial concentration of PS is about 1.0 wt%. Once the solution mixture is exposed to the open atmosphere, the solvents start evaporating, and the PS concentration increases with



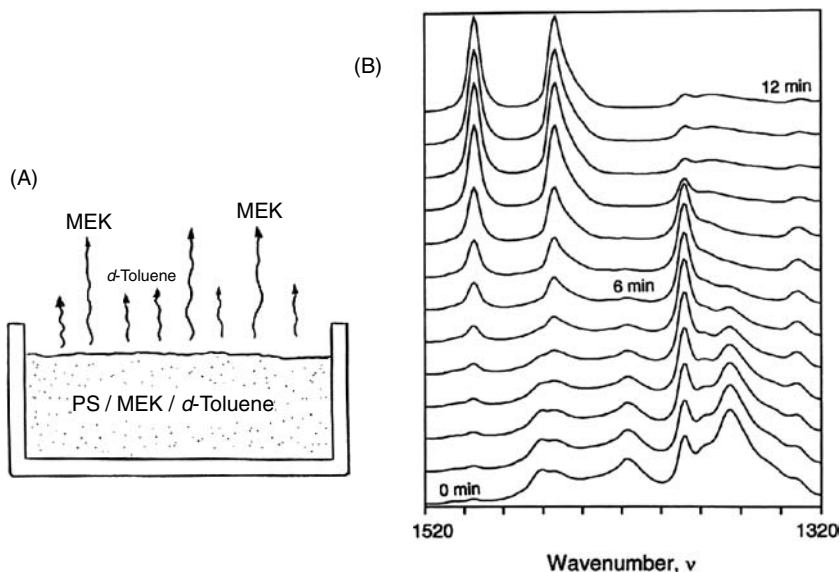


**Figure 4.1** Model solvent evaporation experiment. (A) Schematic representation of the competitive evaporation of MEK and deuterated toluene leaving the PS component behind. (B) Time-dependent IR spectra of a mixture of MEK, deuterated toluene, and PS during the solvent evaporation process

time. However, due to the substantial difference in the volatility of MEK and toluene coupled with their slightly dissimilar affinity to PS, the composition of the solution mixture changes as a function of time in a rather complex manner during the spontaneous evaporation process.

The transient IR spectra were collected as the two solvents evaporated, eventually leaving a PS film behind, as shown schematically in Figure 4.1(A). The measurement was actually made using a horizontal attenuated total reflectance (ATR) prism. Figure 4.1(B) displays some of the transient IR spectra of the solution mixture during the solvent evaporation process collected at the interval of every 9 s. As expected, the intensities of bands at 2980 and 1720  $\text{cm}^{-1}$  due to volatile MEK and those of bands at 2275 and 820  $\text{cm}^{-1}$  assigned to perdeuterated toluene gradually decrease, while those of PS bands at 3020 and 1450  $\text{cm}^{-1}$  increase with time.

#### 4.1.2 2D SPECTRA GENERATED FROM EXPERIMENTAL DATA

A synchronous 2D IR correlation spectrum corresponding to the transient IR data of Figure 4.1(B) is shown in Figure 4.2. All 2D spectra are obtained by the calculation method (Equations 3.2 and 3.5) described in Chapter 3. The reference spectrum provided at the top and left side of the 2D spectrum is the time-averaged spectrum (Equation 3.15). Autopeaks appearing at the diagonal positions of the

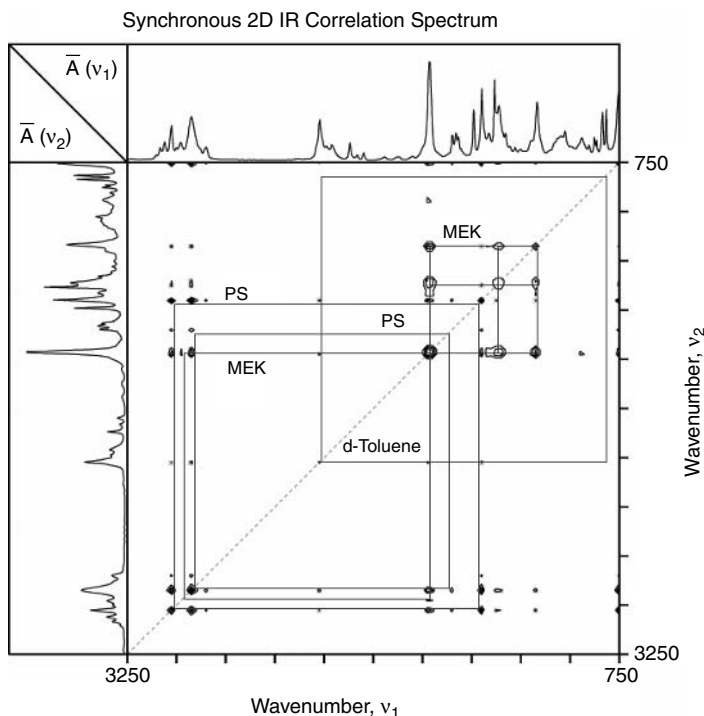


**Figure 4.2** Synchronous 2D IR correlation spectrum of a mixture of MEK, deuterated toluene, and PS during the solvent evaporation process

synchronous 2D spectrum represent the spectral intensity changes for these IR bands during the observation period. Individual autopeaks can be readily assigned to bands arising from contributions of PS, MEK, and toluene. By combining the synchronous cross peaks and autopeaks located at similar spectral coordinates, one can draw a set of correlation squares. Note that each correlation square groups the band intensity changes due to the same component. No positive cross peak is apparent in this 2D spectrum among different species.

Figure 4.3 is a close up view of the synchronous correlation map. Again, strong correlation is observed for sets of IR bands assignable to the same component. However, the development of some cross peaks is observable among different species, e.g., between PS and toluene bands, in the close up map. The signs of cross peaks between toluene bands and PS bands are all negative (marked by the presence of shading). Negative cross peaks indicate that band intensities (e.g., 1490 and 1450  $\text{cm}^{-1}$ ) of one species (PS in this case) are increasing, while the intensity at 1385  $\text{cm}^{-1}$  for the other species (toluene) is decreasing. Because of the high spectral resolution in the 2D spectrum, one can readily sort out and classify some of the overlapped bands between MEK and toluene, or between PS and toluene.

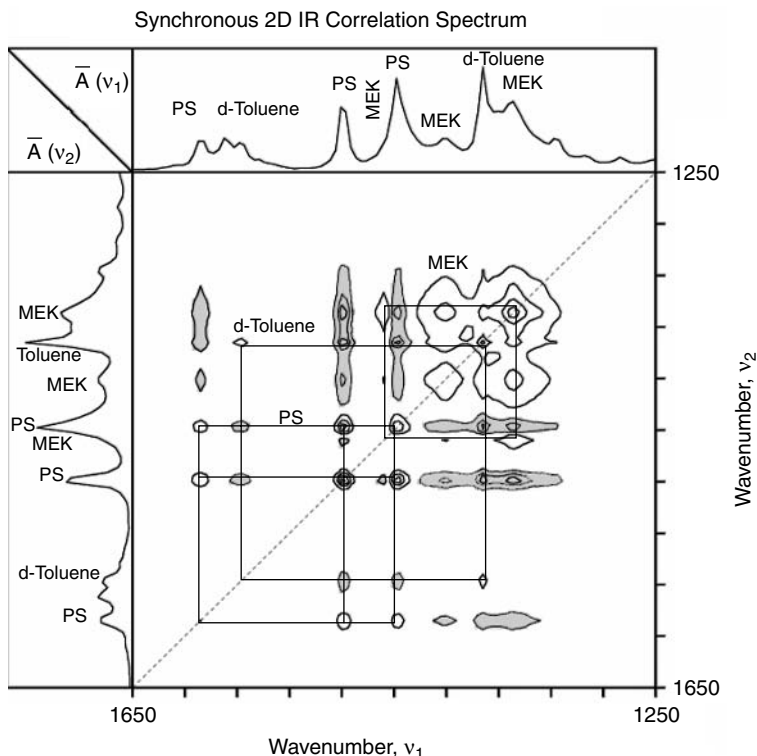


**Figure 4.3** Synchronous 2D IR correlation spectrum of a mixture of MEK, deuterated toluene, and PS during the solvent evaporation process. Negative peaks are marked by shading

The corresponding asynchronous 2D IR correlation spectrum is shown in Figure 4.4. It provides an even clearer picture of the time-dependent changes of IR intensities of component bands of this solution mixture. In the asynchronous spectrum, cross peaks are observed between pairs of IR bands assignable to different species. Thus, even bands located very close to each other, such as MEK and PS bands near  $1450\text{ cm}^{-1}$ , can be readily discriminated. This interesting feature of an asynchronous 2D correlation spectrum to be able to discriminate overlapped bands originating from different species or molecular moieties behaving distinctively under a given perturbation is especially useful in obtaining high-resolution spectra.

#### 4.1.3 SEQUENTIAL ORDER ANALYSIS BY CROSS PEAK SIGNS

The sequential order of events that occurs during the observation period can be determined by the analysis of signs of asynchronous cross peaks. By convention,

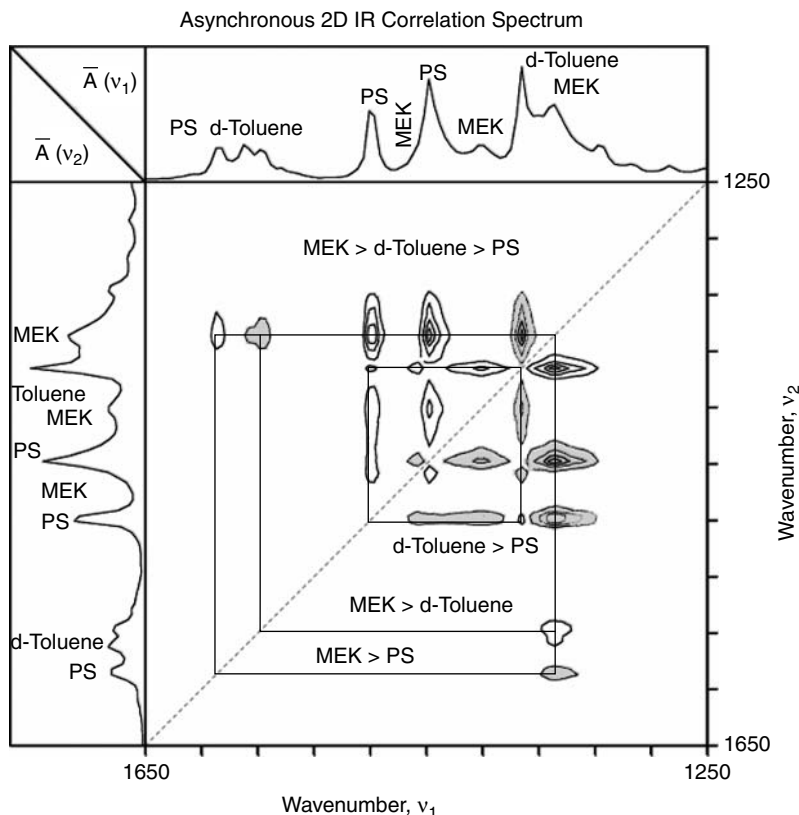


**Figure 4.4** Asynchronous 2D IR correlation spectrum of a mixture of MEK, deuterated toluene, and PS during the solvent evaporation process. Negative peaks are marked by shading

negative asynchronous peaks are all marked by shading, while positive peaks are plotted without. As discussed in Section 2.3, the appearance of a positive asynchronous peak located at the spectral coordinate  $(\nu_1, \nu_2)$  indicates that the IR intensity variation observed during the evaporation process at wavenumber  $\nu_1$  occurs predominantly before that at  $\nu_2$ . Likewise, the appearance of a negative asynchronous peak marked with shading indicates that the IR intensity variation at wavenumber  $\nu_1$  occurs predominantly after that at  $\nu_2$ . The sequential order, however, is reversed in both cases if the sign of the corresponding synchronous correlation intensity at the same spectral coordinate  $(\nu_1, \nu_2)$  becomes negative.

For example, the appearance of a negative asynchronous cross peak with shading located at  $(1360, 1600 \text{ cm}^{-1})$  in Figure 4.4, coupled with the presence of a negative synchronous peak at the same coordinate in Figure 4.3 indicates that the intensity change at  $1360 \text{ cm}^{-1}$  (MEK) occurs predominantly before the change at  $1600 \text{ cm}^{-1}$  (PS). In other words, the rapid disappearance of the volatile

MEK component is occurring predominantly in the earlier stage of evaporation compared to the gradual accumulation of PS. Similarly, the presence of a positive asynchronous cross peak at  $(1360, 1385 \text{ cm}^{-1})$ , coupled with the positive synchronous correlation intensity at the same coordinate, suggests that the intensity change of the  $1360 \text{ cm}^{-1}$  band (MEK) occurs before that of the  $1385 \text{ cm}^{-1}$  band (toluene). It becomes apparent that the band intensity changes of MEK, which is more volatile, occur before those of toluene.

The system analyzed here is a relatively simple model case, but the result clearly demonstrates that generalized 2D correlation analysis can be used to differentiate the subtle differences in the time-dependent intensity variations of IR spectral intensities of bands assignable to different species. Spectral signals having different patterns of changes with respect to time, or as a matter of fact any variables such as temperature, concentration, spatial position *etc.*, can be readily distinguished by the 2D correlation analysis, even if the band positions are relatively close.

## 4.2 PRETREATMENT OF DATA

### 4.2.1 NOISE REDUCTION METHODS

Noise often causes artifacts particularly in asynchronous spectra, because the intensities of noises change out of phase with each other. Noise arises from various interfering physical or chemical processes. We usually think of noise as the high-frequency noise associated with the instrument's detector and electronic circuits. However, there are other forms of noise as well; for example, low-frequency noise and localized noise. Low-frequency noise is caused, for example, by instrument drift during the scanning measurements. Usually, it is more difficult to reduce the low-frequency noise because it often resembles the real information in the data. Noise is a common obstacle for spectral analysis, so that noise reduction methods have been extensively investigated.

The most commonly used noise reduction method is smoothing. Particularly, the Savitzky–Golay method is very popular as a smoothing method. The Savitzky–Golay method came from the idea that, in the vicinity of a measurement point, a spectrum can be fitted by low-degree polynomials. We do not explain the detail of the Savitzky–Golay method, but an example of its application will be shown later. A problem in smoothing is that, if one tries to increase the effect of smoothing by increasing the number of the points of the convolution weights, a band shape would be distorted. This distortion may lead to the decrease of spectral resolution and band intensity. The Savitzky–Golay method was proposed with the aim of reducing the distortion. However, care must still be taken when one uses smoothing methods.

There are other methods for denoising data as well. Wavelets, eigenvector reconstruction and artificial neural networks (ANN) are all used for noise reduction.

Berry and Ozaki demonstrated that wavelets are useful in noise reduction for 2D correlation spectroscopy.<sup>2</sup> Wavelets can eliminate both high- and low-frequency noise as well as localized noise due to phenomena such as scattering, while smoothing removes only high frequency noise. Wavelets operate by taking a spectrum and transforming it into the wavelet domain and returning it to the spectral domain. This is similar in practice to applying the Fourier transform to data to reduce noise, except that wavelets use a much more sophisticated function for modeling the data than the sine/cosine of Fourier analysis. Jung *et al.* used principal component analysis (PCA) to minimize the contribution of noise to 2D spectra.<sup>3</sup> It was demonstrated that the PCA reconstruction of spectral data provides a robust noise filter, capable of preserving the pertinent feature of 2D spectra. This particular approach is discussed in Section 6.3 of Chapter 6.

#### 4.2.2 BASELINE CORRECTION METHODS

Major causes for baseline fluctuations in transmittance spectra are light scattering and changes in density, while those in diffuse reflectance spectra are intensity changes in light scattering and normal reflection light induced by the variations in average particle size, distribution of particle shape, the density of packing of a sample in a cell, and so on. Baseline fluctuations are also induced by the effect of an optical fiber cable. The most popular baseline correction method is the use of derivative spectra. However, it is not a good idea to use derivative methods for the pretreatment of spectral data for 2D correlation spectroscopy, because ripples in derivative spectra may produce artificial peaks in 2D maps.

Multiplicative scatter (or signal) correction (MSC) is a very useful pretreatment method for eliminating the additive scatter factor (offset deviation) and the multiplicative scatter factor (amplification factor) in a spectrum.<sup>4</sup> The idea of MSC lies in the fact that light scattering typically has a wavelength dependence different from that of chemically based light absorbance. Therefore, one can utilize data from many wavelengths to distinguish between light absorption and light scattering. MSC corrects spectra according to a simple linear univariate fit to a standard spectrum and is estimated by least squares regression using the standard spectrum. As the standard spectrum, a spectrum of a particular sample or an average spectrum is used. Let us give an example of effective pretreatments.

Figure 4.5 displays NIR spectra in the 1100–2500 nm region of 165 milk samples.<sup>5</sup> It is noted that the signal-to-noise ratio of the spectra is not high in the 2000–2500 nm region, and the baseline changes from one spectrum to another. The spectra are very similar to that of water. A broad feature near 1450 nm is due to the combination of OH symmetric and antisymmetric stretching modes of water, while an intense band near 1930 nm is assigned to the combination of OH bending and symmetric stretching modes of water. It is difficult to extract useful information about milk components directly from the NIR spectra. Thus, 2D correlation analysis was applied to the 1100–1900 nm and 2000–2400 nm regions.

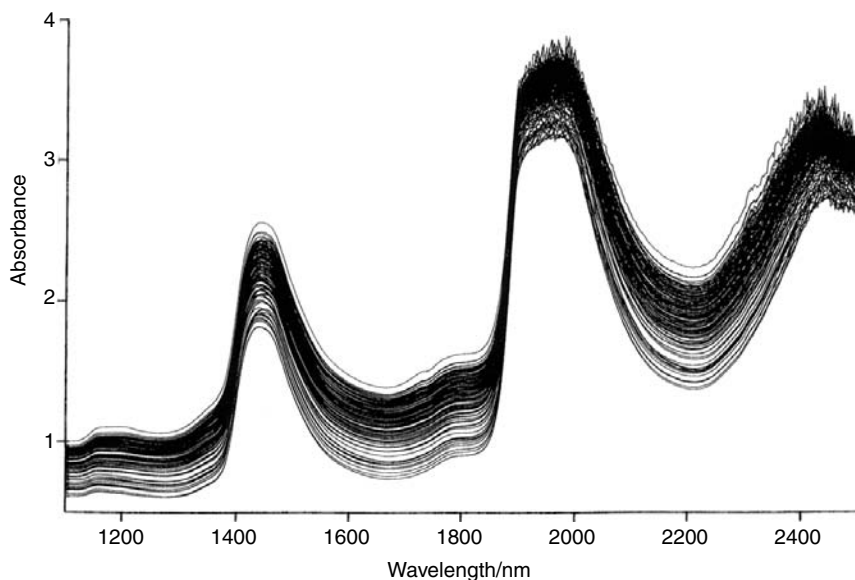


**Figure 4.5** NIR spectra in the 1100–2500 nm region of the milk samples. (Reproduced with permission from B. Czarnik-Matusewicz *et al.*, *Appl. Spectrosc.*, **53**, 1582 (1999). Copyright (1999) Society for Applied Spectroscopy.)

Comparison of the two figures reveals that the baseline changes are much larger for the fat than for the protein concentration-dependent spectral variations. Milk contains light-scattering particles in the form of fat globules and protein micelles. The direct calculation of 2D correlation spectra from the raw spectra in Figures 4.6(Aa) and (Ba) yields a very strange looking synchronous spectrum and a very noisy asynchronous spectrum. The synchronous spectra showed only positive peaks probably because of the increasing baseline change. Thus, Czarnik-Matusewicz *et al.* applied MSC and smoothing as pretreatment procedures of the milk spectra selected for the calculation of 2D NIR correlation.<sup>5</sup> Figures 4.6(Ab) and (Bb) depict, respectively, the spectra obtained after the applications MSC and smoothing to the spectra shown in Figures 4.6(Aa) and (Ba). The results in Figs. 4.6(Ab) and (Bb) demonstrate the usefulness of MSC and smoothing. More detailed discussion on the 2D correlation NIR study of milk based on protein and fat concentrations will be given in Chapter 13.

#### 4.2.3 OTHER PRETREATMENT METHODS

Many other data pretreatment methods have been used in 2D correlation spectroscopy. While they are generally good techniques, care must always be exercised because the indiscriminate use of preprocessing methods may generate unwanted artifacts. For example, Fabian *et al.* used Fourier self-deconvolution

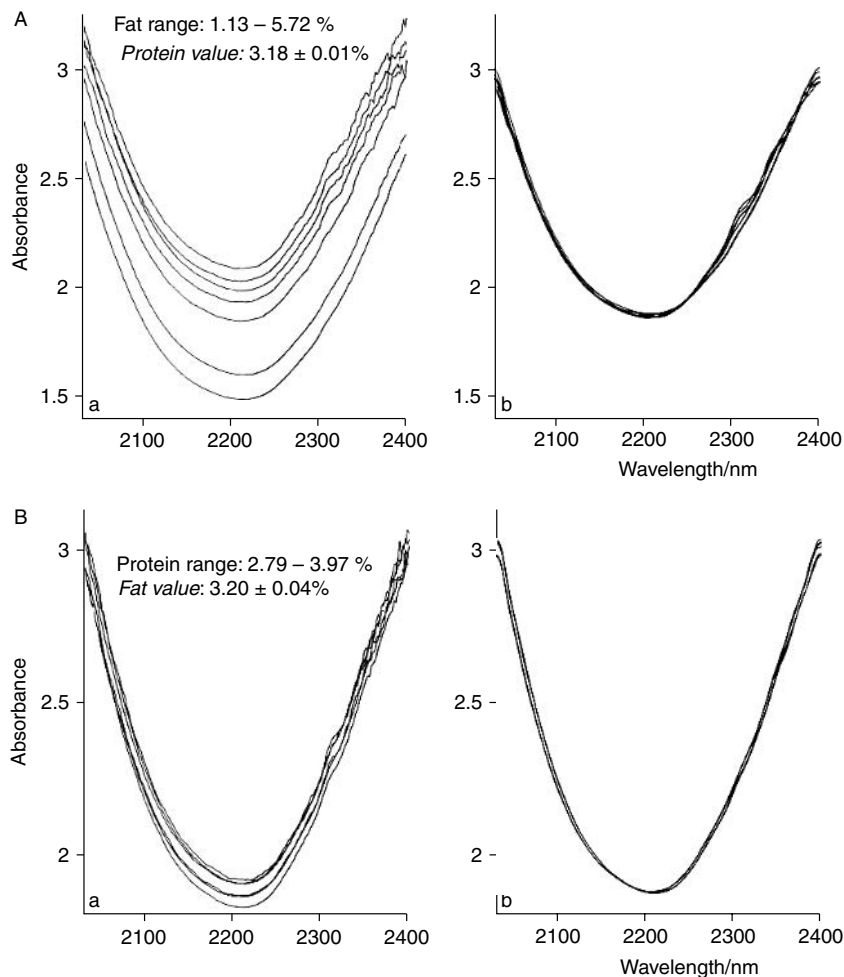


**Figure 4.6** (A) Fat concentration-dependent NIR spectral variations in the 2000–2400 nm region of milk samples before (a) and after (b) pretreatments. (B) Protein concentration-dependent NIR spectral variations in the 2000–2400 nm region of milk samples before (a) and after (b) pretreatment. (Reproduced with permission from B. Czarnik-Matusewicz *et al.*, *Appl. Spectrosc.*, **53**, 1582 (1999). Copyright (1999) Society for Applied Spectroscopy.)

(FSD) as a pretreatment for IR and NIR spectra of proteins to further enhance the spectral resolution of highly overlapped bands.<sup>6</sup> Czarnik-Matusewicz *et al.* employed four kinds of pretreatment procedures to construct 2D correlation spectra from concentration-dependent attenuated total reflection (ATR) IR spectral changes of  $\beta$ -lactoglobulin (BLG) buffer solutions, including ATR correction, subtraction of the spectrum of buffer solution, smoothing, and normalization over the concentration.<sup>7</sup>



### 4.3 FEATURES ARISING FROM FACTORS OTHER THAN BAND INTENSITY CHANGES

In this section, we present a systematic way to handle some complex features appearing in 2D correlation spectra due to factors not related to the straightforward spectral intensity changes. In particular, the effect of band position shift and line broadening on 2D spectra will be discussed. Features generated from such effects belong to special cases, which cannot be readily interpreted in terms of the simple cross peak sign rules established in Section 2.3 of Chapter 2.

#### 4.3.1 EFFECT OF BAND POSITION SHIFT AND LINE SHAPE CHANGE

The 2D correlation scheme described in this book is focused on the analysis of changes in spectral features induced by an external perturbation. The most common variations in the spectral features are a simple increase or decrease in the band intensities. Such intensity variations can be easily interpreted as a reflection of some perturbation-induced population change in moieties contributing to the spectral intensities. However, there are many other cases where more complex changes in spectral features beyond simple intensity variations are involved.<sup>8–18</sup> Two important examples of complex spectral feature variations are: band position *shift* and line *broadening* phenomena. Both cases are very commonly observed when the perturbation used in 2D correlation analysis strongly affects the system environment such that the nature of the constituent response to the spectral probe itself is altered. Typical examples include the composition- or temperature-induced changes in the wavenumber position and line shape of IR bands caused by the altered strength of hydrogen bonding interactions which affect molecular vibrations.

The effect of spectral variations other than intensity changes cannot be analyzed in a simple manner based on the basic assumption of one-to-one correspondence between the position of a correlation peak and spectral band. Very often, surprisingly complex (but fortunately very characteristic) features comprising a cluster of multiple correlation peaks may appear from a single band with varying position or width in the corresponding 2D correlation spectra. Such 2D spectral features are not an artifact but legitimate signature patterns of complex spectral changes manifested in the correlation spectra. However, overlooking the possible existence of multiple correlation peaks assignable to a single spectral band often leads to erroneous interpretation of 2D spectra. This section, therefore, provides a guide to the detection and interpretation of such complex spectral features.

The existence of a characteristic cluster of multiple correlation peaks in 2D spectra arising from the position shift of a single band was well recognized from the very early days of 2D correlation spectroscopy.<sup>8</sup> The so-called four-leaf-clover cluster pattern of synchronous peaks generated from X-ray scattering data was interpreted as the result of a position shift of the scattering maximum.

Gericke *et al.* reported the first systematic study of the effect of position shift and broadening of an IR band on 2D correlation spectra using simulated data.<sup>9</sup> Similar simulation studies were carried out by Czarnecki,<sup>10,11</sup> Elmore and Dluhy,<sup>12,13</sup> and others<sup>14,15</sup> to identify the characteristic features of 2D peak patterns arising from the position shift and broadening of bands. Kim and Jeon compiled an extensive list of simulation results for cases where multiple band shift and broadening possibilities were combined.<sup>16</sup> These studies based on simulation analysis all point to the fact that the characteristic cluster patterns of 2D spectra arising from band shift and broadening are distinct and well recognizable. The pitfall of over-interpretation of multiple correlation peak clusters arising from a single band as the false indication of a multiple band feature is discussed.<sup>17,18</sup>

#### 4.3.2 SIMULATION STUDIES

The effects of complex spectral feature variations, such as band shift and line broadening, are best visualized with simulated model data emphasizing the specific aspect of spectral changes. Figure 4.7 shows four examples of such simulated spectral data comprising Lorentzian peaks in an arbitrary spectral region between 1000 and 2000  $\text{cm}^{-1}$ . Fig. 4.7(A) represents the case for spectral variations arising from the classical intensity changes of two highly overlapped bands with fixed band position and relative line shape. One band (located at 1450  $\text{cm}^{-1}$ ) decreases in the intensity quickly, while the other band (at 1550  $\text{cm}^{-1}$ ) increases in intensity much more gradually, as indicated by the two arrows. For this simulation study, less than 20 % of intensities are changed for any part of the spectrum.

Figure 4.7(B) shows the case where the position of an isolated single band, with a fixed intensity and line shape, is gradually shifted along the spectral axis in the direction of the arrow. For illustrative purpose, the extent of band shift in this figure is carried out to about 25 % of the band width. It should be pointed out that even a much lower extent of band shift, say less than 0.5 % of the band width, will also generate detectable characteristic features of band shift in the corresponding 2D spectra. Such a small level of band shift cannot be readily detected in a typical stack of 1D spectra such as that shown in Fig. 4.7(B). The high sensitivity of 2D spectra to band shift is not really a burden, but actually a useful asset to be exploited for the detection of subtle features, as long as the expected characteristic pattern of 2D spectra is known.

Figure 4.7(C) depicts a somewhat more realistic but substantially more complicated case, where the band position shift is coupled with a simultaneous intensity increase. This type of response is often observed for spectra of solution mixtures of strongly associated fluids with varying compositions. The degree of molecular level interactions such as hydrogen bonding in a mixture, which often influence the band position of the spectrum, is often affected by the composition of the mixture. Thus, changing the composition of a mixture comprising strongly interacting species will result in not only the intensity change due to the composition but also the position shift of some of the component bands.

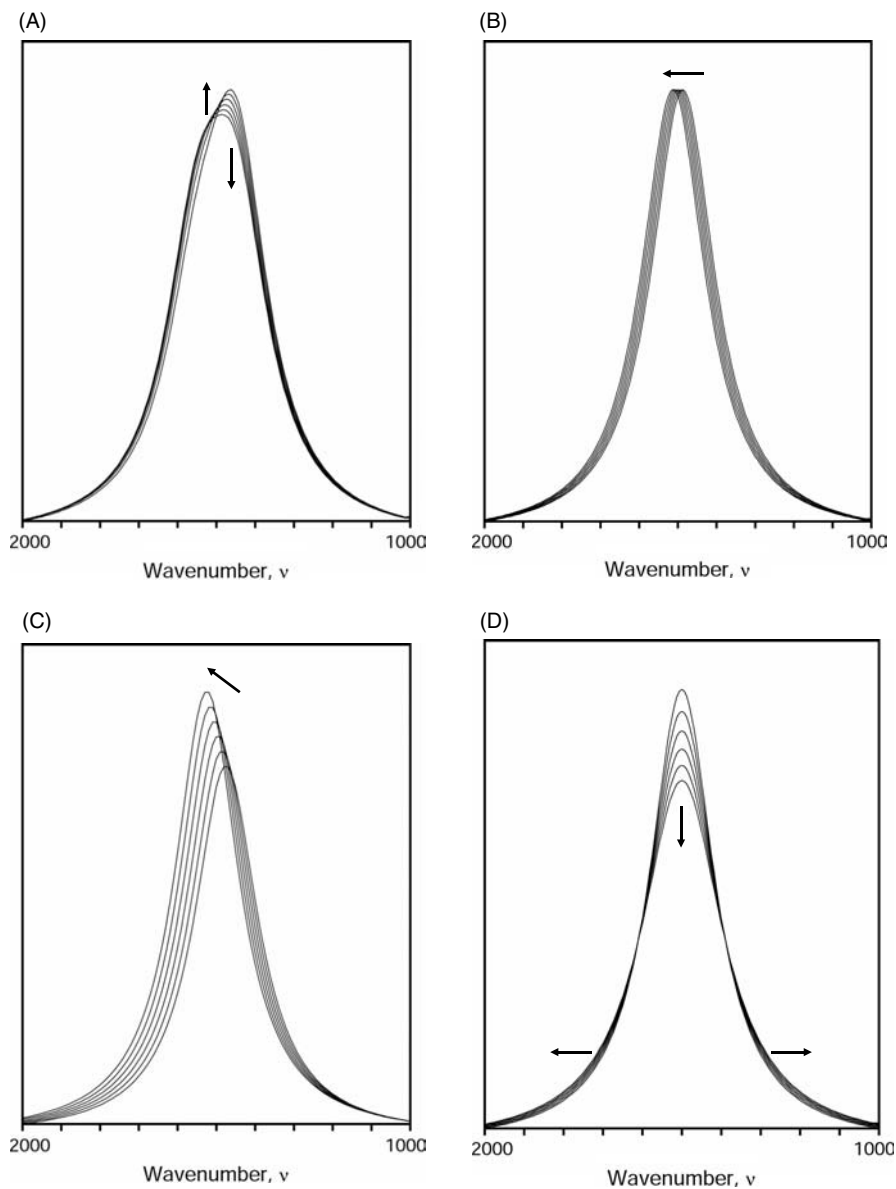


**Figure 4.7** Simulated model data for spectral feature variations: (A) two overlapped bands changing in intensity in opposite directions; (B) a single band shifting in position from a lower to a higher wavenumber; (C) a single band simultaneously shifting in position and increasing in the intensity; and (D) line broadening of a single band

Figure 4.7(D) represents a series of spectra for the line broadening case. The integral intensity of the peak is kept at a constant value, so the peak height decreases gradually as the band width is increased. The broadening in this example was carried out to about 25 % increase in the band width, again purely for illustrative purpose. As in the case of band position shift, a much smaller extent of line broadening, e.g., less than 0.5 % of the increase in band width, will produce the same characteristic signature pattern of line broadening in 2D spectra discussed later.

#### 4.3.3 2D SPECTRAL FEATURES FROM BAND SHIFT AND LINE BROADENING

The set of simulated model data listed in Figure 4.7 are now converted to 2D correlation spectra by the standard procedure. The resulting 2D spectra show very distinct and recognizable patterns which can be used as diagnostic tools for the existence of complex spectral feature changes.

##### 4.3.3.1 Two Separate Bands with Intensity Changes

Figure 4.8 shows the 2D spectra generated from model spectral data where two highly overlapped but separate bands change their intensities, one increasing and the other decreasing. The synchronous spectrum (Fig. 4.8(A)) shows a cluster of 2D peaks comprising two autopeaks and two negative cross peaks. This particular form of peak cluster is commonly known as the *four-leaf-clover* pattern cluster. For a two-band system, the cluster pattern is not always four-way symmetric, as the size of the two autopeaks may not be the same. The corresponding asynchronous spectrum (Fig. 4.8(B)) shows two well-resolved cross peaks without any further complicating features. This simplicity of asynchronous 2D spectra is the most reliable indicator that no other complicating factors such as band position shift or line broadening are in operation.

The interpretation of such 2D spectra is straightforward. According to the rules established in Section 2.3, the presence of two autopeaks indicates that intensity change occurs at two different wavenumbers, ca 1450 and 1550  $\text{cm}^{-1}$ . The sign of synchronous cross peaks are both negative, indicating that the intensity change of the band pair occurs in the opposite direction as expected. The sign of asynchronous cross peaks provides the sequential order information such that the intensity decrease at 1450  $\text{cm}^{-1}$  occurs before the intensity increase at 1550  $\text{cm}^{-1}$ .

##### 4.3.3.2 Band Position Shift

Figure 4.9 shows the 2D spectra corresponding to the band position shift to the higher wavenumber direction, as described in Figure 4.7(B). The synchronous

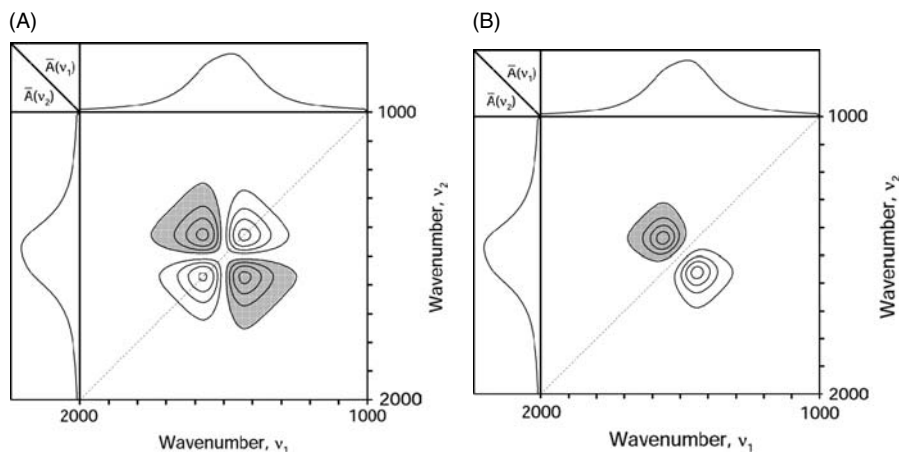


**Figure 4.8** (A) Synchronous and (B) asynchronous 2D correlation spectrum based on two overlapped bands changing in intensity in opposite directions

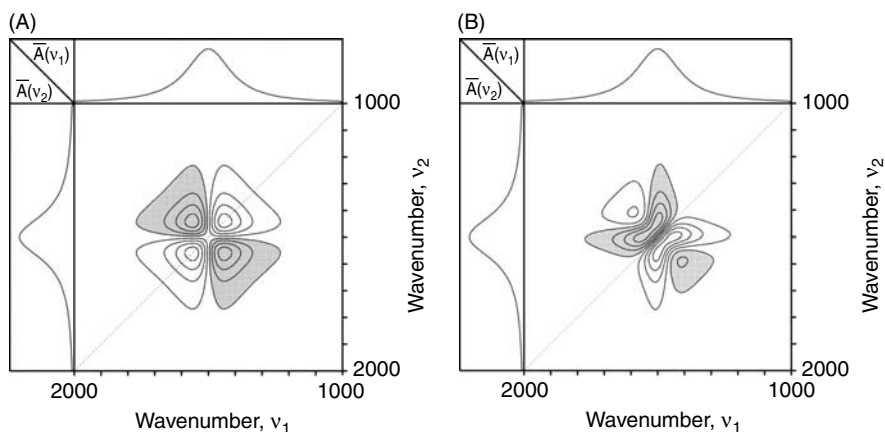


**Figure 4.9** (A) Synchronous and (B) asynchronous 2D correlation spectrum based on a single band shifting in position from a lower to a higher wavenumber

spectrum (Figure 4.9(A)) shows the very characteristic four-way symmetric four-leaf-clover cluster pattern, comprising two autopeaks and two negative cross peaks. The center of the cluster is located near the spectral coordinate corresponding to the peak maximum position of the average spectrum. It is noted that the clover pattern observed in the synchronous spectrum for two overlapped bands (Fig. 4.8(A)) and that for a single band with position shift (Fig. 4.9(A)) are somewhat similar. It is usually difficult to distinguish these two cases by the simple observation of the synchronous spectrum alone.

Fortunately, the distinction can be made in a relatively straightforward manner when asynchronous spectra are examined. The asynchronous spectrum obtained for the case of band position shift is shown in Fig. 4.9(B). This very characteristic pattern of an asynchronous peak cluster is known as the *butterfly* pattern. The cluster consists of a pair of elongated cross peaks of opposing signs located very close to the diagonal of the 2D spectrum. Next to the main pair of elongated cross peaks are another set of weaker cross peaks, which are confined to smaller parts and located slightly away from the diagonal. This weaker pair is sometimes overlooked, especially if the contour level of the 2D map is not appropriately set. Readjustment of the contour level after the initial observation of an elongated cross peak pair often reveals the existence of the second pair. This butterfly pattern is a very distinct and easily recognizable indicator for the existence of a shift in the band position. No simple combinations of intensity changes of multiple bands generate such a characteristic cluster pattern.

The interpretation of the region of 2D correlation spectra showing the asynchronous butterfly pattern can no longer be handled in the manner described in Section 2.3. The presence of multiple correlation peaks in the asynchronous spectrum may give an erroneous impression that there may be multiple hidden bands involved in the formation of such clusters. In reality, only a single band with varying position is involved. Furthermore, the elongated asynchronous cross peak lies in a broad region of the 2D correlation map encompassing both positive and negative synchronous correlation intensity regions, so the simple assignment of sequential order based on the sign rule is not possible for this peak. An alternative form of sequence rule is developed here specifically for a cross peak cluster showing the butterfly pattern. Whenever such a pattern is observed, one may assume that there is a possibility of a single band shifting in position. The direction of the shift is determined by the signs of the asynchronous cross peaks within the butterfly cluster. If the elongated cross peak above the diagonal is negative, and that below the diagonal is positive, the band position is shifting from right to left along the horizontal axis of the 2D spectrum and from top to bottom along the vertical axis. Opposite signs of the cross peaks indicate the opposite direction of band shift. In the simulation example of Figure 4.9(B), the band is moving from the lower wavenumber side to the higher wavenumber side.

It is interesting to point out that the location of peaks found in both the synchronous four-leaf-clover pattern cluster and the asynchronous butterfly pattern cluster are surprisingly insensitive to the extent of the band position shift. Only the correlation intensity level is affected uniformly by the extent of the band shift. The overall graphic appearance of the cluster pattern is dictated only by the cross peak positions and their spread, which are primarily determined by the intrinsic width of the shifting band. Thus, even a slight shift in the band position, as small as 0.5 % of band width, will generate the distinct pattern of butterfly-shaped cluster in 2D correlation spectra, as long as the signal-to-noise ratio is good enough to capture the systematic spectral feature change. In other

words, if you do not see any butterfly pattern in the asynchronous 2D spectrum, the chance is high that there is no band position shift.

#### 4.3.3.3 Band Position Shift Coupled with Intensity Change

Figure 4.10 shows the 2D spectra corresponding to the band position shift in the higher wavenumber direction coupled with some intensity increase, as depicted in Fig. 4.7(C). The cluster pattern in the synchronous spectrum (Fig. 4.10(A)) is no longer four-way symmetric, as one autpeak becomes disproportionately large compared to the other. Such a pattern is sometimes referred to as the *angel* pattern with cross peak wings. A similar angel pattern can also be observed in the case of two overlapped bands with intensity changes, so this pattern cannot be used as an unambiguous indicator for a position shift coupled with an intensity change.

The corresponding asynchronous spectrum (Fig. 4.10(B)) is also distorted from the standard butterfly pattern when the intensity change is coupled with the band position shift. The elongated asynchronous cross peaks near the diagonal are now distributed closer to the stronger autpeak side, i.e., the leg side of the angel. The pair of main cross peaks almost looks as if they represent two distinct bands. Indeed, this case is probably the most difficult one to interpret unambiguously, as it can be easily confused with the two overlapped band case. However, one may notice that the spread of the elongated asynchronous cross peaks near the diagonal actually covers 2D spectral regions of both positive and negative synchronous correlation intensities (i.e., the body and wings of the angel). Thus, the simple sign rules cannot be applied unequivocally to the elongated asynchronous cross peaks. This observation provides a clear indication that these asynchronous cross

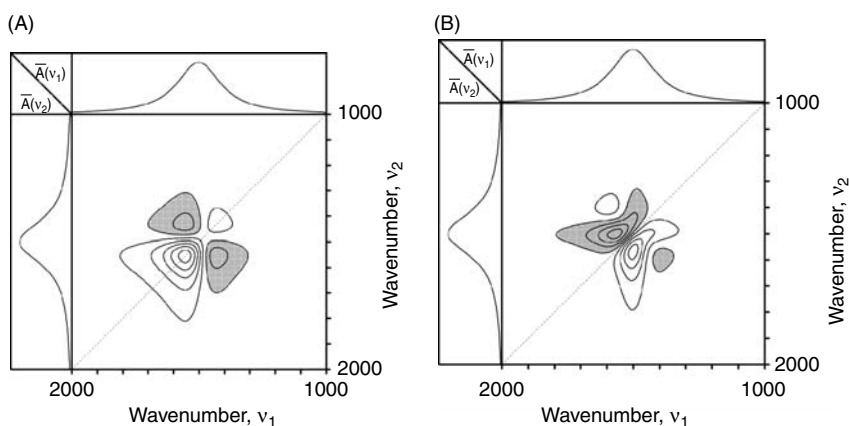


**Figure 4.10** (A) Synchronous and (B) asynchronous 2D correlation spectrum based on a single band simultaneously shifting in position from a lower to a higher wavenumber and increasing in intensity

peaks are generated from a much more complex process than simple intensity changes of two overlapped bands.

Another possible clue indicating that the origin of the peak cluster is a single shifting band with accompanying change in intensities is the presence of the weak secondary pair of cross peaks in the asynchronous spectrum. Unlike the strong elongated cross peaks near the diagonal, the position of the secondary asynchronous cross peaks are not influenced much by the accompanying increase in the intensity of the moving band. Unfortunately, these weak cross peaks are often overlooked because of their low intensity compared to the strong neighboring cross peaks. Appropriate adjustments of the contour level for 2D spectrum plots often reveal the presence of such weak cross peaks.

#### 4.3.3.4 Line Broadening

Figure 4.11 shows the 2D spectra for the broadening of a spectral line, as depicted in Fig. 4.7(D). The synchronous spectrum (Fig. 4.11(A)) shows a characteristic four-way symmetric pattern, consisting of the dominant central autopeak and minor surrounding autopeaks and negative cross peaks. The main autopeak is so strong that other peaks are often overlooked, unless the contour level of the 2D map is adjusted accordingly. The asynchronous spectrum (Fig. 4.11(B)) shows the four-way symmetric cross-like cluster pattern, comprising two positive and two negative cross peaks. Note that the symmetry axes of the 2D peak clusters are aligned with the spectral axes of the 2D correlation spectra, so the pairs are formed between cross peaks in either the vertical or the horizontal direction. Huang *et al.* refer to this pattern as the *rotated four-leaf-clover* pattern.<sup>17,18</sup> Others simply call it the *cross* pattern.

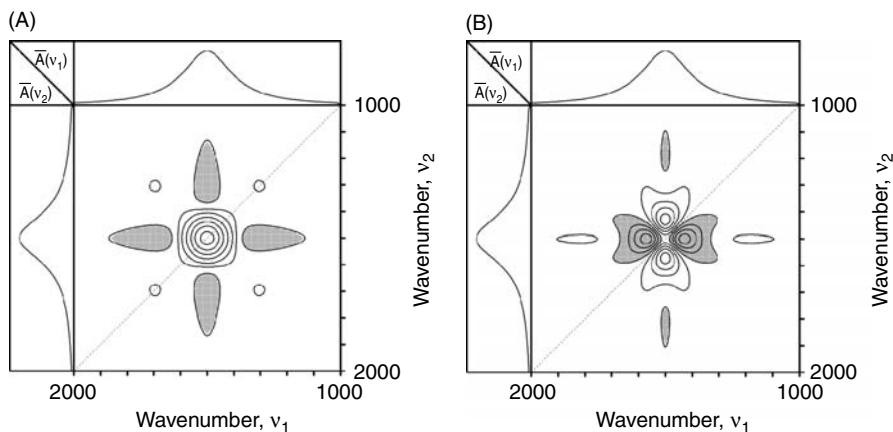


**Figure 4.11** (A) Synchronous and (B) asynchronous 2D correlation spectrum based on the line broadening of a single band



The cluster patterns of 2D spectra corresponding to the line broadening are reasonably distinct and recognizable. However, it is often easy to confuse the 2D peak cluster pattern of a line broadening or narrowing process with the existence of multiple overlapped hidden bands, unless the characteristic pattern is recognized at the beginning of the analysis. The interpretation of 2D spectra for line broadening is again straightforward. If the sign of the vertical pair of the asynchronous cross peaks is positive, or alternatively the horizontal pair is negative, the process is that of line broadening. On the other hand, if the sign of the vertical pair of cross peaks is negative, the process is line narrowing.

Similar to the case of band position shift, the extent of line broadening has little effect on the appearance of the characteristic peak cluster patterns associated with the line broadening. Even a slight amount of change in the line width will result in the development of a cross pattern in 2D spectra. Unlike the band position shift case, however, the resulting 2D cross peaks are much more easily confused with those arising from overlapped multiple bands. The presence of the relatively symmetric vertical pair of asynchronous cross peaks with the same signs, accompanied by the similar horizontal pair with the sign opposite from the vertical pair, will be a good clue to the identification of features associated with changes in the line width.

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## 5 Further Expansion of Generalized Two-dimensional Correlation Spectroscopy – Sample–Sample Correlation and Hybrid Correlation

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We now discuss two relatively new forms of generalized 2D correlation schemes, which extend the original concept of 2D correlation spectroscopy to much broader applications. One is referred to as *sample–sample* 2D correlation spectroscopy,<sup>1,2</sup> and the other is called *hybrid* 2D correlation spectroscopy.<sup>3,4</sup> These two developments are actually very closely related to each other, so it makes sense to treat them together here.

### 5.1 SAMPLE–SAMPLE CORRELATION SPECTROSCOPY

#### 5.1.1 CORRELATION IN ANOTHER DIMENSION

The basic concept of the variant correlation scheme, now commonly known as sample–sample correlation, was first discussed by Zimba in late 1990s.<sup>5</sup> This idea was later refined and reformulated by Šašić *et al.*,<sup>1,2</sup> who carried out most of the subsequent research on this topic.<sup>6,7</sup> Šašić is also credited with coining the term ‘sample–sample’ correlation,<sup>1</sup> as opposed to more conventional variable–variable correlation. In sample–sample correlation, generalized 2D correlation analysis is applied to a set of *transposed* data to yield a new type of 2D spectrum having two *sample* axes. Samples are indicated by the state of a chosen external physical variable, such as concentration, along which the spectral intensities are measured. In the traditional *variable–variable* 2D correlation, the relationship between patterns of spectral band intensity changes along different samples is systematically examined. In sample–sample correlation, on the other hand, the relationship between different samples observed under different states of perturbation is studied by examining the similarity or difference of their spectral trace patterns along the spectral variable.

Although the composition of mixtures is the most often chosen perturbation variable in sample–sample correlation analysis, the applicability of this interesting scheme is not at all limited to a set of spectra obtained from samples

having different compositions. As discussed in previous chapters, data examined by the generalized 2D correlation scheme may be any reasonable set of systematically varying analytical measurement traces, such as time-dependent spectra, temperature-dependent chromatograms, and so on. Therefore, a sample–sample correlation spectrum may be obtained as a plot of correlation intensities mapped on any appropriate 2D perturbation–perturbation plane, such as temperature–temperature or pressure–pressure spectrum. Furthermore, it is natural to extend this concept further to construct *hetero sample–sample* correlation spectra, such as concentration–temperature correlation spectra, when more than one perturbation variables are involved. This extension of sample–sample correlation to multiple perturbation cases forms the basis for the development of the hybrid 2D correlation concept.

### 5.1.2 MATRIX ALGEBRA OUTLOOK OF 2D CORRELATION

The concept of sample–sample correlation is best understood by treating 2D correlation spectra in terms of matrix algebra notation. It can be easily shown that a variable–variable synchronous spectrum may be related to the *covariance matrix* of the observed experimental result, if the sampling measurement is taken at a fixed interval. The expression for a 2D spectrum in terms of vector notation has already been provided in Chapter 3, so it is quite straightforward to express the entire 2D correlation scheme in the form of matrix representation.

Given a set of dynamic spectra  $\tilde{y}(\nu, t)$ , the spectral data matrix  $\mathbf{Y}$  is given as

$$\mathbf{Y} = \begin{bmatrix} \tilde{y}(\nu_1, t_1) & \tilde{y}(\nu_2, t_1) & \cdots & \tilde{y}(\nu_n, t_1) \\ \tilde{y}(\nu_1, t_2) & \tilde{y}(\nu_2, t_2) & \cdots & \tilde{y}(\nu_n, t_2) \\ \cdots & \cdots & \cdots & \cdots \\ \tilde{y}(\nu_1, t_m) & \tilde{y}(\nu_2, t_m) & \cdots & \tilde{y}(\nu_n, t_m) \end{bmatrix} \quad (5.1)$$

This matrix consisting of  $m$  rows of spectral traces (each measured at a different perturbation variable  $t = t_1, t_2, \dots, t_m$ ) with  $n$  columns of spectral intensity variations along the external variable (at a given spectral variable  $\nu = \nu_1, \nu_2, \dots, \nu_n$ ). It is noted that the data matrix  $\mathbf{Y}$  represents dynamic spectra, so the reference spectrum has already being subtracted along the column according to Equation (2.1). The operation is often referred to as *mean-centering* if the average spectrum is used for the reference.

Each column of the matrix  $\mathbf{Y}$  is now being viewed as a vector, as in Equation (3.3), representing the characteristic dynamic change of the spectral intensity at a given spectral variable  $\nu$  along the perturbation axis. To compare these vectors, i.e., to examine the correlations between intensity changes along  $t$  for any particular pair of spectral variables  $\nu_1$  and  $\nu_2$ , the covariance matrix  $\Phi_{\nu\nu}$  is calculated.

$$\Phi_{\nu\nu} = \frac{1}{m-1} \mathbf{Y}^T \mathbf{Y} \quad (5.2)$$

This form of covariance matrix  $\Phi_{vv}$  is a symmetric  $m \times m$  square matrix obtained from the product of data matrix  $\mathbf{Y}^\top \mathbf{Y}$ , sometimes known as the *dispersion product*, scaled by the degree of freedom term  $1/(m - 1)$ . Each element of  $\Phi_{vv}$  represents similarity between a particular pair of intensity variations measured at different wavenumbers. Diagonal elements of the covariance matrix are the autocorrelation or *variance* of data  $\mathbf{Y}$  along  $t$  at a given  $v$ . It is noted that  $\Phi_{vv}$  contains all the features of the synchronous spectrum. In fact, for any data set sampled at a constant increment along the variable  $t$ , the element at the  $i$ th row and  $j$ th column of  $\Phi_{vv}$  becomes mathematically equivalent to the synchronous correlation intensity  $\Phi(v_i, v_j)$  calculated by generalized 2D correlation. In this way, the synchronous spectrum can be considered simply as a table of variance and covariance among vectors of a given dynamic spectral matrix. In a similar manner, the matrix form of an asynchronous spectrum is given by multiplying the data matrix  $\mathbf{Y}$  and the orthogonal counterpart of  $\mathbf{Y}$  obtained by the Hilbert transformation.

$$\Psi_{vv} = \frac{1}{m - 1} \mathbf{Y}^\top \mathbf{N} \mathbf{Y} \quad (5.3)$$

The  $\mathbf{N}$  ( $m \times m$ ) is the *Hilbert–Noda transformation matrix* (Equation 3.9), which by premultiplication provides the expression equivalent to a pertinent segment of the Hilbert transform of each column vector of  $\mathbf{Y}$ .

### 5.1.3 SAMPLE–SAMPLE CORRELATION SPECTRA

The sample–sample correlation is based on a simple exchange (i.e., transposition) of rows and columns of data matrix  $\mathbf{Y}$ , when 2D spectra are calculated by using the above matrix algebra notation. Thus, the corresponding synchronous sample–sample correlation spectrum  $\Phi_{ss}$  is related to the *association product* of data matrix  $\mathbf{Y} \mathbf{Y}^\top$ , which serves in a complementary role to the dispersion product  $\mathbf{Y}^\top \mathbf{Y}$ . In a manner similar to the case of variable–variable correlation, one can obtain the correlation among different sample traces by calculating the covariance of transposed data matrix  $\mathbf{Y}^\top$ .

$$\Phi_{ss} = \frac{1}{n - 1} \mathbf{Y} \mathbf{Y}^\top \quad (5.4)$$

The form of the covariance matrix yields a new type of 2D synchronous correlation spectrum with samples on both axes. Each point in the 2D map represents a correlation between a given pair of sample traces, such as those measured at different concentration or temperature. The covariance matrix given by Equation (5.4) is well suited for analyzing features characteristic to samples, such as concentration dynamics of components, while the previous form given by Equation (5.2) is more convenient for elucidating detailed spectral features. The two types of covariance matrices, thus, represent different but complementary aspects of spectral data and their dependence on the external perturbation.

Closer examination of the structure of association product  $\mathbf{Y}\mathbf{Y}^T$  used in sample–sample correlation provides the insight into how this scheme actually works. Each element of the association product matrix is obtained by calculating the dot (inner) product of two spectral vectors, i.e., different rows of  $\mathbf{Y}$ . In other words, the sample–sample correlation effectively carries out the detection of similarity or collinearity of two spectra. The synchronous sample–sample correlation intensity becomes significant only if the spectra of two samples are similar in shape. If, on the other hand, the spectral shapes of two samples are substantially different,  $\Phi_{ss}$  becomes small. Thus, the underlying concept of synchronous sample–sample correlation is essentially identical to the classical spectral matching method based on the calculation of inner products of spectral vectors.<sup>8</sup> Such a comparison method has been extensively used in spectral library searches.

It is obviously tempting to set the corresponding asynchronous sample–sample correlation spectrum in the form

$$\Psi_{ss} = \frac{1}{n-1} \mathbf{Y} \mathbf{N}_v \mathbf{Y}^T \quad (5.5)$$

The  $n \times n$  matrix  $\mathbf{N}_v$  performs the orthogonal transformation of spectral traces in the wavenumber domain in a manner similar to the Hilbert–Noda transformation matrix  $\mathbf{N}$ . The above equation, at least purely from a mathematical viewpoint, seems like a reasonable extension of the relationships already provided in Equations (5.2)–(5.4). However, unlike the well-established asynchronous wavenumber–wavenumber correlation case, the indiscriminate use of asynchronous sample–sample correlation can potentially be a problematic exercise.

The physical significance of asynchronous sample–sample correlation intensities, especially those generated from a set of optical spectra, is unfortunately not established. In asynchronous sample–sample correlation, one spectrum is converted to the orthogonal form by calculating the Hilbert transform along the wavenumber axis before being cross-correlated against another spectrum. The Hilbert transformation of an absorbance spectrum, which is also commonly known as the Kramers–Kronig transformation in optical spectroscopy, merely produces the corresponding refractive index spectrum. Simplistic comparison of absorbance and refractive index by direct cross-correlation obviously does not carry much interpretable information, other than an indication if two spectra compared are different. For example, Wu *et al.* reported that asynchronous sample–sample correlation spectra they generated for their data did not show any regular feature except fluctuations.<sup>9</sup>

The possible saving utility of asynchronous sample–sample correlation may lie in the fact that asynchronous correlation is often sensitive to the dissimilarity between two spectral traces belonging to different samples. Thus, one can expect the value of  $\Psi_{ss}$  to become small if two spectral traces measured for different samples are nearly matched. The mismatch of spectral patterns usually generates

some intensity for  $\Psi_{ss}$ . Unlike the conventional variable–variable 2D correlation, however, one cannot analyze the sequential order of events directly from the sign of  $\Psi_{ss}$  and  $\Phi_{ss}$ . The sign of  $\Psi_{ss}$  simply indicates which side of the spectral region the mismatch resides for different sample traces. Finally, the fundamental constraint imposed on the structure of dynamic spectra (Equation 2.1), which makes the link between the Hilbert transformation (Equation 2.48) and the generalized 2D correlation function (Equation 2.5) possible via the Wiener–Khinchine theorem, actually does not apply to the wavenumber domain, as implicitly assumed in Equation (5.5). Thus, the mathematical interpretation of  $\Psi_{ss}$ , at least in the form given by Equation (5.5), remains ambiguous from the view point of classical correlation analysis.

In order to emphasize the true dissimilarity of spectral traces associated with individual samples, it is probably more fruitful to consider the construction of sample–sample correlation spectra based on disrelation analysis. The sample–sample disrelation matrix  $\Lambda_{ss}$  is constructed such that the  $i$ th row and  $j$ th column element of the disrelation is given by

$$\Lambda_{ss}(i, j) = \sqrt{\Phi_{ss}(i, i) \cdot \Phi_{ss}(j, j) - \Phi_{ss}^2(i, j)} \quad (5.6)$$

where  $\Phi_{ss}(i, j)$  is the  $i$ th row and  $j$ th column element of the synchronous sample–sample correlation matrix (Equation 5.4). Here, the dissimilarity between two spectral features is most prominently analyzed, more so than asynchronous analysis. Any slight discrepancies between two spectral traces belonging to different samples can be detected by the positive intensity of disrelation spectra.

#### 5.1.4 APPLICATION OF SAMPLE–SAMPLE CORRELATION

In the previous chapter (Section 4.1), we examined the IR spectra of a polymer solution mixture system undergoing spontaneous evaporation of volatile components using the standard variable–variable correlation analysis. Coordinated changes of band intensities arising from the same species were depicted in the synchronous 2D correlation spectrum (Figure 4.3), and the sequential order of the compositional changes was sorted out in the asynchronous spectrum (Figure 4.4). It was found that highly volatile methyl ethyl ketone (MEK) disappears first, followed by the gradual evaporation of deuterated toluene (*d*-toluene), which leads to the accumulation of nonvolatile polystyrene (PS). We now revisit the same solution mixture system, but this time using the sample–sample correlation analysis.

A series of time-dependent IR spectra of the solution mixture undergoing evaporation is presented again in Figure 5.1. Based on this set of spectral data, a sample–sample 2D correlation spectrum (Figure 5.2) is calculated using Equation (5.4). The time-averaged spectrum is used as the reference to obtain so-called mean-centered dynamic spectra. Although this selection of reference is somewhat

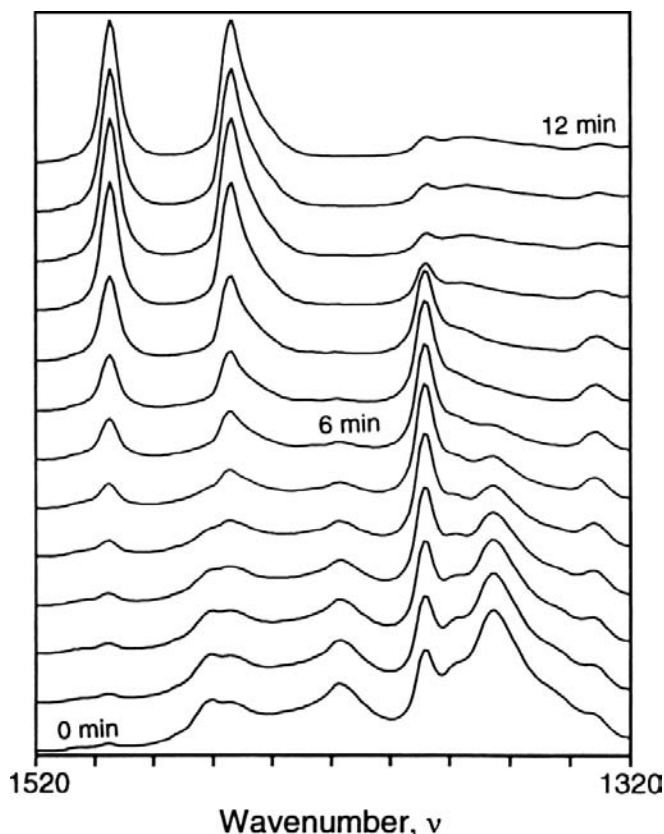


**Figure 5.1** Time-dependent IR spectra of a mixture of MEK, *d*-toluene, and PS during the spontaneous evaporation process

arbitrary, it often brings out more detailed features of sample–sample correlation spectra. Note that axes of the 2D correlation spectrum are no longer in the wavenumber scale as in Figures 4.3 or 4.4 but are replaced by the two orthogonal time axes. The autopower spectrum, which corresponds to the correlation intensity at the main diagonal position, is provided at the top and left side of the 2D spectrum for reference.

In this example (Figure 5.2), the correlation intensity is the highest at the bottom left corner of the 2D spectrum, corresponding to the early stage of the evaporation process. For the first few minutes of the evaporation process, the correlation intensity is high even at the off-diagonal position, as the sample spectra collected during this period all have very similar spectral features dominated by the contribution from MEK. As the evaporation process continues, new spectral features show up with more contributions from *d*-toluene and PS. Thus, the correlation intensity becomes much weaker between the MEK-rich samples collected after only several minutes of evaporation and those after 6 or 7 min with

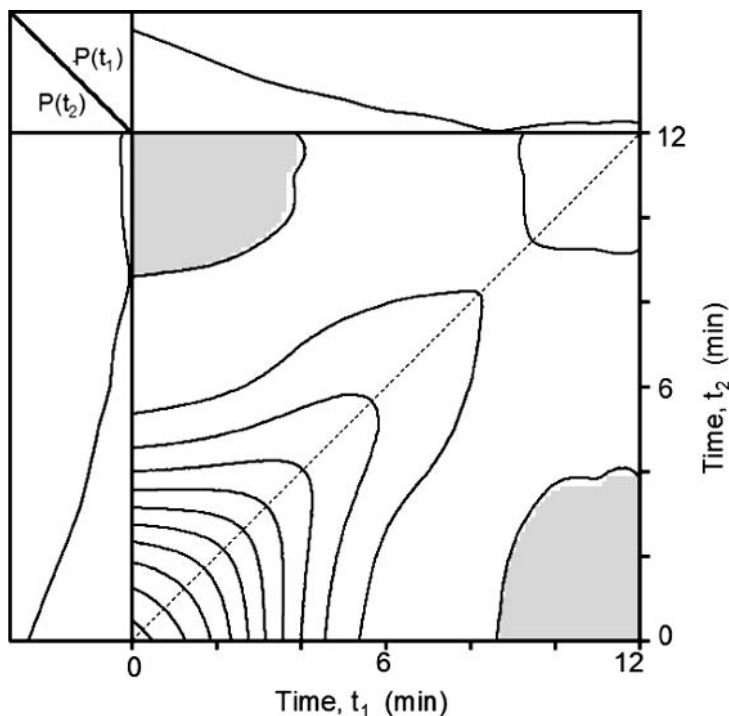


**Figure 5.2** Synchronous sample-sample 2D correlation spectrum for a solution mixture of MEK, *d*-toluene, and PS undergoing evaporation

higher relative content of *d*-toluene. Samples collected after 9 min are negatively correlated with the rest of the region of the 2D spectrum, reflecting the fact that the solvents are mostly gone leaving only the residual PS component with spectral features very different from those of the original solution mixture. As apparent from this example, sample-sample correlation is especially useful in detecting trends or transitions along a series of spectral traces.

There have been increasing numbers of the application of sample-sample correlation. In this book one can find examples of sample-sample correlation spectroscopy in Chapters 7 and 9. The sample-sample correlation has been used for the analysis of temperature-induced NIR changes of oleic acid to detect the existence of two phase transition temperatures (see Section 9.2).<sup>2</sup> Other sample-sample correlation applications include 2D IR and NIR studies of polycondensation reaction of bis(hydroxyethylterephthalate) (see Section 7.2.2),<sup>10</sup> raw milk,<sup>6</sup> and other reaction kinetics studies.<sup>7</sup> Sample-sample correlation was used by Wu *et al.*<sup>9</sup> to study the temperature effect on intermolecular hydrogen bonding for a supramolecular assembly of azobenzene derivatives. Normalized sample-sample correlation for field-induced reorientation dynamics of ferroelectric liquid crystals was reported on the 2D analysis based on polarization angle dependence.<sup>11</sup>



## 5.2 HYBRID 2D CORRELATION SPECTROSCOPY

### 5.2.1 MULTIPLE PERTURBATIONS

Hybrid correlation deals with the 2D correlation analysis between two separately obtained data matrices.<sup>3,4</sup> It may be regarded as one of several useful variants of heterocorrelation methods.<sup>12</sup> Another example of heterocorrelation, heterospectral correlation, has already been discussed in Section 2.2.4. In heterospectral correlation, two sets of data matrices are obtained by making two different types of spectroscopic measurements under the same perturbation. In hybrid correlation, on the other hand, a single type of spectroscopic measurement is usually carried out under multiple perturbation variables. Most importantly, hybrid 2D correlation spectroscopy is often concerned with both variable–variable and sample–sample 2D correlation spectroscopy. If coupled with sample–sample correlation, hybrid correlation furthermore can potentially explore the latent correlation between different perturbation variables.

Suppose spectroscopic measurements are carried out with two independent perturbations (for example, time  $t$  and temperature  $T$ ). The data thus obtained will be a three-way array with the  $(i, j, k)$ th element denoting the spectral value at the  $i$ th time, the  $j$ th temperature and the  $k$ th wavenumber. A reasonable approach to such complex data, for example, would be to keep one of the perturbation variables constant, and apply the standard 2D correlation analysis to the data now reduced to a two-way array matrix. There are many different options for obtaining two-way slices from three-way data arrays, so that one would inevitably end up with multiple data matrices when dealing with this type of data.

### 5.2.2 CORRELATION BETWEEN DATA MATRICES

When two data matrices are obtained under separate perturbation conditions, it becomes possible to carry out the hybrid correlation analysis directly between these matrices.<sup>3</sup> Synchronous variable–variable and sample–sample hybrid 2D correlation spectra may be represented by using the following equations.

$$\Phi_{vv}(v_1, v_2) = \frac{1}{m-1} \mathbf{Y}_1^\top \mathbf{Y}_2 \quad (5.7)$$

$$\Phi_{ss}(s_1, s_2) = \frac{1}{n-1} \mathbf{Y}_1 \mathbf{Y}_2^\top \quad (5.8)$$

The dummy variables  $s_1$  and  $s_2$  represent generic perturbation variables, e.g., time, temperature, or pressure, governing the two separate data matrices  $\mathbf{Y}_1$  and  $\mathbf{Y}_2$ .

For variable–variable correlation (Equation 5.7), the multiplication of  $\mathbf{Y}_1$  and  $\mathbf{Y}_2$  is possible only when the sample number  $m$  in the two experiments is equal. This condition is not always satisfied, so the truncation of one of the data matrices

may become necessary. The multiplication is allowed in many cases for the sample–sample correlation spectra (Equation 5.8), since the spectral range measured for a system under different perturbations can often be selected to be the same. Synchronous sample–sample hybrid correlation  $\Phi_{ss}(s_1, s_2)$  indicates that spectral changes induced by these two different external variables show similar trends. Variable–variable correlation  $\Phi_{vv}(v_1, v_2)$  provides the complementary information, indicating which wavenumber pairs are responding to the two different perturbations in a similar manner.

The corresponding, asynchronous variable–variable and sample–sample 2D correlation spectra may be computed by the following equations.

$$\Psi_{vv}(v_1, v_2) = \frac{1}{m-1} \mathbf{Y}_1^\top \mathbf{N} \mathbf{Y}_2 \quad (5.9)$$

$$\Psi_{ss}(s_1, s_2) = \frac{1}{n-1} \mathbf{Y}_1 \mathbf{N}_v \mathbf{Y}_2^\top \quad (5.10)$$

Caution must be exercised for the possible over-interpretation of asynchronous hybrid correlation as two data matrices compared in hybrid correlation are governed by different external perturbations. Thus, the search for apparent asynchronicity between two independently chosen external effects has little physical basis. Asynchronous hybrid correlation should be used primarily just for the verification of the mismatch of spectral change patterns to assist synchronous correlation.

### 5.2.3 CASE STUDIES

A synchronous sample–sample hybrid 2D correlation spectrum  $\Phi_{ss}(s_1, s_2)$ , which correlates perturbation variables  $s_1$  and  $s_2$ , may take different forms depending on the applications. There may be several interesting cases for the application of hybrid 2D correlation spectra: (1) two separate sets of data obtained under two kinds of perturbations; (2) one set of data obtained under physically coupled perturbation or related perturbations; and (3) two sets of data obtained on the basis of the similar perturbation but under different conditions. Let us consider these cases in more detail.

*Case I.* 2D correlation spectra are calculated by using two independent spectral data sets obtained under two kinds of perturbations; for example, temperature–pressure hybrid 2D correlation spectroscopy. For this example, experimental data collected under temperature variation ( $\mathbf{Y}_T$ ) and pressure variation ( $\mathbf{Y}_P$ ) perturbations can be represented as follows.

$$\mathbf{Y}_1 = \mathbf{Y}_T \quad (5.11)$$

$$\mathbf{Y}_2 = \mathbf{Y}_P \quad (5.12)$$

The hybrid sample–sample correlation  $\Phi_{ss}(T, P)$  now represents the correspondence between temperature effect and pressure effect, which produces similar

results on the spectral intensity changes, for example, during a phase transition process.

*Case II.* 2D correlation spectra are generated by using one spectral data set obtained under conjugated or related perturbations. Hybrid 2D correlation of time- and temperature-dependent spectral modifications, where temperature  $T$  is varied as a function of time  $t$ , is an example. In this case, one can represent the experimental data as follows.

$$\mathbf{Y}_1 = \mathbf{Y}_t \quad (5.13)$$

$$\mathbf{Y}_2 = \mathbf{Y}_{t,T} \quad (5.14)$$

The hybrid sample–sample correlation  $\Phi_{ss}(t, T)$  may be used to map out the time dependence of temperature change by using the spectral pattern as a latent indicator.

*Case III.* 2D correlation spectra are constructed by using two spectral data sets obtained based on the same type of perturbation but under different conditions. For example, hybrid 2D correlation spectra of similar chemical reactions using different catalysts belong to this class. For this case the experimental matrix can be represented as follows.

$$\mathbf{Y}_1 = \mathbf{Y}_{t1} \quad (5.15)$$

$$\mathbf{Y}_2 = \mathbf{Y}_{t2} \quad (5.16)$$

Both data matrices contain time-dependent spectra, but their time dependence will be different. The correlation  $\Phi_{ss}(t_1, t_2)$  obtained for two chemical reactions may, for example, compare the equivalent state of reaction process for two different rates.

### 5.3 ADDITIONAL REMARKS

Sample–sample correlation and hybrid 2D correlation are very interesting examples of a generalized 2D correlation scheme directed toward applications beyond the regular boundary of conventional spectroscopic analysis. In both cases, spectral intensity variations are used only as a form of latent variables, implicitly relating other physical variables of interest. These soft modeling techniques relying heavily on inferences are most effectively utilized in conjunction with concrete scientific reasoning and physical reality. Over-interpretation of correlation results without sound justification should be carefully avoided.

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## 6 Additional Developments in Two-dimensional Correlation Spectroscopy – Statistical Treatments, Global Phase Maps, and Chemometrics

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In this chapter some additional developments pertinent to generalized 2D correlation spectroscopy are discussed. The basic idea of generalized 2D correlation spectroscopy is intimately related to various well-known quantities found in classical statistical analysis, such as coherence, correlation coefficient, standard deviation, and covariance matrix. A combination of some of the statistical measures leads to a parameter called the correlation phase angle. This concept is further extended to the development of global 2D phase maps. Another useful development in 2D correlation spectroscopy is the incorporation of multivariate chemometrics techniques. An example of combining factor analysis and 2D correlation is presented.

### 6.1 CLASSICAL STATISTICAL TREATMENTS AND 2D SPECTROSCOPY

The basic concept of generalized 2D correlation spectroscopy originally evolved from the classical time-series analysis of transient variations of spectral signal intensities measured during dynamic IR experiments.<sup>1,2</sup> Therefore, the evolution of 2D correlation spectroscopy is from the beginning based on a sound foundation of statistical theory that could be exploited further to make additional conceptual developments.<sup>3</sup> It is natural to revisit the standard statistical treatment of spectral data set to position ourselves for further discussion.<sup>4</sup>

#### 6.1.1 VARIANCE, COVARIANCE, AND CORRELATION COEFFICIENT

As already mentioned in Chapter 2, the correlation intensity at the diagonal position of a synchronous 2D correlation spectrum corresponds to the maximum autocorrelation of the dynamic fluctuation of spectral intensity signals with

respect to time. Thus, the correlation intensity along the diagonal line is nothing but a power spectrum of signal fluctuations (deviations) around the time-averaged reference (or estimate of expected value). In other words, the first statistical interpretation of 2D correlation intensities is that autotopics along the diagonal represent the *variance* of spectral signal fluctuations sampled over a fixed period of time at a given wavenumber point.

One can, of course, easily extend this statistical interpretation to cross peaks located at off-diagonal positions that compare the intensities of fluctuating signals at two different spectral variables,  $\nu_1$  and  $\nu_2$ . The calculation of 2D correlation intensities at different wavenumbers is equivalent to the computation of the *covariance* between the spectral intensity fluctuations measured at  $\nu_1$  and  $\nu_2$ . Therefore, a synchronous correlation spectrum may be regarded as a continuous form of a variance–covariance matrix having an infinite number of matrix elements, each element corresponding to the correlation intensity at some incremental coordinates of the 2D map. Unlike an ordinary variance–covariance matrix, however, the neighboring points of a synchronous 2D spectrum are highly correlated.

Now, one can compute the *correlation coefficient*  $\rho(\nu_1, \nu_2)$  by using the standard formula of variance and covariance.

$$\rho(\nu_1, \nu_2) = \frac{\Phi(\nu_1, \nu_2)}{\sqrt{\Phi(\nu_1, \nu_1) \cdot \Phi(\nu_2, \nu_2)}} \quad (6.1)$$

Note that the above expression is equivalent to the form used in the 2D correlation spectroscopy proposed by Barton II *et al.*<sup>5</sup> (We shall describe the details of 2D correlation spectroscopy by Barton II *et al.* in Chapter 7.)

A continuous 2D map of correlation coefficients is conceptually attractive, since it contains the purest form of normalized correlation information with no interference by the magnitude of signal fluctuations. On the other hand, the direct mapping of the continuous 2D correlation coefficients often suffers from two practical limitations. One is that such a map often looks excessively noisy, especially in the spectral region where the signal level is small, due to the artificially amplified nonzero value of the correlation coefficient. The other is that the lack of spectral intensity information exhibited by the constant value of unity along the diagonal line skews the overall shape of correlation maps. In spite of these limitations, the use of the 2D correlation coefficients for further analysis holds intrinsic potential, as the interfering effect of signal magnitude is effectively eliminated by the normalization.

### 6.1.2 INTERPRETATION OF 2D DISRELATION SPECTRUM

The use of a heuristic disrelation spectrum  $\Lambda(\nu_1, \nu_2)$  is an excellent substitution for an asynchronous spectrum  $\Psi(\nu_1, \nu_2)$  for many practical applications. We can provide a simple interpretation of this quantity based on a classical statistical theory.

For a given 2D correlation coefficient  $\rho(v_1, v_2)$ , one can consider a complementary quantity called the *disrelation coefficient*  $\zeta(v_1, v_2)$ , such that

$$\rho^2(v_1, v_2) + \zeta^2(v_1, v_2) = 1 \quad (6.2)$$

The synchronous and disrelation spectra,  $\Phi(v_1, v_2)$  and  $\Lambda(v_1, v_2)$ , then become

$$\Phi(v_1, v_2) = \rho(v_1, v_2) \sqrt{\Phi(v_1, v_1) \cdot \Phi(v_2, v_2)} \quad (6.3)$$

$$\Lambda(v_1, v_2) = \zeta(v_1, v_2) \sqrt{\Phi(v_1, v_1) \cdot \Phi(v_2, v_2)} \quad (6.4)$$

Note that the term  $[\Phi(v_1, v_1) \cdot \Phi(v_2, v_2)]^{\frac{1}{2}}$  is simply a product of *standard deviations* of spectral intensity fluctuations over time measured independently at  $v_1$  and  $v_2$ . This quantity corresponds to the magnitude of the total variance of two signals. The total *joint variance* may be expressed in a compact form using a complex number notation.

$$\Phi(v_1, v_2) + i\Lambda(v_1, v_2) = \sqrt{\Phi(v_1, v_1) \cdot \Phi(v_2, v_2)} [\rho(v_1, v_2) + i\zeta(v_1, v_2)] \quad (6.5)$$

Multiplying the above expression by its complex conjugate, one obtains the defining equation of disrelation spectrum discussed in Chapter 2.

$$\Phi^2(v_1, v_2) + \Lambda^2(v_1, v_2) = \Phi(v_1, v_1) \cdot \Phi(v_2, v_2) \quad (2.49)$$

The above result provides a simple statistical interpretation of the disrelation intensity. It is the portion of the total joint variance of signal fluctuations measured at  $v_1$  and  $v_2$ , which is not accounted for by the *covariance*. In other words, it corresponds to the *disvariance* component of the total variance, where fluctuations are not coincidental with each other but occurring separately in time, i.e., asynchronously.

### 6.1.3 COHERENCE AND CORRELATION PHASE ANGLE

The importance of relative phase in the 2D correlation is well documented in Noda's original paper on 2D correlation spectroscopy for sinusoidal systems.<sup>1</sup> The discussion on the idea of utilizing exclusively the relative phase relationship separated from magnitude is given by Müller *et al.*<sup>6</sup> A benefit of such a relative phase concept was that one can cancel amplitude information. Noda expanded the idea of relative phase to nonperiodic systems in generalized 2D correlation spectroscopy and named it as a *global correlation phase angle*.<sup>4</sup> The global correlation phase angle  $\Theta(v_1, v_2)$  between two fluctuating signals is represented by

$$\tan \Theta(v_1, v_2) = \Psi(v_1, v_2) / \Phi(v_1, v_2) \quad (6.6)$$

Thus, the global phase angle is directly related to the *coherence* of signals, i.e., the ratio between the asynchronous and synchronous 2D correlation intensity. For



a simple pair of sinusoidally varying spectral intensities, the correlation phase angle is identical to the actual phase difference between the two signals. For signals having more complex waveforms, however, the global correlation phase angle loses the physically interpretable meaning of a simple phase shift. It still serves as a convenient quantitative index to sort out the sequence of events. In general,  $\Theta(\nu_1, \nu_2) > 0$  is observed if the spectral intensity changes at  $\nu_1$  occur before those at  $\nu_2$ . On the other hand,  $\Theta(\nu_1, \nu_2) < 0$  will be the result if the sequence is reversed.

One can easily recognize that the correlation coefficient and correlation phase angle are intimately related by

$$\rho(\nu_1, \nu_2) = \cos \Theta(\nu_1, \nu_2) \quad (6.7)$$

For sinusoidal signals, one also has

$$\zeta(\nu_1, \nu_2) = \sin \Theta(\nu_1, \nu_2) \quad (6.8)$$

For signals having more complex waveforms, however, the above simple relationship between the disrelation coefficient and global correlation phase no longer holds, so each quantity has to be obtained separately from the asynchronous and disrelation spectra. By linking the correlation phase angle with the correlation coefficient, one can separate the distinct contributing factors to the 2D correlation intensity, which can be further manipulated to improve the discriminating power of the 2D correlation spectrum. Buchet *et al.*<sup>7</sup> presented an idea similar to the global phase angle for discriminating physically meaningful correlation peaks from artifactual correlation peaks. Morita *et al.*<sup>8,9</sup> demonstrated the practical usage of the global phase map by proposing a filtering scheme based on the standard deviation spectrum of dynamic spectra (see Section 6.2).

#### 6.1.4 CORRELATION ENHANCEMENT

Inspection of Equation (6.3) suggests that the synchronous 2D correlation intensity is separated into two distinct contributing factors: the correlation coefficient  $\rho(\nu_1, \nu_2)$ , which represents the normalized extent of signal coherence, and the term related to the magnitude of total intensity variation  $[\Phi(\nu_1, \nu_1) \cdot \Phi(\nu_2, \nu_2)]^{1/2}$ . This separation offers the intriguing possibility of artificially enhancing the discriminating power of the correlation intensity by simply raising the correlation coefficient to a higher power as  $\rho^\# = \rho^{2n+1}(\nu_1, \nu_2)$ .<sup>4</sup> For large  $n$ , only very strongly correlated signal pairs will have significant value for  $\rho^\#$ . Thus, the correlation enhancement factor  $K_n$  and a modified synchronous 2D spectrum  $\Phi^\#(\nu_1, \nu_2)$  with enhanced discriminating power are given by

$$K_n = \left( \frac{\Phi^2(\nu_1, \nu_2)}{\Phi(\nu_1, \nu_1) \cdot \Phi(\nu_2, \nu_2)} \right)^n \quad (6.9)$$

and

$$\Phi^\#(v_1, v_2) = K_n \Phi(v_1, v_2) \quad (6.10)$$

## 6.2 GLOBAL 2D PHASE MAPS

### 6.2.1 FURTHER DISCUSSION ON GLOBAL PHASE

We now start the detailed discussion of global phase with a simple case of correlating two sinusoidally varying signals first. The sinusoidal responses with the period of  $T$  at the spectral frequencies of  $v_1$  and  $v_2$  are represented by the following equations

$$y_1 = a(v_1) \cos[2\pi t/T + \theta_1] + b(v_1) \quad (6.11)$$

$$y_2 = a(v_2) \cos[2\pi t/T + \theta_2] + b(v_2) \quad (6.12)$$

where  $a(v_j)$ ,  $t$ ,  $\theta_j$ , and  $b(v_j)$  are amplitude, time, time lag, and offset value of the sinusoidal responses at a given spectral frequency of  $v_j$ , respectively.<sup>8,9</sup> The synchronous and asynchronous correlation intensities for sinusoidal systems can then be written as<sup>1</sup>

$$\Phi(v_1, v_2) = \frac{1}{2}a(v_1)a(v_2) \cos(\theta_1 - \theta_2) \quad (6.13)$$

$$\Psi(v_1, v_2) = \frac{1}{2}a(v_1)a(v_2) \sin(\theta_1 - \theta_2) \quad (6.14)$$

To combine these correlation functions with a concept of correlation phase angle, normalized synchronous and asynchronous functions are introduced. In sinusoidal systems, these amplitudes,  $a(v_1)$  and  $a(v_2)$ , can be regarded as radii of assumed uniform circular motions of response vectors on a complex phase plane derived from the sinusoidal responses. Thus, the normalized synchronous and asynchronous functions can be calculated as

$$\Phi_n(v_1, v_2) = \frac{\Phi(v_1, v_2)}{\frac{1}{2}a(v_1)a(v_2)} = \cos(\theta_1 - \theta_2) \quad (6.15)$$

$$\Psi_n(v_1, v_2) = \frac{\Psi(v_1, v_2)}{\frac{1}{2}a(v_1)a(v_2)} = \sin(\theta_1 - \theta_2) \quad (6.16)$$

These representations of  $\Phi_n(v_1, v_2)$  and  $\Psi_n(v_1, v_2)$  are a straightforward extension of the basic concept of 2D correlation. However, from this seemingly simple result, it becomes obvious to consider the importance of a fundamental concept intrinsic to the 2D correlation: the concept of phase angle. The phase difference between the two responses at the frequencies,  $v_1$  and  $v_2$  can be determined as follows:

$$\Theta(v_1, v_2) = \arctan \left\{ \frac{\Psi_n(v_1, v_2)}{\Phi_n(v_1, v_2)} \right\} = \theta_1 - \theta_2 \quad (6.17)$$

Thus, it is found that one can obtain  $\Phi_n(\nu_1, \nu_2)$ ,  $\Psi_n(\nu_1, \nu_2)$ , and  $\Theta_n(\nu_1, \nu_2)$  with the simple normalization of amplitude from Equations (6.15), (6.16), and (6.17). However, this process of obtaining  $\Theta(\nu_1, \nu_2)$  is not straightforward. The following process of calculating  $\Theta(\nu_1, \nu_2)$  may be effective as will be discussed later:

$$\Theta(\nu_1, \nu_2) = \arctan \left\{ \frac{\Psi(\nu_1, \nu_2)}{\Phi(\nu_1, \nu_2)} \right\} \quad (6.18)$$

Equation (6.18) implies that  $\Phi(\nu_1, \nu_2)$  and  $\Psi(\nu_1, \nu_2)$  with rather complicated amplitude information can be directly reduced to  $\Theta(\nu_1, \nu_2)$  without any conscious normalization of amplitude.

The above result can be readily extended to the case for nonperiodic signals. For nonperiodic signals having complicated waveforms, the global correlation phase angle no longer has any simple physical meaning such as a fixed time delay. Rather it will serve as the ensemble average of the phase delay effects of multiple frequency responses. However, it still serves as a convenient index to estimate similarity between the two nonperiodic signals, as long as the signal of interest varies monotonically with respect to time. This is the case for most of our analysis.

### 6.2.2 PHASE MAP WITH A BLINDING FILTER

The major reason why a practical application of global 2D phase maps has not been successfully carried out is the appearance of unintentionally enhanced noise, especially, in the baseline region. The cancellation of amplitude information results in the exaggeration of the noise contribution in some parts of the global 2D phase map. To generate a more information-rich global 2D phase map, the use of a standard deviation spectrum was proposed as a convenient basis for developing a noise filter.

Following Equation (6.18), a global 2D phase map can be calculated as follows,

$$\Theta(\nu_1, \nu_2) = \begin{cases} \arctan \left\{ \frac{\Psi(\nu_1, \nu_2)}{\Phi(\nu_1, \nu_2)} \right\} & \text{if } \sqrt{\Phi(\nu_1, \nu_1)} > \delta \text{ and } \sqrt{\Phi(\nu_2, \nu_2)} > \delta \\ \text{blind (i.e. not defined)} & \text{otherwise} \end{cases} \quad (6.19)$$

where  $\delta$  is a small threshold value of mathematical blinding filter. Here, the parameter  $\delta$  is selected as the standard deviation of the signal measured at wavenumber  $\nu$ . Note that the standard deviation spectrum  $SD(\nu)$  is given by the square root of  $\Phi(\nu, \nu)$ . The standard deviation spectrum filter is conservative such that the potentially artifactual influence from the contribution of any weak signal regions will be rejected. This approach has the merit of pragmatic simplicity and clarity, which should secure the robustness of operation.

### 6.2.3 SIMULATION STUDY

Let us explain the procedure for calculating a global phase map from the generalized 2D synchronous and asynchronous correlation maps by using simulated spectra in Figure 6.1.<sup>8</sup> In Figure 6.1 three bands, **A**, **B**, and **C**, exhibit exponential decay along the time axis. In this simulation a Gaussian form is assumed to represent the band shapes of these bands. The bands are then represented by

$$y_j(v, t) = I_j \exp \left\{ \frac{(v - m_j)^2}{s^2} \right\}^{-1} \exp(-k_j t) \quad j = A, B, \text{ and } C \quad (6.20)$$

where  $I_j$  is the initial value of peak maximum intensity,  $m_j$  is the peak maximum position, and  $k_j$  is the characteristic rate constant of decay process. For bands **A**, **B**, and **C**, the initial values of peak maximum intensity and the rate constants were set as 1.0, 0.20, and 0.50, and 0.1, 0.2, 0.1, respectively. It is noted that  $I_j$  represents the scaling factor to determine only the amplitude, while  $k_j$  reflects exclusively the exponential decaying waveform of the band independent of the amplitude. A uniformly distributed random noise was added to any intensity variation  $y_j(v, t)$  whose standard deviation was about 0.001.

Figure 6.2(A) and (B) depicts synchronous and asynchronous 2D correlation maps calculated by the conventional method, respectively.<sup>8</sup> It is noted that these

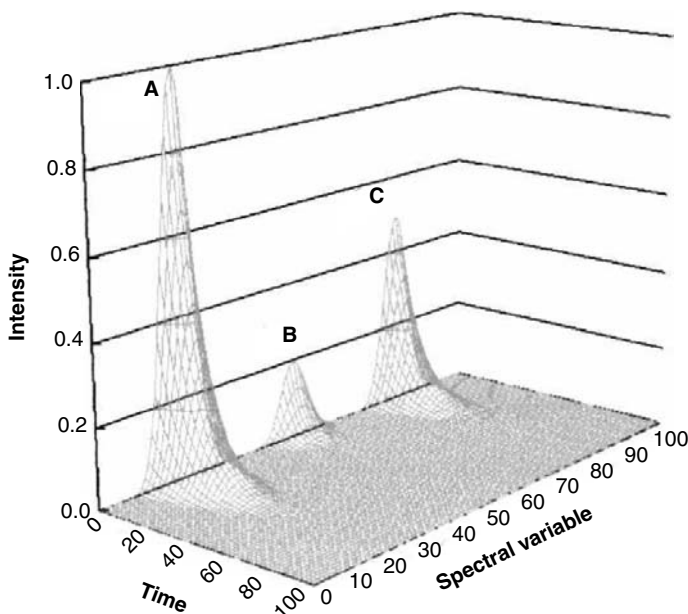


**Figure 6.1** Simulated spectra for calculating a phase map from the generalized 2D correlation spectra. The three bands have a Gaussian form and show exponential decay along the time axis

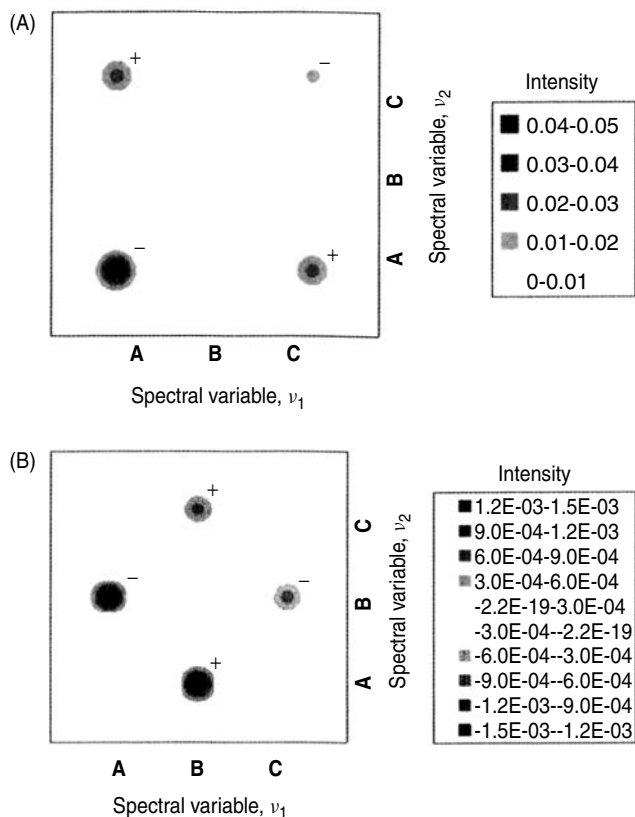


**Figure 6.2** (A) Synchronous and (B) asynchronous 2D correlation spectra calculated by the conventional method

two maps contain the amplitude information. Therefore, the detailed quantitative feature of nonlinear responses are found in the asynchronous map. Actually in Figure 6.2(A), none of the synchronous correlation peaks corresponding to band **B** are detected due to the small amplitude effect of band **B**. Moreover, it is impossible to discuss the significance of peak heights in both the synchronous and the asynchronous maps. To calculate a global 2D phase map masked with Equation (6.19), the standard derivation spectrum was calculated as shown in Figure 6.3.<sup>8</sup> Then, finally the global 2D phase map was obtained as shown in Figure 6.4.<sup>8</sup>

Compared to conventional 2D correlation maps, it is much more straightforward to extract pure correlation information from a global 2D phase map. The global 2D phase map in Figure 6.4 provides the real correlation information of time-dependent behavior without being obscured by any amplitude influence. Degrees were used as a unit of global phase angle. Note that the masked region, determined by the blinding condition, is distinguished from the correlated region

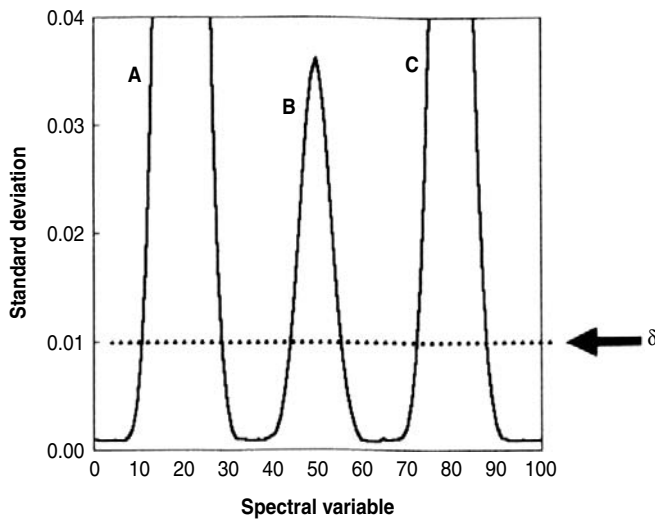


**Figure 6.3** Calculated standard deviation spectrum. The parameter  $\delta$  is set as shown in this figure

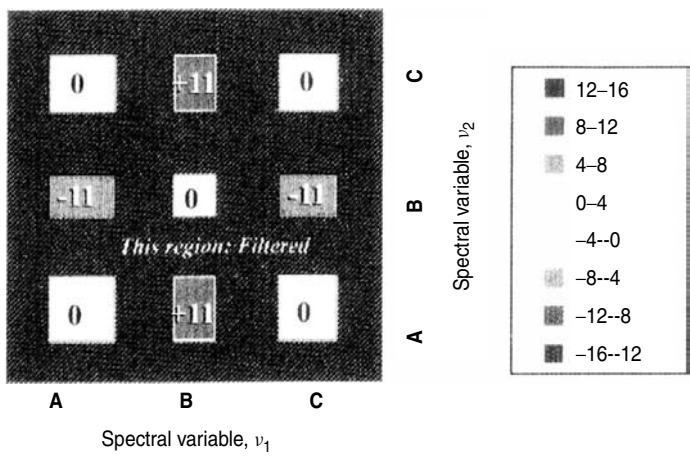


**Figure 6.4** Global 2D phase map

as not having a defined value. For a nonperiodic system, the asynchronous axis does not usually present any tangible physical meaning beyond mathematical projection. For the specific example used here, where simple exponential decays are compared, it is possible to combine the global phase angle with the characteristic rate constants. The mathematical description of the synchronous and asynchronous functions for the exponential decays were already reported.<sup>10</sup> In general, it is impossible to discuss the rigorously quantitative meaning of values on the global phase angle intensities aside from certain specialized cases.

However, one can still use the global phase angle as a convenient semiquantitative index to detect the nature of nonlinear responses.

Morita and Ozaki<sup>11</sup> have recently demonstrated the potential of global 2D phase mapping as a pattern recognition method for distinguishing between band shifting, overlapping, and broadening. In order to obtain the distinct patterns, the concept of scaling by the ‘assumed perturbation’ of spectra has been proposed. After such pretreatment, the global 2D phase map method yields the titling of a correlation plane, four flat terraces, and an asymmetric curved plane with respect to the diagonal line for the band shifting, overlapping, and broadening, respectively.

### 6.3 CHEMOMETRICS AND 2D CORRELATION SPECTROSCOPY

In this section, a potentially fruitful combination of multivariate chemometrics techniques and generalized 2D correlation spectroscopy is discussed. *Chemometrics* is defined as the chemical discipline that uses mathematical and statistical methods to design or select optimal measurement procedures and experiments and to provide maximum chemical information by analyzing chemical data.<sup>12–14</sup> Various tools of chemometrics have become popular in the field of spectroscopic studies, and it seems useful to extend the application to 2D correlation. As a specific example, the use of a reconstructed data matrix based on the *principal component analysis* (PCA) is discussed.

#### 6.3.1 COMPARISON BETWEEN CHEMOMETRICS AND 2D CORRELATION

It is interesting first to compare some similarities and differences between various techniques used in chemometrics and generalized 2D correlation spectroscopy. While both chemometrics and 2D correlation share the same mathematical operations based on the manipulation of data using standard matrix algebra, each tends to focus on somewhat different aspects of spectral data structure. For example, conventional chemometric techniques often treat each spectrum of a data set as a whole entity defined within some chosen spectral region and represent them as linear combinations of a set of representative loading vectors. In contrast, 2D correlation treatment historically dealt with the dynamics of local spectral features, such as individual peaks and bands. Thus, 2D correlation spectroscopy and chemometrics are often used as complementary but essentially independent data analysis techniques.<sup>15–19</sup>

Some direct comparisons between features of outcomes from chemometrics and 2D correlation analyses have also been made.<sup>15,16</sup> For example, it has been pointed out that the autopower spectrum located at the diagonal position of a synchronous 2D correlation spectrum often resembles the first PCA loading vector of mean-centered data. Likewise, some similarity was noted between certain

slices of asynchronous 2D correlation spectra and the second loading vector of mean-centered data. There have also been some more detailed discussions about the similarity and difference between the two approaches, but the extent of discussion has been limited to the analysis of synchronous spectra.

Truly meaningful comparison between 2D correlation spectroscopy and chemometrics must eventually delve into the fundamental concept of asynchronicity, which is so central to the practice of 2D correlation analysis. Evolving factor analysis (EFA) and related techniques,<sup>20,21</sup> for example, may carry some conceptual commonality with 2D correlation analysis. Both techniques place emphasis on the order and sequence of spectral data collection. However, there still exist significant differences in the fundamental approach to exploiting the sequentially ordered spectral data set. Future development of chemometrics techniques capable of sorting out the sequential order of spectral signal changes would certainly bring the two fields much closer.

### 6.3.2 FACTOR ANALYSIS

In a well-established chemometrics technique of *factor analysis*, one decomposes the original spectral data matrix into a set of a small number of underlying *factors*, often expressed as a product of score and loading vector matrices.<sup>12–14</sup> Factors are separated into a significant set representing the linear combinations of spectral contributions of actual components of interest and remaining factors dominated by noise or contributions insignificant to the analysis. The basic hypothesis of factor analysis is that the improved proxy of the original data matrix can be reconstructed from only a limited number of significant factors. Three direct practical outcomes are: (1) determination of the number of components (i.e., number of significant factors) involved in the description of data matrix; (2) rejection of noise and insignificant information by discarding the interfering factors; and (3) reduction of the information into a compact set of factors.

We will explore a simple example for a combination of a chemometrics technique and 2D correlation by using an abstract factor analysis known as *principal component analysis* (PCA).<sup>22</sup> This technique is especially well suited for the identification of the number of factors governing the data structure and also for effective identification and rejection of noise components from a raw spectral data set prior to 2D correlation analysis. The latter feature is especially interesting for asynchronous 2D correlation analysis. Asynchronous spectra based on noisy raw data are often contaminated by artifactual peaks, which are attributed to the fortuitous correlation of noise. The reconstructed data matrix derived only from the significant factors significantly reduces this problem.

### 6.3.3 PRINCIPAL COMPONENT ANALYSIS (PCA)

Let us consider a raw data matrix **A**, comprising the original set of perturbation-induced dynamic spectra, to be an  $n \times m$  matrix with  $n$  dynamic spectra and



$m$  wavenumber points. The loading vector matrix  $\mathbf{V}$  is an  $m \times r$  matrix, where each column is the loading (i.e., the eigenvector of the *dispersion matrix*  $\mathbf{A}^T \mathbf{A}$ ) obtained by PCA. Here,  $\mathbf{A}^T$  stands for the transpose of  $\mathbf{A}$ . It should be pointed out that the dispersion matrix is proportional to the covariance matrix,  $1/(n-1) \mathbf{A}^T \mathbf{A}$ , which in turn is equivalent to the discrete case of a synchronous 2D correlation spectrum. The total number of loading vectors  $r$  selected for the analysis must be less than or equal to  $n$ . It is customary to normalize each column of  $\mathbf{V}$  (i.e., each loading), such that the product  $\mathbf{V}^T \mathbf{V}$  is an identity matrix. Associated with the PCA loading vectors are the scores (sometime called latent variables). The score matrix,  $\mathbf{W} = \mathbf{A} \mathbf{V}$ , is a relatively small  $n \times r$  matrix.

The basic idea of PCA is that the significant part of the data matrix can be expressed as the product of dominant score and loading vectors matrices

$$\mathbf{A} = \mathbf{W} \mathbf{V}^T + \mathbf{E} = \mathbf{A}^* + \mathbf{E} \quad (6.21)$$

where  $\mathbf{E}$  is the residual matrix often associated with noise. Thus, the matrix product  $\mathbf{A}^*$  can be regarded as the noise-suppressed reconstructed data matrix of the raw data matrix  $\mathbf{A}$ .

$$\mathbf{A}^* = \mathbf{W} \mathbf{V}^T \quad (6.22)$$

This reconstructed data set  $\mathbf{A}^*$ , instead of the raw data  $\mathbf{A}$ , may be used for the 2D correlation analysis to minimize the effect of noise.<sup>22</sup> A similar concept of noise reduction by using PCA as a filter to reconstruct less noisy data was mentioned by Gillete and Koenig.<sup>23</sup>

### 6.3.4 NUMBER OF PRINCIPAL FACTORS

The selection of the number of significant principal factors representing the complexity of the data matrix is an important consideration, and there are ample methods in the field of chemometrics to estimate the optimal number of factors.<sup>12-14</sup> The number of factors, which reflects the rank of the data matrix for dynamic spectra, also plays an important role in 2D correlation analysis. If the total number of significant factors is only one (i.e., rank-one data matrix), we are dealing with a trivial case of a collection of spectral data essentially comprising the same spectrum component with some noise. Such a data matrix will yield a strictly synchronous response if 2D correlation analysis is applied. All the spectral features will vary in a well-coordinated fashion such that there will be no discernible difference in the sequential order of changes (i.e., asynchronicity) among detectable spectral intensities.

Typically, however, the number of factors needed to describe a dynamic spectral data set will be more than one. Most interestingly, any spectral data set having the raw matrix rank of three or more, and thus requiring more than two factors for description in addition to the average spectrum, may have an asynchronous 2D

correlation spectrum with significant features. Any strictly synchronous system, on the other hand, can be adequately and completely described by the linear combination of only two independent spectra. Thus, the number of significant eigenvalues (or number of principal components) deduced from classical chemometrics analysis will immediately tell us about the presence or lack of asynchronicity and thus the potential benefit of constructing an asynchronous 2D correlation spectrum.

### 6.3.5 PCA-RECONSTRUCTED SPECTRA

One of the important benefits derived from chemometric analysis combined with 2D correlation is the ability to rationally reject the irrelevant information and interfering noise in the spectral data. Thus, the quality of 2D correlation spectra may be improved by introducing the advantage of chemometrics to the analysis. The 2D correlation analysis of a PCA-reconstructed data matrix, for example, should selectively accentuate the most important features of synchronicity and asynchronicity without being hampered by noise.

Figure 6.5(A) shows a set of spectra corrected during the evaporation of solvents from a mixture of methyl ethyl ketone (MEK), deuterium-substituted toluene, and polystyrene. To accentuate the effect of noise, additional synthetic noise was injected to create simulated very noisy raw data. The noisy spectral data set was decomposed into the scores and loading vectors by the standard PCA

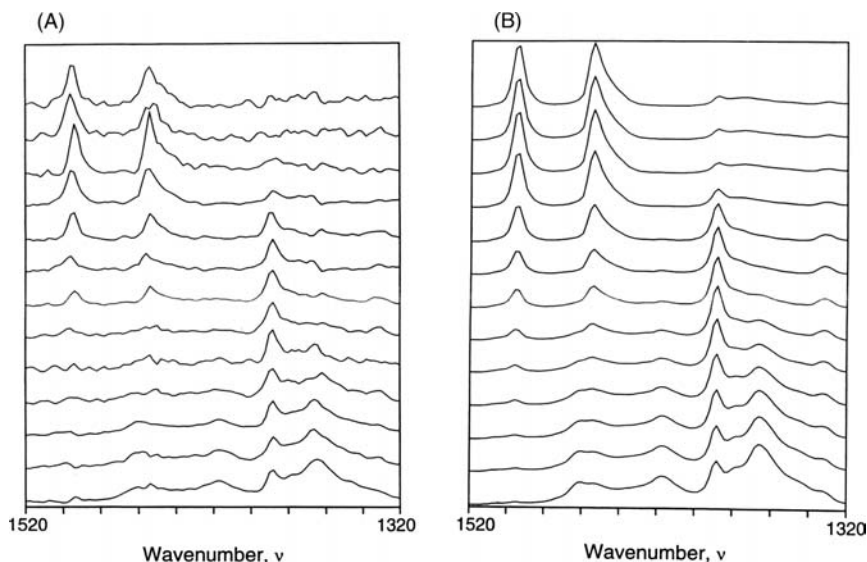


**Figure 6.5** Time-dependent spectra of a solution mixture of methyl ethyl ketone, deuterium-substituted toluene, and polystyrene during the solvent evaporation: (a) raw data with additional synthetic noise; (b) PCA-reconstructed data with three principal components

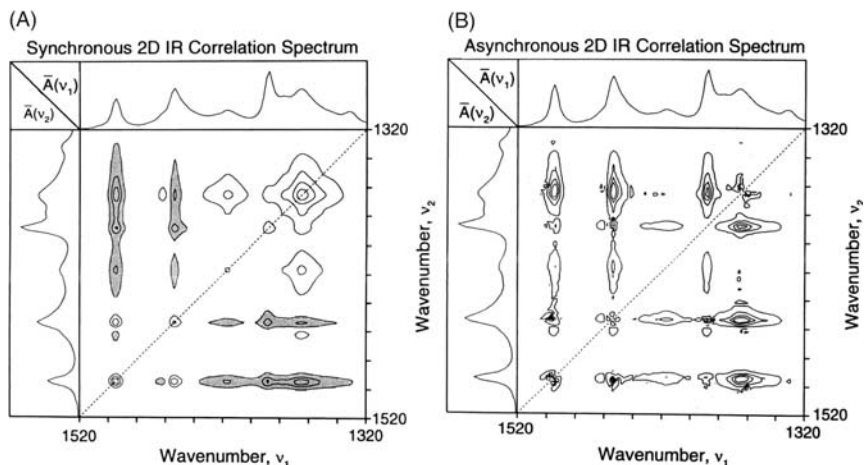


**Figure 6.6** 2D correlation spectra derived from the noisy data shown in Figure 6.5(a): (A) synchronous spectrum; (B) asynchronous spectrum

method. Several factors are derived from the raw data set to capture the pertinent information contained in the original noisy spectra. Figure 6.5(B) depicts spectra of the PCA-reconstructed data represented as  $\mathbf{A}^*$  (Equation 6.22) from the first three dominant PC factors. It is apparent that the reconstructed spectra contain a substantially lower level of noise.

Figure 6.6(A) and (B) shows synchronous and asynchronous 2D correlation spectra constructed directly from the noisy dynamic spectra (Figure 6.5(A)). While the synchronous spectrum (Figure 6.6(A)) shows relatively little effect of noise, the corresponding asynchronous spectrum (Figure 6.6(B)) is noticeably contaminated with numerous speckles of superfluous correlation intensities spikes, clearly caused by the unintended correlation of noise contributions.

Figure 6.7(A) and (B) displays synchronous and asynchronous 2D correlation spectra derived from the PCA-reconstructed data. The main features of the 2D correlation spectra are all well preserved without distortion, and peaks are present in a manner similar to those directly obtained from the original data but with much less effect of noise. The effect of noise suppression is not so obvious in the synchronous spectrum (Figure 6.7(A)), but the improvement is dramatic in the case of the asynchronous spectrum (Figure 6.7(B)). Noise-induced speckles observed in Figure 6.6(B) are virtually all removed. It is thus shown that we can successfully obtain noise-suppressed 2D correlation spectra by reconstructing dynamic spectra using PCA factors.

If we decrease the number of PCs below three, more changes are observed for the resulting 2D correlation spectra. Eventually, the 2D correlation spectra display the feature as if the system is behaving more or less synchronously. We can clearly create less noisy 2D spectra by simply using a smaller number of PCs. However, since we are discarding some real information from the raw data,

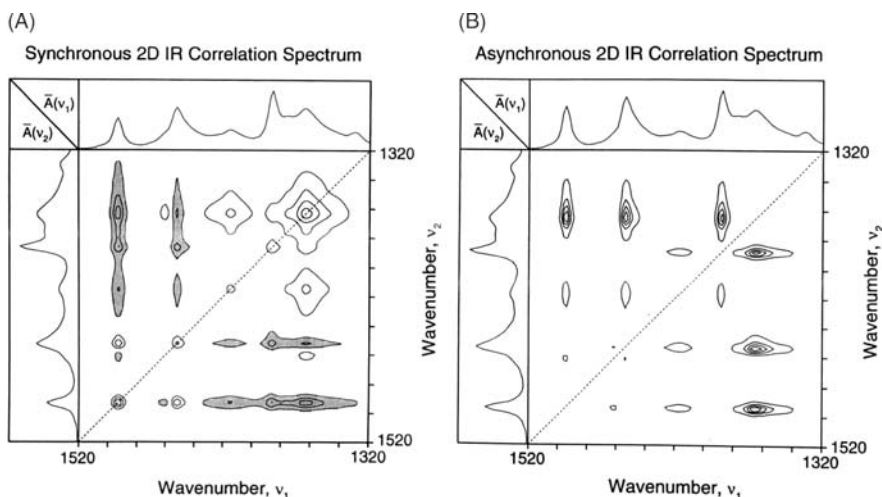


**Figure 6.7** 2D correlation spectra derived from the PCA-reconstructed data shown in Figure 6.5(b): (A) synchronous spectrum; (B) asynchronous spectrum

we may experience a certain loss of subtle feature from 2D correlation spectra. It is noted that the interpretation of such simplified 2D spectra is often much easier. In the extreme, the asynchronicity of dynamic spectra is preserved even for a data matrix comprising only two principal components. This result is consistent with the expectation from the basic 2D correlation theory, which requires only the existence of nominal nonlinearity in the sample dimension to exhibit detectable asynchronicity.

### 6.3.6 EIGENVALUE MANIPULATING TRANSFORMATION (EMT)

We introduced the idea of using a reconstructed data matrix from scores and loading vectors obtained by applying PCA to raw data before 2D correlation analysis. The 2D correlation analysis of such a reconstructed data matrix should more effectively accentuate the most important features of synchronicity and asynchronicity without being hampered by noise or insignificant minor components. We now explore another PCA-based chemometrics technique useful for 2D correlation analysis, known as *eigenvalue manipulation transformation* (EMT).<sup>24,25</sup>

It is well known that the matrix product  $\mathbf{L} = \mathbf{W}^T \mathbf{W}$ , obtained from the PCA score  $\mathbf{W}$ , is a diagonal matrix where each diagonal element corresponds to the eigenvalue of a principal component. The eigenvalues are all positive real numbers and arranged in descending order of magnitude. It is often useful to express the score matrix in the form  $\mathbf{W} = \mathbf{U}\mathbf{S}$ . Here the square matrix  $\mathbf{S} = \mathbf{L}^{1/2}$  is another diagonal matrix, where the diagonal elements are now the positive square roots of eigenvalues, or singular values. The orthonormal matrix  $\mathbf{U}$  can be obtained from  $\mathbf{W}$  by normalizing each column of  $\mathbf{W}$ . Alternatively, the matrix  $\mathbf{U}$  can be obtained

directly from the original data matrix  $\mathbf{A}$ , since columns of  $\mathbf{U}$  correspond to the eigenvectors of the so-called *association matrix*  $\mathbf{A}\mathbf{A}^\top$ . It is noted here that the association matrix is proportional to the sample–sample correlation synchronous 2D correlation spectrum.

By combining the above results, the reconstructed data matrix  $\mathbf{A}^*$  can now be expressed in the familiar form known as the singular value decomposition (SVD)<sup>12–14</sup>,

$$\mathbf{A}^* = \mathbf{U}\mathbf{S}\mathbf{V}^\top \quad (6.23)$$

As we discussed earlier, the separation of the original data  $\mathbf{A}$  into a significant portion of the data  $\mathbf{A}^*$  and residual noise  $\mathbf{E}$  is somewhat arbitrary, and determined by choosing exactly how many eigenvectors one would like to use to reconstruct  $\mathbf{A}^*$  to represent the original data  $\mathbf{A}$ . One can, for example, employ a very large number of eigenvectors to reconstruct  $\mathbf{A}^*$ , but such additional eigenvectors often tend to contain no useful information but noise, and the corresponding eigenvalues are very small.

The singular values are arranged in the order of their size, so the first singular value, located at the upper left corner of  $\mathbf{S}$ , is the largest. As we move down the rows and columns of  $\mathbf{S}$ , the diagonal elements become smaller. If we replace smaller diagonal elements of  $\mathbf{S}$  beyond a select point with zero, the contribution of the corresponding eigenvectors from  $\mathbf{U}$  and  $\mathbf{V}$  to the reconstruction of  $\mathbf{A}^*$  disappears. In other words, replacement of minor eigenvalues or singular values with zero is equivalent to the truncation of minor factors consisting mainly of noise contribution.

The above realization that the commonly practiced PCA-based noise truncation can be regarded as an intentional manipulation or replacement of certain (in this case, smaller magnitude) eigenvalues, leads to the idea designed to further transform a data set to extract useful information. The concept of *eigenvalue manipulation transformation* (EMT) of spectral data involves the systematic substitution of individual eigenvalues associated with the original data set. This process will generate a new set of transformed data with a very different emphasis placed on specific information content.

For our first attempt of producing a new reconstructed data matrix by EMT, we calculate a modified diagonal matrix  $\mathbf{S}^q$  where the diagonal elements are now given by raising the corresponding singular values from  $\mathbf{S}$  by the power of  $q$ , where  $q$  is a real number. Thus, the new data matrix will be obtained by

$$\mathbf{A}^{**} = \mathbf{U}\mathbf{S}^q\mathbf{V}^\top \quad (6.24)$$

We can then construct a new 2D correlation spectra based on this new transformed data matrix  $\mathbf{A}^{**}$  obtained by replacing eigenvalues according to Equation (6.24) instead of the usual PCA-reconstructed data matrix  $\mathbf{A}^*$ .

In general, the parameter  $q$  has the effect of changing the emphasis placed by different PC factors. If  $q$  is much larger than 1, the dominant PC factor of

the data matrix will be accentuated at the expense of the contributions from minor factors. This operation will result in the effective suppression of noise often encoded in the minor factors. However, the subtle asynchronous nature of dynamic spectra will also be lost, as the major factors and finally only a single factor will dominate the structure of the data matrix, as  $q$  becomes large. In the extreme, the EMT-reconstructed data with a very large  $q$  will yield only the synchronous correlation.

On the other hand, if the value of  $q$  becomes much smaller than 1, the contribution of minor factors to the reconstructed data becomes important. Such minor factors associated with small eigenvalues contain the fine asynchronous structure of dynamic spectra. Thus, by accentuating the minor factors, much more interesting asynchronous relationships between various spectral signals become apparent. Lowering the value for parameter  $q$  also brings out the contribution of noise from minor factors. In order to minimize this effect, one has to limit the number of PC factors to reconstruct the data matrix to the minimum. As the parameter  $q$  becomes very small, and eventually near zero, the diagonal matrix  $\mathbf{S}^q$  is essentially replaced by the identity matrix  $\mathbf{I}$ . In this case, the EMT-based discrete synchronous 2D spectrum becomes  $1/(n-1)\mathbf{V}^T\mathbf{V}$  instead of the original form  $1/(n-1)\mathbf{A}^T\mathbf{A}$  calculated from the original data. Note the matrix  $\mathbf{V}^T\mathbf{V}$  is the projection matrix spanning the principal factor space.

Figure 6.8 shows synchronous 2D correlation spectra derived from the EMT-reconstructed data  $\mathbf{A}^{**}$  using Equation (6.24) where the parameter  $q$  is set to be  $\frac{1}{2}$  for Figure 6.8(A) and zero for Figure 6.8(B). The PCA-reconstructed data (Figure 6.5b) with three factors was used prior to the EMT operation to minimize the effect of over accentuating the noise contribution. It is apparent that

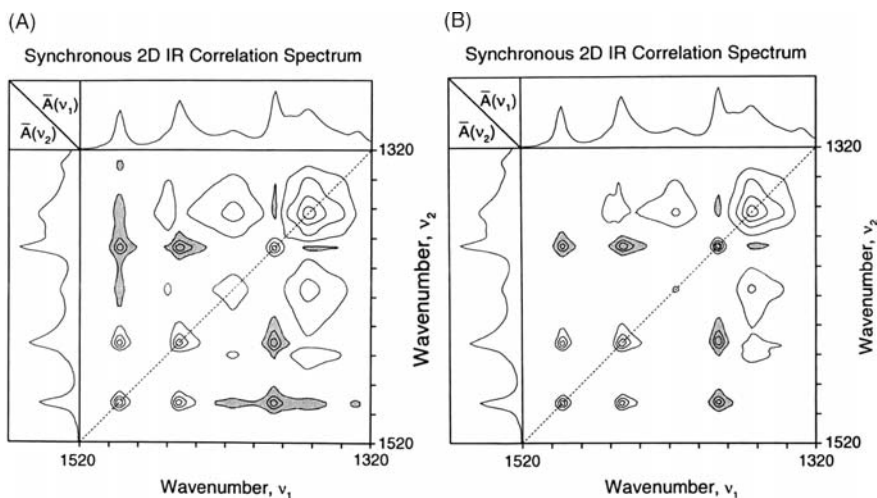


**Figure 6.8** Synchronous 2D correlation spectra obtained from the EMT-reconstructed data  $\mathbf{A}^{**} = \mathbf{US}^q\mathbf{V}^T$ : (a)  $q = 0.5$ ; (b)  $q = 0$

the reduced value of  $q$  results in a noticeable change in the appearance of the synchronous 2D correlation spectrum. Features associated with small variations (i.e., lower intensity autopeaks) in the original 2D spectrum (Figure 6.7(A)) are now accentuated much more so in the EMT-based 2D spectra.

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## 7 Other Types of Two-dimensional Spectroscopy

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As we have already noted earlier, there have been many attempts with varying degrees of success to develop different types of 2D correlation spectroscopy schemes utilizing various forms of optical spectroscopic probes, such as IR, NIR and Raman. These efforts may be currently classified into two extensive but distinct streams of major scientific research activities. One such stream is concerned with the development of nonlinear optical 2D spectroscopy techniques based on some pulsed laser excitations.<sup>1–7</sup> The advancement in the field is creating an exciting possible link between optical spectroscopy and well-established multiple pulse approaches adopted by 2D NMR.<sup>8</sup> There are many reviews written on this type of optical 2D spectroscopy,<sup>9–14</sup> so we will only briefly discuss this subject in this chapter.

The second stream, which is more relevant to the theme of this book, has its origin in a simple correlation scheme introduced in late 1980s to the analysis of dynamic IR spectra obtained under a sinusoidal perturbation.<sup>15–18</sup> Chapter 8 discusses numerous examples of such dynamic 2D IR spectroscopy and related topics. Another form of 2D correlation technique, utilizing well-known statistical tools, soon appeared for the analysis of different types of spectral data.<sup>19–21</sup> Such statistical and chemometrical techniques are still active parts of the 2D correlation spectroscopy field.<sup>22–36</sup> The early development of statistical 2D correlation,<sup>20,21</sup> which demonstrated an important departure from the original dynamic 2D IR method bound by sinusoidal waveforms, led to the introduction of the formal generalized 2D correlation concept in 1993.<sup>37</sup> We will examine some of the ideas not fully explored in Chapter 6 behind the development of the statistical 2D correlation methods.<sup>21–24,32–36</sup>

Many attempts have also been made to greatly refine the original 2D correlation method. We will discuss some of the interesting variant form of 2D correlation methods. Moving window 2D correlation introduced by Richardson *et al.*,<sup>38</sup> based on the idea of segmenting data matrix into small subsets, was further utilized by Šašić *et al.*<sup>39,40</sup> Model-based 2D correlation methods, such as those introduced by Dluhy *et al.*<sup>43–45</sup> or that of Eads and Noda,<sup>46</sup> are also emerging as a new and powerful alternative to the conventional 2D correlation analysis.



## 7.1 NONLINEAR OPTICAL 2D SPECTROSCOPY

Only a brief mention is made here on the newly emerging field of nonlinear optical 2D spectroscopy techniques based on ultrafast femtosecond laser pulses.<sup>1-7</sup> These techniques, including various nonlinear vibrational spectroscopic methods such as nonlinear 2D Raman and 2D IR measurements, utilize a combination of multiple short optical pulses, often with prescribed delay times among them defining additional time scales. Multiple time-domain data thus collected may be converted to 2D frequency domain plots. As such, they are conceptually much closer to original 2D experiments developed in NMR field based on the use of radio frequency pulses.<sup>8</sup>

### 7.1.1 ULTRAFAST LASER PULSES

Modern progress in laser technology has made it possible to produce experimentally accessible ultrafast optical pulses in the time scale of femtosecond ranges. The ultrafast impulsive excitations of such pulses are actually shorter than the vibrational periods or dephasing time scales of molecules. Measurements on such a short time scale have also enabled researchers to probe real-time dynamics of molecules in a complex and mutually interacting system such as a liquid solution. Third order nonresonant Raman spectroscopy based on the threefold interactions of visible laser pulses, for example, is now routinely employed in the study of the vibrational mode of solutions. Higher order nonlinear experiments, such as fifth order nonresonant Raman measurements, may even be extended to multi-dimensional optical spectroscopy experiments using the delay time between the optical pulses as the second independent time variable in the study. Such multiple time experiments will lead to measurements physically analogous to those in the conventional 2D NMR spectroscopy.

The theoretical foundation for the possibility of carrying out a multidimensional nonlinear optical experiment was laid out by the classic paper published by Tanimura and Mukamel.<sup>1</sup> They showed, among other things, the possibility of differentiating between homogeneous and inhomogeneous vibrational line broadening if a fifth order, instead of a third order, nonresonant spectroscopy experiment is carried out. Following the publication of this direction-setting theoretical paper, Tominaga and Yoshihara reported the first measurement of fifth order signals detected from the intermolecular low frequency vibrational mode of a CS<sub>2</sub> solution using ultrafast nonresonant six-wave mixing with five different pulses.<sup>2</sup> This development was followed by the other experimental work of Steffen and Duppen.<sup>3</sup> Tokmakoff and Flemming soon reported the first 2D Raman spectral map of a nonlinear optical measurement.<sup>4</sup> Thus, the era of nonlinear optical 2D spectroscopy based on ultrafast laser pulses started.

The experimental setups for ultrafast multiple laser pulse experiments are not trivial to construct. Ordinary spectroscopic instrumentations found in conventional laboratories, such as a commercial FT IR instrument equipped with a

Michelson interferometer, are not suitable for such measurements. This requirement tends to limit the activities in this field to a few specialized academic laboratories with access to ultrafast laser pulses. No routine commercial instrumentation is currently available for nonlinear optical 2D spectroscopy measurements. New experimental developments, including the incorporation of the heterodyne detection method,<sup>5</sup> are obviously helpful in the advancement of the field, while other major difficulties in this class of measurements such as the effect of third order cascades persist. The definitive demonstration of the utility of nonlinear optical 2D spectroscopy techniques, not only in scientifically significant research work but also in practical applications of general interest, will undoubtedly accelerate the broader activities in this field. The field of nonlinear optical 2D spectroscopy is still very rapidly evolving. The discussion on the direction of the development, as well as the potential impact on broad optical spectroscopy in general, is well beyond the scope or intent of this book. Readers are directed to some of the review articles describing the recent progress in this field.<sup>9–14</sup>

### 7.1.2 COMPARISON WITH GENERALIZED 2D CORRELATION SPECTROSCOPY

Conceptually, ultrafast laser pulse based nonlinear optical 2D spectroscopy techniques are, in a sense, true optical analogues of earlier two-dimensional experimental methods developed in the field of NMR.<sup>8</sup> Plurality of time scales is incorporated directly into the optical measurement by the use of multiple light frequencies or intervals of laser pulses. The evolution of population changes of a given vibrational state and the relaxation of the coherence state or phase are probed and analyzed in a manner parallel to the procedures used in 2D NMR.

The generalized 2D correlation spectroscopy discussed throughout this book, on the other hand, employs a very different approach. The multidimensional nature of generalized 2D correlation analysis arises from the use of an external perturbation from an independent source and time scale, which usually does not have the same magnitude of the ultrafast time scale of the frequency of light or pulse intervals. The time scales of the external perturbations are usually restricted to those of much slower processes of chemical interactions involving multiple atoms and nuclei and even larger submolecular functional groups. In other words, the experimental scope of nonlinear optical 2D spectroscopy and that of generalized 2D correlation spectroscopy are substantially different from the start.

The apparent similarity of plotting spectral intensities along two independent spectral variable axes suggests the possible commonality of the two techniques. Such fortuitous visual similarity, however, may be quite misleading, as the origins of 2D peaks are based on fundamentally different treatments of physical observations in those experiments. Ironically, research activities in both nonlinear optical 2D spectroscopy and generalized 2D correlation spectroscopy started around the same time,<sup>1,15</sup> and consequently the terminologies, such as 2D IR

or 2D Raman spectroscopy, are used extensively among research groups from both sides, but referring to very different techniques. In this book, *2D correlation spectroscopy* refers to our basic method of analyzing a set of dynamic spectra obtained under an external perturbation.

### 7.1.3 OVERLAP BETWEEN GENERALIZED 2D CORRELATION AND NONLINEAR SPECTROSCOPY

Some fundamental differences clearly exist between nonlinear optical 2D spectroscopy and generalized 2D correlation spectroscopy, as pointed out above. The former followed the fruitful path already defined by the success of 2D NMR spectroscopy, while the latter had to venture the uncharted path of coupling conventional spectroscopic measurements with an external physical perturbation to yield 2D correlation spectra. The two fields seem so far apart in the experimental design that one may prematurely conclude that no overlap should exist between the two except for the superficial similarity of the 2D display schemes used for the final construction of spectral maps. Actually, there are some possibilities where the two fields can substantially overlap and even merge to create useful experimental methods.

It was pointed out earlier in the book that the generalized 2D correlation scheme is truly a universally applicable technique without any restriction to the type, waveform, and most importantly *time scale* of the experiment. Thus, it is possible to apply the basic concept of generalized 2D correlation directly to the analysis of data obtained in an ultrafast laser pulse experiment. Pulsed experiments usually consist of a train of multiple pulses to excite the system. The combination of different pulse sequences yields a set of signals encoded with multiple time scales defined by the intervals between pulses and the duration after the (usually the last) probe pulse. If one considers the entire train of exciting pulses as a set of external perturbations, the set of spectral signals thus collected can be treated as a dynamic spectrum in a manner familiar to generalized 2D correlation spectroscopy. Thus, any sets of nonlinear optical data obtained under multiple pulse conditions may potentially be analyzed by the same formal treatment used in generalized 2D correlation spectroscopy.

The key difference in the approaches lies only at the final stage of the data conversion. In conventional pulse-based 2D spectroscopy, the 2D frequency domain spectra are usually obtained by the double Fourier transformation of multiple time domain data. In generalized 2D correlation, on the other hand, the conversion of at least one of the time domain axes to the frequency domain will be carried out not by the straightforward Fourier transformation but by the generalized 2D correlation function (Equation 2.5). In other words, the same pulse-based experimental measurement can actually yield both types of 2D spectra, depending on the specific mathematical conversion method (i.e., double FT and generalized correlation) of time domain variations emphasizing different aspects of information gathered by the experiment.

It is also important to point out that the concept of asynchronous correlation, which occupies the central position in generalized 2D correlation spectroscopy, does not at this point have the equivalent counterpart in double FT-based 2D spectroscopy. The introduction of asynchronous 2D correlation analysis to pulse-based nonlinear optical experiments should provide an exciting new possibility of extracting more information from the data. While this type of application of generalized 2D correlation in pulsed experiments has already been successfully implemented in the field of 2D NMR,<sup>46</sup> the same is not yet true for nonlinear optical spectroscopy. The possible limitation here is that, unlike the 2D NMR counterpart, nonlinear optical spectroscopy is a relatively young and also highly specialized field with an obvious need for further experimental and theoretical development. As the field matures, the implementation of generalized 2D correlation analysis even in ultrafast laser pulse-based experiments may become possible.

## 7.2 STATISTICAL 2D CORRELATION SPECTROSCOPY

2D correlation spectroscopy methods based purely on classical statistical analysis have been known for some time. For example, the technique of *2D covariance mapping* was applied to time-of-flight mass spectrometry data by Frasinski *et al.*<sup>19</sup> and also to IR analysis of detergent samples by Marcott *et al.*<sup>20</sup> Statistical 2D correlation spectroscopy based on *correlation coefficient mapping* was first proposed by Barton II *et al.*<sup>21,22</sup> A similar technique was also used by Šašić and coworkers.<sup>23,24</sup> A method based on the *analysis of variance* (ANOVA) by Barros *et al.*<sup>25</sup> is another example of statistical 2D correlation.

The chemometric approach to 2D correlation has also been popular. Windig *et al.*<sup>26</sup> introduced the partial least squares chemometrics concept to 2D correlation coefficient mapping. Sample–sample correlation promoted by Šašić *et al.*<sup>27,28</sup> and hybrid correlation introduced by Wu *et al.*<sup>29,30</sup> have already been examined in Chapter 5. The effective combination of 2D correlation with various chemometrics techniques, such as PCA-2D by Jung *et al.*,<sup>31</sup> has also been discussed earlier in Chapter 6. These statistical 2D techniques are now becoming a part of the mainstream 2D correlation analysis.

### 7.2.1 STATISTICAL 2D CORRELATION BY BARTON II *et al.*

The 2D correlation method proposed originally by Barton II *et al.* employs cross-correlation based on the least-squares linear regression analysis to assess spectral changes in two regions, such as the NIR and mid-IR regions, that arise from variations in sample composition.<sup>21,22</sup> The simple idea of statistical 2D correlation, not restricted to sinusoidally varying dynamic signals, has provided an important clue to the development of a generalized 2D correlation spectroscopy scheme.

The statistical 2D correlation of Barton II *et al.* starts with the computation of Pearson's product-moment correlation coefficient. The correlation coefficient  $r$  between two sets of values  $\mathbf{x}\{x_1, x_2, \dots, x_m\}$  and  $\mathbf{y}\{y_1, y_2, \dots, y_m\}$  is given by

$$r = \frac{\{m \sum xy - (\sum x)(\sum y)\}}{\left[\left\{m \sum x^2 - (\sum x)^2\right\} \left\{m \sum y^2 - (\sum y)^2\right\}\right]} \quad (7.1)$$

where  $\mathbf{x}$  is an array of  $m$  measurements being regressed on an array  $\mathbf{y}$ , also containing the  $m$  corresponding measurements. This equation provides a single correlation coefficient for the linear regression of the line described by the  $\mathbf{x}$ - $\mathbf{y}$  pairs in the two arrays. This process can be readily extended into another dimension by regressing the columns of two separate matrices with the same number of rows, but different numbers of columns. Let us consider two data matrices  $\mathbf{X}$  and  $\mathbf{Y}$  given by

$$\mathbf{X} = \begin{bmatrix} x_{11} & x_{12} & \cdots & x_{1n_x} \\ x_{21} & x_{22} & \cdots & x_{2n_x} \\ \cdots & \cdots & \cdots & \cdots \\ x_{m1} & x_{m2} & \cdots & x_{mn_x} \end{bmatrix} \quad (7.2)$$

and

$$\mathbf{Y} = \begin{bmatrix} y_{11} & y_{12} & \cdots & y_{1n_y} \\ y_{21} & y_{22} & \cdots & y_{2n_y} \\ \cdots & \cdots & \cdots & \cdots \\ y_{m1} & y_{m2} & \cdots & y_{mn_y} \end{bmatrix} \quad (7.3)$$

The matrices  $\mathbf{X}$  and  $\mathbf{Y}$  may, for example, be regarded as an  $m \times n_x$  matrix of IR measurements and an  $m \times n_y$  matrix of corresponding NIR measurements, respectively. Here,  $m$  represents the number of spectra in each matrix, while  $n_x$  and  $n_y$  are, respectively, the numbers of data points in the IR and NIR spectra. A column of the  $\mathbf{X}$  or  $\mathbf{Y}$  matrix represents the spectral responses at a single wavelength in the IR or NIR. The matrices have the same number of elements in each column ( $m$ ), so that a column of  $\mathbf{X}$  can be regressed upon any column of  $\mathbf{Y}$  to calculate a correlation coefficient. Regressing all possible combinations of the columns of  $\mathbf{X}$  against the columns of  $\mathbf{Y}$  creates a new  $n_x \times n_y$  matrix  $\mathbf{R}$ , which contains the correlation coefficient for each combination, as

$$\mathbf{R}(i, j) = \frac{n \sum x_j y_i - (\sum x_j)(\sum y_i)}{\left(\left[n \sum x_j^2 - (\sum x_j)^2\right] \left[n \sum y_i^2 - (\sum y_i)^2\right]\right)} \quad (7.4)$$

The subscripts  $i$  and  $j$  are the column indices of the  $\mathbf{X}$  and  $\mathbf{Y}$  matrices ranging from 1 to  $n_x$  and 1 to  $n_y$ , respectively. They also indicate the corresponding element in the  $\mathbf{R}$  matrix of correlation coefficients. Therefore, the calculated matrix element  $\mathbf{R}(i, j)$  represents the correlation coefficient of the IR responses

at wavenumber  $i$  with the NIR responses at wavelength  $j$ . One can construct the correlation coefficient spectrum of all IR wavenumbers with the individual NIR wavelength at index  $i$  by extracting a single row  $i$  from the  $\mathbf{R}$  matrix. Likewise, by extracting a single column  $j$  from the  $\mathbf{R}$  matrix one can obtain the correlation spectrum of all NIR wavelengths with the individual IR wavenumber at index  $j$ .

Figure 7.1 shows an example of a 2D contour map plot of the NIR versus mid-IR against the coefficient of determination ( $r^2$ ), generated from the spectra of complex agricultural samples that differ in wax (cuticle), carbohydrate, protein, and lignin content.<sup>21</sup> One-dimensional IR and NIR spectra of the samples are placed on the top and right side of the contour map, respectively. A high  $r^2$  value at a point of the map indicates the presence of a strong correlation between mid-IR and NIR band intensities. It is noted that  $r^2$  at 2130 nm (0.5) increases across the OH and CH stretching band regions of the IR spectra from about  $3700\text{ cm}^{-1}$  to a maximum at  $2915\text{--}2850\text{ cm}^{-1}$ ; then it decreases down to the minimum (0.1) around  $1850\text{ cm}^{-1}$ . Although the OH stretching bands are broader and stronger in the spectra (see the top spectrum in Figure 7.1), the C–H stretching bands are correlated more intensely. The fingerprint region of the IR spectra yields similar results, but the overall correlations are smaller below

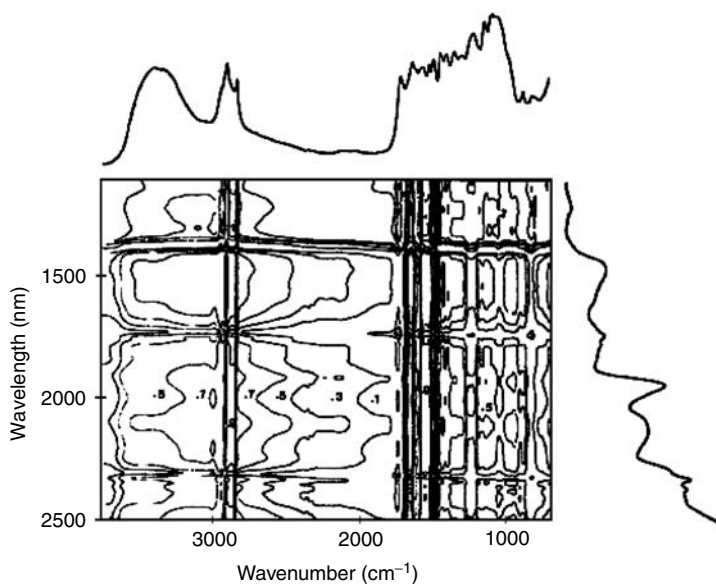


**Figure 7.1** Contour map plot of the NIR versus mid-IR against the coefficient of determination ( $R^2$ ). The numerals on the contours are  $R^2$  rounded to the nearest tenth. The map in this figure is a broad-range map which depicts the general shape of the correlation over the entire regions (NIR and IR). The number contours in this case is 5, which shows the effects without appearing overly 'busy'. The  $R^2$  values for the contours are 0.1, 0.3, 0.5, 0.7 and 0.9, respectively (Reproduced with permission from F.E. Barton II *et al.*, *Appl. Spectrosc.*, 46, 420 (1992) (Ref. 21). Copyright (1992) Society for Applied Spectroscopy.)

1500  $\text{cm}^{-1}$ . The NIR regions where most correlation activity is seen are 1385, 1730, and 2200–2450 nm. The IR regions correlating with the above regions are the 2900–2700  $\text{cm}^{-1}$ , 1700–1500  $\text{cm}^{-1}$ , and 1100–800  $\text{cm}^{-1}$  regions. These IR regions correspond to those for CH stretching band region, C=O and C=C stretching, and C–C, C–O, and C–N stretching band region, respectively.

In 2D correlation spectroscopy, slice spectra play important roles in interpreting the correlations between the two spectral regions.<sup>21</sup> The slices, obtained by holding a wavelength in one region constant, and letting the wavelengths in the other vary, can give a lot of information about the relationship between an absorber in one region and many absorbers in another. Barton II *et al.*<sup>21</sup> employed the CH stretching modes of the ‘waxy’ material in the samples as the most prominent example to show how the correlation patterns from one region can be used to interpret the other region of the spectrum. Figure 7.2(A) and (B) shows NIR and mid IR spectra of beeswax, respectively.<sup>21</sup> The three bands at 2307, 1726, and 1396 nm in the NIR spectrum are assigned to the CH combination mode, the first overtone of the CH stretching mode, and its second overtone. These bands have the major correlations to the IR bands at 2919 and 2854  $\text{cm}^{-1}$  due to the CH stretching modes, as shown in the correlation slices in Figure 7.3(A) and (B).<sup>21</sup> The correlation pattern is virtually identical for the two IR slices, as it is for the three NIR slices (Figure 7.4(A), (B), and (C)). It is clear from Figure 7.4 that the patterns at all three wavelengths, 1390, 1729, and 2312 nm, respectively, are identical to the major correlations at 2919 and 2850  $\text{cm}^{-1}$ .

This type of 2D correlation spectroscopy has two advantages. First, based on an NIR correlation slice of a spectrum in another region (for example, a mid-IR spectrum), one can examine which component in a sample contributes to a particular NIR band. Second, 2D correlation spectroscopy assists one to develop or interpret a chemometrics model. With the aid of an IR correlation slice of an NIR spectrum, it may be possible to predict useful wavelengths for a chemometrics model. This 2D approach has been used to construct NIR and Raman heterocorrelation 2D maps as well as NIR and IR ones. This type of 2D correlation spectroscopy does not have an asynchronous spectrum, so that one cannot investigate the dynamic behavior of spectral changes.

## 7.2.2 STATISTICAL 2D CORRELATION BY ŠAŠIĆ AND OZAKI

The idea of statistical 2D correlation spectroscopy used by Šašić and Ozaki,<sup>23,24</sup> constructing 2D correlation coefficient maps, is essentially the same as the approach originally introduced by Barton II *et al.*<sup>21</sup> Šašić and Ozaki used a concise matrix algebra notation to describe the derivation of 2D correlation coefficient spectra. They noted that the scope of statistical 2D correlation strictly based on product-moment correlation coefficients is different from the full-scale generalized 2D correlation, but such maps can be interpreted in an analogous way to those obtained by a synchronous 2D spectrum in the generalized correlation. However, an asynchronous

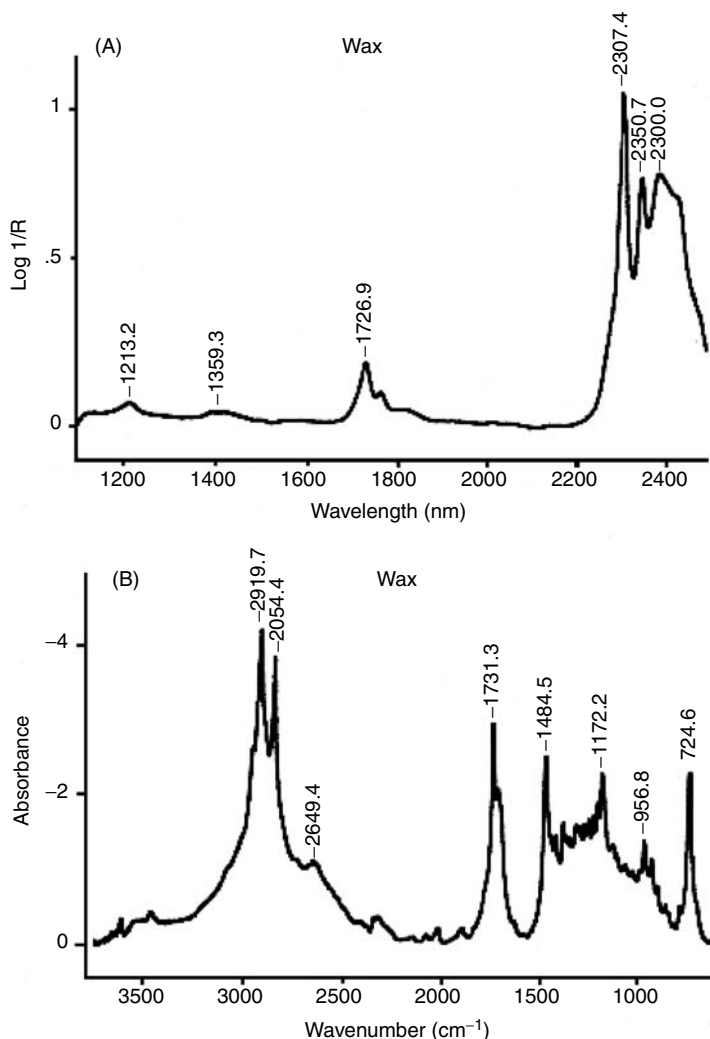


**Figure 7.2** (A) NIR spectrum of beeswax from the 1100 to 2500 nm region. (B) IR spectrum of the same sample from the 4000 to 600  $\text{cm}^{-1}$  region (Reproduced with permission from F.E. Barton II *et al.*, *Appl. Spectrosc.*, **46**, 420 (1992) (Ref. 21). Copyright (1992) Society for Applied Spectroscopy.)

2D spectrum or even the basic concept of asynchronicity does not exist in statistical 2D correlation spectroscopy. As in the case of the generalized 2D correlation spectroscopy, one can develop both variable–variable and sample–sample 2D correlation spectra for the statistical 2D correlation spectroscopy.

In the statistical 2D correlation of Šašić and Ozaki, the correlation coefficient matrix is calculated directly from the experimental data matrices, **X** and **Y**, by



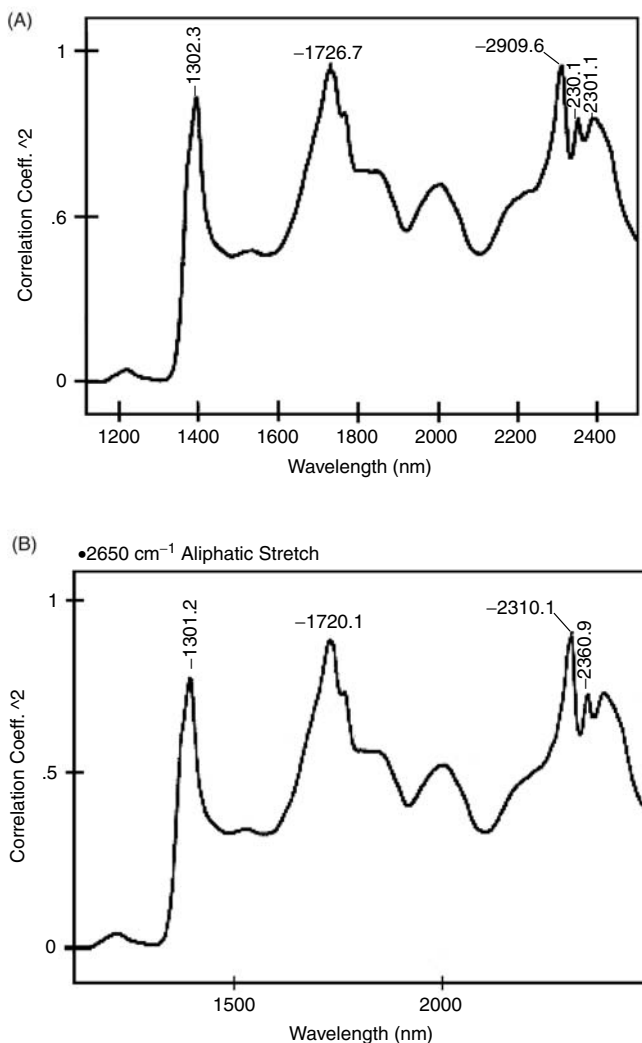


**Figure 7.3** (A) IR correlation slice of the NIR spectrum at 2919 cm<sup>-1</sup> in the 1100–2500 nm region. (B) IR correlation slice of the NIR spectrum at 2850 cm<sup>-1</sup> in the 1100–2500 nm region (Reproduced with permission from F.E. Barton II *et al.*, *Appl. Spectrosc.*, **46**, 420 (1992) (Ref. 21). Copyright (1992) Society for Applied Spectroscopy.)

a simple pretreatment operation called autoscaling. The autoscaling of a data matrix column is given by the form

$$d_{ij}' = \frac{(d_{ij} - \bar{d}_j)}{s_j} \quad (7.5)$$

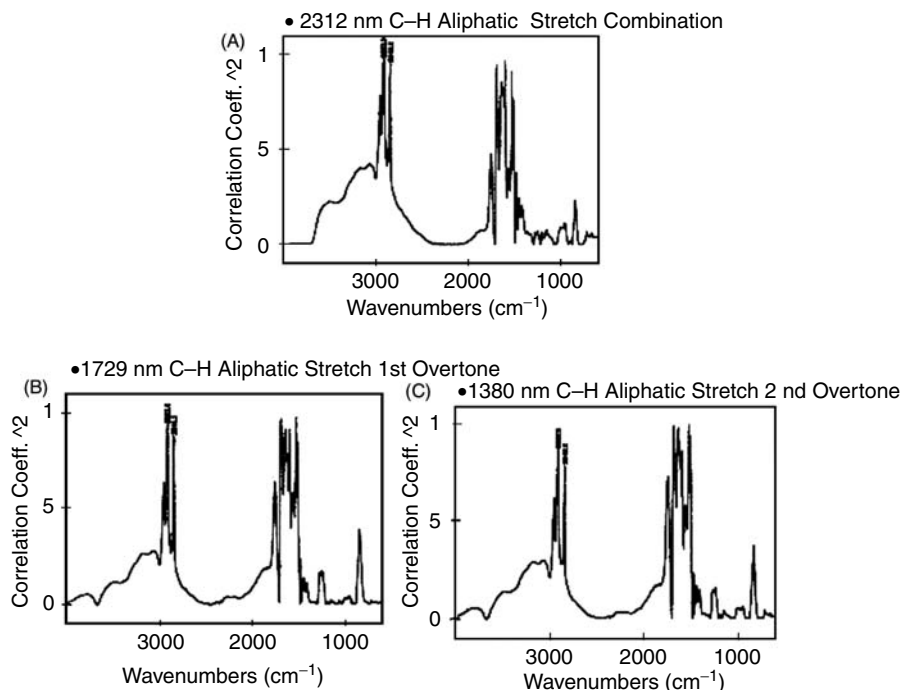


**Figure 7.4** (A) NIR correlation slice of the IR spectrum at 2312 nm in the 4000–600 cm<sup>-1</sup> region. (B) NIR correlation slice of the IR spectrum at 1729 nm in the 4000–600 cm<sup>-1</sup> region. (C) NIR correlation slice of the IR spectrum at 1390 nm in the 4000–600 cm<sup>-1</sup> (Reproduced with permission from F.E. Barton II *et al.*, *Appl. Spectrosc.*, **46**, 420 (1992) (Ref. 21). Copyright (1992) Society for Applied Spectroscopy.)

where  $d_{ij}'$  represents the autoscaled element of the  $i$ th row and  $j$ th column of the original data matrix. Column parameters,  $\bar{d}_j$  and  $s_j$ , are the mean value and standard deviation of the  $j$ th column. Autoscaling equalizes variances of all variables and transforms the spectra into shapes that are visually very far from the common spectral shapes. Once the autoscaling has been carried out on the data matrices to generate a new set of data matrices  $\mathbf{X}_{\text{scaled}}$  and  $\mathbf{Y}_{\text{scaled}}$ , the correlation coefficient matrix is given simply as a matrix product.

$$\mathbf{R} = \mathbf{X}_{\text{scaled}}^{\top} \mathbf{Y}_{\text{scaled}} \quad (7.6)$$

The autoscaling makes all the variances comparable and limits the vectors to the unit lengths, and thus, all possible scalar products between these vectors take the values between 1 and  $-1$ . These two figures mean perfect correlation, while 0 corresponds to the absence of correlation. It is also noted the correlation

coefficients can be directly transformed into angles. For variable–variable correlation maps, correlation coefficients (or angles) between concentration profiles are measured. On the other hand, for sample–sample correlation maps, the similarities of autoscaled spectra are measured. Since all the correlation coefficients are displayed in one map, one must choose the ranges of the highest and smallest coefficients to be investigated.

Statistical 2D correlation spectroscopy was applied to short wave NIR spectra of raw milk,<sup>23</sup> IR spectra measured during polymerization of bis-(hydroxyethyl terephthalate) (BHET),<sup>23</sup> and model chemical reactions.<sup>24</sup> Here, we outline statistical 2D correlation spectroscopy study of the IR spectra measured during the polymerization of BHET. Figure 7.5(A) shows IR spectra measured on line during polymerization of BHET that yields poly(ethylene terephthalate) (PET) as a final product and one molecule of ethylene glycol (EG) per each condensation reaction.<sup>23</sup> This polymerization reaction is described in more detail in Chapter 11. EG is expelled from the system, and it is expected that the spectra contain a very weak contribution from EG mainly due to the difference between the rates of EG production and evacuation. The model shown in Figure 7.5(A) consists of 44 spectra. Spectra of pure components, BHET, PET, and EG, shown in Figure 7.5(B) illustrate the degree of overlapping of bands.<sup>23</sup> The only available spectrum of PET was that of the cast film, which does not correspond truly to the spectrum of PET produced during the reaction, hence having limited usefulness.

It can be seen from the variable–variable 2D correlation map in Figure 7.6(A) that almost all the intensity changes perfectly correlate with each other. There is a regular alteration of the fields with levels of either 1 or  $-1$  that refer to the variable regions where intensities increase or decrease. The correlation

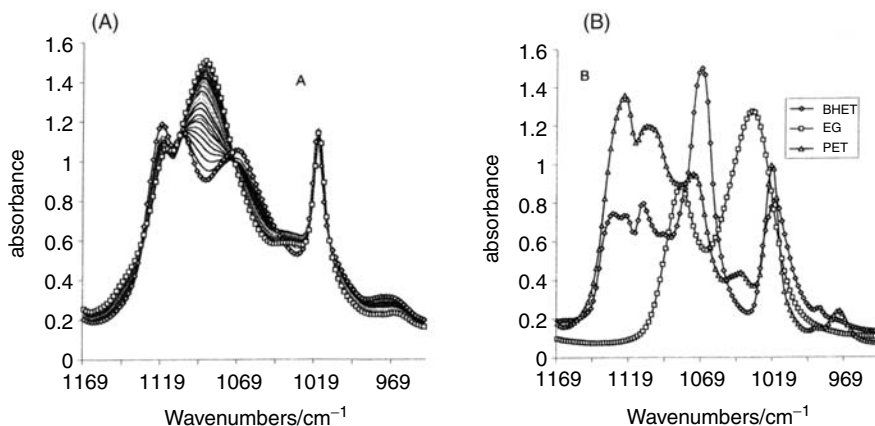


**Figure 7.5** (A) Experimental IR spectra measured on line during the polymerization of BHET. The first spectrum (diamonds) and the last spectrum (squares) are marked. (B) Spectra of BHET, PET, and EG (Reproduced with permission from Ref. No. 23. Copyright (2001) American Chemical Society.)

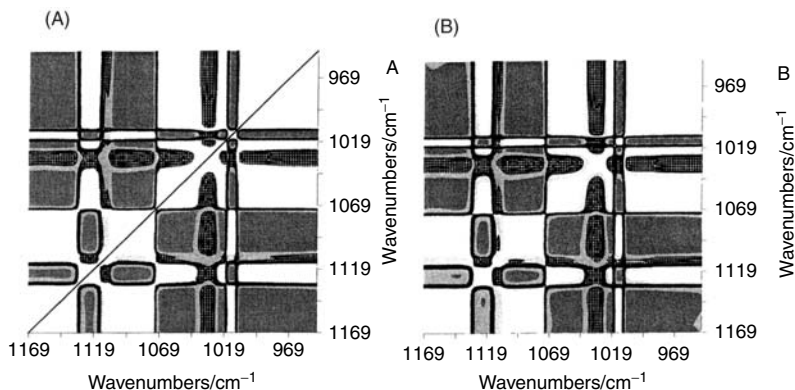


**Figure 7.6** Maps of (A) variable–variable correlation coefficients and (B) related angles obtained from the spectra in Figure 7.5. White and light gray: correlation coefficients 0.6–1 and angles 0–50°. Gray: correlation coefficients –0.6 to –1 and angles 120–180°. Squares: correlation coefficients 0.4 to –0.4 and angles 50–120° (Reproduced with permission from Ref. No. 23. Copyright (2001) American Chemical Society.)

map in Figure 7.6 (A) shows that changes in band intensity in the 1170–1134, 1108–1073 and 1015–1009  $\text{cm}^{-1}$  regions occur in the same direction whereas in the regions of 1129–1117, 1067–1038, 1023–1018, and 1005–947  $\text{cm}^{-1}$  they take place in opposite direction. The intensities in the former regions increase all the time whereas those in the latter four regions decrease. A subtle difference in the vicinity of the peak at 1019  $\text{cm}^{-1}$  can be clearly seen in the variable–variable correlation coefficient map. The difference is observable between the spectra of BHET and PET (see Figure 7.5), but it is rather difficult to monitor spectral changes near 1019  $\text{cm}^{-1}$  clearly in the spectra measured during the polymerization (Figure 7.5(A)). The boundaries of the regions roughly correspond to the positions of the isosbestic points. There is an exception, however, for the variables in the 1038–1023  $\text{cm}^{-1}$  region. The spectral changes along these wavenumbers do not show high correlation with the dynamics of both previous groups of variables. The smallest correlation coefficients are found at the positions where clearly defined spectral changes of other species occur (e.g., variables in the regions of 1170–1134 and 1005–947  $\text{cm}^{-1}$ ). Weak correlation along the 1038–1023  $\text{cm}^{-1}$  region may come from the presence of EG bands, which can contribute evidently only to that region, disarranging perfect correlation existing for all other variables. The PCA of mean-centered data reveals that the peak at 1030  $\text{cm}^{-1}$  is very prominent in PC2. This is in good agreement with the results obtained here because the PCA of mean-centered two components of the polymerization reaction system should show only one factor. EG has a strong peak around 1080  $\text{cm}^{-1}$  (Figure 7.5(B)), but its intensity is very weak compared to the strong bands due to the reactant and product in its vicinity.

Figure 7.6(B) shows the angles between the vectors that represent intensity changes. Entries in Figure 7.6(B) are just arccosines of the values in Figure 7.6(A).

The angles of  $0^\circ$  and  $180^\circ$  mean perfect correlation, while the angle of  $90^\circ$  indicates the isosbestic point. The angles between concentration vectors are potentially useful for the determination of pure variables in a manner similar to that shown by Windig *et al.*<sup>26</sup> Šašić and Ozaki have also found that correlation maps with angles displayed are slightly easier to analyze since the changes of angles are more illustrative when plotted than the correlation coefficients are.

Parts A and B of Figure 7.7 show respectively sample–sample 2D correlation maps with correlation coefficients and corresponding angles calculated from the spectra in Figure 7.5(A). It seems that the concentrations of BHET and PET become equal at around 80 min while the eventual influence of the EG concentration vector onto the correlation maps is hard to evaluate. The sample region of 65–85 min shows a correlation with all other samples that have correlation coefficients less than 0.7 and higher than  $-0.7$ . The reason for that could be due to the precision of the calculation. The threshold for the correlation coefficients (and angles related to them) to be displayed is difficult to evaluate. The values of 0.7 and  $-0.7$  are chosen arbitrarily to indicate noise-free lower boundaries of similarity between the spectra viewed as unit vectors. It is generally very likely that, for highly overlapped spectra, sample–sample correlation maps are less sensitive than variable–variable correlation maps, simply because the former are calculated from a highly similar array of numbers with no preferences on a given range, as is case for the  $1038\text{--}1023\text{ cm}^{-1}$  region in Figure 7.6. Samples at 65 and 80 min were found as those where BHET and PET have equal concentrations in self-modeling curve resolution (SMCR) studies by Simplisma (65 min) and orthogonal projection approach (OPA) (85 min), respectively.<sup>47</sup> Considering that simple multiplication

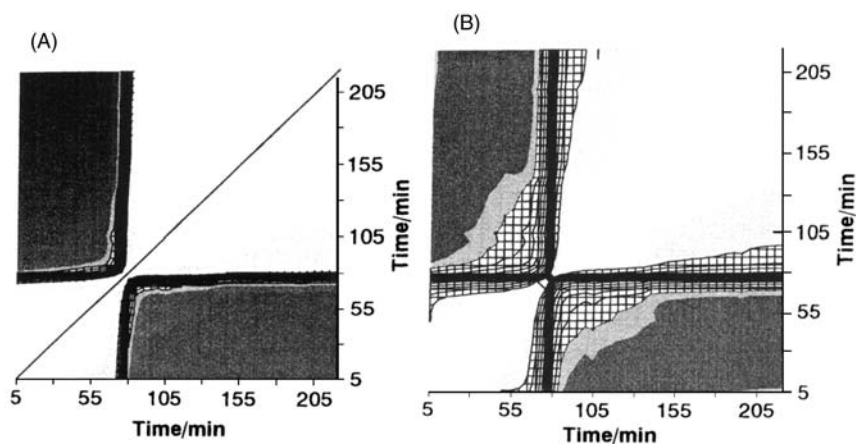


**Figure 7.7** Maps of (A) sample–sample correlation coefficients and (B) related angles obtained from the spectra in Figure 7.5. White and light gray: correlation coefficients 0.7–1 and angles  $0\text{--}30^\circ$ . Gray: correlation coefficients  $-0.7$  to  $-1$  and angles  $150\text{--}180^\circ$ . Squares: correlation coefficients 0.6 to  $-0.6$  and angles  $40\text{--}140^\circ$  (Reproduced with permission from Ref. No. 23. Copyright (2001) American Chemical Society.)

of previously autoscaled matrices is carried out here only, the calculation of the regions where BHET and PET prevail can be estimated as rather good.

### 7.2.3 OTHER STATISTICAL 2D SPECTRA

There are other types of 2D mapping methods based on statistical analysis of data, which are substantially different from the conventional statistical 2D correlation approaches, such as mapping of covariance or correlation coefficient. Two noteworthy examples in this category are 2D maps of maximum likelihood and outer product of spectral vectors.

#### 7.2.3.1 Maximum Likelihood 2D Maps

Wentzell and Lohnes<sup>32</sup> applied maximum likelihood principal component analysis (MLPCA) to correlate measurement errors and measurement uncertainty in multivariate data analysis. MLPCA is a decomposition method like PCA, but less emphasis is placed on measurements with large variances. Full covariance is often too large to be tractable. Pooled error covariance and the inverse of covariance matrix can be plotted as 2D maps to bring out the pattern of correlated errors. The technique is used to differentiate patterns for heteroscedastic noise from that for correlated noise.<sup>33</sup>

#### 7.2.3.2 Outer Products

The outer product of spectral vectors forms a second order tensor, which can be displayed as a form of a 2D map. For example, Šašić and Ozaki discussed the 2D map from the outer product matrix of loading vectors versus the variance–covariance matrix.<sup>34</sup> This operation will lead to a set of bilinear rank-one projection matrices of each loading. Summing up these matrices with appropriate weights (i.e., eigenvalues) brings back the dispersion matrix proportional to the synchronous spectrum. 2D mapping is possible for any pairs of two vectors by simply obtaining the outer product, resulting in the mutual weighting of vector signals. The bilinear map can be, for example, unfolded to a one-dimensional vector for further chemometric manipulation (e.g., PLS) and then folded back later. Although this type of 2D map may not be the same as typical 2D correlation spectra,<sup>35</sup> outer product analysis is capable of fusing two different data vectors for multiway analysis, e.g., PARAFAC.<sup>36</sup>

### 7.2.4 LINK TO CHEMOMETRICS

Statistical 2D analysis of various types and forms discussed in this section offers a very promising link between the field of 2D correlation spectroscopy and chemometrics. The former originated from a simple time-series analysis

of transient data in dynamic IR experiments to eventually become a generally applicable analytical technique, while the latter evolved from the beginning as an encompassing branch of chemical science including broad applications, such as multivariate calibration, pattern recognition, data processing, and the like. By using the common language of statistical tools, the two disciplines should be able to interact and exchange valuable ideas more effectively.

### 7.3 OTHER DEVELOPMENTS IN 2D CORRELATION SPECTROSCOPY

In addition to the nonlinear optical 2D spectroscopy and statistical 2D techniques discussed in this and previous chapters, such as covariance maps, correlation coefficient plots, coherence plots, and phase angle plots, there have been many new or variant forms of 2D spectroscopy proposed in recent years, which are worthy of our attention. We select two of the noteworthy developments: moving-window and model-based 2D correlation methods.

#### 7.3.1 MOVING-WINDOW CORRELATION

Moving-window 2D correlation analysis is an extension of the data subdivision technique already used widely in many 2D correlation studies.<sup>38–40</sup> The interpretation of 2D correlation spectra based on a large set of spectral data sometimes becomes difficult, due to the inclusion of too many underlying processes influencing the spectral changes. Subdivision of the full data set into smaller blocks circumvents this problem.<sup>41,42</sup> Individual small subsets of data are then separately analyzed by 2D correlation. 2D spectra based on the specific subset data are then analyzed and compared to provide an overall picture. If a small subset is chosen sequentially by incrementally shifting the position along the perturbation axis, the moving-window 2D correlation protocol is achieved.

The first 2D correlation analysis based on a moving window was reported by Thomas and Richardson on a temperature-resolved 2D IR study of a phase transition of liquid crystals.<sup>38</sup> In their study, autocorrelation analysis was carried out by (1) partitioning spectral data by a small moving window (e.g., four spectra), (2) calculating the 2D spectrum and extracting the power spectrum, and (3) plotting autopeaks versus the average temperature of the moving window to determine the region of significant signal changes. This approach was found to be useful in focusing the 2D correlation only on selected regions for unambiguous analysis. The moving window combined with sample–sample correlation has also been pursued by Šašić *et al.*<sup>39,40</sup>

#### 7.3.2 MODEL-BASED 2D CORRELATION SPECTROSCOPY

Elmore and Dluhy introduced a new and intriguing form of 2D correlation analysis called  $\beta\nu$  correlation,<sup>43</sup> based on the correlation between the spectral data

and a set of kernel functions with varying phase shift. The kernel is a single sinusoid corresponding to the lowest Fourier component of the signal, such that one quarter cycle of the sinusoid is set to be the entire observation period. This is probably the first report on a 2D correlation analysis based purely on a model function. 2D IR  $\beta\nu$  correlation has been applied to IRRAS data of a binary phospholipid mixture at an interface.<sup>44</sup> 2D IR  $\beta\nu$  correlation analysis of a pulmonary surfactant at the air–water interface with surface pressure effect has also been reported.<sup>45</sup>

Eads and Noda formally put forward the concept of the model-based 2D correlation analysis.<sup>46</sup> The technique is sometimes referred to as the 2D *waveform correlation* analysis. Their method may be regarded as a form of heterocorrelation analysis, where one of the data sets is a collection of model calculations with systematically varying adjustable parameters. The highest correlation is achieved when the model parameter matches closest to the function resembling the actual data. The resulting 2D spectrum then becomes an intuitively understandable visual representation of the least squares curve fitting of the model function to experimental measurements. The technique is robust and generally applicable to a wide ranges of studies.

2D waveform correlation analysis was applied by Eads and Noda to a set of NMR spectra obtained during the diffusion of multiple components in a solution mixture. The Fickian diffusion of small molecules is known to lead to Gaussian-like relaxation profiles of NMR signals. When the diffusivity of component molecules is used as an adjustable parameter, the 2D waveform correlation analysis based on a set of model diffusion profiles immediately yielded individual diffusivities of the component species, even in a complex mixture.

Model-based 2D correlation is a very promising development, which can provide a major departure from the current limitation of the scope of correlation analysis. Unlike the strictly phenomenological data treatment of conventional 2D correlation analysis, model-based 2D correlation can bring in the idea of hypothesis testing and even the determination of causal relationship to the data analysis, at least within the boundary of the proposed model structure. In the past, 2D correlation analysis provided an unbiased model-free procedure to directly extract pertinent information from a spectral data set. This time, however, the user of 2D correlation spectroscopy must think of and propose a hypothesis before carrying out the data analysis. It could be an advantage or disadvantage, depending on the level of prior information we have on the system to be studied.

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## 8 Dynamic Two-dimensional Correlation Spectroscopy Based on Periodic Perturbations

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This chapter provides illustrative application examples of 2D correlation analysis to various spectroscopic studies, especially those systems studied under the influence of a *periodically varying* dynamic perturbation. There are many ways that a system can be stimulated with a periodic repetitive external perturbation. For example, sinusoidal fields of electrical, mechanical, thermal, acoustic, and many other perturbations have been successfully utilized to stimulate different systems for subsequent 2D correlation spectroscopic analysis. Among those, rheo-optical *dynamic infrared linear dichroism* (DIRLD) experiment is a well-known example, holding a special place in the early development of 2D correlation spectroscopy. Numerous interesting examples of DIRLD-based 2D IR studies have been reported, and the research activity in this field is still rapidly growing. Some of the work carried out in the 2D correlation analysis of DIRLD-based data and related topics of various periodic perturbations are presented here.

### 8.1 DYNAMIC 2D IR SPECTROSCOPY

The *dynamic* 2D IR spectroscopy based on a *sinusoidally* varying external perturbation is almost a field in itself among the numerous emerging family members of 2D correlation spectroscopy techniques. In this section, the fundamental concept of dynamic 2D correlation spectroscopy is examined. As the most representative example of this class of 2D correlation spectroscopy, 2D correlation studies based on rheo-optical IR dichroism measurement for film samples undergoing sinusoidal deformation are described first. Related topics using other sinusoidal perturbations will be treated in the later sections. Readers are directed to the in-depth treatment of the subject of 2D spectroscopy based on repetitive perturbation in several pertinent published reports.<sup>1–5</sup>

#### 8.1.1 SINUSOIDAL SIGNALS

A simple system under a repetitive external stimulus in the form of sinusoidal signal of fixed frequency, amplitude, and phase is of great interest for 2D

correlation spectroscopy. The dominant component of the dynamic response of such a system, i.e., the perturbation-induced variation of spectral intensities measured at a given spectral variable such as wavenumber to the applied perturbation, often also becomes a well-defined sinusoidal function having the same frequency as the perturbation.

There are numerous examples of dynamic spectroscopic experiments where a sample is stimulated by an externally determined small-amplitude sinusoidal perturbation. Dynamic infrared linear dichroism (DIRLD) measurement, where a polymer film is sinusoidally deformed repeatedly with a mechanical stretcher while being probed with a polarized IR beam, is a classical rheo-optical experiment for polymer characterization.<sup>6</sup> Spectroscopic analysis of dynamic reorientation under an alternating electric field is a well-known method in the study of liquid crystals.<sup>7</sup> Depth-profiling photo-acoustic IR spectroscopy is another example of a spectroscopic technique that uses a sinusoidal external perturbation.<sup>8</sup>

The measurement of a repetitive sinusoidal signal is often favored for dynamic or time-dependent experiments, as the measurement accuracy of the signal can be enhanced by the co-additive observation over an extended period of time covering many cycles of signal fluctuations. The well-known phase-sensitive modulation method can accurately detect the signal component of interest, even in the presence of large interfering background signals and noise.<sup>6</sup> In addition to the experimental ease of signal detection, sinusoidal signals have other intrinsic advantages over other waveforms for analysis by the 2D correlation approach. As already discussed in the earlier chapters, the 2D correlation analysis of sinusoidal signals is especially straightforward, as closed form analytical expressions for the synchronous and asynchronous spectrum are available (see Equations 2.19–2.22). Furthermore, in the case of sinusoidal perturbations, the mathematical expression for the heuristic disrelation spectrum (Equation 2.50) is found to be identical to that for the asynchronous spectrum. This fact makes it especially simple to compute the complete set of 2D correlation spectra for sinusoidal signals. Finally, it is also worth pointing out that the sinusoidal functions are the basic building blocks of the Fourier transform operations, which in turn are central to the formal definition of the generalized 2D correlation function given in Equation (2.5). Thus, understanding the basic form of the 2D correlation procedure based on sinusoidal signals provides valuable insight into the more universal form of generalized 2D correlation spectroscopy.

### 8.1.2 SMALL-AMPLITUDE PERTURBATION AND LINEAR RESPONSE

As a starting point, let us consider a case of time-dependent IR absorbance intensity variations induced by an arbitrary external perturbation. To simplify the analysis, the level of perturbation applied to the system is assumed to be relatively small, so that the induced spectral variations are reversible, superposable with previous and future variations, and proportional to the magnitude of the

applied perturbation. In other words, the formal treatment based on a simple linear response theory is applicable. In general, any time-dependent IR absorbance signal of a perturbed system, which is measured at a wavenumber  $\nu$  under a small dynamic stimulus, can be treated as a combination of two distinct components.

$$A(\nu, t) = \overline{A}(\nu) + \tilde{A}(\nu, t) \quad (8.1)$$

The first term  $\overline{A}(\nu)$  is the *reference* or quasi-static component, corresponding to the normal absorbance of the system, which is observed without the external perturbation. The second term  $\tilde{A}(\nu, t)$  corresponds to the perturbation-induced *fluctuation* of absorbance, i.e., *dynamic absorbance spectrum* of the system.

So far, the specific mechanism of absorbance fluctuations by an imposed perturbation has not been discussed. There are actually many different possibilities of inducing dynamic absorbance changes, depending on the physical nature of the applied perturbation. As a convenient example with potential practical utility, sinusoidal fluctuations of IR absorbance intensities arising from the orientational change of submolecular constituents of a system under a dynamic mechanical or acoustic perturbation is studied here.<sup>6</sup> The dynamic rheo-optical IR measurement for a solid polymer sample is especially suited for 2D correlation analysis, since the typical rate of spectral variations for such a system is well within the experimentally accessible range of a conventional IR spectrometer coupled with a dynamic mechanical stretcher.

### 8.1.3 DYNAMIC IR LINEAR DICHROISM (DIRLD)

While it is possible to analyze dynamic absorbance intensity variations monitored with an ordinary IR beam, much more interesting information can be gathered if a pair of linearly polarized IR beams are used to make a directional absorbance measurement. The application of a uniaxial perturbation can induce a well-defined change in the spatial distribution of various submolecular structures constituting the system. Such a change specifically results in the reorientation of dipole transition moments responsible for the absorption of light, and in turn can be manifested as a change in the optical anisotropy, i.e., IR linear dichroism intensity. The detection of dynamic variations of *directional* absorbances,  $\tilde{A}_{\parallel}(\nu, t)$  and  $\tilde{A}_{\perp}(\nu, t)$ , i.e., those measured with IR beams polarized parallel and perpendicular to the given deformation direction of a sample, leads to the determination of the dynamic dichroic difference,  $\Delta\tilde{A}(\nu, t) = \tilde{A}_{\parallel}(\nu, t) - \tilde{A}_{\perp}(\nu, t)$ . This quantity represents the time-dependent average reorientation of dipole transition moment population associated with the specific chemical functional groups contributing to the molecular vibrations at  $\nu$ .

Suppose a small-amplitude *sinusoidal* strain (deformation) with a fixed angular frequency  $\omega$  is applied to a system, such as a thin plastic film, as described schematically in Figure 8.1.

$$\tilde{\epsilon}(t) = \hat{\epsilon} \sin \omega t \quad (8.2)$$

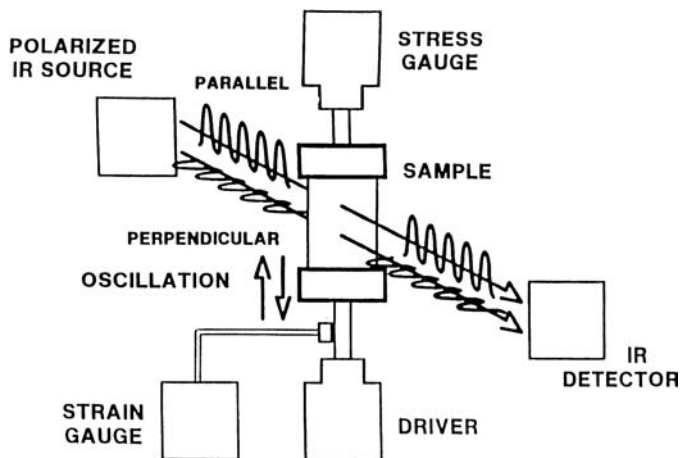


**Figure 8.1** Schematic diagram of a dynamic IR linear dichroism (DIRLD) spectrometer. Dynamic reorientation of molecular constituents induced by a small-amplitude repetitive strain is monitored with a pair of polarized IR beam. (Reproduced with permission from I. Noda *Appl. Spectrosc.*, **44**, 550 (1990). Copyright (1988) Society for Applied Spectroscopy.)

The resulting change in the orientation distribution of submolecular constituents of the system induced by the macroscopic strain perturbation will manifest itself as dynamic variations of IR dichroism intensities.

$$\Delta \tilde{A}(\nu, t) = \Delta \hat{A}(\nu) \sin[\omega t + \beta(\nu)] \quad (8.3)$$

The dynamic dichroism signal  $\Delta \tilde{A}(\nu, t)$  is usually not in phase with the applied strain signal  $\tilde{\epsilon}(t)$ , and there is a finite phase angle difference  $\beta(\nu)$  reflecting the rate-dependent nature of the reorientation processes experienced for submolecular constituents induced by a macroscopic perturbation (Figure 8.2). In general, not only the amplitude  $\Delta \hat{A}(\nu)$  of the response signal but also the phase angle  $\beta(\nu)$  is dependent on the IR wavenumber  $\nu$ , indicating the local independence of the reorientational processes of submolecular constituents. As it is possible to trace the time-dependent variations of dichroism intensities at any different wavenumber  $\nu$ , the measured dynamic IR dichroism may be regarded as a form of time-resolved bilinear spectrum (Figure 8.3). The dynamic IR dichroism (Equation 8.3) may be expressed in terms of the sum of two spectral components orthogonally varying with time

$$\Delta \tilde{A}(\nu, t) = \Delta \hat{A}(\nu) \cos \beta(\nu) \sin \omega t + \Delta \hat{A}(\nu) \sin \beta(\nu) \cos \omega t \quad (8.4a)$$

$$= \Delta A'(\nu) \sin \omega t + \Delta A''(\nu) \cos \omega t \quad (8.4b)$$

where  $\Delta A'(\nu)$  and  $\Delta A''(\nu)$  are commonly known as the *in-phase spectrum* and *quadrature spectrum* of the dynamic dichroism. The overall reorientability of

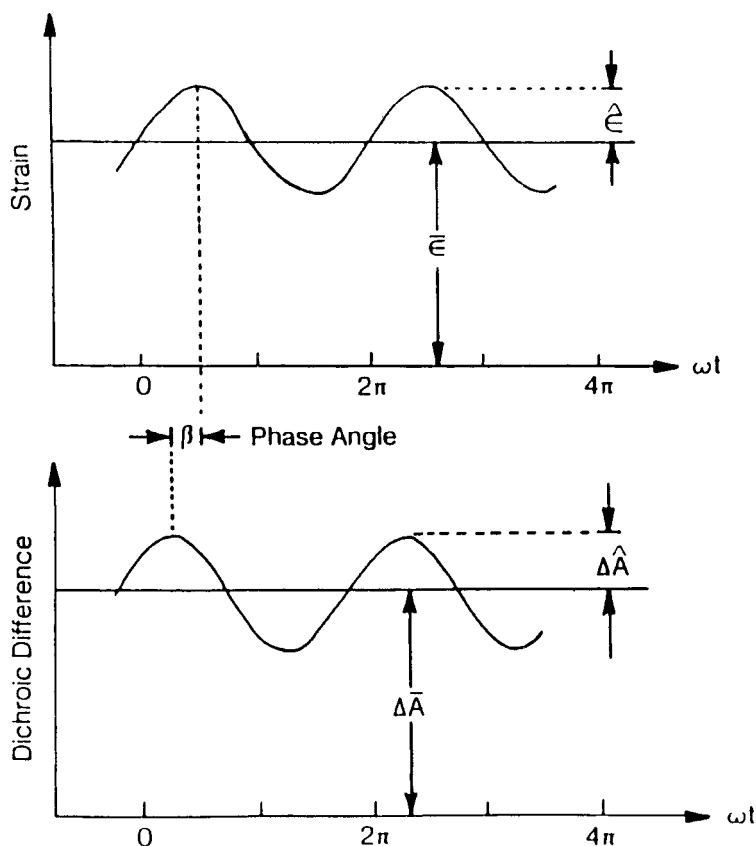


**Figure 8.2** Comparison between dynamic strain  $\tilde{\epsilon}(t)$  and dynamic IR dichroism  $\Delta\tilde{A}(\nu, t)$ . The dynamic dichroism response is not fully synchronized with the applied strain, such that there is a finite phase angle  $\beta(\nu)$  between the two signals. (Reproduced with permission from Ref. No. 4. Copyright (1999) Wiley-VCH.)

dipole transition moments, which relates to the mobility of submolecular groups contributing to the molecular vibrations at the wavenumber  $\nu$ , is characterized by the so-called *power spectrum*  $P(\nu)$  of the dynamic dichroism

$$P(\nu) = \frac{1}{2}[\Delta A'^2(\nu) + \Delta A''^2(\nu)] = \frac{1}{2}\Delta\hat{A}^2(\nu) \quad (8.5)$$

which is proportional to the square of the amplitude of dichroism fluctuation.

#### 8.1.4 2D CORRELATION ANALYSIS OF DYNAMIC IR DICHROISM

A proper manipulation of time-dependent optical anisotropy data such as DIRLD spectra yields an estimate of change in the ensemble average of submolecular



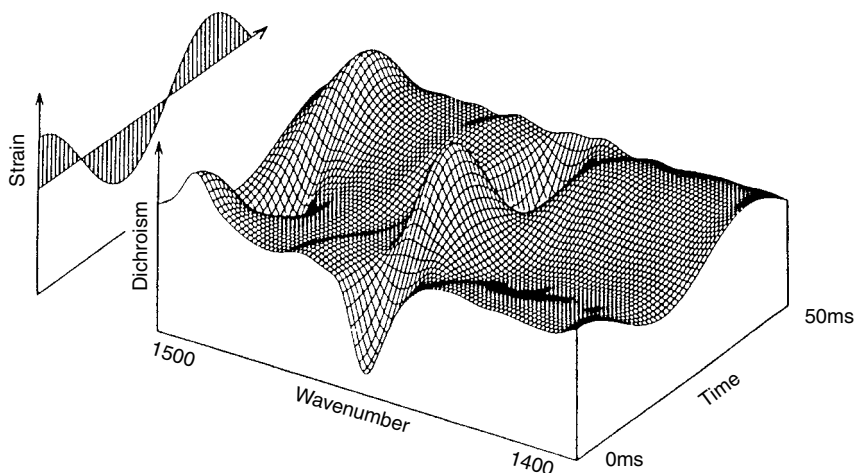


**Figure 8.3** Strain-induced time-resolved dynamic IR linear dichroism (DIRLD) spectrum as a function of time and wavenumber. (Reproduced with permission from I. Noda *et al.*, *Appl. Spectrosc.*, **42**, 203 (1988). Copyright (1988) Society for Applied Spectroscopy.)

structure orientations. The reorientability (i.e., local rotational mobility) of chemical functional groups is strongly influenced by the presence of various inter- and intramolecular interactions. The reorientational rate of electric dipole transition moments thus provides valuable information on the local chemical environment, as well as the structure of functional groups contributing to the molecular vibrations detected by IR.

Specific reorientation rates of individual dipole transition moments, which are determined by the type and local environment of functional groups contributing to the molecular vibrations, can be used as convenient spectroscopic labels to differentiate highly overlapped IR bands. Wavenumber-dependent variations of dynamic dichroism intensities reflecting the non-uniformity of reorientation rates among various dipole transition moments are analyzed quite effectively by a 2D correlation method. Fortunately, 2D correlation spectra of dynamic spectral signals having a well-defined sinusoidal waveform are readily calculated from the closed form analytical expressions derived previously (Equations 2.19–2.22). For a pair of dynamic IR dichroism signals measured at two different wavenumbers,  $\Delta\tilde{A}(\nu_1, t)$  and  $\Delta\tilde{A}(\nu_2, t)$ , the synchronous (coincidental) and asynchronous (quadrature) correlation intensities,  $\Phi(\nu_1, \nu_2)$  and  $\Psi(\nu_1, \nu_2)$ , of the dynamic IR dichroism signals are given by<sup>2</sup>

$$\Phi(\nu_1, \nu_2) = \frac{1}{2} \Delta\hat{A}(\nu_1) \cdot \Delta\hat{A}(\nu_2) \cos[\beta(\nu_1) - \beta(\nu_2)] \quad (8.6a)$$

$$= \frac{1}{2} [\Delta A'(\nu_1) \cdot \Delta A'(\nu_2) + \Delta A''(\nu_1) \cdot \Delta A''(\nu_2)] \quad (8.6b)$$

and

$$\Psi(\nu_1, \nu_2) = \frac{1}{2} \Delta \hat{A}(\nu_1) \cdot \Delta \hat{A}(\nu_2) \sin[\beta(\nu_1) - \beta(\nu_2)] \quad (8.7a)$$

$$= \frac{1}{2} [\Delta A''(\nu_1) \cdot \Delta A'(\nu_2) - \Delta A'(\nu_1) \cdot \Delta A''(\nu_2)] \quad (8.7b)$$

Close observation of Equations (8.6a) and (8.7a) reveals the basic nature of synchronous and asynchronous 2D correlation intensities in terms of the phase angle relationship, at least for simple sinusoidally varying signals. The synchronous correlation intensity  $\Phi(\nu_1, \nu_2)$  represents the degree of coherence between two separate signals measured simultaneously, and the intensity becomes a maximum if the variations of two dynamic dichroism signals studied by the correlation analysis are totally in phase with each other (i.e.,  $\beta(\nu_1)$  and  $\beta(\nu_2)$  are the same) and a minimum if they are antiphase (i.e.,  $\pi$  out of phase) with each other. Signals nearly orthogonal to each other (i.e.,  $\pm\pi/2$  out of phase) should give out little synchronous correlation intensity. The asynchronous correlation intensity  $\Psi(\nu_1, \nu_2)$ , on the other hand, characterizes the degree of coherence between signals measured at two different instances separated by a phase shift of  $\pm\pi/2$ . Thus, the asynchronous correlation intensity becomes either maximum or minimum when the dynamic signals are  $\pi/2$  out of phase with each other and vanishes for a pair of signals exactly in phase or antiphase with each other. 2D IR spectra are obtained as usual by plotting these correlation intensities as function of two independent wavenumbers,  $\nu_1$  and  $\nu_2$ .

## 8.2 DYNAMIC 2D IR DICHROISM SPECTRA OF POLYMERS

Some pertinent examples for 2D IR dichroism spectra, especially those of polymeric materials, are provided here to show how certain useful information can be effectively extracted for different types of applications of the dynamic 2D IR correlation technique. At first, a binary blend mixture system consisting of essentially noninteracting polymer components is studied. Despite the apparent simplicity of this model system, the exercise actually highlights several pertinent features of 2D IR correlation analysis applied to dynamic dichroism experiment. Next examined in detail are single-phase amorphous glassy polymers: one of the simplest form of solid-state systems. This study demonstrates the unique capability of dynamic 2D IR analysis in differentiating between highly overlapped IR bands. The improved spectral resolution arises primarily from the fortuitous submolecular level selectivity of IR dichroism responses to a dynamic mechanical perturbation. It is truly remarkable to be able obtain high-resolution mid-IR spectra for condensed-state systems by coupling a mechanical perturbation applied to a sample with a simple correlation method. The comparison between results obtained from 2D IR dichroism studies and independent IR absorbance measurements of a

selectively deuterium-substituted specimen provides strong experimental support for the physical validity of high-resolution 2D IR dichroism spectra.

The apparent high-resolution capability of 2D IR dichroism spectra may be combined effectively with IR spectral band correlations among common molecular structures to probe some of the most exciting but also very complex systems, such as proteins and other biomolecules. Here, the gross overlap of IR spectral lines of amide vibration bands of protein-rich specimens can be experimentally deconvolved into individual spectral components, and the characteristic molecular vibrations of various conformational features of proteins may be studied.

### 8.2.1 POLYSTYRENE/POLYETHYLENE BLEND

We start with a very simple system, a binary mixture of atactic polystyrene (PS) and low-density polyethylene (PE),<sup>1</sup> to illustrate the unique features of dynamic 2D IR spectra. It is well known that a blend mixture of polymers is often immiscible and spontaneously separates into phases because of the much reduced entropy contribution to the free energy of mixing. Such an immiscible mixture serves as an excellent model system, where molecular level interactions between components are believed to be very small. Each component of an immiscible polymer blend spontaneously segregates into relatively coarse ( $>10\text{ }\mu\text{m}$ ) dispersed phase domains. The volume fraction of the interfacial region is often negligibly small. Therefore, little true interaction at the molecular level will be expected between PS and PE components.

A conventional IR spectrum for this blend film is shown in Figure 8.4. Absorption peaks associated with the semicircle-stretching modes of the PS phenyl ring and  $\text{CH}_2$  deformations of the PE and PS backbones are observed. From this spectrum alone, however, it is rather difficult to determine the state of molecular level interactions between PS and PE components. The corresponding 2D IR dichroism correlation spectra (Figures 8.5–8.7) were derived from the strain-induced dynamic IR spectra measured with a simple dispersive time-resolved IR spectrometer.<sup>6</sup> The dynamic IR measurement was carried out by mechanically perturbing the PS/PE blend sample at the room temperature with a 23 Hz small-amplitude oscillatory tensile strain (about 0.1 % amplitude) and recording the time-dependent fluctuations of IR absorbance induced by the perturbation at a spectral resolution of  $4\text{ cm}^{-1}$ . For this particular study, dynamic absorbance signals were used, instead of dichroism signals, for experimental simplicity.

Figure 8.5 shows a pseudo-three-dimensional fishnet representation of the synchronous 2D IR spectrum. The spectral resolution is clearly enhanced by spreading the peaks over the second dimension. While the relative magnitude of correlation intensity may be best represented by a 3D fishnet plot, it is usually more convenient to use contour map representation (Figures 8.6 and 8.7) to determine the location of peaks in 2D IR spectra. Autopeaks observed on the diagonal positions near  $1454$  and  $1495\text{ cm}^{-1}$  in the synchronous 2D spectrum (Figure 8.6)

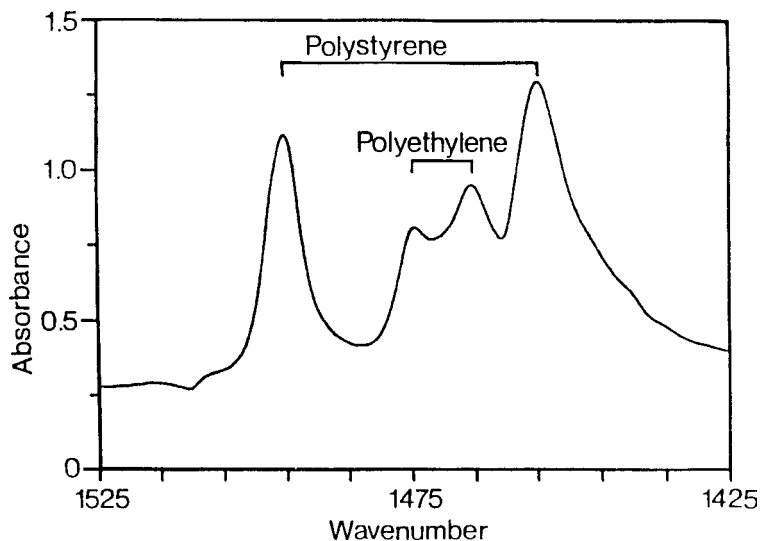


**Figure 8.4** Conventional (1D) IR absorbance spectrum of a mixture of atactic polystyrene and low-density polyethylene obtained at  $4\text{ cm}^{-1}$  resolution. (Reproduced with permission from Ref. No. 1. Copyright (1989) American Chemical Society.)

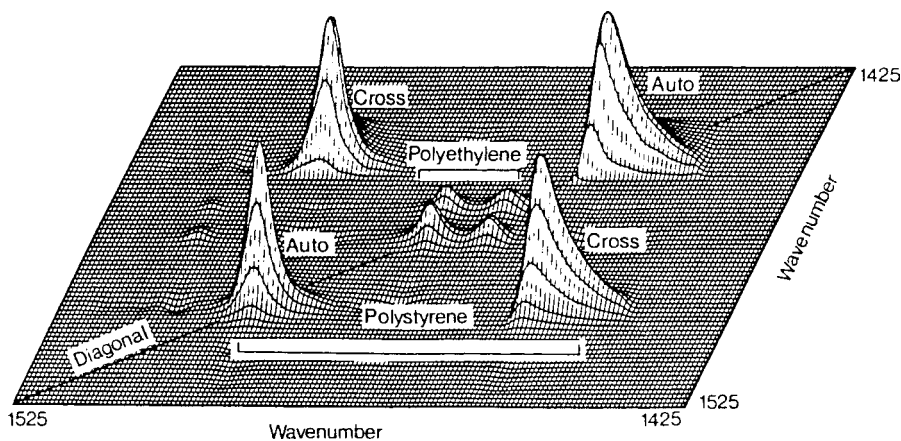


**Figure 8.5** Fishnet representation of the synchronous 2D IR correlation spectrum of a mixture of polystyrene and polyethylene. (Reproduced with permission from Ref. No. 1. Copyright (1989) American Chemical Society.)

represent the perturbation-induced local reorientation of PS phenyl rings. The  $1454\text{ cm}^{-1}$  band may also contain a contribution from  $\text{CH}_2$  deformation in the backbone of PS. A pair of intense cross peaks appearing at the off-diagonal positions of the spectral plane near  $1454$  and  $1495\text{ cm}^{-1}$  indicate the existence of a strong synchronous correlation between the two PS bands.

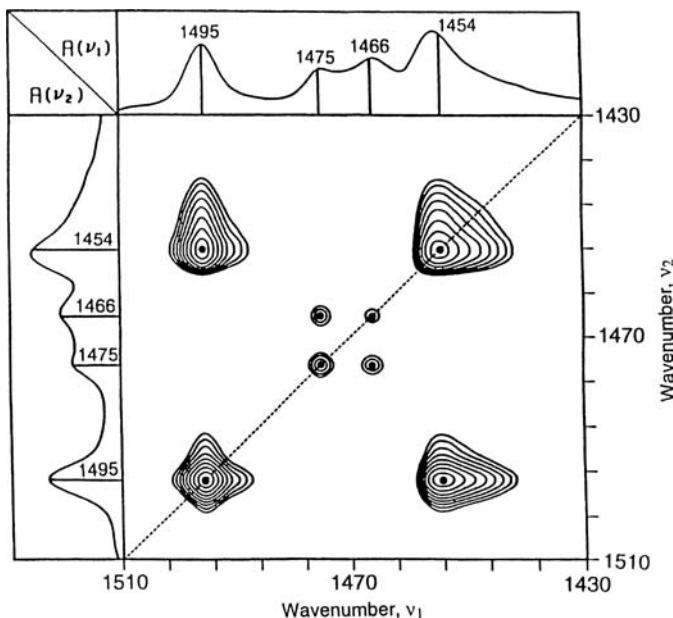


**Figure 8.6** Contour-map representation of the synchronous 2D IR correlation spectrum of a mixture of polystyrene and polyethylene. Conventional absorbance spectra are provided at the top and side of the map for reference purpose. (Reproduced with permission from Ref. No. 1. Copyright (1989) American Chemical Society.)

Similarly, autopeaks corresponding to the dynamic intensity fluctuation of IR bands associated with  $\text{CH}_2$  deformations in the PE are located near  $1466$  and  $1475\text{ cm}^{-1}$ . These autopeaks arise from the reorientation of molecular chains in the amorphous and crystalline regions of PE. A pair of cross peaks clearly correlate with the IR bands originating from the PE component. Interestingly, there is little development of synchronous cross peaks correlating PS and PE bands. The fluctuation rates of IR signals from PS and PE bands are substantially different, since strain-induced motions of fully phase-separated PS and PE components need not be highly coordinated.

The asynchronous 2D spectrum (Figure 8.7) shows cross peaks differentiating IR bands of PS and PE. The development of such cross peaks indicates that, even under an identical macroscopic perturbation (i.e., a dynamic tensile strain), the time dependence of the strain-induced IR intensity fluctuation for the PS component of the sample is substantially different from that for PE. Obviously, PS and PE are reorienting independently of each other at the molecular level. This result is not surprising for a pair of essentially immiscible polymers such as PS and PE, where the molecular-level interaction between the components is not strong enough to coordinate their reorientational responses.

Another notable observation in Figure 8.7 is the appearance of asynchronous cross peaks correlating the  $1459\text{ cm}^{-1}$  band to the  $1454$  and  $1495\text{ cm}^{-1}$  bands.

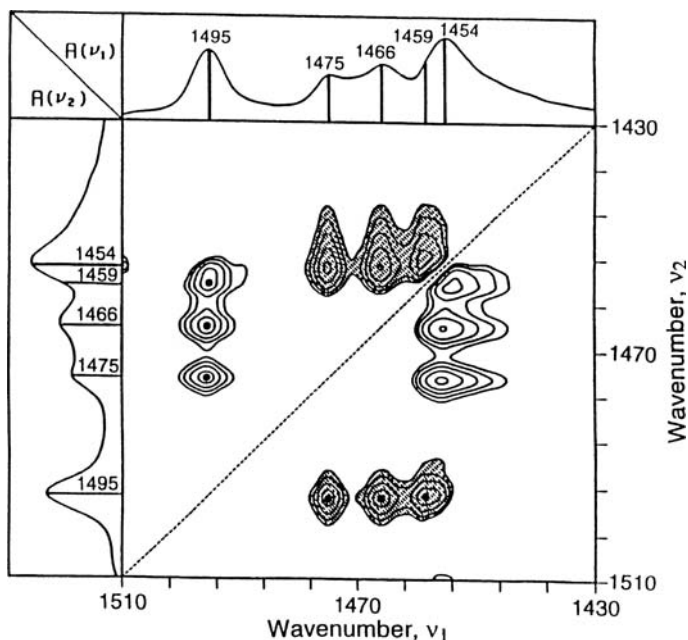


**Figure 8.7** Contour-map representation of the asynchronous 2D IR correlation spectrum of a mixture of polystyrene and polyethylene. (Reproduced with permission from Ref. No. 1. Copyright (1989) American Chemical Society.)

Interestingly, these bands all belong to the PS component of the blend. The asynchronous correlation indicates the existence of different types of reorientational motions occurring at slightly different rates within the same polymer. Bands at 1495 and 1454  $\text{cm}^{-1}$  arise from semicircle-stretching vibrations of the phenyl side groups, while the 1459  $\text{cm}^{-1}$  band is attributed to the  $\text{CH}_2$  deformation modes of the PS main chain. The fact that the synchronous cross peaks (Figure 8.6) are correlating between 1495 and 1454  $\text{cm}^{-1}$ , rather than 1495 and 1459  $\text{cm}^{-1}$ , also supports this assignment. The apparent asynchronicity among IR signals from these bands must, therefore, reflect a difference in the local rotational mobility of backbone and side-group functionalities of the polymer constituent under a dynamic perturbation. This observation is probably the very first 2D IR demonstration of the now well-established deconvolution capability of highly overlapped bands arising from different molecular vibrations.

The existence of overlapped IR bands of PS at 1454 and 1459  $\text{cm}^{-1}$  detected by this 2D IR experiment can be independently verified by selective deuterium substitution. Figure 8.8 compares the spectrum of atactic PS around 1455  $\text{cm}^{-1}$  with that of selectively deuterium-substituted PS. This particular sample has its hydrogen atoms of the methyne and methylene groups of the backbone replaced with deuterium. By selectively deuterium substituting these groups, the vibrational contributions of CH deformation modes of the PS backbone are eliminated from

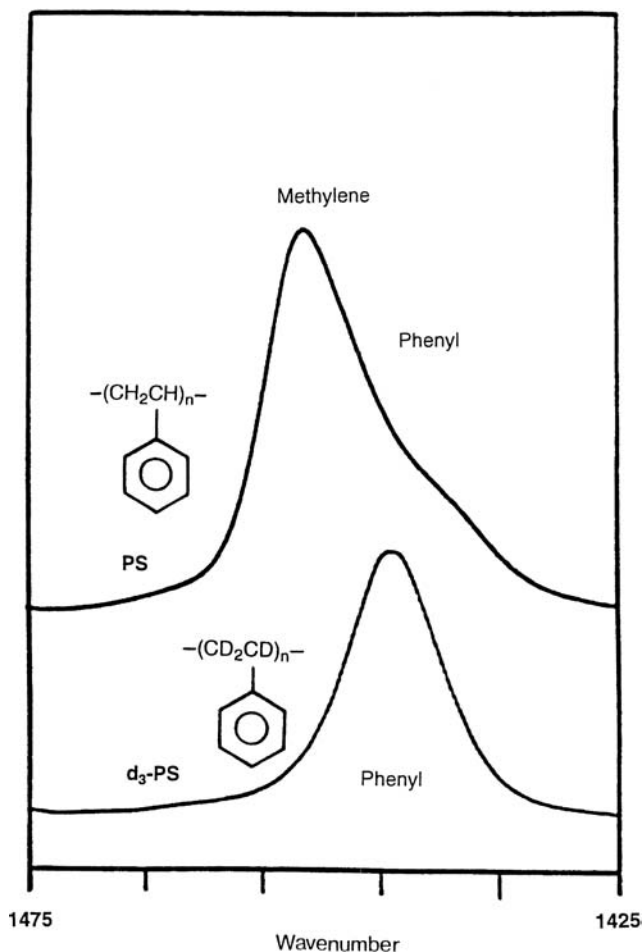


**Figure 8.8** IR spectra of regular polystyrene and backbone-deuterated ( $d_3$ ) polystyrene

this spectral region. The semicircle-stretching vibration of the phenyl side group at  $1454\text{ cm}^{-1}$ , on the other hand, is not affected by the deuterium substitution. The result provides the unambiguous assignments for the individual group contributions of PS in this spectral region and adds credibility to the remarkable resolving power of 2D IR spectroscopy.

In conclusion, the application of 2D IR spectroscopy to the study of a mixture of atactic PS and low-density PE has demonstrated several important advantages of this technique. The spectroscopic evidence clearly shows PS and PE in this blend are segregated at the molecular level, allowing the components to respond to an applied external perturbation independently of each other. A surprising level of difference is observed in the local mobility of the backbone and side group functionalities of PS, manifested in the asynchronous behavior of corresponding

bands under a common mechanical perturbation. Based on this observation, it is possible to assign the  $1459\text{ cm}^{-1}$  component of the broad IR band centered around  $1454\text{ cm}^{-1}$  to the PS backbone  $\text{CH}_2$  deformation, even without selective deuterium substitution.

### 8.2.2 POLYSTYRENE

A simple demonstration in the preceding section reveals the powerful capability of dynamic 2D IR spectroscopy to detect highly localized semi-independent motions of individual submolecular constituents. A more detailed study is carried out for a pure atactic polystyrene (PS) sample to further explore the submolecular responses of this system to a small-amplitude mechanical perturbation.<sup>2</sup> A simple homogeneous one-phase system consisting of a single, noncrystallizing homopolymer provides an excellent opportunity to test the applicability of the dynamic 2D IR technique to the study of local molecular dynamics of polymers. By using 2D IR, it was found that surprisingly rich information can be extracted even from such an elementary system.

Dynamic IR dichroism data used for the 2D correlation analysis was obtained by applying a 23 Hz dynamic strain with nominal amplitude of 0.1 % to a thin solution-cast film of atactic polystyrene (Mw of ca 150 000) at room temperature. The room-temperature DIRLD spectra in the CH stretching and CH deformation region are measured. While the time-dependent reorientations of transition dipoles associated with the molecular vibrations of backbone and side groups are observable in the original dynamic dichroism spectra (not shown), much more detailed features are better recognized in the corresponding dynamic 2D correlation spectrum.

Figure 8.9 shows a contour map representation of the synchronous dynamic 2D IR correlation spectrum of a PS film in the CH stretching region. Autopeaks at the diagonal positions of Figure 8.9 represent the reorientational motions of electric dipole transition moments of the PS sample. Peaks at  $2855$  and  $2925\text{ cm}^{-1}$  are, respectively, assignable to the symmetric and asymmetric  $\text{CH}_2$  stretching vibrations of the backbone methylene groups, while those above  $3000\text{ cm}^{-1}$  represent the reorientation of phenyl side groups. The appearance of positive synchronous cross peaks at the spectral coordinates corresponding to the two methylene bands indicates that the transition dipoles associated with the two wavenumbers reorient at a similar rate. Such synchronized responses are more or less expected, since both IR bands originate from vibrations of the same submolecular structural unit.

The positive sign of the methylene cross peaks at  $2855$  and  $2925\text{ cm}^{-1}$  suggests the electric dipole transition moments for the symmetric and asymmetric methylene vibrations are both realigning in similar relative directions, i.e., perpendicular to the direction of the applied stretch. The molecular architecture dictates that these two dipole transition moments are locally aligned more or less perpendicular to the backbone of the polymer segment.<sup>9,10</sup> It is therefore not difficult to conclude that the molecular chain segment of PS must be realigning



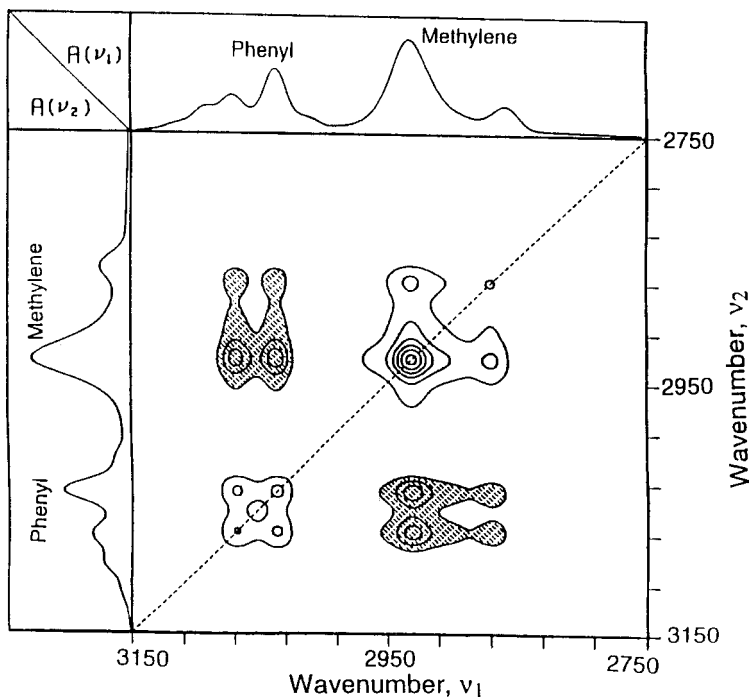


**Figure 8.9** Synchronous 2D IR spectrum of polystyrene in the CH stretching region. (Reproduced with permission from Ref. No. 4. Copyright (1999) Wiley-VCH.)

in the direction parallel to the applied dynamic tensile strain, leading to the perpendicular reorientations of 2855 and 2925  $\text{cm}^{-1}$  transition moments. Negative synchronous cross peaks between the methylene and phenyl bands indicate that some of the dipole transition moments for phenyl side groups are reorienting in the direction parallel to the applied dynamic strain direction.

Figure 8.10 shows the asynchronous dynamic 2D IR dichroism spectrum of the same sample comparing the reorientation dynamics of electric dipole transition moments for backbone methylene and side-group phenyl. The spectral region corresponding to the methylene stretching vibrations below 2800  $\text{cm}^{-1}$  is not shown, since no significant asynchronous cross peaks are observed. The appearance of strong asynchronous cross peaks between phenyl and methylene bands suggests the existence of rather complex reorientation dynamics involving side groups of PS. The rate of reorientational motion of the side groups under a strain is apparently quite different from that of the backbone of this polymer. The signs of asynchronous cross peaks shown in this 2D spectrum are all positive. Coupled with the fact that the synchronous correlation intensities at corresponding coordinates (Figure 8.9) are all negative, one can easily conclude that the phenyl side groups of this glassy PS sample complete the realignment induced by the dynamic strain well before the polymer main chain.

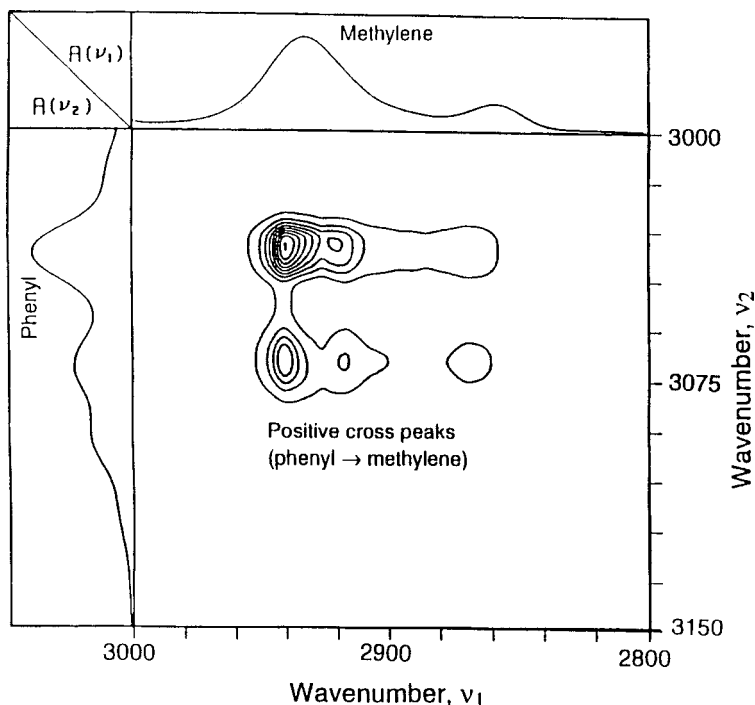


**Figure 8.10** Asynchronous 2D IR spectrum of polystyrene comparing the dynamic responses of the CH stretching and semicircle ring stretching regions. (Reproduced with permission from Ref. No. 4. Copyright (1999) Wiley-VCH.)

### 8.2.3 POLY(METHYL METHACRYLATE)

Another common glassy amorphous polymer, atactic poly(methyl methacrylate) or PMMA, has also been studied by dynamic 2D IR dichroism analysis.<sup>11</sup> This system is used as an important *test case* to verify the validity of the claimed high spectral resolution capability of 2D IR technique. The enhanced spectral resolution of a 2D IR spectrum should make it possible to differentiate highly overlapped IR bands associated with different chemical groups present in PMMA (e.g.,  $\alpha$ -methyl groups attached to the main chain and ester methyl groups). By using the deconvolved IR bands, individual reorientational motions of the functional groups can be monitored during the dynamic deformation of PMMA. From such information, highly localized submolecular reorientation mechanisms governing the macroscopic deformation of a glassy polymer may be elucidated.

Figure 8.11 shows the regular (i.e., one-dimensional) IR absorption spectrum of PMMA between 3100 and 2800  $\text{cm}^{-1}$ . This spectral region of PMMA is dominated by contributions from CH stretching modes of molecular vibrations. These CH stretching vibrations of PMMA are attributable to three distinct constituent groups: the  $\alpha$ -methyl group directly attached to the main chain carbon,

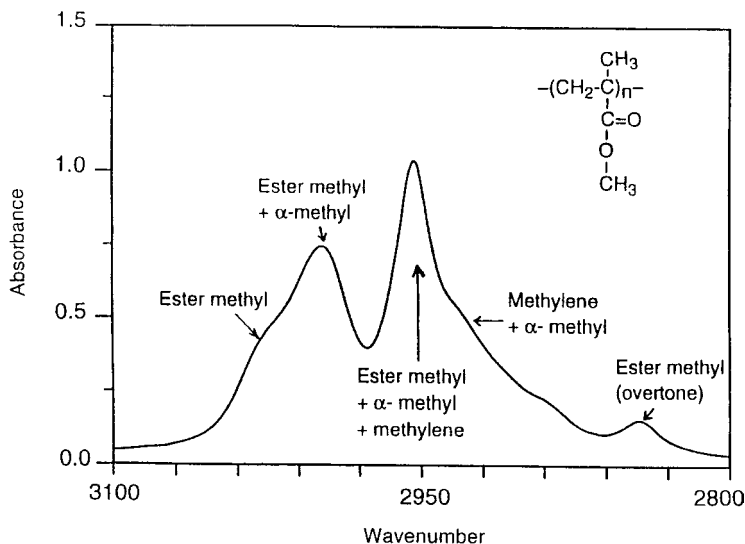


**Figure 8.11** IR spectrum of atactic poly(methyl methacrylate)

the side-chain ester methyl group, and the backbone methylene group. Absorption bands corresponding to the specific contributions from the molecular vibrations of these three constituent groups of PMMA are broad and highly overlapped. Interestingly, however, the assignments for individual IR absorption bands and the precise band positions of PMMA are well understood and verified.<sup>12</sup> Thus, this material is ideally suited for a model system to be used for the confirmation of the apparent high-resolution feature of 2D IR dichroism spectra.

The unambiguous band assignments for the CH stretching vibrations were provided by Dirlikov and Koenig, who carried out a systematic IR study of selectively deuterium-substituted PMMA samples in the late 1970s.<sup>12</sup> More specifically, they prepared three separate PMMA samples. In the first sample, the hydrogen atoms are all replaced with deuterium except for the ester methyl groups. The second sample was fully deuterium substituted except for the α-methyl groups. Likewise, the deuterium substitution of hydrogen atoms except for those of the methylene groups produced the third sample. As expected, the IR spectra of these deuterium-substituted PMMA samples in the CH stretching region are quite different from the spectrum of normal PMMA. Amazingly, however, when the three IR spectra of the selectively deuterated samples were combined, the spectrum of the CH stretching region of regular PMMA containing no deuterium was fully reconstructed. In short, the three IR spectra of selectively deuterium-substituted PMMA samples represent the pure group frequency spectra for individual constituent groups of PMMA contributing separately to the CH stretching vibrations.

Figure 8.12 shows the contour map representation of the synchronous 2D IR spectrum of atactic PMMA. The asynchronous 2D IR spectrum of the same

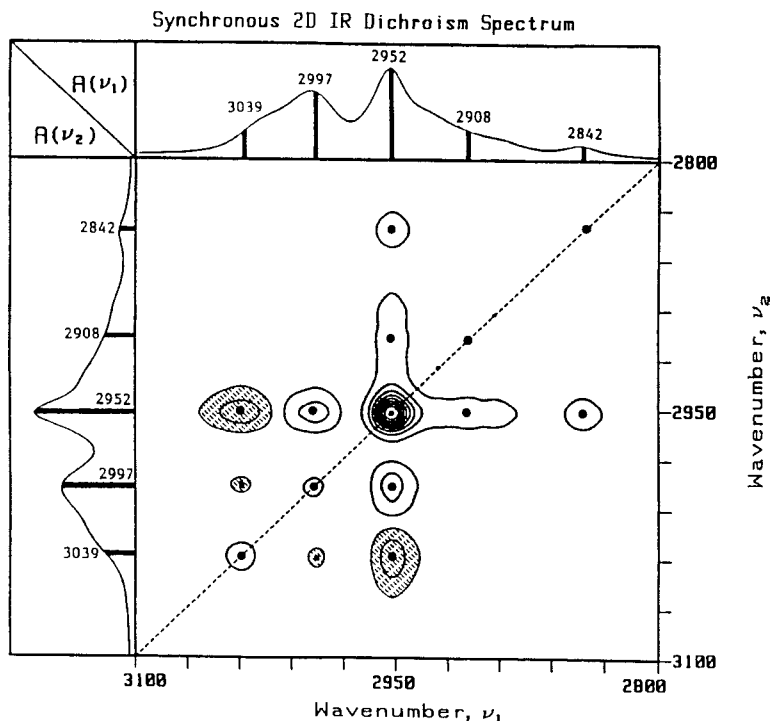


**Figure 8.12** Synchronous 2D IR spectrum of atactic PMMA film under dynamic deformation. (Reproduced from Ref. No. 11. Copyright (1990) I. Noda *et al.*)

sample is given in Figure 8.13. As usual, normal IR absorbance spectrum is provided at the top and side of each 2D map for reference. Dynamic dichroism data used to generate the 2D spectra were obtained with a time-resolved IR spectrometer previously described.<sup>6</sup> The IR dichroism measurement was carried out by mechanically stretching the sample of a thin PMMA film at room temperature with a 23 Hz small-amplitude (ca 0.1 %) oscillatory strain and recording the time-dependent fluctuations of directional IR absorbances induced by the perturbation at a spectral resolution of  $8\text{ cm}^{-1}$ .

Autopeaks observed on the diagonal of the synchronous spectrum (Figure 8.12) near  $2952$  and  $2997\text{ cm}^{-1}$  represent the perturbation-induced local reorientation of  $\alpha$ - and ester-methyl groups of PMMA. The peaks near  $2842$  and  $3039\text{ cm}^{-1}$ , on the other hand, arise exclusively from the contribution of ester-methyl groups. Peaks attributed to the backbone methylene groups are not clearly discernible. Intense cross peaks appearing at the off-diagonal positions of the spectral plane in Figure 8.12 indicate the existence of strong synchronous correlations between various methyl bands. The existence of correlation with the  $2842$  and  $3039\text{ cm}^{-1}$  ester-methyl bands implies that the dichroism fluctuation of the  $2952$  and  $2997\text{ cm}^{-1}$  bands also arises mostly from the reorientation of ester-methyl groups. Thus,

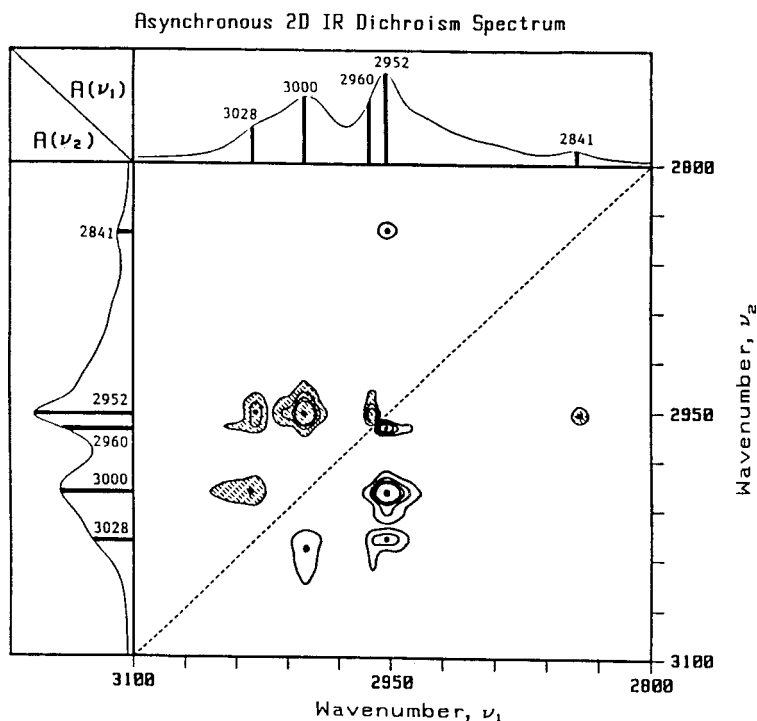


**Figure 8.13** Asynchronous 2D IR spectrum of atactic PMMA film under dynamic deformation. (Reproduced from Ref. No. 11. Copyright (1990) I. Noda *et al.*)

submolecular-level orientation of glassy PMMA under a small-amplitude tensile strain is observed predominantly for ester-methyl groups rather than  $\alpha$ -methyl groups attached to the main chain.

The asynchronous 2D spectrum (Figure 8.13) shows cross peaks differentiating several methyl bands. A set of asynchronous cross peaks, appearing at the spectral coordinates of 2952 and 3028  $\text{cm}^{-1}$ , indicate that these two IR bands belong to different methyl groups. Since the 3028  $\text{cm}^{-1}$  band is assigned to ester methyl, the band at 2952  $\text{cm}^{-1}$  must be from the  $\alpha$ -methyl groups of PMMA. Another set of cross peaks at 2952 and 2960  $\text{cm}^{-1}$  also indicate that the peak is a doublet consisting of the overlapped ester-methyl and  $\alpha$ -methyl bands. We may thus assign the 2952  $\text{cm}^{-1}$  band to  $\alpha$ -methyl groups, which can be differentiated from ester-methyl bands at 2841, 2960, 3000, and 3028  $\text{cm}^{-1}$ . These results are consistent with the assignments based on deuterium-substituted PMMA samples.<sup>12</sup>

The development of asynchronous cross peaks indicates that, even under an identical macroscopic perturbation, the time-dependent behavior of the IR dichroism fluctuation for the  $\alpha$ -methyl component of PMMA at room temperature is substantially different from that for ester-methyl groups. Obviously, the different functional groups in glassy PMMA are reorienting semi-independently of each

other at the submolecular level. The sequential order analysis of the asynchronous 2D IR spectrum based on the sign of cross peaks indicates that the dynamic fluctuation of the IR dichroism signal of  $\alpha$ -methyl precedes that of ester-methyl groups. In other words, the local reorientational motion of ester side groups of PMMA is lagging behind the main chain reorientation process, even though the final extent of reorientation of side groups is much greater.

#### 8.2.4 HUMAN SKIN STRATUM CORNEUM

The above example of a PMMA film demonstrates that 2D IR spectroscopy provides an exciting possibility of obtaining a true high-resolution mid-IR spectrum for a condensed-phase sample based purely on a physical stimulus, without resorting to mathematical manipulation techniques such as Fourier self-deconvolution of a spectrum. This feature is especially promising in the study of very complex biomolecules such as proteins and peptides. Various conformations of polypeptides (e.g.,  $\alpha$ -helix,  $\beta$ -sheet,  $3_1$  helix, and random coil) result in characteristic absorption bands in the Amide I and II regions.<sup>13–15</sup> These bands are usually highly overlapped, so that unambiguous band assignment is difficult. 2D IR analysis with its high spectral resolution capability should provide an excellent opportunity to study such convoluted spectra.

The 2D IR analysis of protein was first attempted for the study of protenaceous component of a human skin stratum corneum specimen.<sup>2</sup> The sample of human skin was mounted over an IR-transparent thin fluorocarbon substrate film. This composite sample was then acoustically stimulated with a small-amplitude strain at 80 Hz to induce the dynamic IR dichroism to be analyzed by the 2D correlation method. Figures 8.14 and 8.15 show the Amide I and II regions of the 2D IR dichroism spectra for stratum corneum.

The synchronous 2D IR spectrum of the human skin (Figure 8.14) clearly indicates the existence of temporal correlations between certain components of IR bands belonging to the Amide I and Amide II modes of vibrations (e.g., 1635 and 1530  $\text{cm}^{-1}$  bands). The composite nature of the amide peaks is well demonstrated by the asynchronous spectrum (Figure 8.15), which shows the decoupling of the Amide I peak into several independent bands. For example, the asynchronous peaks at 1646 and 1635  $\text{cm}^{-1}$  indicate the existence of separate component bands at these wavenumbers. While the dipole transition moments for the different component bands of the Amide I peak are reorienting independently of each other, some are coupled with the reorientation of certain Amide II component bands.

It is reasonable to assume that the correlated bands in human stratum corneum probably originate from common local conformations of polypeptide chains. Thus, it can be speculated that the  $3_1$  helix component of the Amide I band, for example, will couple with the  $3_1$  helix component of the amide II band. Similar arguments may hold for other conformations. By systematically developing 2D spectra of model polypeptides with known conformations, it may

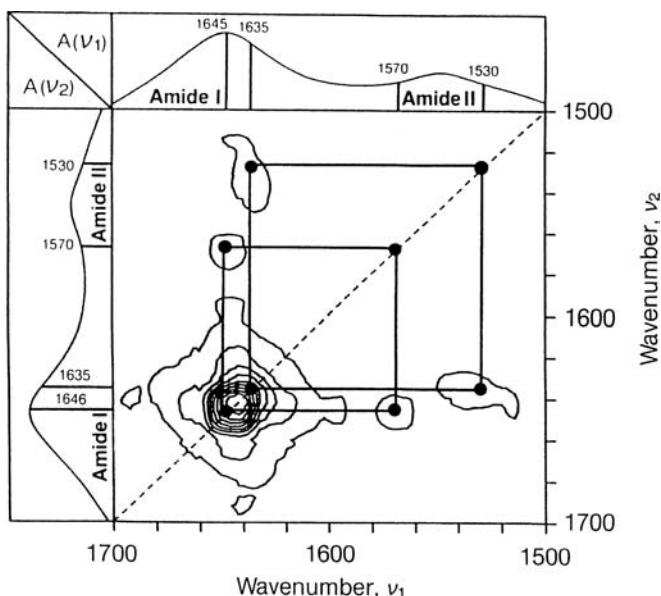


**Figure 8.14** Asynchronous 2D IR spectrum of human stratum corneum. (Reproduced with permission from I. Noda, *Appl. Spectrosc.*, **44**, 550 (1990). Copyright (1990) Society for Applied Spectroscopy.)

eventually become possible to identify well-resolved *two-dimensional vibrational fingerprints* of protein conformations. 2D IR spectroscopy alone obviously will not provide a complete answer to the fundamental questions of vibrational spectroscopy of a complex biological sample. The technique, however, is a powerful analytical tool, which should augment other spectroscopic studies to provide submolecular-level understanding of complex systems.

### 8.2.5 HUMAN HAIR KERATIN

2D IR spectroscopy has been used to study another protein-based system: the microstructure of human hair keratin by carrying out a correlation analysis of the time-dependent changes under small-amplitude oscillatory strain.<sup>16</sup> It is well known that the mechanical properties of human hair are very sensitive to changes in environmental conditions. For example, climatic changes in temperature and relative humidity can have a dramatic effect on hair set. Cosmetic treatments for hair such as shampooing, conditioning, and permanent waving are also known to affect the mechanical properties of hair fibers. However, little is known about the mechanism by which these changes in physical properties occur.

Features in a 2D IR spectrum of keratin are very sensitive to molecular-level changes induced by temperature, relative humidity (RH), and the addition of hair treatments. 2D IR spectra also provide an attractive resolution enhancement

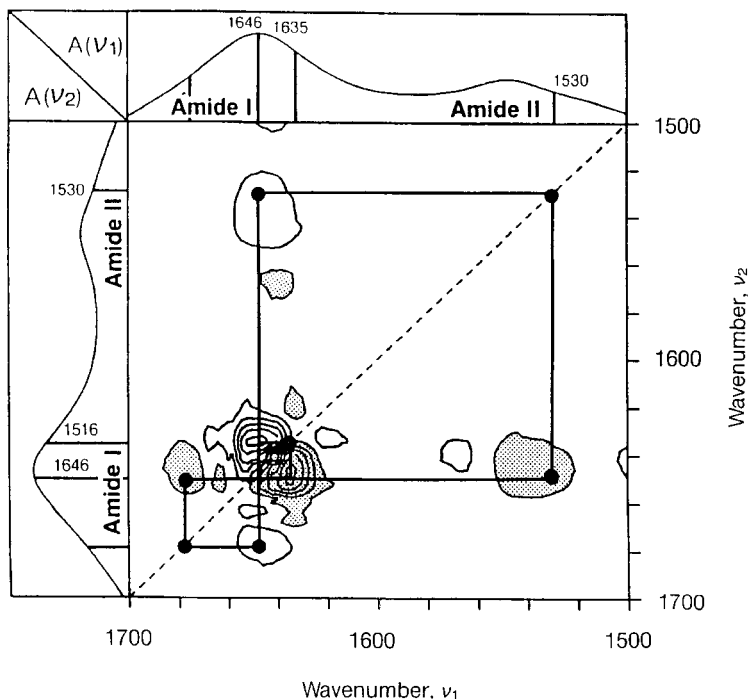


**Figure 8.15** Synchronous 2D IR spectrum of human stratum corneum. (Reproduced with permission from I. Noda, *Appl. Spectrosc.*, **44**, 550 (1990). Copyright (1990) Society for Applied Spectroscopy.)

of naturally broad IR absorbance bands in the Amide I and Amide II regions of proteins. The net result is an improved molecular-level understanding of the mechanical properties of human hair and identification of factors affecting these properties. Thus, 2D IR spectroscopy is used to study the molecular-level origin of the physical properties of human hair.

A clean-blended European blonde hair sample (National Hair, New York) was solubilized by pulverizing it first, and then breaking the disulfide bonds using 8 M urea and 50 mM dithiothreitol. After filtering, the keratin sulfhydryls were alkylated to form *S*-carboxymethyl keratin (SCMK). The SCMK was dialyzed and thin films were cast from a 10 mg ml<sup>-1</sup> 50/50 water/isopropanol solution onto an IR-transparent polyhalocarbon substrate. Finished samples typically measured 4 × 2 cm with a thickness of 25–100 μm (not including substrate). A 23 Hz dynamic tensile strain with an amplitude of 50 μm was applied to the sample during the measurement. The sample was located inside an environmental control chamber. This chamber allows temperature control within 0.5 °C, and was modified to allow humidity control within 2 % RH. Keratin films were analyzed at temperatures from 30 to 60 °C and at various humidity levels up to about 90 % RH. All IR spectra were collected at a nominal resolution of 8 cm<sup>-1</sup>.



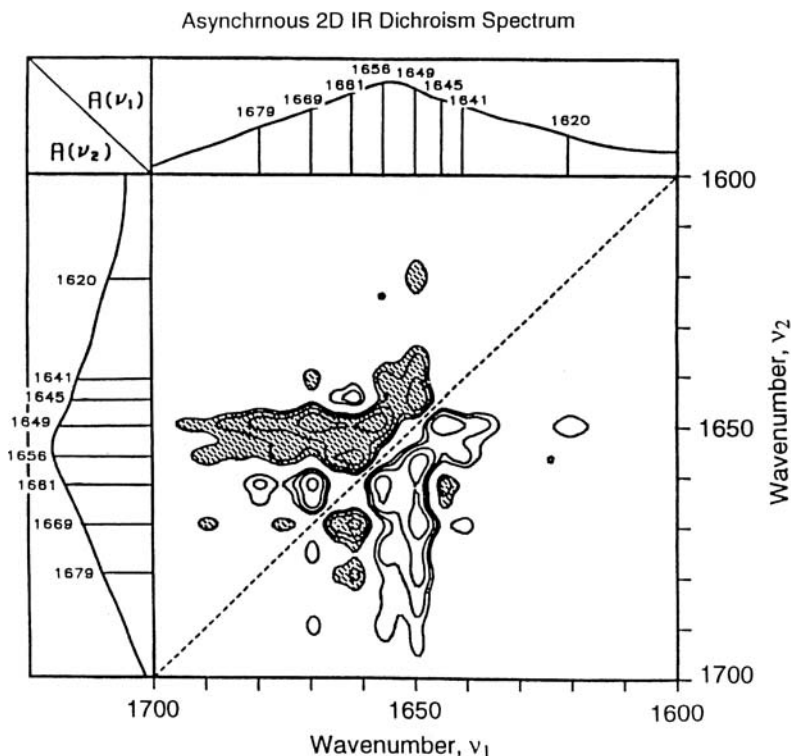


**Figure 8.16** Asynchronous 2D IR spectrum of human-hair keratin in the Amide I region

Figure 8.16 shows the 2D IR asynchronous correlation spectrum of a thin keratin (SCMK) film cast from solubilized hair in the Amide I region at ambient temperature and humidity. Cross peaks represent individual submolecular components reorienting at different rates in response to the externally applied perturbation. A clear resolution enhancement of this naturally broad IR absorbance band is achieved. The bands resolved can be readily assigned to  $\alpha$ -helices (1661 and 1649  $\text{cm}^{-1}$ ),  $\beta$ -like extended chains and turns (1679, 1669, 1645, 1641, and 1620  $\text{cm}^{-1}$ ) as well as disordered structures (1656  $\text{cm}^{-1}$ ).<sup>16</sup>

Figure 8.17 shows the corresponding synchronous 2D IR spectrum of the same system. The synchronous spectrum in the amide I region is dominated by a characteristic four-leaf-clover pattern consisting of two autopeaks with adjacent negative cross peaks which is characteristic of  $\alpha$ -helical responses. The amide I region of 2D IR spectra of the keratin changes as a function of temperature. The changes observed correlate well with DSC (differential scanning calorimetry) data of actual hair showing an irreversible thermal transition near 45 °C. The  $\alpha$ -helical four-leaf-clover feature near 1650  $\text{cm}^{-1}$  in the synchronous spectra remains relatively unchanged as a function of temperature, suggesting other components such as random or  $\beta$ -sheet-like structures are responsible for those differences seen.

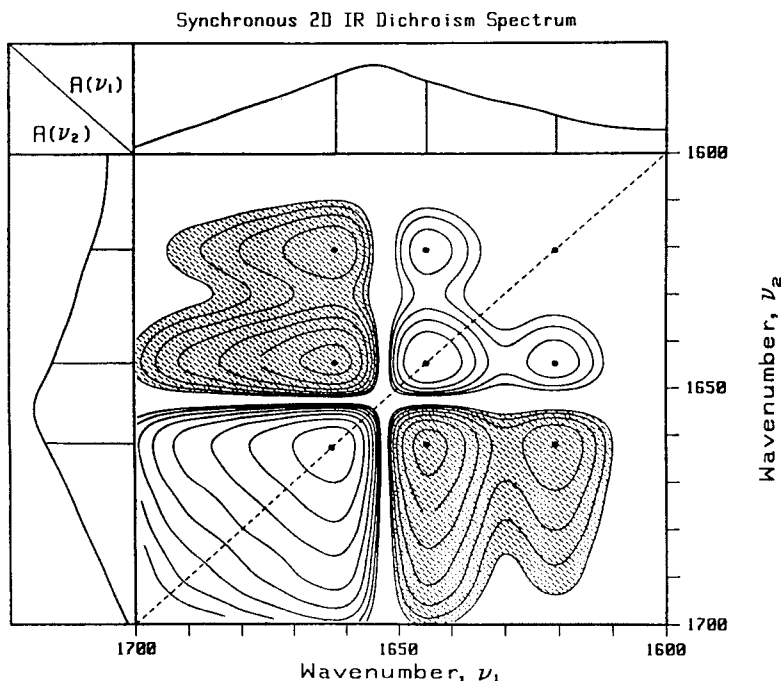


**Figure 8.17** Synchronous 2D IR spectrum of human-hair keratin in the Amide I region

The effects of high relative humidity and treatment with naphthalene sulfonic acid (NSA) on hair-keratin films have also been examined by 2D IR spectroscopy. We found a significant increase in dynamic IR signal intensity for bands characteristic of an  $\alpha$ -helical response at high RH and after NSA treatment.

#### 8.2.6 TOLUENE AND DIOCTYLPHTHALATE IN A POLYSTYRENE MATRIX

The understanding of the origin and specific nature of miscibility in multicomponent mixtures of different chemical compounds is of great scientific interest, from both fundamental research and practical technological points of view. The macroscopic thermodynamic description of solutions cannot provide adequate molecular level insight into the underlying mechanisms of solubilization and miscibility, necessary for proper design of various mixtures. 2D IR dichroism spectroscopy, which is sensitive to specific submolecular scale interactions of chemical groups, may provide useful background information for the study of miscible multicomponent systems.

The first example of the miscible blend study is a mixture system consisting of a small amount ( $<10$  wt%) of toluene added to a matrix of atactic polystyrene. The polystyrene used for this experiment is perdeuterated to minimize the overlap

of the spectral features of toluene and polystyrene. Toluene is a good solvent for polystyrene, and at a low level of addition, it serves as an effective plasticizer for polystyrene to reduce the glass transition temperature. The sample was prepared from a dilute solution of polystyrene in toluene. The solvent was evaporated gradually for a given amount of time in open air to form a uniform film. The film was then placed in a container saturated with toluene vapor until later use. The polystyrene sample film containing a small amount of toluene was analyzed with a dynamic spectrometer at  $-30^{\circ}\text{C}$  under a dynamic deformation applied at a frequency of 23 Hz and a strain amplitude of 0.1 %.

The synchronous 2D IR spectrum (Figure 8.18) of the toluene/polystyrene mixture system shows that the strain-induced molecular reorientation of toluene is fully synchronized with the motion of polystyrene. The entire spectral plane is filled with strong synchronous correlation peaks. On the other hand, the corresponding asynchronous 2D IR spectrum (not shown) has few correlation intensities beyond the noise level of the instrumental detection limit. At this temperature, the asynchronicity among motions of constituent groups of polystyrene, such as side-chain phenyl and backbone methylene, is rather weak. The whole system, including toluene molecules, is reorienting under the applied strain as a coordinated unit with few local independent motions. While this finding by itself

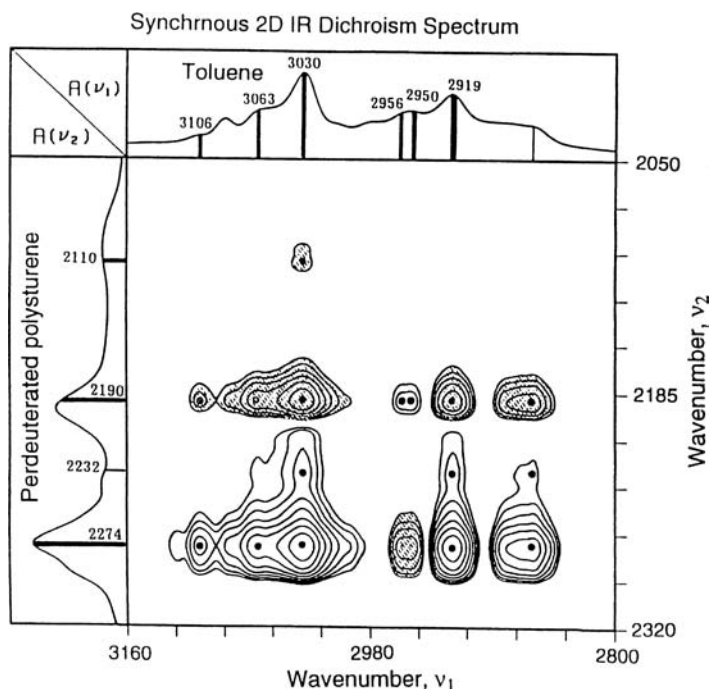


**Figure 8.18** Synchronous 2D IR spectrum of PS/toluene blend. (Reproduced from I. Noda *et al.*, *Appl. Spectrosc.*, 54, 236A (2000). Copyright (2000) Society for Applied Spectroscopy.)

is not very exciting, it is the first demonstration that strain-induced reorientational motions of small molecules can be observed by dynamic IR measurement and analyzed with 2D correlation analysis.

The second mixture system studied here provides a more interesting result.<sup>17</sup> This time, a small amount of dioctylphthalate (DOP) is mixed with deuterium-substituted atactic polystyrene. The new sample was made by adding a small amount of DOP to a toluene solution of polystyrene. The toluene was then fully removed by a prolonged evaporation to produce a thin uniform film with residual DOP. The DOP-containing sample film was analyzed with the dynamic spectrometer under conditions similar to the case for the toluene/PS mixture. DOP is a commonly used plasticizer for various polymers due to its affinity to a wide variety of polymeric materials. The chemical structure of DOP has a slight overlap with toluene; DOP also has an aromatic ring. However, instead of a single methyl group as in toluene, DOP has two long fatty octyl ester chains attached to the ring. The presence of ester chains makes this compound much less volatile, which is an important requirement for a good plasticizer.

Unlike the case for the toluene/polystyrene mixture, the asynchronous 2D IR spectrum of the DOP/polystyrene mixture (Figure 8.19) shows the development

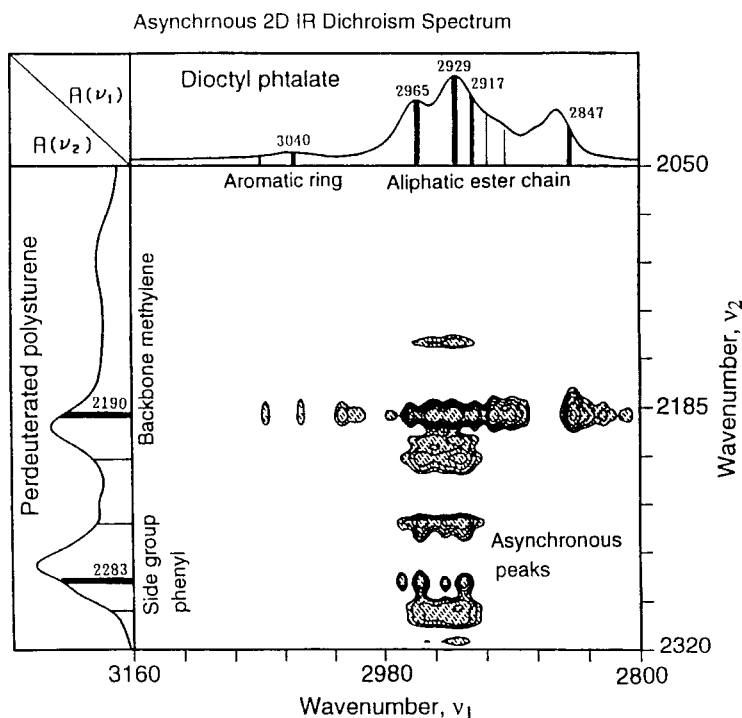


**Figure 8.19** Asynchronous 2D IR spectrum of PS/DOP blend. (Reproduced from I. Noda *et al.*, *Appl. Spectrosc.*, 54, 236A (2000). Copyright (2000) Society for Applied Spectroscopy.)

of numerous asynchronous cross peaks with significant correlation intensities between bands assignable to the ester side chains of DOP and those for backbone groups of polystyrene. According to this result, DOP molecules are moving more or less independently of the reorientational motions of polystyrene under the applied dynamic deformation. The result is somewhat surprising since such strong asynchronicity among different compounds is more commonly observed among the components of phase-separated systems.

Considering the affinity of DOP for polystyrene, it is hard to imagine the system is phase separated into DOP-rich and polystyrene-rich domains. The key to answering this puzzling question lies in the notable absence of an asynchronous cross peak between the band assignable to the aromatic ring of DOP and the phenyl side group of polystyrene. The synchronous 2D IR spectrum (Figure 8.20) clearly indicates the development of a distinct cross peak at the same spectral coordinate between the two aromatic groups from DOP and polystyrene. Unlike the rest of the system, the strain-induced reorientational motions of these two aromatic groups are fully synchronized with each other. One may thus speculate on

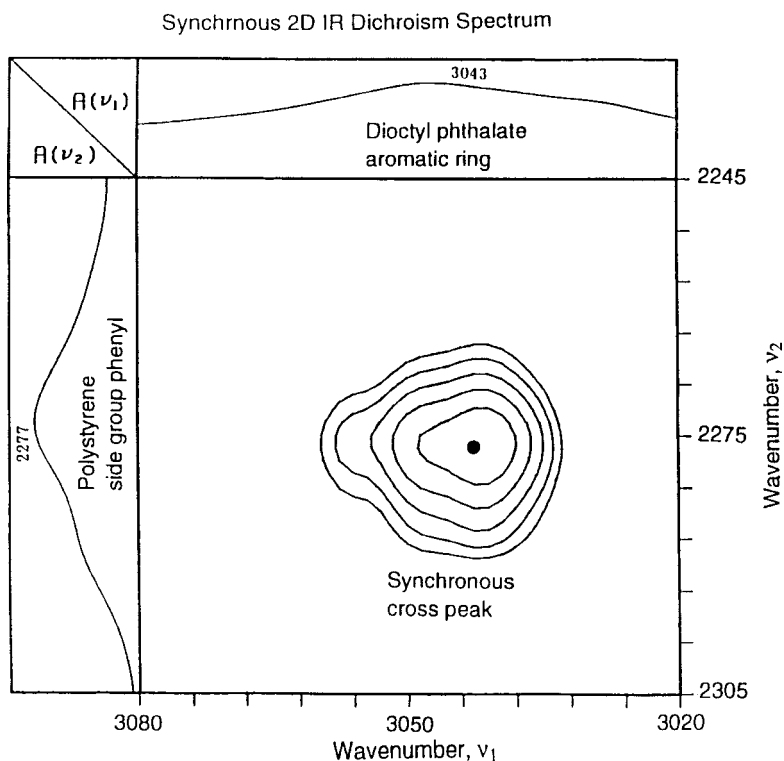


**Figure 8.20** Synchronous 2D IR spectrum of PS/DOP blend. (Reproduced from I. Noda *et al.*, *Appl. Spectrosc.*, 54, 236A (2000). Copyright (2000) Society for Applied Spectroscopy.)

the existence of a specific interaction between these aromatic groups, most likely facilitated by the presence of  $\pi$  electrons, which coordinates the submolecular reorientational motions of aromatic rings under a common strain.

By combining the observations made for the molecular motions of the DOP/polystyrene system probed by dynamic 2D IR spectroscopy, a potential hypothetical picture emerges for the mechanism of plasticization of polystyrene by DOP. It is quite possible that DOP molecules form a short-lived association complex with the host polystyrene. The site of specific interactions driving such associated behavior is confined locally to the aromatic groups. The aliphatic ester groups of DOP, on the other hand, are free to move around without being restricted by the presence of polystyrene. An association complex of this type should have a temporal structure similar to a comb (i.e., short-chain branched) polymer. The additional free volume provided by the comb structure results in the increased molecular chain mobility, which leads to the observed plasticization. The duration of the complex formation between DOP and polystyrene may not be very long. If indeed the complexation between the aromatic groups takes place, it probably does not last much longer than the characteristic time scale of the experimental observation. In the case of our dynamic dichroism spectroscopy experiment, where a subacoustic-range mechanical perturbation is used to stimulate the system, the characteristic time of the observation is on the order of milliseconds. In order to study a system with much shorter relaxation times, such as molecular interactions in a common liquid solution, faster excitation schemes capable of probing nanosecond-range responses must be employed.

### 8.2.7 POLYSTYRENE/POLY(VINYL METHYL ETHER) BLEND

While most polymer blends are immiscible with each other because of the very low entropy of mixing of large molecules, certain exceptional classes of polymer blends are truly miscible and form homogeneous one-phase mixtures. A specific interaction at the submolecular level, e.g., hydrogen bonding, is usually involved in such miscible blends to overcome the tendency to phase segregate. 2D IR spectroscopy is especially suited to the investigation of the molecular level description of such specific interactions. One of the best-known miscible systems, a blend of atactic polystyrene and poly(vinyl methyl ether), is investigated here by using the dynamic 2D IR technique to elucidate the molecular origin of the miscibility of these polymers.<sup>18</sup>

Polystyrene and poly(vinyl methyl ether) are very different polymers. Polystyrene is a hard hydrophobic plastic resin, while poly(vinyl methyl ether) is a soft water-soluble polymer. It is quite surprising that a pair of such dissimilar polymers could produce a molecularly homogeneous one-phase system. The specific origin of the miscibility of this polymer pair is not well understood, although some level of interaction is thought to occur between the methoxyl groups of poly(vinyl methyl ether) and the phenyl groups of polystyrene.<sup>19</sup>

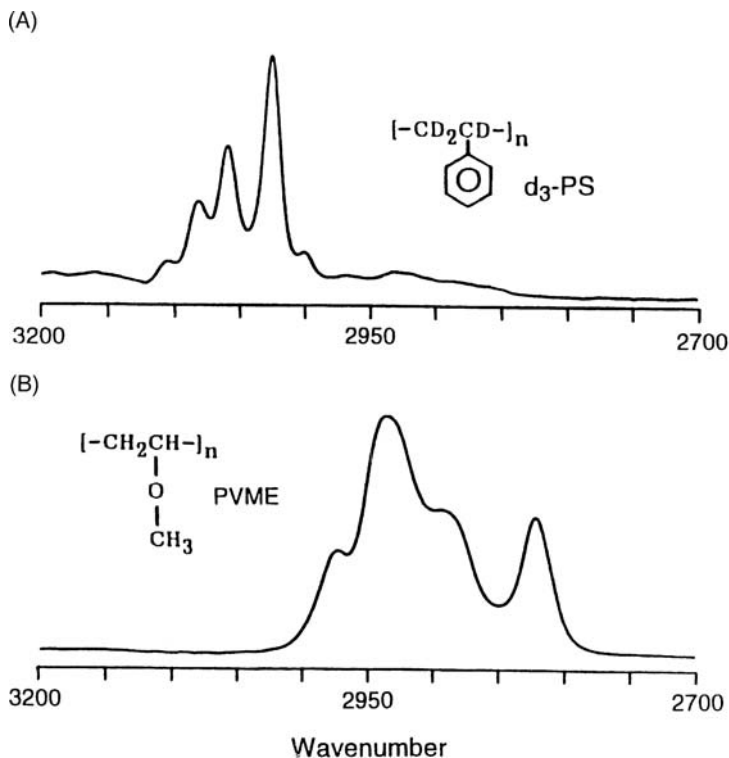


**Figure 8.21** IR spectrum of PS/PVME blend. The spectrum is well resolved to regions for (A) backbone-deuterated PS and (B) PVME. (Reprinted from *Polymer Solutions, Blends, and Interfaces*, M. M. Satkowski, J. T. Grothaus, S. D. Smith, A. Ashraf, C. Marcott, A. E. Dowrey, and I. Noda, pp. 89–108, Copyright (1992), with permission from Elsevier.)

To simplify the spectral assignment, polystyrene which had been selectively deuterium substituted to eliminate the backbone contribution (Figure 8.21) was blended with poly(vinyl methyl ether). Special attention was paid to the spectral features of the methoxyl peak of the poly(vinyl methyl ether) component near  $2820\text{ cm}^{-1}$ . Figure 8.22 shows the asynchronous 2D IR spectrum of poly(vinyl methyl ether) in this region. The presence of a pair of asynchronous cross peaks at the spectral coordinates of  $2825$  and  $2813\text{ cm}^{-1}$  indicates there are two distinct populations of methoxyl groups belonging to the poly(vinyl methyl ether) component of this blend, each having a different IR absorption band and unique reorientation rate under the dynamic strain.

The synchronous spectrum (Figure 8.23) shows that the methoxyl band of poly(vinyl methyl ether) at  $2813\text{ cm}^{-1}$  is correlated with the IR bands located at  $3055$  and  $3024\text{ cm}^{-1}$  assignable to the phenyl side groups of the polystyrene component of this blend. The presence of such synchronous correlation cross peaks suggests the existence of a specific interaction to coordinate the local

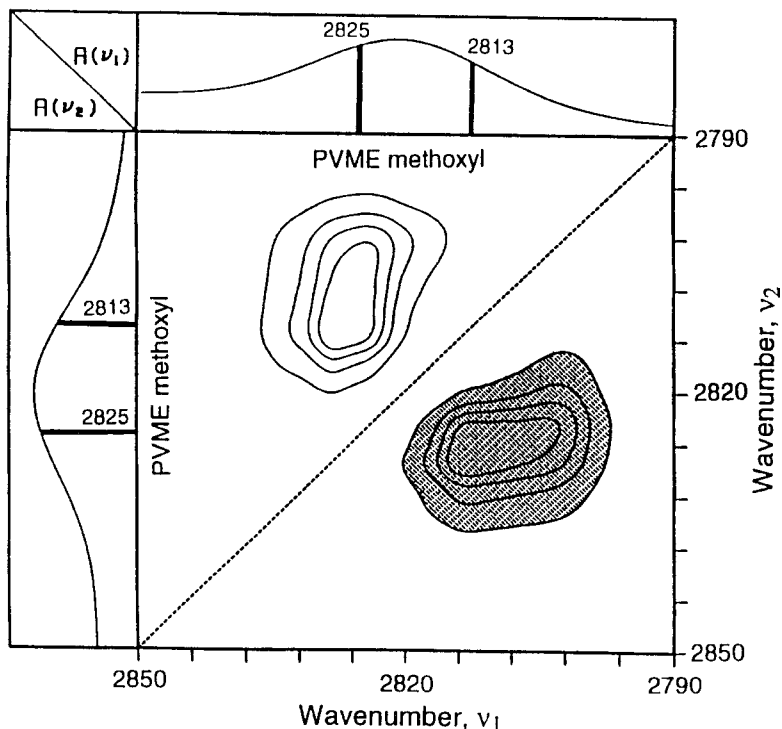


**Figure 8.22** Asynchronous 2D IR spectrum of methoxylate groups in PS/PVME blend. (Reprinted from *Polymer Solutions, Blends, and Interfaces*, M. M. Satkowski, J. T. Grothaus, S. D. Smith, A. Ashraf, C. Marcott, A. E. Dowrey, and I. Noda, pp. 89–108, Copyright (1992), with permission from Elsevier.)

motions between the methoxyl groups of poly(vinyl methyl ether) and polystyrene phenyl groups. A model has been proposed in the past in which there exists a specific molecular interaction involving the lone-paired electron of the methoxyl oxygen of poly(vinyl methyl ether) and  $\pi$  electrons of the polystyrene phenyl groups.<sup>19</sup> The current 2D IR result of coordinated motions of phenyl and methoxyl groups supports this hypothesis.

Interestingly, however, Figure 8.23 indicates only one methoxyl band is interacting with the polystyrene phenyl, even though there are two distinct IR contributions of poly(vinyl methyl ether) methoxyl groups as already shown in Figure 8.22. The other methoxyl band of poly(vinyl methyl ether) at  $2824\text{ cm}^{-1}$  is asynchronously correlated with the phenyl bands of the polystyrene component (Figure 8.24). This result suggests that, while the methoxyl groups of the poly(vinyl methyl ether) component represented by the  $2813\text{ cm}^{-1}$  band are strongly interacting with the polystyrene phenyl groups, the other methoxyl groups represented by the  $2824\text{ cm}^{-1}$  band are not strongly interacting with the phenyls.



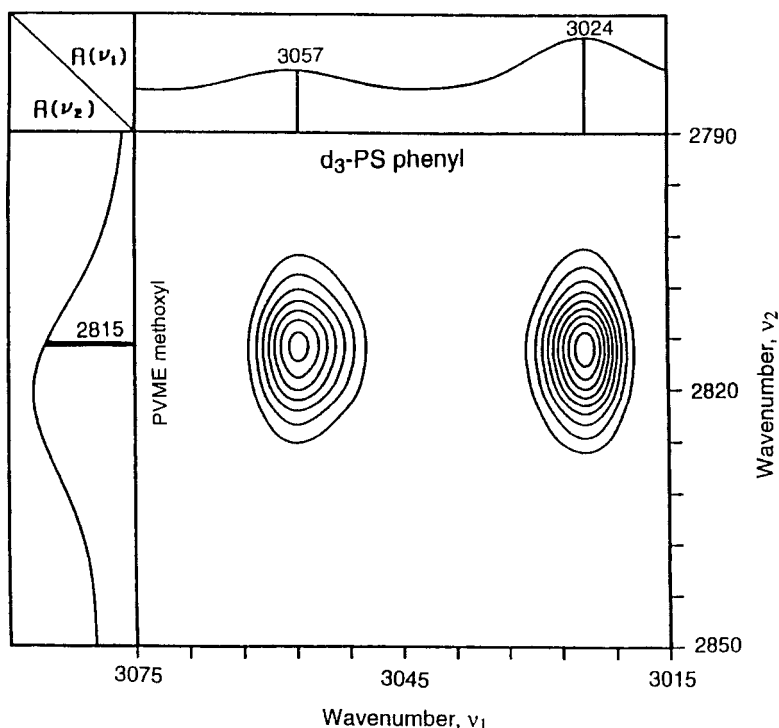


**Figure 8.23** Synchronous 2D IR spectrum of PS/PVME blend. (Reprinted from *Polymer Solutions, Blends, and Interfaces*, M. M. Satkowski, J. T. Grothaus, S. D. Smith, A. Ashraf, C. Marcott, A. E. Dowrey, and I. Noda, pp. 89–108, Copyright (1992), with permission from Elsevier.)

There are many other examples of dynamic 2D IR studies of miscible blend mixtures involving specific submolecular interactions of the components. The miscible polymer pair of polystyrene and poly(phenylene oxide), for example, was studied by the 2D FT-IR technique in an effort to understand the molecular origin of the miscibility of this system.<sup>20</sup> Specific interactions between polymers and various additives such as plasticizers may also be studied by 2D IR. The importance of localized submolecular level interactions among chemical groups of the mixture components is often demonstrated.

### 8.2.8 LINEAR LOW DENSITY POLYETHYLENE

Multiphase systems, such as those consisting of crystalline and amorphous phases, provide very interesting opportunities for dynamic 2D IR analysis. Because of differences in mechanical properties of coexisting phase domains, even under a common macroscopic deformation, each phase is often subjected to a different level of mechanical stimuli. The molecular reorientational responses of individual

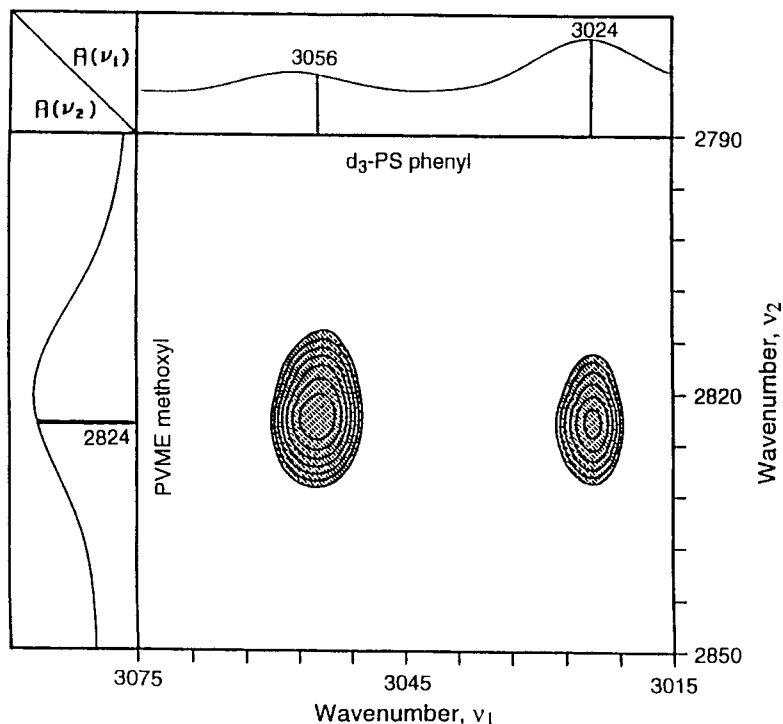


**Figure 8.24** Asynchronous 2D IR spectrum of PS/PVME blend. (Reprinted from *Polymer Solutions, Blends, and Interfaces*, M. M. Satkowski, J. T. Grothaus, S. D. Smith, A. Ashraf, C. Marcott, A. E. Dowrey, and I. Noda, pp. 89–108, Copyright (1992), with permission from Elsevier.)

phase components also are quite different. Thus, it is often possible to differentiate dynamic IR signals arising from different phases, even if their spectral coordinates are only several wavenumbers apart.

Semicrystalline polymers, such as polyethylene and polypropylene, may also be studied by using the 2D IR technique. By taking advantage of the enhanced spectral resolution of 2D IR, overlapping IR bands assignable to the coexisting crystalline and amorphous phases of semicrystalline polymer samples can be distinguished. Such differentiation is especially useful, for example, in the study of blends of high-density polyethylene and low-density polyethylene.<sup>21</sup> It was found that blends of polyethylenes are mixed at the molecular scale only in the amorphous phase, while each component crystallizes separately. In this section, a 2D IR analysis applied to a film of linear low-density polyethylene is discussed.<sup>22</sup>

Figure 8.25 shows the asynchronous 2D IR spectrum of a thin film of linear low-density polyethylene consisting mainly of ethylene repeating units with a small amount (ca 3 mol%) of deuterium-substituted 1-octene comonomer units. The octene comonomer is incorporated into the polyethylene chain to produce

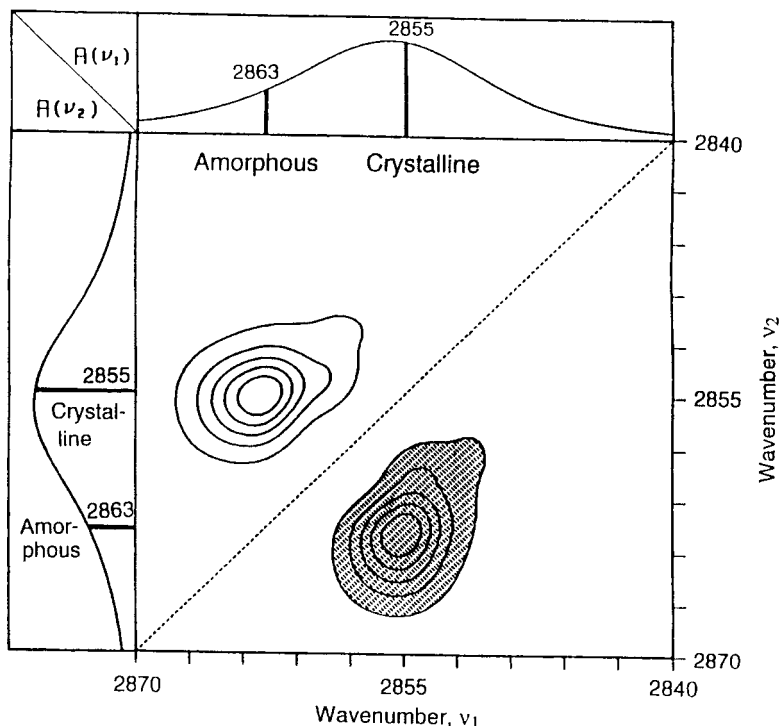


**Figure 8.25** Asynchronous 2D IR spectrum of LLDPE in the  $\text{CH}_2$  symmetric stretching region. Crystalline and amorphous bands around  $2855$  and  $2863\text{ cm}^{-1}$  are split. (Reprinted from *Polymer Solutions, Blends, and Interfaces*, R. S. Stein, M. M. Satkowski, and I. Noda, pp. 109–131, Copyright (1992), with permission from Elsevier.)

short (six-carbon) side branches, which reduce the melt temperature and crystallinity of the polymer. By selectively labeling the octene units with deuterium, one can unambiguously differentiate the dynamics of the main chain and short side branches. The spectral region shown in Figure 8.25 represents IR signals arising exclusively from the polyethylene chain, with little contribution from the octene side branches.

The presence of asynchronous peaks in the  $\text{CH}_2$  stretching vibration of backbone methylene groups indicates that there are two distinct bands in this region of the IR spectrum of the linear low-density polyethylene sample. The band located near  $2855\text{ cm}^{-1}$  is assignable to the contribution from the crystalline phase, while the band near  $2863\text{ cm}^{-1}$  is due to the amorphous component of this semicrystalline polymer. The above assignment can be easily verified by heating the sample above its melt temperature; the IR spectral contribution from the crystalline component of the specimen disappears. Because of the significant difference in the reorientational responses of polymer chains located in the crystalline and amorphous phase domains, 2D IR dichroism spectroscopy can separate

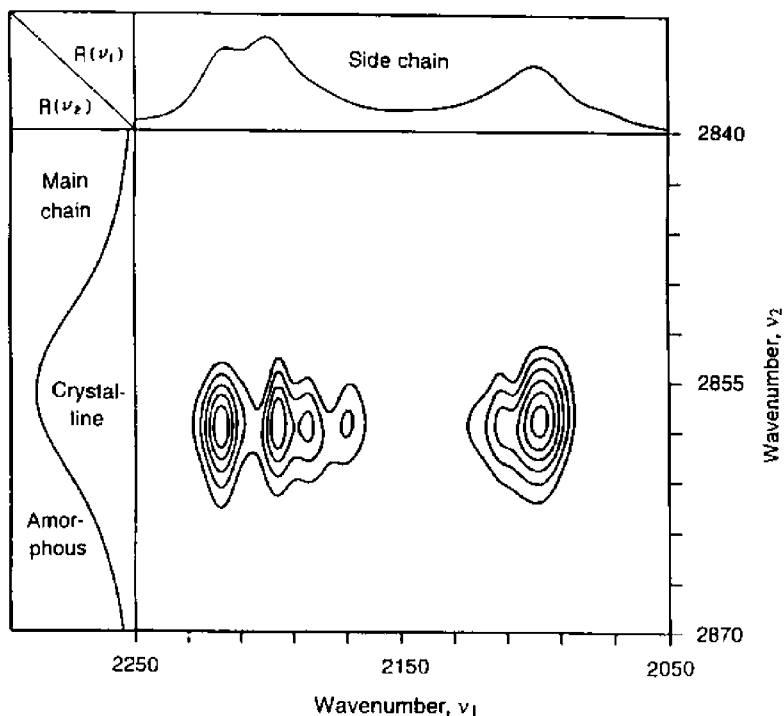


**Figure 8.26** Asynchronous 2D IR spectrum of side-chain-deuterated LLDPE. Cross peaks develop between the side-chain bands and the backbone crystalline band. (Reprinted from *Polymer Solutions, Blends, and Interfaces*, R. S. Stein, M. M. Sattkowski, and I. Noda, pp. 109–131, Copyright (1992), with permission from Elsevier.)

IR bands associated with the two-phase domains, even though these bands are highly overlapped. Asynchronous cross peaks (Figure 8.26) develop between the bands for the polyethylene backbone located in either the crystalline or the amorphous domains and bands for deuterium-substituted octene side branches. Such asynchronicity indicates that short side branches can move independently of the polyethylene main chain located in the crystalline phase of the sample. No noticeable asynchronicity, however, is observed between the reorientation dynamics of side-branch and amorphous components of the system.

The above observation suggests that the octene side branches are excluded from the crystalline lattice and accumulate preferentially in the noncrystalline region of the polyethylene. This result agrees with the view that the depression of the melt temperature and crystallinity of linear low-density polyethylene is caused by the inability of the crystalline lattice to incorporate chain branches above a certain length. It is not possible, however, to determine conclusively from this 2D IR data if the short branches of linear low-density polyethylene are uniformly distributed within the amorphous phase domain, or preferentially accumulate near the interface between the amorphous and crystalline regions.

## 8.2.9 POLY(HYDROXYALKANOATES)

The high resolution capability of 2D IR spectroscopy in differentiating overlapped crystalline and amorphous IR bands has been successfully used for characterizing many other semicrystalline polymers. Biodegradable poly(hydroxyalkanoate)s, for example, have been studied by this technique.<sup>23</sup> It was shown that molecular defects created by the incorporation of branched comonomer units tend to accumulate in the amorphous regions in a manner similar to the case of linear low-density polyethylene. Additives, such as plasticizers which are miscible in the molten state of semicrystalline polymers, also tend to be excluded from the crystal lattice and preferentially accumulate in the amorphous phase, once the system is brought to a temperature below the melt temperature.

Figures 8.27 and 8.28 show 2D IR correlation spectra for the CH stretching region of poly(3-hydroxybutyrate), or PHB. PHB is a biodegradable semicrystalline aliphatic polyester produced by microorganisms.<sup>24</sup> PHB films were cast from

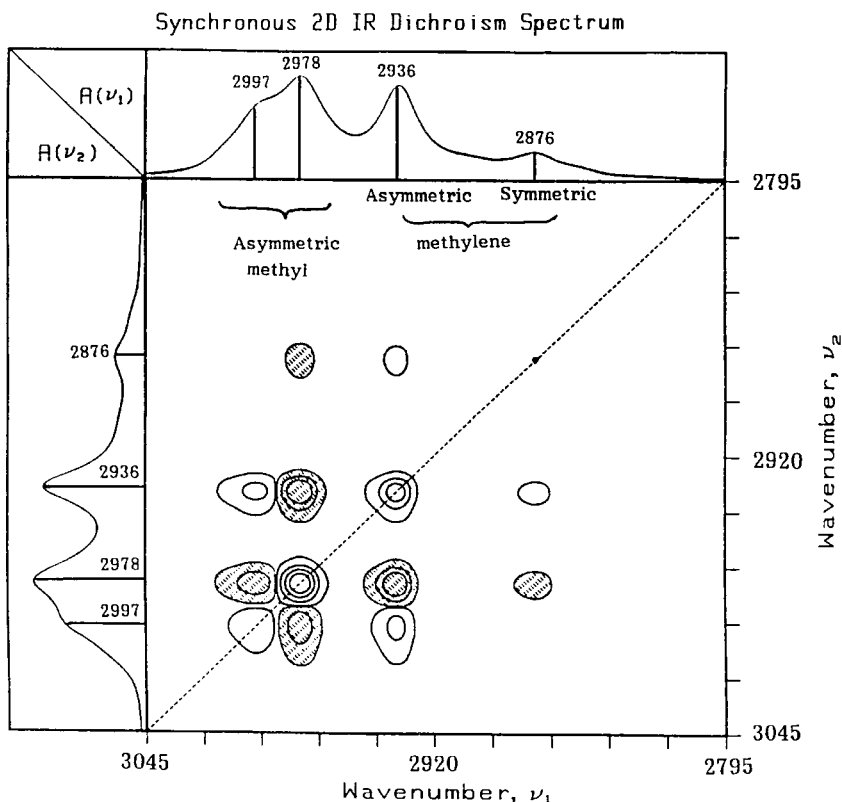


**Figure 8.27** Synchronous 2D IR spectrum of PHB. (Reprinted from *Analytica Chimica Acta*, **250**, C. Marcott, I. Noda, and A. E. Dowrey, p. 131, Copyright (1991), with permission from Elsevier.)

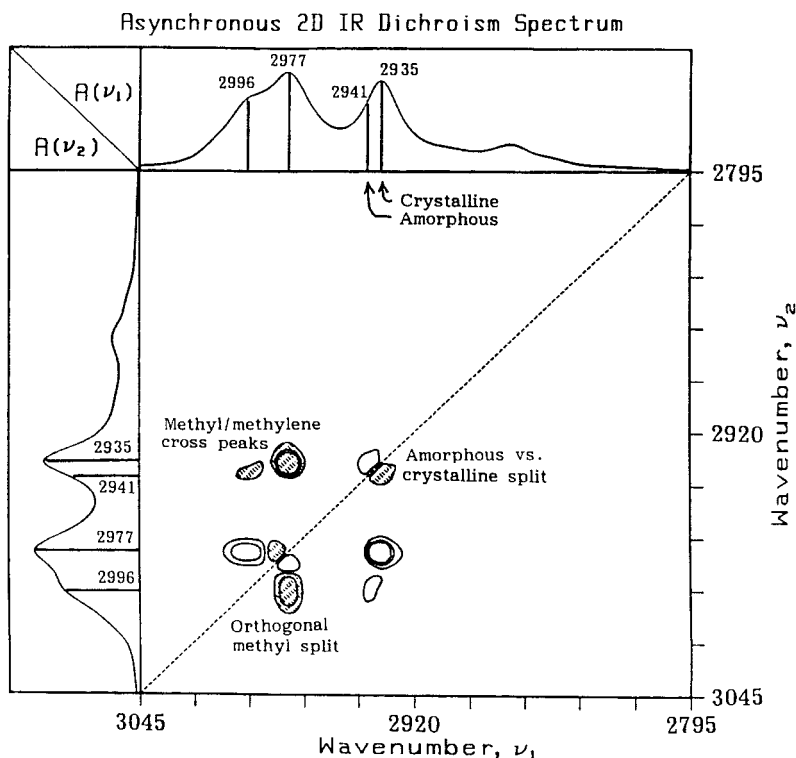


**Figure 8.28** Asynchronous 2D IR spectrum of PHB. (Reprinted from *Analytica Chimica Acta*, **250**, C. Marcott, I. Noda, and A. E. Dowrey, p. 131, Copyright (1991), with permission from Elsevier.)

chloroform solution, vacuum dried, and annealed at 60 °C overnight. The samples were analyzed at room temperature with a dynamic IR spectrometer with a low-amplitude (0.1 %) oscillatory (23 Hz) strain. In this spectral region, well-defined correlation peaks for symmetric and asymmetric CH stretching vibrations of the side-group methyl and backbone methylene groups are observed in the synchronous 2D IR spectrum (Figure 8.27). The negative cross peaks at 2978 and 2997  $\text{cm}^{-1}$  indicate the two dipole transition moments for the doubly degenerate asymmetric methyl stretching bands are reorienting in the directions perpendicular to each other. Peaks at 2936  $\text{cm}^{-1}$  indicate the dipole transition moment for the 2997  $\text{cm}^{-1}$  band reorients along with the methylene transition moments in the direction perpendicular to the axis of the applied dynamic strain.

An asynchronous 2D IR spectrum of the asymmetric methylene stretching mode of PHB is shown in Figure 8.28. Highly overlapped methylene bands are now well resolved into the amorphous and crystalline components at 2941 and 2935  $\text{cm}^{-1}$  respectively. This assignment can be verified by dissolving the sample, which causes the crystalline band to disappear. The temporal analysis shows that

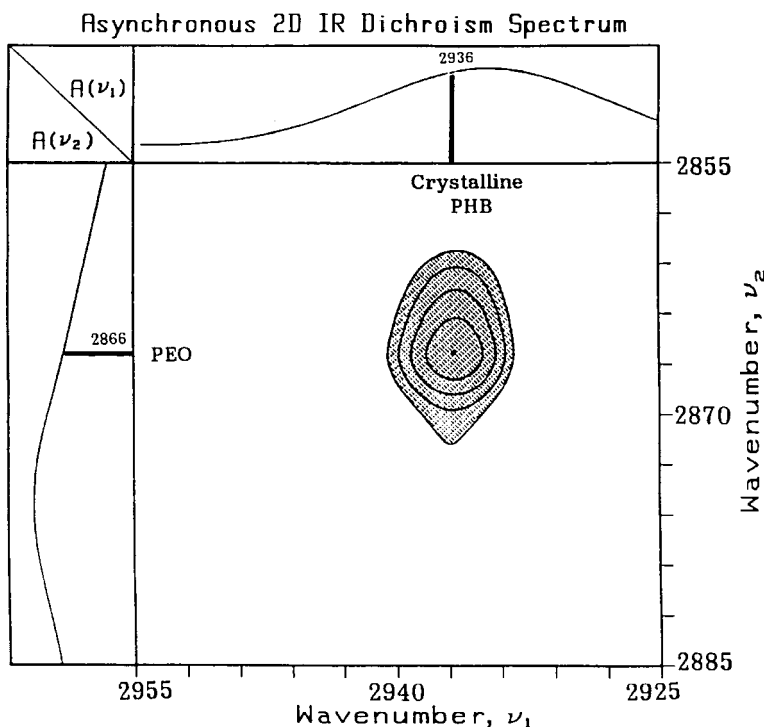


**Figure 8.29** Asynchronous 2D IR spectrum of PHB/PEO blend

the strain-induced reorientation of PHB starts in the amorphous region followed by the slower crystallite orientation.

An asynchronous 2D IR spectrum (Figure 8.29) is shown for a 95:5 blend of PHB and low-molecular weight poly(ethylene oxide) or PEO. In the molten state, they are known to form a miscible blend.<sup>25</sup> PEO acts as an effective polymeric plasticizer and improves the mechanical properties of PHB. Since PEO is a biodegradable polymer, the blend is also believed to be biodegradable. At 60 °C where this spectrum was collected, a part of the PHB is crystallized. The development of an asynchronous peak between the crystalline PHB band (2936  $\text{cm}^{-1}$ ) and PEO band (2866  $\text{cm}^{-1}$ ) indicates that PEO is no longer miscible with the crystalline portion of PHB and is excluded from the crystal lattice. Most likely, PEO is still mixed with the amorphous component of PHB. Such selective partition of PEO should lower the  $T_g$  of the amorphous phase and profoundly affect the physical properties (e.g., toughness and flexibility) of the blend.

#### 8.2.10 BLOCK COPOLYMERS

Block copolymers made of dissimilar polymer segments joined together by a covalent bond are interesting systems to be studied by 2D IR spectroscopy.

Because of the repulsive interaction between the dissimilar segments, block copolymers often phase separate at the submolecular scale to form microdomains. It has been speculated for some time that the composition of block segments making up such microdomains does not change sharply at the domain interface.<sup>26,27</sup> Instead, a diffuse interlayer is thought to exist where substantial mixing of dissimilar block segments actually takes place. 2D IR may be used to probe the diffuse interphase region between microdomains.

A thin solution-cast film of a diblock copolymer consisting of a polystyrene block ( $M_W = 50\,000$ ) and a deuterium-substituted polyisoprene block ( $M_W = 100\,000$ ) was stimulated at room temperature with a 0.1 % amplitude, 23 Hz dynamic tensile strain. In this composition range, diblock copolymers of styrene and isoprene produce a hexagonally packed regular array of rod-like polystyrene microdomains embedded in a continuous matrix of the polyisoprene block. The diameter of these polystyrene microdomains is well below  $0.1\,\mu\text{m}$ .

Figure 8.30 shows the synchronous 2D IR spectrum of the block copolymer in the CH stretching region. Since the polyisoprene block is fully deuterium substituted, responses shown in Figure 8.30 represent exclusively the contribution from the polystyrene segment. Intuitively, one expects the applied strain on the block

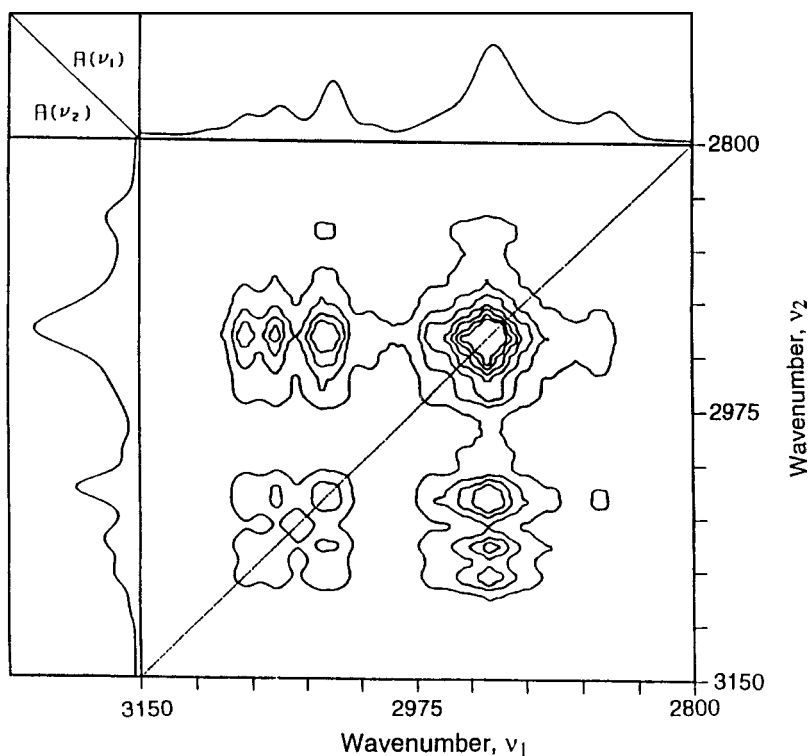


**Figure 8.30** Synchronous 2D IR spectrum of SI block copolymer



copolymer to be distributed predominantly in the soft and rubbery polyisoprene phase, since the dispersed polystyrene microdomains are in the rigid glassy state at room temperature. It is, therefore, rather surprising to find autopeaks representing substantial molecular mobility of polystyrene segments under dynamic strain at this temperature. The signs of cross peaks correlating the motion of side-group phenyl and backbone methylene bands are all positive. Experience shows positive cross peaks appear at these spectral coordinates only if polystyrene is in the rubbery state. Thus, the polystyrene segments contributing to the dynamic IR signals are in the rubbery state, even though the measurement was made at room temperature. This result may be interpreted as an indication that the polystyrene segments showing rubber-like behavior are located in the diffuse interlayer region between the micro-domains. The glass transition temperature of polystyrene segments in this region is substantially depressed due to mixing with the polyisoprene segment.

This hypothesis was independently verified by selectively deuterium substituting a part of the polystyrene segment.<sup>28,29</sup> The region near the junction between the two blocks (most likely to be located within the interphase region) was found to be rubbery even at room temperature. This part of the polystyrene segment reoriented to a large extent for a given dynamic deformation and contributed strongly to the dynamic IR dichroism signals. On the other hand, the tail end of the polystyrene segment (believed to distribute uniformly within the polystyrene microdomain) remained predominantly glassy. The local submolecular mobility of the tail end of polystyrene was noticeably limited.

### 8.2.11 SUMMARY

Dynamic 2D IR dichroism spectroscopy study of polymers based on the correlation analysis of the time dependence of localized reorientational motions of various submolecular moieties constituting a system has proven to be a very powerful tool for a broad range of applications, especially in the study of complex macromolecules, such as polymers and proteins. It was noted from the early stage that the peak resolution of 2D IR correlation spectra is substantially enhanced by spreading the overlapped IR bands along the second dimension. The presence or lack of strong chemical interactions or connectivity among submolecular groups located in various parts of the polymer system is readily detected by the appearance of correlation cross peaks at appropriate spectral coordinates. Furthermore, the relative reorientation directions of intensity changes and the sequential order of realignment of submolecular structural units are also conveniently provided by the signs of the cross peaks appearing in 2D IR spectra.

Information obtained from specific applications of 2D IR dichroism spectroscopy include the local dynamics of an amorphous polymer, where side groups reorient independently of the polymer main chain and abrupt changes in the side-group realignment mechanism above and below the glass transition temperature. By using dynamic 2D IR, it is possible to probe the microscopic spatial

distribution of molecular components, especially those found in phase-separated systems. The degree of specific interactions between components in mixtures is effectively identified. The application certainly is not limited to the characterization of traditional synthetic polymers. Complex macromolecules of biological origin can be readily studied by this technique. 2D IR spectroscopy thus greatly expands the scope of possible spectroscopic studies of materials.

### 8.3 REPETITIVE PERTURBATIONS BEYOND DIRLD

Dynamic 2D IR analysis based on rheo-optical DIRLD measurement using sinusoidal mechanical perturbation has been enormously successful in the study of complex polymer systems. The basic scheme for obtaining 2D correlation spectra using a form of repetitive perturbation can be readily expanded to encompass a much broader range of spectroscopic measurements. The excitation of system constituents induced by an arbitrary external stimulus may be monitored by any type of electromagnetic probe. The universal applicability of the 2D correlation approach based on the perturbation-induced spectral variation is demonstrated in this section.

#### 8.3.1 TIME-RESOLVED SMALL ANGLE X-RAY SCATTERING (SAXS)

*Small angle X-ray scattering* (SAXS) analysis is a powerful technique to probe the spatial heterogeneity of systems in the range of nano-to micrometer scales. This technique has been especially useful in the morphological characterization of microphase-segregated domain structures of block polymers. We now extend 2D correlation analysis to the dynamic SAXS studies.

In SAXS studies, the X-ray scattering intensity  $I(q)$  is measured as a function of *scattering vector*  $q$  defined as

$$q = \frac{4\pi \sin \theta_B}{\lambda} \quad (8.8)$$

where  $\theta_B$  is the *Bragg diffraction angle* (i.e., half of the scattering angle), and  $\lambda$  is the wavelength of the X-rays.

If the system is placed under a time-dependent perturbation such as a sinusoidal strain with a fixed angular velocity  $\omega$ , the scattering intensity also becomes a function of time, reflecting the dynamic changes in the spatial distributions of the constituents of the system.

$$\begin{aligned} I(q, t) &= \bar{I}(q) + \tilde{I}(q, t) \\ &= \bar{I}(q) + I'(q) \sin \omega t + I''(q) \cos \omega t \end{aligned} \quad (8.9)$$

Thus, the synchronous and asynchronous 2D SAXS correlation spectra should be obtained as

$$\Phi(q_1, q_2) = \frac{1}{2}[I'(q_1) \cdot I'(q_2) + I''(q_1) \cdot I''(q_2)] \quad (8.10)$$

and

$$\Psi(q_1, q_2) = \frac{1}{2}[I''(q_1) \cdot I'(q_2) - I'(q_1) \cdot I''(q_2)] \quad (8.11)$$

Unlike the 2D IR spectrum, the correlation intensity of a 2D SAXS spectrum is plotted as a function of two independent scattering vector axes,  $q_1$  and  $q_2$ .

Figure 8.31 shows the schematic diagram of the dynamic SAXS instrument developed by Professor Takeji Hashimoto's group at Kyoto University.<sup>30</sup> All the measurements were carried out with this instrument at room temperature. Dynamic tensile strain was applied continuously to the sample at a rate of 0.47 Hz and an amplitude of 1.1 %. The dynamic strain was superposed onto a static strain of 10 %. The dynamic scattering intensity was measured by dividing the deformation period into 64 sectors and coadding the intensity to the histogram memory of the multichannel analyzer. Fourier analysis was applied to the results, and the first-order terms were used to calculate the static and dynamic scattering intensities.

The material studied was a styrene–butadiene–styrene (SBS) triblock copolymer with 23 % styrene content. This system is known to form microphase-separated regularly spaced domain structures with hexagonally packed cylindrical morphology. The sample was prepared by melt pressing at a temperature above the glass transition temperature of polystyrene (ca 100 °C) but below their order–disorder transition temperature (ca 180 °C). Under such a condition, the microphase-separated cylindrical domains are oriented in a preferential direction.

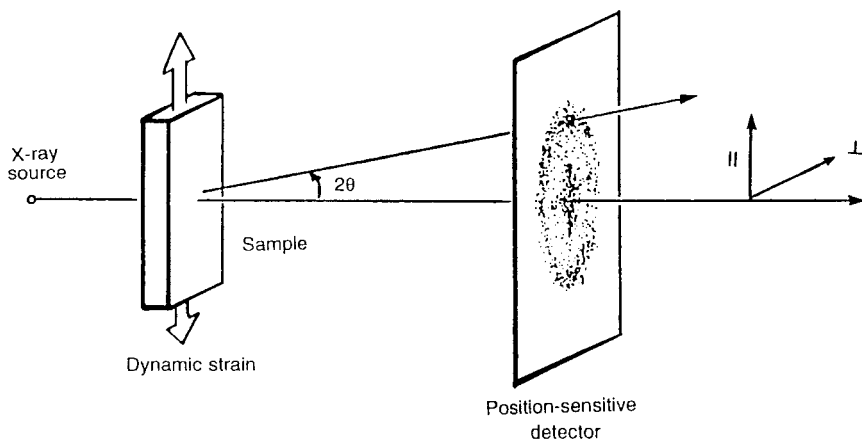


**Figure 8.31** Schematic description of a dynamic small-angle X-ray scattering (DSAXS) experiment

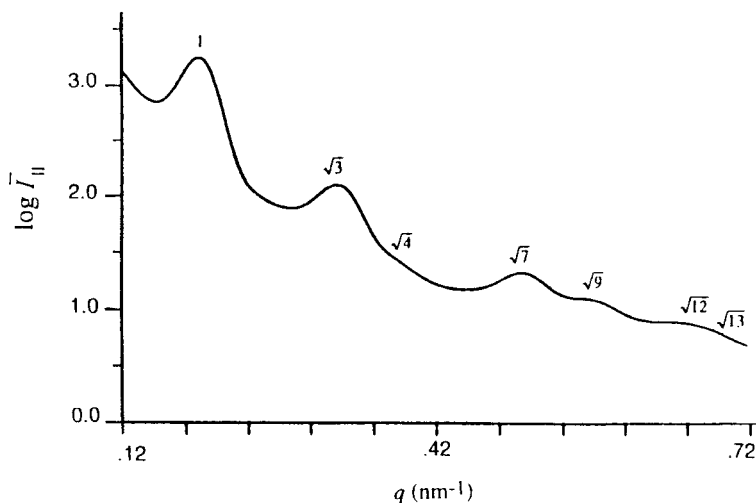


**Figure 8.32** Static SAXS pattern of microphase-separated SBS triblock copolymer

This direction is set to be perpendicular to the stretch direction for a dynamic SAXS experiment.

Figure 8.32 shows the static SAXS spectrum of the microphase-separated SBS block polymer system. A well-defined set of diffraction peaks to very high orders can be observed, indicating the presence of a regular domain structure. The local scattering peaks for the triblock copolymer arise from the Bragg diffraction of the regular hexagonal array of microphase-separated domains. The relative peak positions with respect to the lowest angle peak position are given by the ratios,  $1 : \sqrt{2} : 2 : \sqrt{7} : 3 : \sqrt{12} : \sqrt{13}$ . This series matches well with the scattering pattern of hexagonally close-packed cylinder morphology. Each diffraction peak corresponds, respectively, to the  $(hk0)$  plane of (100), (110), (200), (210), (300), (220), and (310).

Dynamic variation of meridian scattering intensities induced by a small-amplitude oscillatory tensile deformation applied to the block copolymer sample was analyzed using Equations (8.10) and (8.11) to construct the 2D correlation map. Figure 8.33 shows a synchronous 2D SAXS spectrum. A very characteristic peak cluster consisting of two positive and two negative correlation peaks forming a *four-leaf-clover*-like pattern is observed throughout the synchronous 2D SAXS spectrum. The center of each four-peak cluster is located at the spectral coordinate corresponding to the scattering vector  $q_{\max}$  of the local scattering intensity maximum observed in a normal static SAXS spectrum. In the close-up view (Figure 8.34) of the synchronous 2D SAXS spectrum, a peak cluster is found at the spectral coordinate,  $q_{\max} = 0.19 \text{ nm}^{-1}$ , which corresponds to the  $d_{100}$  reflection plane of the microphase-separated block copolymer sample.

The development of a four-leaf-clover pattern of the correlation peak clusters in a synchronous 2D SAXS correlation spectrum of block copolymers is explained

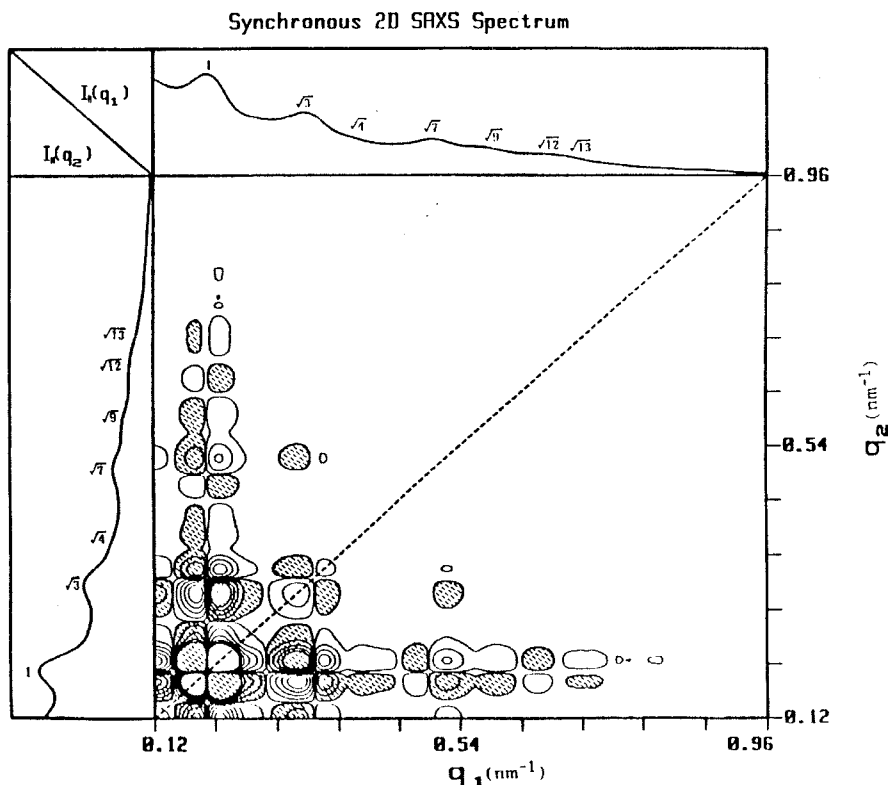


**Figure 8.33** Synchronous 2D SAXS correlation spectrum of microphase-separated SBS triblock copolymer. (Copyright © 1990 by Chemtracts, originally published in *Chem-Tracts: Macromolecular Chemistry*, 1(2): 89–105 and reproduced here with permission.)

by the dynamic shift in the position of a scattering peak. The result, in turn, reflects the strain-induced dynamic change in the interdomain Bragg distance between microphase-separated domains as shown in Figure 8.35(A) and (B). The dynamic shift of a diffraction peak toward the lower  $q$  direction, for example, will result in a local increase in scattering intensity at a coordinate  $q < q_{\max}$  coupled with a decrease in intensity at  $q > q_{\max}$ . The cross-correlation of the bisignate dynamic SAXS spectrum arising from a shift in peak position will result in the formation of the characteristic four-peak pattern, where the simultaneous increase in scattering intensity above  $q_{\max}$  and decrease below  $q_{\max}$  are correlated. Such a characteristic cross peak pattern in synchronous 2D SAXS correlation spectra is especially useful in identifying the location of higher order scattering peaks, where only weak local maxima and shoulders are usually observed.

It turned out that the source of dynamic SAXS signals is not limited to strain-induced changes in the Bragg distances between microdomains. The asynchronous 2D SAXS correlation spectrum (Figure 8.36) shows that the dynamic

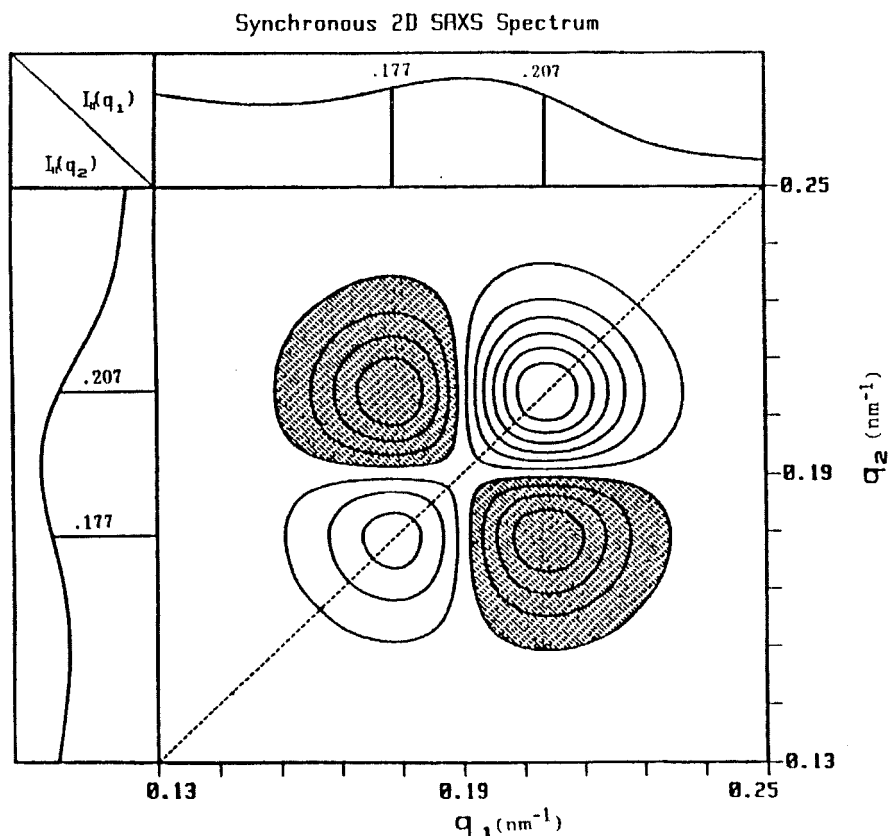


**Figure 8.34** Synchronous 2D SAXS correlation spectrum of microphase-separated SBS triblock copolymer for the  $d_{100}$  peak. (Copyright © 1990 by Chemtracts, originally published in *ChemTracts: Macromolecular Chemistry*, 1(2): 89–105 and reproduced here with permission.)

increase in scattering intensity at  $0.18 \text{ nm}^{-1}$  and decrease at  $0.20 \text{ nm}^{-1}$  are not completely synchronized. Additional changes in the scattering intensity, i.e., the overall decrease of the meridian scattering under a tensile strain due to the reorientation of microphase-separated cylindrical styrene domains, is superimposed onto the coupled intensity changes caused by the shift in the scattering peak position. This additional reorientational effect of microdomains will result in an apparent acceleration of the dynamic change in SAXS intensity at lower  $q$  ( $0.18 \text{ nm}^{-1}$ ) and deceleration at higher  $q$  ( $0.20 \text{ nm}^{-1}$ ).

The above results can be readily explained if one assumes that changes in the interdomain Bragg spacing (i.e., spreading) under a dynamic deformation occur at slightly different rates from the reorientation (i.e., rotation or realignment) of cylindrical microdomains. The reorientation process lags behind the spreading as shown schematically in Figure 8.37(a), (b), and (c). Such differentiation of

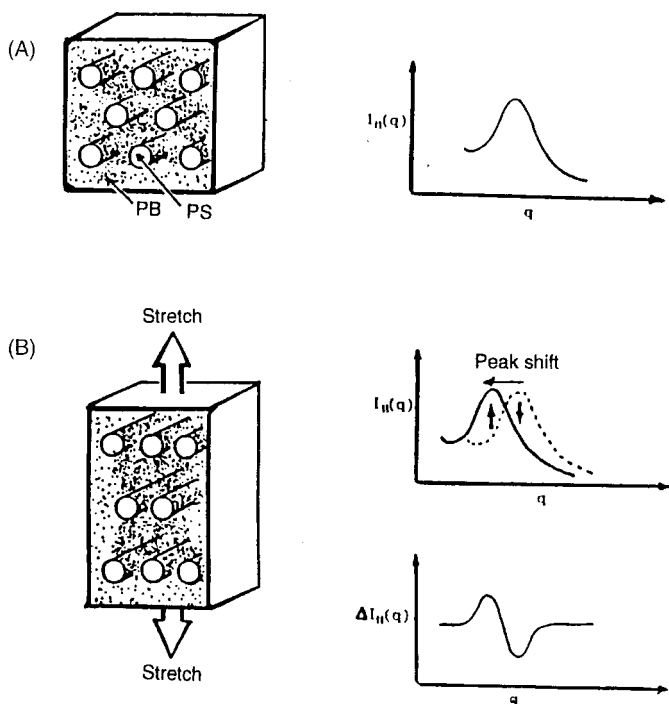


**Figure 8.35** Schematic representation of the deformation-induced changes in the interdomain Bragg distance and scattering peak shift: (A) the shorter interdomain distance along the deformation axis in the compressed sample results in the up-shift of the scattering angle; (B) the longer interdomain distance in the elongated sample, on the other hand, results in the down-shift of the peak maximum to a lower scattering angle. The difference is a bisignate signal

multiple dynamic reorganizational processes is rather difficult to carry out based on the direct analysis of original-time resolved SAXS data. Thus, even though the 2D correlation by itself may not directly add new physical information to rheo-optical data, the technique provides the opportunity to gain rapid insight into the identification of dynamics and interactions among system constituents often not apparent in the original dynamic spectral data.

### 8.3.2 DEPTH-PROFILING PHOTOACOUSTIC SPECTROSCOPY

Infrared *photoacoustic* spectroscopy (PAS) can be used to profile the nonuniform distribution of molecular constituents of a layered sample along the depth axis normal to the surface.<sup>31</sup> In photoacoustic spectroscopy (Figure 8.38), the incident IR beam absorbed at a certain depth position of a sample is converted to heat, which in turn results in the local thermal expansion of the sample. By modulating the beam which results in the repetitive thermal expansion and subsequent

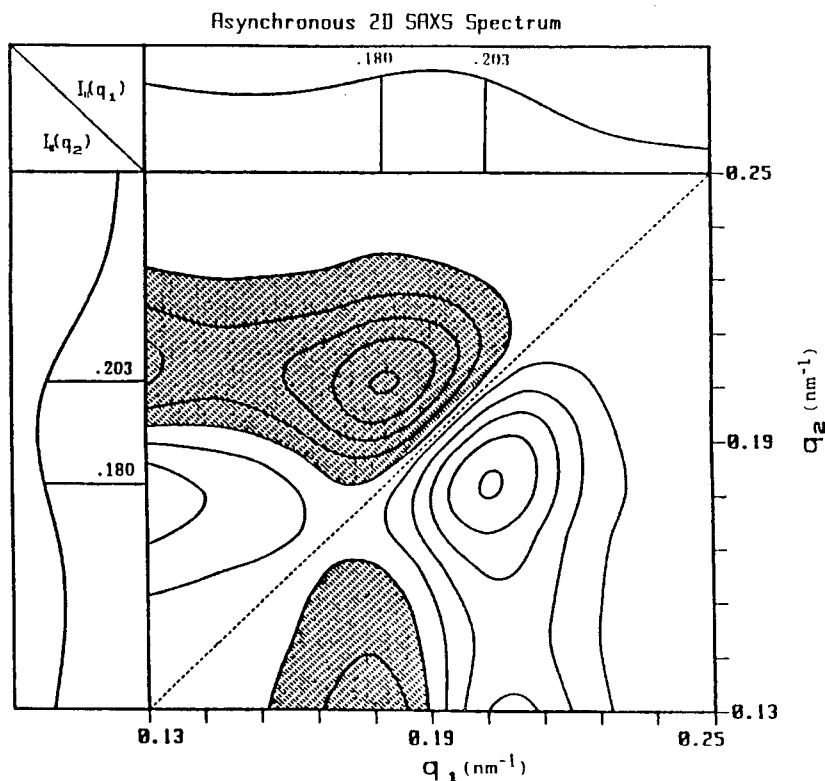


**Figure 8.36** Asynchronous 2D SAXS correlation spectrum of microphase-separated SBS triblock copolymer for the  $d_{100}$  peak. (Copyright © 1990 by Chemtracts, originally published in *ChemTracts: Macromolecular Chemistry*, 1(2): 89–105 and reproduced here with permission.)

contraction, photo-acoustic sound signals detectable with a microphone are generated. Since each photo-acoustic signal arising from the individual molecular constituent located at a specific depth layer must travel for a fixed distance before reaching at the surface of the sample, there is a finite phase delay for each photo-acoustic signal; the delay is characteristic to the depth where the signal is originated. Thus, by sorting out the individual phase delays of photo-acoustic signals generated at different IR wavenumbers, one should be able to profile the depth distribution of constituents associated with photo-acoustic signals.

2D correlation analysis is ideally suited for organizing and visualizing such PAS signals arising from different depth layers. Because of the characteristic phase-delay signature associated with the depth origin of individual PAS signals, it is quite straightforward to identify the similarity or difference among PAS signals detected at different wavenumbers. By applying the correlation analysis to time-dependent photoacoustic spectra, one obtains *depth-correlated 2D IR PAS spectra*. Autopeaks located at the diagonal positions of a synchronous 2D



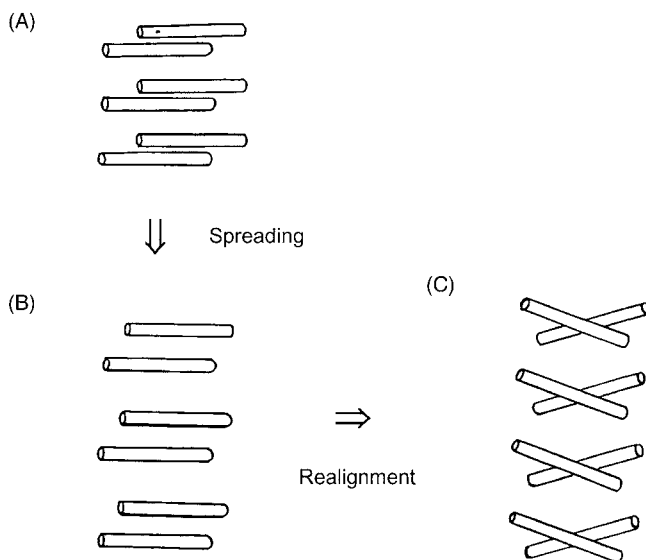


**Figure 8.37** Deformation mechanism consisting of spreading followed by reorientation of cylindrical microdomains

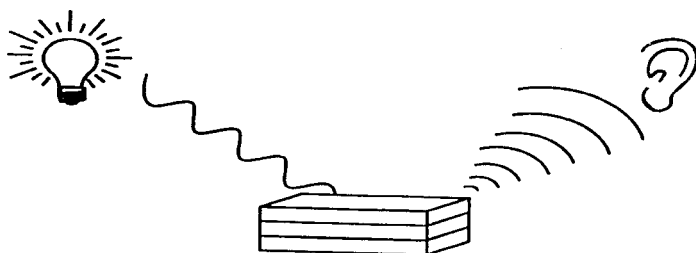


**Figure 8.38** Depth-profiling infrared photoacoustic spectroscopy (PAS)

photo-acoustic spectrum represent the magnitude of each photo-acoustic signal. The photo-acoustic synchronous cross peaks located at off-diagonal positions indicate the similarity of time (phase) signature of PAS signals measured at corresponding wavenumbers. Such synchronicity arises if the two signals originate from the same depth layer. The asynchronous 2D PAS spectrum, on the other hand, represents the dissimilarity of the time signatures among PAS signals. If an asynchronous correlation peak located at a given spectral coordinate ( $\nu_1$ ,  $\nu_2$ ) is positive, the PAS signal assignable to the band at wavenumber  $\nu_1$  arises from a layer shallower than that for  $\nu_2$ . For a negative asynchronous cross peak, the depth relationship is reversed.

The probe depth and characteristic phase delay of photoacoustic signals vary considerably with the modulation frequency of the IR beam used in a PAS experiment. A photo-acoustic measurement under a higher modulation frequency

tends to emphasize the contribution of signals originating from shallower layers, while deeper layers are better probed with a lower frequency modulation experiment. One of the difficulties encountered in depth-profiling photo-acoustic spectroscopy with a conventional rapid-scanning FT-IR spectrometer is that the actual amplitude-modulation frequency of the IR beam is intimately coupled with the interferometric Fourier frequency used to label the individual wavenumber component of the incident photon. The probe depth, therefore, is not constant for different wavenumbers, which made the interpretation of depth-profiling FT-IR spectra difficult. The introduction of step-scanning FT-IR instrumentation has greatly reduced the difficulty of the multiple modulation frequency problem inherent in rapid-scanning spectrometers. By stepping the mirror position of the interferometer and separately applying amplitude or phase modulation with a single fixed frequency to the entire beam, a step-scanning FT-IR spectrometer can yield a quantitative photoacoustic spectrum probed at a constant sampling depth regardless of the wavenumber. Thus, it has become possible to utilize the phase delay information of signals arising from the depth distribution of different molecular constituents to its fullest potential through 2D correlation analysis.

All of the measurements were performed on a Bio-Rad FTS-60A FT-IR spectrometer operating in step-scan mode. A phase modulation frequency of 400 Hz with an amplitude of 1.0 HeNe laser wavelength was applied through the dynamic alignment piezoelectric devices of the spectrometer. The 400 Hz phase modulation signal was demodulated using a lock-in amplifier supplied by Bio-Rad with the FTS-60A spectrometer. The PAS measurements were carried out by aligning an MTEC 200 PAS cell in the sample compartment of the spectrometer. The instrument response phase was determined using a standard carbon black sample equipped with an optical screen supplied by MTEC. The PAS cell was purged with helium gas. Full double-sided interferograms were collected at a spectral resolution of  $8\text{ cm}^{-1}$ . Interferograms were under sampled by a factor of two (i.e., every other HeNe laser zero crossing) and zero filled two times. Approximately 16000 data points in each channel were integrated, and the average value was stored. Sample interferograms were computed using the carbon black in-phase stored phase array which contains only instrument response function contributions.

The first sample studied was a model laminate made of approximately  $25\text{ }\mu\text{m}$  of low-density polyethylene (PE) on top of a  $25\text{ }\mu\text{m}$  thick layer of polystyrene (PS) with a thin (ca  $3\text{ }\mu\text{m}$ ) coating of polydimethylsiloxane (PDMS) spread over the PE surface. The second sample was a *surface hydrophilic elastomer latex* (SHEL) film. SHEL is a novel polymeric alloy consisting of styrene-butadiene rubber (SBR) and an amphiphilic block oligomer containing a short polyethyleneoxide segment.<sup>32</sup> SHEL is prepared by the emulsion copolymerization of styrene and butadiene in the presence of the amphiphilic block oligomer. The latex spontaneously forms a film having a surface which has exceptionally high surface energy and consequently water wettability. It is believed that the ethoxylate block chain segments are rejected from the bulk SBR phase and accumulate at the surface

to render it hydrophilic. The enthalpic penalty of forming such a high energy surface is most likely compensated by the unfavorable entropic energetics of creating high-curvature inclusions of polymeric moieties within the bulk phase.

The depth-correlated synchronous 2D photoacoustic FT-IR spectrum of the model laminate sample is shown in Figure 8.39. Each set of cross peaks and corresponding autopeaks corresponds exclusively to a particular layer component. For example, peaks arising from the contributions of PDMS layers are synchronously correlated with other PDMS bands. However, no cross peaks are observed between PDMS bands and PS or PE bands. Clear differentiation between PAS signals from different depth layers is demonstrated.

The close up view of the asynchronous spectrum (Figure 8.40) reveals very useful information. By examining the signs of the cross peak intensities, it is possible to determine the depth sequence of the laminate. Thus, the positive asynchronous peaks on the far right of the 2D PAS spectrum correlate the small PDMS band at  $1405\text{ cm}^{-1}$  with a PE band at  $1460\text{ cm}^{-1}$  and with PS bands at

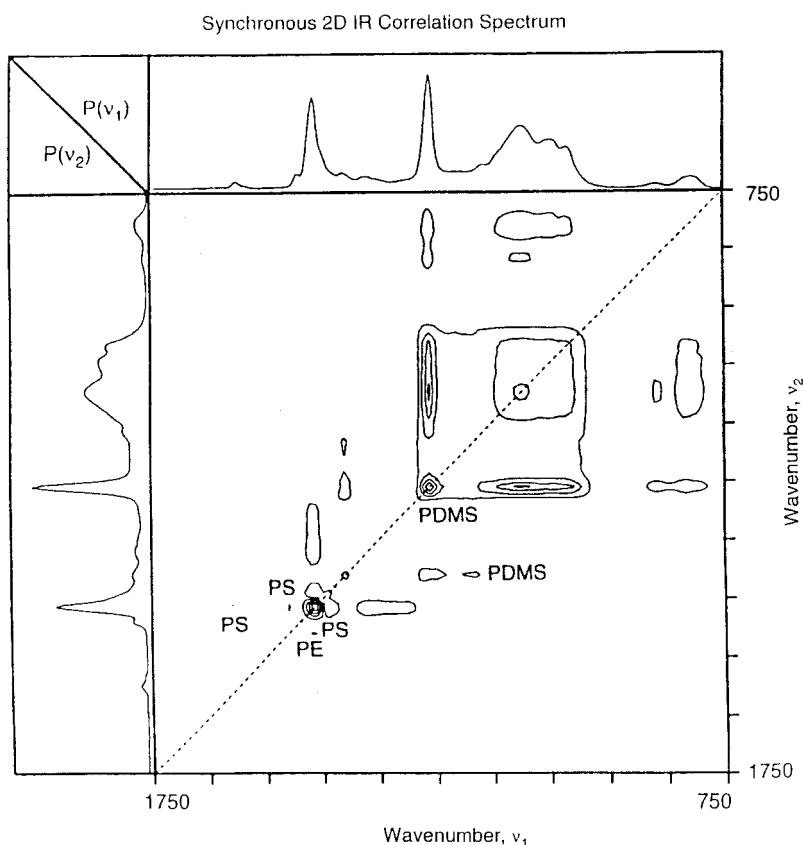


**Figure 8.39** Synchronous 2D PAS correlation spectrum of a DMPS/PS/PE laminate film

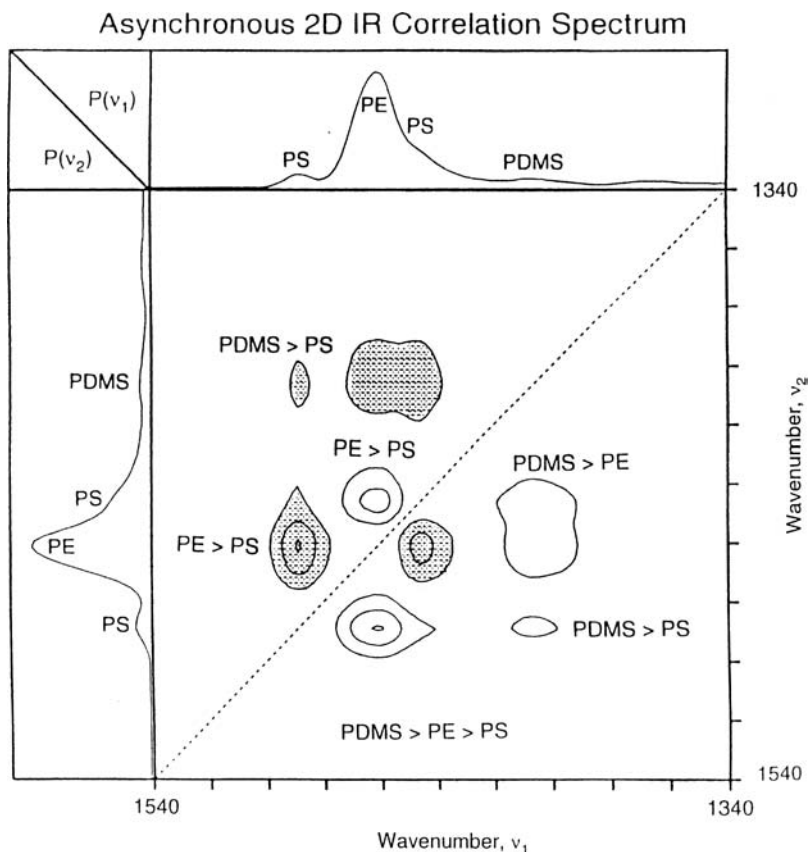


**Figure 8.40** Asynchronous 2D PAS correlation spectrum of a DMPS/PS/PE laminate film

1450 and  $1490\text{ cm}^{-1}$ . This result indicates that the PDMS photo-acoustic signals detected at the microphone precedes those of PS and PE. In other words, the PDMS signal originates from the layer nearer to the surface of the sample than the other two layers. The other two signals below the diagonal line (one positive and one negative) correlate PE and PS bands and indicate that the PS signal originates from a deeper layer in the sample. By combining the above information, one can readily deduce that the sample consists of layers in the depth order of  $\text{PDMS} < \text{PE} < \text{PS}$ .

Figure 8.41 shows the depth-correlated synchronous 2D photo-acoustic spectrum of the SHEL film. The presence of cross peaks clearly indicates that the ethoxylate moieties of the block oligomer are located at some depth substantially different from that of SBR. The synchronous spectrum correlates all bands assignable to SBR components with each other. However, no synchronous correlation is observed between ethoxylate and SBR bands. The signs of the asynchronous peaks (not shown) indicate that ethoxylate moieties are located

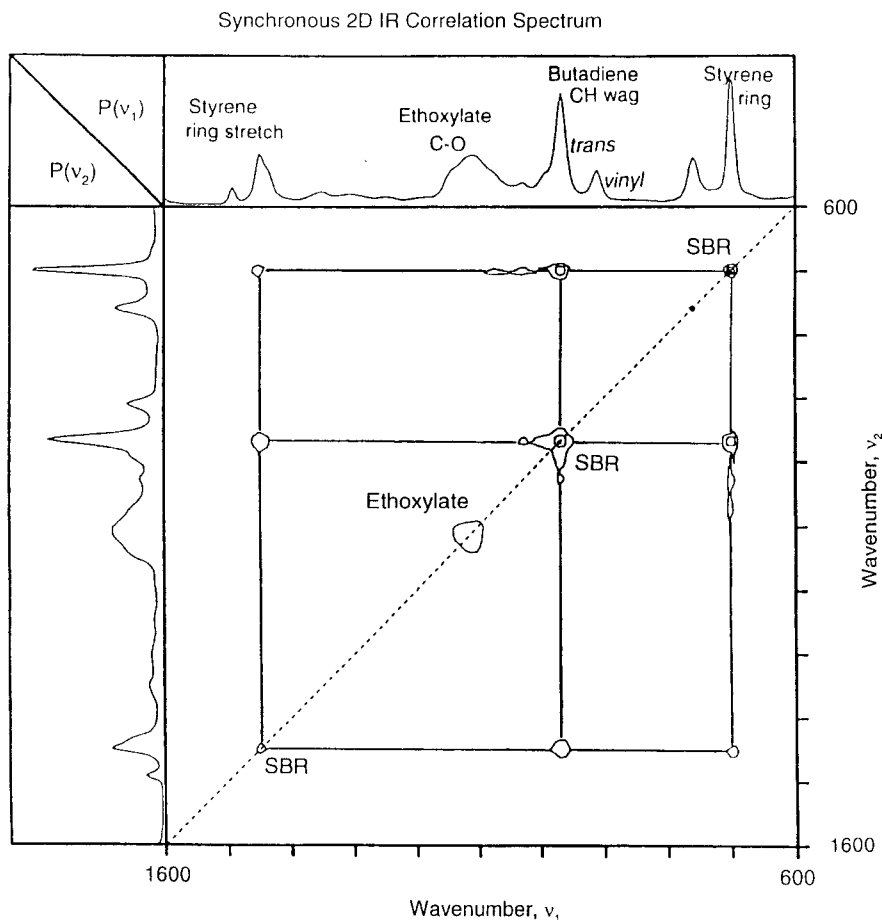


**Figure 8.41** Synchronous 2D PAS correlation spectrum of a SHEL film

at the surface, while SBR is buried at a much deeper position. This fact can be independently verified by comparing the regular transmission spectrum in Figure 8.42(A) with the attenuated total reflectance (ATR) spectrum of a SHEL film in Figure 8.42(B). While the transmission spectrum has strong peaks of SBR copolymer bands, the corresponding ATR spectrum is dominated by the signals assignable to the C–O stretching vibrations of ethoxylate moieties of the amphiphilic block oligomer. Thus, the ATR result of this system agrees well with the conclusion drawn from the depth-correlated 2D IR PAS spectroscopy.

The advantage of depth-correlated 2D IR PAS over conventional ATR analysis is the ability to analyze systems comprising multiple layers. As demonstrated earlier in this chapter, 2D PAS provides the depth sequence of multiple layers. At this stage, however, 2D PAS generates only qualitative information about the depth distribution of chemical moieties. The quantitative depth profiling must wait for

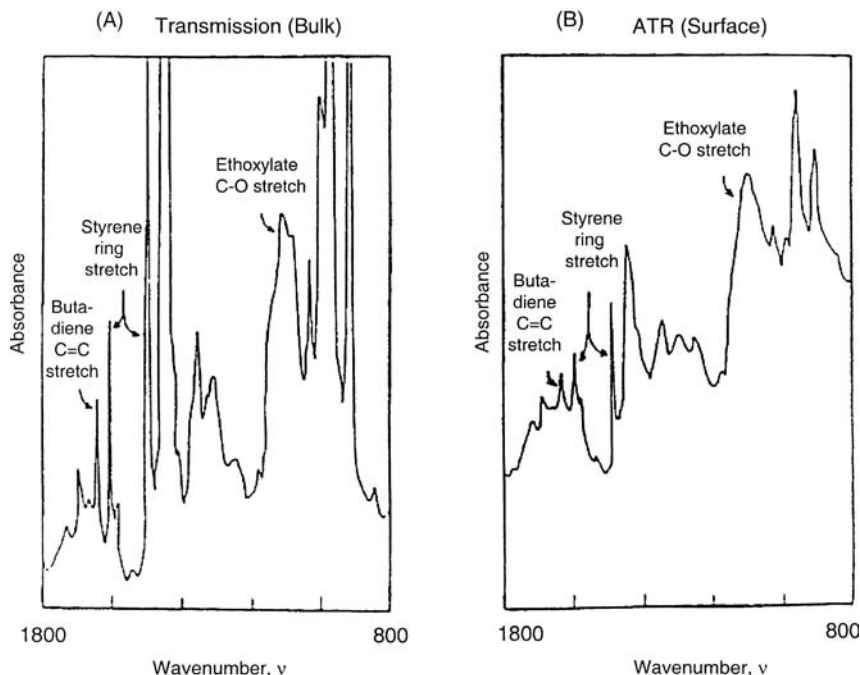


**Figure 8.42** Comparison of IR spectra of a SHEL film: (A) transmission spectrum probing the entire sample and (B) ATR spectrum probing primarily the layer near the surface

the development of more rigorous derivation of thermal wave propagation in the system. Unfortunately, such derivation in turn requires *a priori* knowledge of the composition-sensitive thermal diffusivity of the system. Additional complications arise when separate layers having similar compositions, such as adhesive layers, are present in the system. In PAS analysis, such multiple layers with identical compositions must be represented as a fictitious single layer corresponding to the spatial linear combination of individual layers. Finally, unlike DIRLD spectroscopy, the phase delay of individual PAS signals can exceed well over  $\pi/2$ . Some signals originating from a very deep layer can be delayed by several cycles compared to those coming from much shallower layers. There is no way of distinguishing sinusoidal signals spread apart by the phase difference of a multiple of  $\pi$ .

### 8.3.3 DYNAMIC FLUORESCENCE SPECTROSCOPY

Analytical techniques based on periodic modulations of fluorescence signals with external repetitive perturbations have been known for some time. As early as in 1970, for example, Vesolova *et al.* carried out the detection of sinusoidal fluorescence response signals arising from the sample excitation using modulated light.<sup>33</sup>

Importantly, they noted that different fluorescing components of the sample responded to the excitation with different phase angles. McGown and coworkers reported a series of work on similar phase-resolved excitation–emission fluorescence experiments with modulated excitation.<sup>34–36</sup> The phase shift of the dynamic fluorescence response is related to the fluorescence lifetime, which provides additional selectivity to the origin of the signal. The effect of modulation frequencies and associated phase shifts of fluorescence signals were examined with multiway curve resolution analysis of dynamic excitation–emission data.

Although such phase-resolved fluorescence data are well suited for straightforward dynamic 2D correlation analysis, little attempt was made to exploit the 2D technique in the fluorescence field until a much later date. The first application of generalized 2D correlation analysis to fluorescence spectroscopy was carried out by Rosselli *et al.*, using the wavelength of excitation as a perturbation variable.<sup>25</sup> Nakashima *et al.* also reported work on 2D fluorescence correlation spectroscopy based on steady changes in external variables, such as excitation wavelength and sample composition.<sup>37</sup> Specific applications of generalized 2D correlation to fluorescence spectroscopy using such stationary perturbation will be discussed separately in Chapter 9.

Dynamic 2D fluorescence correlation spectroscopy based on phase-sensitive detection of sinusoidally modulated signals was put forward by Geng and coworkers.<sup>38–40</sup> Sinusoidal modulation of laser light field with a frequency range of 5–30 MHz was used to generate fluorescence signals, which can be separated into the in-phase and quadrature components. In a dynamic 2D fluorescence experiment, one can actually generate three different types of 2D correlation spectra: excitation–excitation spectra, emission–emission spectra, and excitation–emission spectra. The combined use of these 2D correlation spectra with associated phase maps provides unprecedented spectral resolution advantage to the identification of fine vibronic structures of highly overlapped fluorescence spectra. For example, the resolution of different microenvironments of a probe molecule in a biological system has become possible without relying on statistical fitting of multi-exponential fluorescence decay curves. The high sensitivity of a fluorescence probe is a major advantage over other optical probes such as IR in the detection of low-concentration samples, if combined with the 2D correlation approach.

### 8.3.4 SUMMARY

The feasibility of extending the 2D correlation technique beyond the analysis of DIRLD data has been demonstrated. It was shown that new types of information, quite different from those obtained by dynamic 2D IR experiments, could be generated. 2D SAXS spectra, obtained for the structural reorganization of a microphase-separated block copolymer system undergoing dynamic deformation, show the deformation-induced spreading of interdomain Bragg distances

followed by the rotation of microdomain structures. The depth-correlated 2D IR PAS spectra of a laminated film sample demonstrates the ability of this technique to identify chemical moieties belonging to the same depth layer, as well as the depth sequences of moieties belonging to different layers. While the number of specific examples cited here may be limited, it should be obvious that the 2D correlation technique can be used for any dynamic spectral variations induced by a periodic external perturbation. As long as the system responds sinusoidally to the external perturbation, and appropriate spectral intensities vary accordingly, dynamic spectral data can be converted to a set of 2D correlation spectra containing useful information.

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## 9 Applications of Two-dimensional Correlation Spectroscopy to Basic Molecules

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2D correlation spectroscopy has been applied extensively to investigate a wide variety of basic molecules. Many of 2D correlation spectroscopy studies of basic molecules are concerned with the investigation of self-associated molecules such as water, alcohols, and amides. Since changes in hydrogen bondings, molecular interactions, and conformations of self-associated molecules are often reflected well in both synchronous and asynchronous spectra, 2D correlation spectroscopy becomes a very powerful tool for the study of such systems. Moreover, 2D correlation spectroscopy of basic molecules provide the solid foundation for further study of more complicated compounds such as polymers and proteins.

A number of published reports are available regarding 2D correlation spectroscopy studies of basic molecules.<sup>1–17</sup> Noda *et al.*<sup>6–9</sup> reported 2D correlation spectroscopy studies of temperature-dependent NIR spectral variations of oleyl alcohol, and IR, NIR, and Raman spectral variations of *N*-methylamide (NMA). They also reported IR–Raman heterospectral analysis of NMA.<sup>10</sup> Czarnecki *et al.*<sup>11–14</sup> studied 2D NIR correlation spectra of various alcohols including deuterated compounds. They investigated detailed band assignments of NIR spectra, thermal dynamics of hydrogen bondings, and rotational isomers of alcohols. IR–NIR heterospectral analysis of polyamide was reported by Czarnecki *et al.*<sup>15</sup> Šašić *et al.*<sup>16</sup> used sample–sample 2D NIR correlation spectroscopy to investigate phase transitions of oleic acid in the pure liquid. Segtnan *et al.*<sup>17</sup> applied both variable–variable and sample–sample 2D NIR correlation spectroscopy to explore the structure of water.

Of note is that besides IR and Raman spectroscopy, NIR spectroscopy has been used extensively. NIR spectroscopy has the following characteristics in studying the hydrogen bonds and molecular interactions of self-associated molecules, such as alcohols, carboxylic acids and amides.<sup>5,9,18</sup> (i) OH and NH stretching bands due to monomeric and polymeric species are better separated in the NIR region than in the IR region. Even bands ascribed to free-terminal OH and NH groups of the polymeric species can be clearly identified. (ii) Because of their larger anharmonicity, bands ascribed to the first overtones of OH and NH stretching modes of monomeric species appear much more strongly than the corresponding

bands arising from hydrogen-bonded OH and NH group. Therefore, it may be easier in the NIR region than in the IR region to monitor the dissociation process from the polymeric species into monomeric ones by means of the first overtone of the OH or NH stretching mode of the monomeric species. (iii) NIR bands have much weaker absorption intensities than IR bands, so that a more convenient pathlength of a cell can be used and the exact volume of the sample can be evaluated. In IR spectroscopy, one must use a very thin cell or an attenuated total reflection (ATR) prism, and therefore one often encounters problem of adsorption.

While NIR spectroscopy has several advantages over IR and Raman spectroscopy for the studies of self-associated molecules, NIR region contains more bands due to overtones and combination modes that are often heavily overlapped. For this reason, it is not easy to make unambiguous assignments for NIR bands and to extract the spectral parameters of the individual bands. The high resolution aspect of 2D NIR correlation spectroscopy should be a powerful tool for unraveling complicated NIR spectra. It has been repeatedly shown that 2D NIR spectra can accentuate useful information obscured in the original spectra in a surprising way. In this chapter, four illustrative examples concerning 2D correlation spectra of simple molecules will be discussed. One of them deals with a 2D fluorescence correlation spectroscopy study.

## 9.1 2D IR STUDY OF THE DISSOCIATION OF HYDROGEN-BONDED *N*-METHYLACETAMIDE

*N*-Methylacetamide (NMA) is a simple but very important model for amide groups of peptides, proteins, and polyamides. A 2D IR study of NMA in the pure liquid state provides new insight into the mechanism of the dissociation of hydrogen-bonded NMA. Figure 9.1 shows IR spectra of NMA in the pure liquid measured at 30, 45, and 65 °C.<sup>6</sup> Hydrogen-bonded associated species of various sizes are expected for NMA in the pure liquid and solutions, so that all the amide bands should consist of several component bands assignable to each species. Nevertheless, no definitive study has been undertaken until lately to fully unravel the overlapping bands.

The synchronous 2D spectrum of the Amide I and II regions of NMA, generated from the IR spectra observed over a temperature range of 30–65 °C, is shown in Figure 9.2.<sup>6</sup> The synchronous spectrum is dominated by two auto-peaks at 1650 and 1565 cm<sup>-1</sup>. While the synchronous spectra do not seem to provide immediately useful information, the corresponding asynchronous spectrum gives rise to valuable information about the band assignments and the mechanism of the dissociation of the hydrogen-bonded associated species of NMA. Figure 9.3(A) and (B), respectively, shows the asynchronous spectra of the Amide I and Amide II regions of NMA. It is easily observed that at least four distinct IR bands at 1685, 1665, 1650, and 1635 cm<sup>-1</sup> are located in the Amide I region (Figure 9.3(A)), and that at least two bands at 1570 and 1545 cm<sup>-1</sup> are

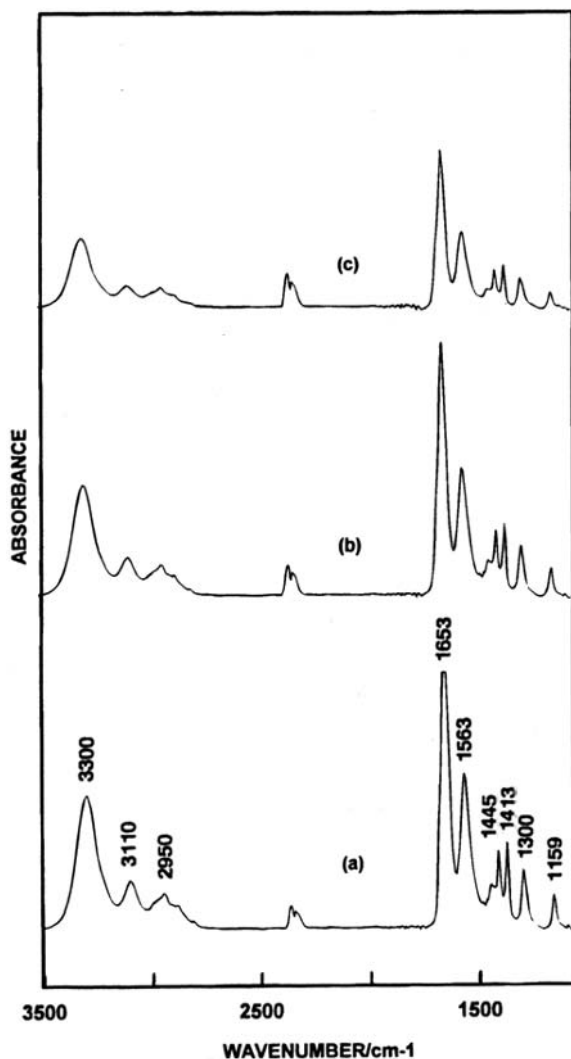


**Figure 9.1** IR spectra of NMA in the pure liquid state at (a) 30, (b) 45, and (c) 65 °C (Reproduced with permission from Ref. No. 6. Copyright (1996) American Chemical Society.)

identified in the Amide II region (Figure 9.3(B)). The Amide I band is largely due to a C=O stretching mode of the amide group, and thus its frequency reflects the strength of the hydrogen bond of the C=O group. It is well known that there is almost no monomeric form of NMA in its pure liquid state at room temperature. Thus, Noda *et al.* assigned the bands at 1685, 1665, 1650, and 1635 cm<sup>-1</sup>, respectively, to the Amide I modes of the dimer and small, medium, and large oligomers of NMA.<sup>6</sup> From the signs of cross peaks in the synchronous and

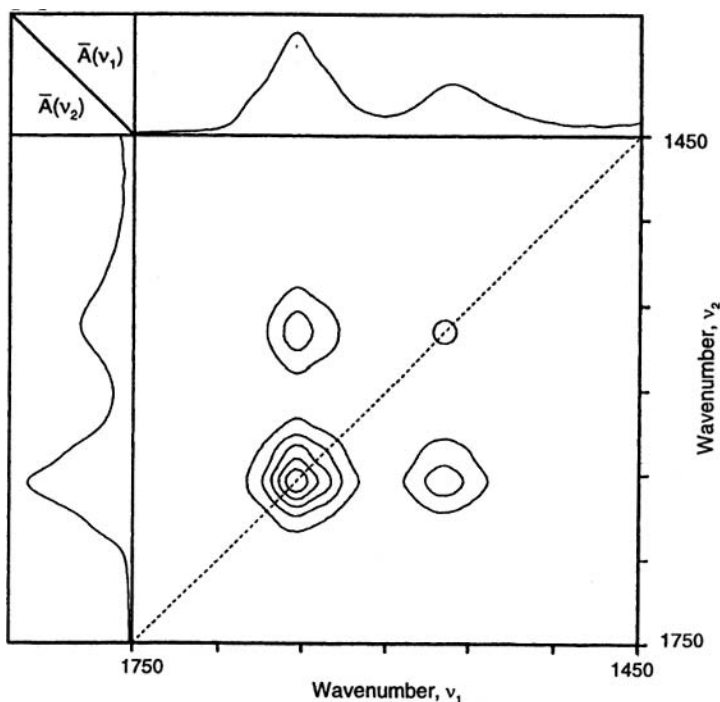


**Figure 9.2** Synchronous 2D FT-IR correlation spectrum of NMA in the 1750–1450  $\text{cm}^{-1}$  region (Reproduced with permission from Ref. No. 6. Copyright (1996) American Chemical Society.)

asynchronous spectra one can investigate the sequence of intensity changes. For example, there are negative asynchronous cross peaks at (1665, 1650) and (1665, 1635)  $\text{cm}^{-1}$ ;  $\Phi$  (1665, 1650)  $< 0$  and  $\Phi$  (1665, 1635)  $< 0$  while  $\Psi$  (1665, 1650)  $> 0$  and  $\Psi$  (1665, 1635)  $> 0$ . Therefore, one can conclude that the intensity change at 1665  $\text{cm}^{-1}$  occurs at a higher temperature than those at 1650 and 1635  $\text{cm}^{-1}$ .

Figure 9.3(C) compares the temperature-dependent variations in the intensities of the bands observed in the Amide I and II regions. From the observations in Figure 9.3 it can be concluded that the intensity changes in ascending order of temperature as follows:

$$1635 \text{ and } 1570 \text{ cm}^{-1} < 1650 \text{ cm}^{-1} < 1665 \text{ and } 1545 \text{ cm}^{-1} < 1685 \text{ cm}^{-1}$$

The pair of bands at 1635 and 1570  $\text{cm}^{-1}$  shows identical temperature-dependent behavior for the intensity variations, suggesting the shared origin of the contributing molecular variations. Comparable similarity is observed for the band pair at 1665 and 1545  $\text{cm}^{-1}$ . The above sequence for the spectral intensity variations leads to the conclusion that the large oligomers are consumed first, the medium-size oligomers follow next (they are produced, on the one hand, and

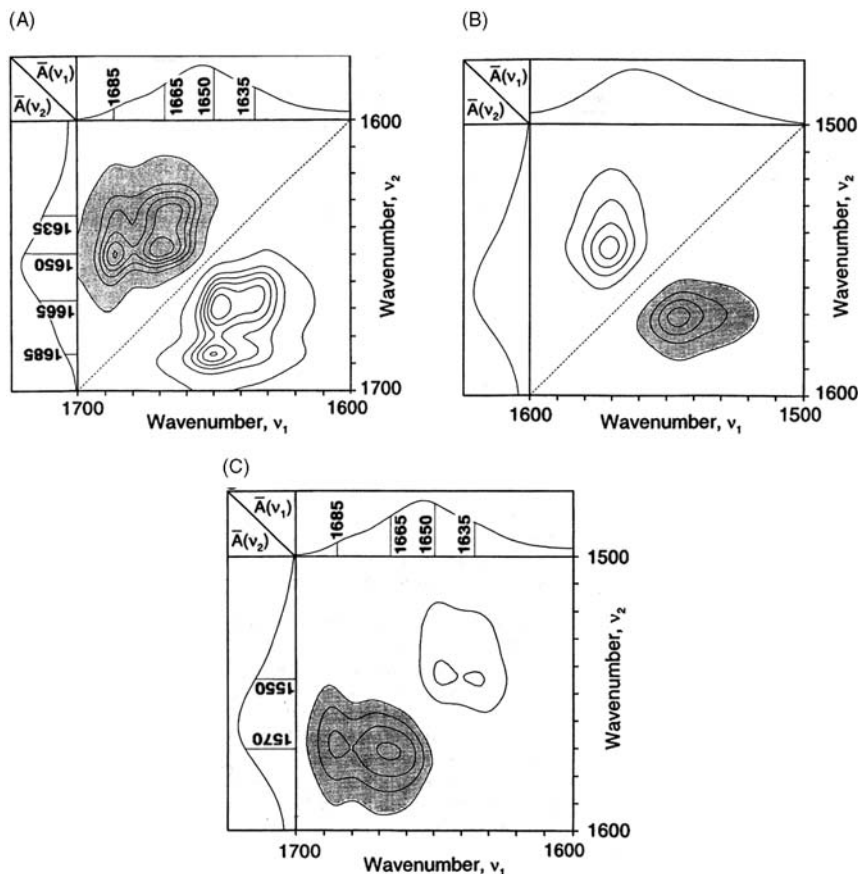


**Figure 9.3** Asynchronous 2D IR correlation spectrum of NMA (A) in the 1700–1600  $\text{cm}^{-1}$  region, (B) in the 1600–1500  $\text{cm}^{-1}$  region, and (C) localized view of the off-diagonal position. (Reproduced with permission from Ref. No. 6. Copyright (1996) American Chemical Society)

then consumed, on the other hand, at lower temperatures), and finally the dimers decrease with temperature. The corresponding 2D NIR study suggested the same mechanism for the dissociation of hydrogen-bonded NMA.<sup>7</sup>

Figure 9.4 illustrates the temperature-dependent spectral intensity variations between the high- and low-frequency regions. It can be seen from Figure 9.4(A) that the lower wavenumber side of the Amide A band at 3275  $\text{cm}^{-1}$  is synchronously correlated with the lower wavenumber side of Amide I at 1650  $\text{cm}^{-1}$  and the higher wavenumber side of Amide II at 1570  $\text{cm}^{-1}$ . The higher wavenumber side of amide A at 3335  $\text{cm}^{-1}$  is synchronized with Amide I at 1670  $\text{cm}^{-1}$  and Amide II at 1545  $\text{cm}^{-1}$ .

The 2D correlation approach between the different spectral regions (sometimes referred to as heteromode correlation) allows one to correlate each component

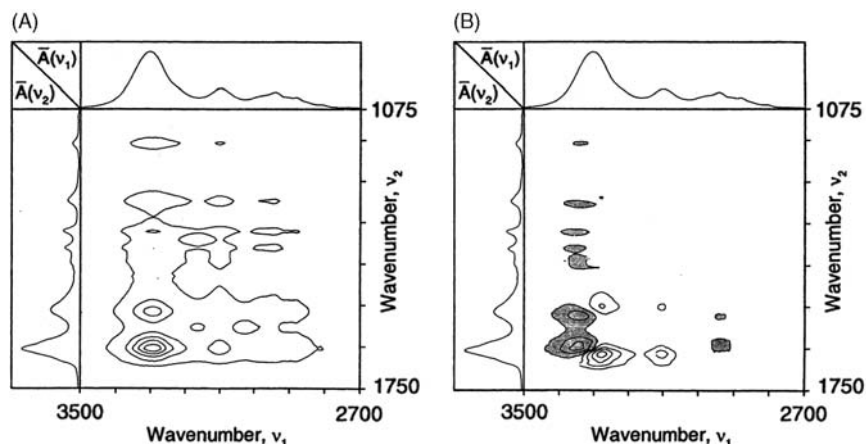


**Figure 9.4** Localized view of the off-diagonal position of (A) synchronous and (B) asynchronous 2D IR spectrum of NMA between the 3500–2700 and 1750–1075  $\text{cm}^{-1}$  regions. (Reproduced with permission from Ref. No. 6. Copyright (1996) American Chemical Society.)

correlation of the Amide A, I, II, and III bands. The following two correlations have been found:

3275 (Amide A)–1635 (Amide I)–1570 (Amide II)–1300  $\text{cm}^{-1}$  (Amide III)

3335 (Amide A)–1665 (Amide I)–1545 (Amide II)–1285  $\text{cm}^{-1}$  (Amide III)

The top series of bands may be attributed to the large-size oligomers of NMA, while the bottom series of bands may be ascribed to the small-size oligomers (or dimer). The intensities of the former bands decrease at a lower temperature than do those of the latter bands. This study has nicely demonstrated the potential of 2D IR correlation spectroscopy in studies of the dissociation process of self-associated molecules.

## 9.2 2D NIR SAMPLE–SAMPLE CORRELATION STUDY OF PHASE TRANSITIONS OF OLEIC ACID

Temperature-dependent NIR spectral variations of oleic acid in the pure liquid state were analyzed in the 7600–6600  $\text{cm}^{-1}$  region by sample–sample 2D correlation spectroscopy to explore its phase transitions.<sup>16</sup> On the basis of a number of spectroscopic and physicochemical studies, Iwahashi *et al.*<sup>19–21</sup> showed that oleic acid probably has three kinds of liquid structures depending on temperature. In the temperature range from the melting point at 15 °C to about 30 °C, the liquid structure consists of clusters having a quasimectic liquid crystal structure,

while the structure in the temperature range between 30 °C and 55 °C is composed of clusters with a less ordered structure. Above 55 °C oleic acid appears to be an isotropic liquid. These conclusions have been drawn from the studies of oleic acid by NIR, differential scanning calorimetry (DSC), density, viscosity, and self-diffusion.

Figure 9.5 shows NIR spectra in the region of 7600–6600  $\text{cm}^{-1}$  of oleic acid in the pure liquid state over a temperature range of 15–80 °C.<sup>16</sup> Bands at 7194 and 7092  $\text{cm}^{-1}$  are assigned to the combination modes of the CH vibrations, while the band at 6920  $\text{cm}^{-1}$  consists of at least two overlapped bands. One is due to the combination mode of CH vibrations, and the other arises from the first overtone of the OH stretching vibration of the monomer. The intensity of the band at 6920  $\text{cm}^{-1}$  increases as a function of temperature, giving good evidence that the acid dimer dissociates into the monomeric species even in the pure liquid state. In a plot of the absorbance at 6920  $\text{cm}^{-1}$  versus temperature (not shown), one can see two break points at 30 and 55 °C. All the conclusions regarding the liquid structure were reached from the intensity change of the band at 6920  $\text{cm}^{-1}$ . However, the obtained temperature-dependent plot of the intensity at 6920  $\text{cm}^{-1}$  did not always give convincing evidence for the existence of the two break points.

The sample-sample 2D correlation spectroscopy study applied to the whole spectral region of 7600–6600  $\text{cm}^{-1}$  yields more unambiguous evidence for the break points.<sup>16</sup> The sample-sample correlation analysis was applied to the NIR spectra in the original form, as well as those after two different kinds of spectral pretreatments. First, the spectra were offset on the higher wavenumber side (around 7500  $\text{cm}^{-1}$ ) and adjusted with respect to the intensity of the band at

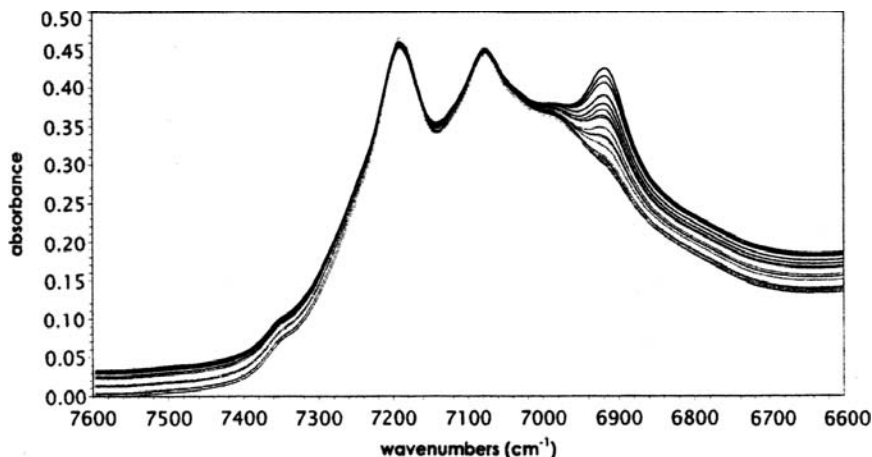


**Figure 9.5** NIR spectra in the region of 7600–6600  $\text{cm}^{-1}$  of oleic acid in the pure liquid state over a temperature range of 15–80 °C. (Reproduced with permission from Ref. No. 16. Copyright (2000) American Chemical Society.)



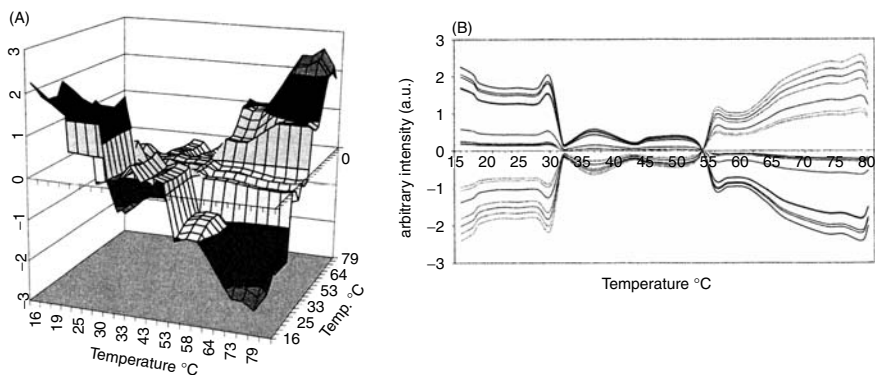


**Figure 9.6** Sample–sample correlation spectrum (A) calculated from the spectra shown in Figure 9.5 and (B) and corresponding slice spectra. (Reproduced with permission from Ref. No. 16. Copyright (2000) American Chemical Society.)

$7182\text{ cm}^{-1}$  measured at  $15^\circ\text{C}$ . In the second pretreatment, both wavenumber sides (the  $7600\text{--}7400$  and  $6700\text{--}6600\text{ cm}^{-1}$ ) were baseline corrected, and only the C–H combination bands and the band due to the first overtone of the monomer O–H group were considered. In the last case the original spectra without any pretreatment were analyzed.

It was found that the analysis based on the raw spectra gives the strongest evidence for two phase transition temperatures at  $32$  and  $55^\circ\text{C}$  in the sample–sample correlation pattern. Figure 9.6(A) illustrates asynchronous sample–sample correlation spectra calculated from the spectra shown in Figure 9.5. Figure 9.6(B) depicts the corresponding slice spectra. Note that the slice spectra yield unambiguous evidence for the two break points at  $32$  and  $55^\circ\text{C}$ . The sample–sample correlation can easily be applied to systems where a process similar to the monomerization of a dimer takes place.

### 9.3 2D NIR CORRELATION SPECTROSCOPY STUDY OF WATER

Studies of water have always been a matter of great interest because the studies are very important not only in basic science but also in a variety of applications. Water is involved in almost all kinds of substances, and the water content and the structure of water in them are often key factors in determining their functions and structure. NIR spectroscopy has been employed to investigate the water content, hydrogen bonds of water, and hydration in various fields such as agricultural and food industries, medical and pharmacological sciences, and polymer and textile industries.<sup>17,18,22–25</sup> However, it is still not easy to understand NIR spectra of water completely because water does not exist in single species and water molecules form various cluster structures.

Figure 9.7 depicts NIR spectra of water in the 900–2500 nm (11 100–4000  $\text{cm}^{-1}$ ) region.<sup>18</sup> The three spectra were measured by using cells with the pathlength of (a) 10, (b) 1.0, and (c) 0.05 mm. An intense tail near 2500 nm is due to the fundamental of the OH stretching modes ( $\nu_1$ ,  $\nu_3$ ; see Figure 9.8). Bands near 1910, 1430, and 960 nm (5235, 6900, and 10 420  $\text{cm}^{-1}$ ) are assigned to  $\nu_2 + \nu_3$ ,  $\nu_1 + \nu_3$ , and  $2\nu_1 + \nu_3$  modes of water, respectively. As shown in Figure 9.7, the intensities of the water bands decrease stepwise with the decrease in the wavelength. This means that one can select the spectral region used or the pathlength of a cell when one investigates aqueous solutions using NIR spectroscopy.

NIR spectra of water are very sensitive to temperature and the ions involved. Figure 9.9(A) presents NIR spectra of water measured over the temperature range 5–85 °C at an increment of 5 °C.<sup>25</sup> It is noted that the spectrum of water changes

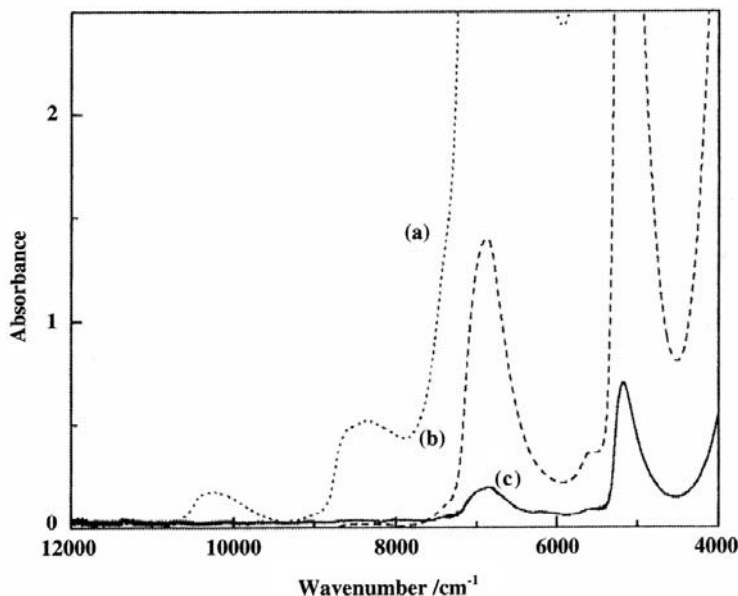


**Figure 9.7** NIR spectra of water in the 900–2500 nm (11 100–4000  $\text{cm}^{-1}$ ) region. (Reproduced with permission from Ref. No. 18. Copyright (2002) Wiley-VCH.)

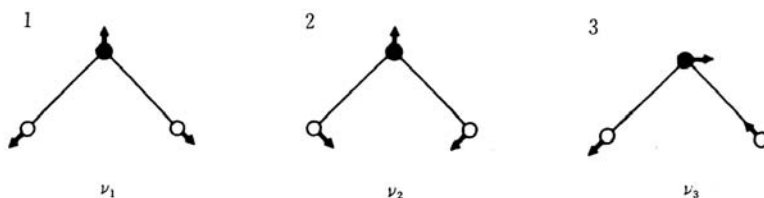


**Figure 9.8** Vibrational modes of water:  $\nu_1$  symmetric OH stretching mode,  $\nu_2$  OH bending mode,  $\nu_3$  antisymmetric OH stretching mode

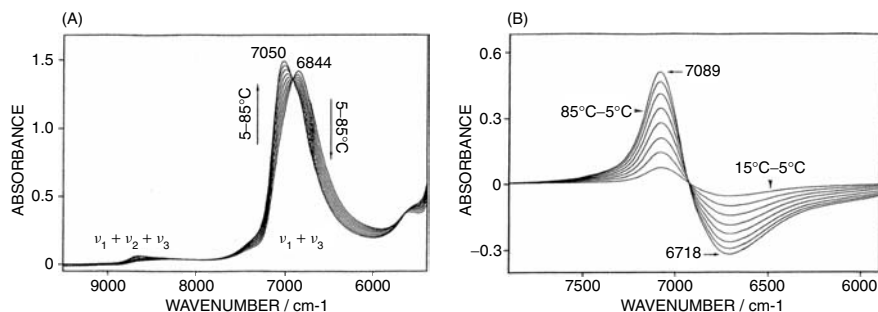


**Figure 9.9** (A) NIR spectra of water measured over a temperature range of 5–85 °C at an increment of every 5 °C. (B) Difference spectra of water calculated by subtracting the spectrum at 5 °C, taken as the reference spectrum, from the other spectra measured at various temperatures. (Reproduced with permission from Ref. No. 25. Copyright (1995) NIR Publications.)

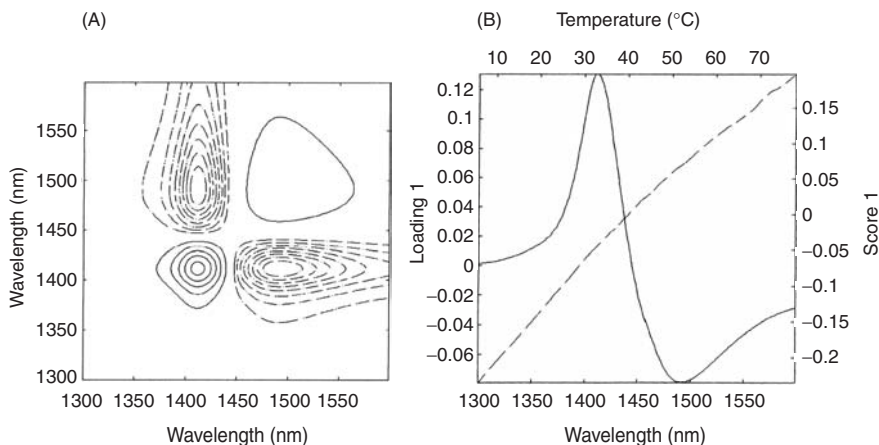


**Figure 9.10** (A) Synchronous 2D correlation spectrum generated from the temperature-dependent spectral variations of water measured over a temperature range of 6–80 °C at 2 °C increments. (B) Loading and scores of the first principal component constructed from the same data. Bottom and left axes represent loadings, plotted in solid lines. Top and right axes represent scores, plotted in dashed line (Reproduced with permission from Ref. No. 17. Copyright (2001) American Chemical Society.)

gradually with temperature. In Figure 9.9(B) is shown a series of difference spectra of water calculated by subtracting the spectrum at 5 °C, taken as a reference spectrum, from the other spectra measured at various temperatures. A band at 7089  $\text{cm}^{-1}$  becomes stronger while that at 6718  $\text{cm}^{-1}$  becomes weaker with temperature.

Segtnan *et al.*<sup>17</sup> investigated the structure of water using 2D correlation spectroscopy and PCA. Figure 9.10(A) and (B), respectively, shows a synchronous 2D correlation spectrum generated from temperature-dependent spectral variations of

water measured over the temperature range 6–80 °C at an increment of 2 °C and loadings and scores of the first principal component constructed from the same data. The synchronous map shows autopeaks at 1412 and 1491 nm, indicating that the spectral features at these positions vary significantly with temperature. The appearance of negative cross peaks between the autopeaks reveals that the intensity changes occur in opposite directions. Of note is that loadings of the first principal component also show peaks at 1412 and 1491 nm. The results of difference spectra, 2D correlation spectroscopy and PCA all suggest that there are two major water species giving the  $\nu_1 + \nu_3$  bands at 7089 (1412 nm) and 6718 (1491 nm)  $\text{cm}^{-1}$ . Segtnan *et al.* also found that the wavelengths of 1412 and 1491 nm account for more than 99 % of the spectral variations. These two wavelengths of 1412 and 1491 nm represent two major water species with weaker and stronger hydrogen bonds, respectively. These two species are perfectly correlated, i.e., the concentration of one species increases at the expense of the concentration of the other when the temperature is varied, and in this sense the results are in accordance with a two-state mixture model.

## 9.4 2D FLUORESCENCE STUDY OF POLYNUCLEAR AROMATIC HYDROCARBONS

2D fluorescence correlation spectroscopy has also been used to investigate simple molecules.<sup>26–28</sup> The first application of the 2D correlation method to fluorescence spectroscopy was reported by Roselli *et al.*, who investigated the metal-binding sites of proteins.<sup>26</sup> They analyzed the fluorescence from  $\text{Yb}^{3+}$  at two different binding sites of transferrin. The perturbation method used by Roelli *et al.* was to change the excitation wavelength.

Nakashima *et al.* reported the analysis of anthracene–phenanthrene (AN–PH) and anthracene–pyrene (AN–PH) mixtures in cyclohexane solutions by employing two perturbation methods: a change in concentration and a change in excitation wavelength.<sup>27</sup> They also used 2D fluorescence correlation spectroscopy to resolve the fluorescence of two tryptophan (Trp) residues in horse heart myoglobin (Mb).<sup>28</sup> Fluorescence quenching is employed as a perturbation mode for causing intensity changes in the fluorescence. Two kinds of quenchers, iodide ion and acrylamide, are used to induce a change in fluorescence intensity. This technique works because the Trp residue located at the seventh position (W7) is known to be easily accessible to the quencher, whereas that located at the 14th position (W14) is not. By using this technique, the fluorescence spectra of the two Trp residues were clearly resolved.

In this section, 2D fluorescence correlation spectroscopy of polynuclear aromatic hydrocarbons in cyclohexane solutions reported by Nakashima *et al.*<sup>27</sup> is outlined. These aromatic hydrocarbons are commonly used as fluorescence probes. They have fine-structured and well-characterized spectra suitable for investigating basic features of the 2D fluorescence correlation spectroscopy.

Nakashima *et al.* employed two types of perturbation to induce the intensity variations of fluorescence spectra: one is a change in concentration and the other is a change in excitation wavelength. Both perturbations gave a series of systematically varying spectra, which developed clear synchronous and asynchronous maps. Based on the 2D correlation maps thus obtained, the vibronic bands in the complicated fluorescence spectra of the mixture of the probes were successfully analyzed.

We focus our attention to the 2D fluorescence correlation spectroscopy study of AN–PY mixtures. The AN–PY pair was examined as a model case where the fluorescence spectra are heavily overlapped, so that the band analysis is not straightforward by a conventional one-dimensional method. Figure 9.11 shows fluorescence spectra of AN and PY observed separately in cyclohexane solutions.<sup>27</sup> The bands are denoted  $A_i$  ( $i = 1-4$ ) for AN and  $Y_i$  ( $i = 0-5$ ) for PY. It is noted that the fluorescence spectra of AN and PY are heavily overlapped. The band Y0 is a hot band which originates from a vibrationally excited state ( $v' = 1$ ) of the first excited single state ( $S_1$ ) of PY. The locations of the PY bands are listed in Table 9.1.

Figure 9.12 displays fluorescence spectra of the mixtures of AN and PY in cyclohexane solutions.<sup>27</sup> The fluorescence spectra of the mixtures with nearly equimolar concentrations of AN and PY (i.e., AY46, AY55, and AY64) are extremely tangled by heavy overlap of the component spectra. Figure 9.13 depicts

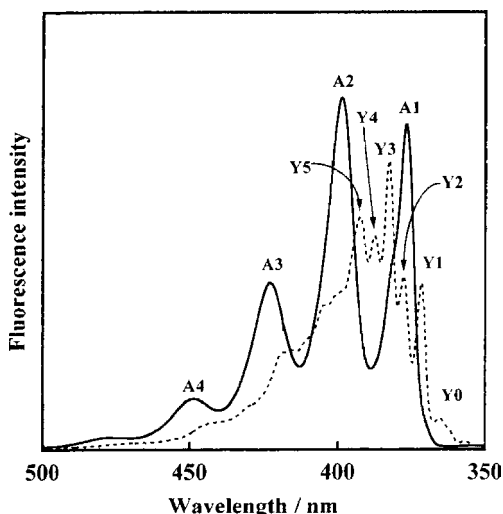


**Figure 9.11** Fluorescence spectra of AN (—) and PY (···) in cyclohexane solutions. Concentrations: [AN] = 10  $\mu$ M, [PY] = 10  $\mu$ M. The samples are excited at 321 nm. Excitation and emission band-passes are 10 and 1.5 nm, respectively. The excitation wavelength, 321 nm, is selected in order that the fluorescence of both probes may become comparable in intensity. (Reproduced with permission from Ref. No. 27. Copyright (2000) American Chemical Society.)

**Table 9.1** Locations and notations of the prominent vibronic bands in the fluorescence spectra of AN and PY (from Ref. No. 27)

| Compound | Location (nm) | Notation |
|----------|---------------|----------|
| AN       | 376           | A1       |
|          | 398           | A2       |
|          | 423           | A3       |
|          | 448           | A4       |
| PY       | 365           | Y0       |
|          | 372           | Y1       |
|          | 378           | Y2       |
|          | 383           | Y3       |
|          | 388           | Y4       |
|          | 392           | Y5       |

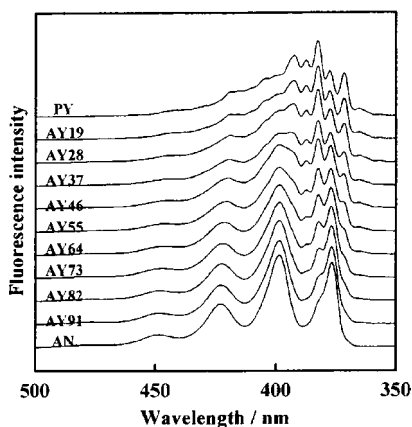


**Figure 9.12** Fluorescence spectra of the mixtures of AN and PY with varying concentrations in cyclohexane solutions. The samples are excited at 321 nm. Excitation and emission band-passes are 10 and 1.5 nm, respectively. The symbols AY $mn$  ( $m, n = 1-9$ ) denote that the concentrations of AN and PY are  $m$  and  $n$   $\mu\text{M}$ , respectively. The top and bottom spectra indicated by PY and AN, respectively, correspond to the samples which contain only PY or AN at the level of 10  $\mu\text{M}$ . (Reproduced with permission from Ref. No. 27. Copyright (2000) American Chemical Society.)

synchronous and asynchronous maps generated from the spectra shown in Figure 9.12. In the synchronous correlation map, the band at 365 nm (Y0) is ascribed to PY, because this band is clearly separated from others as seen from Figure 9.11. The band at 372 nm (Y1) is assigned to PY, because this band has positive correlation with Y0. These two bands are used as clues for further assignment. The peaks at 383 nm (Y3), 388 nm (Y4), 392 nm (Y5), and 412 nm have positive correlation with Y1, and thus are assigned to PY. The peak at 412 nm corresponds

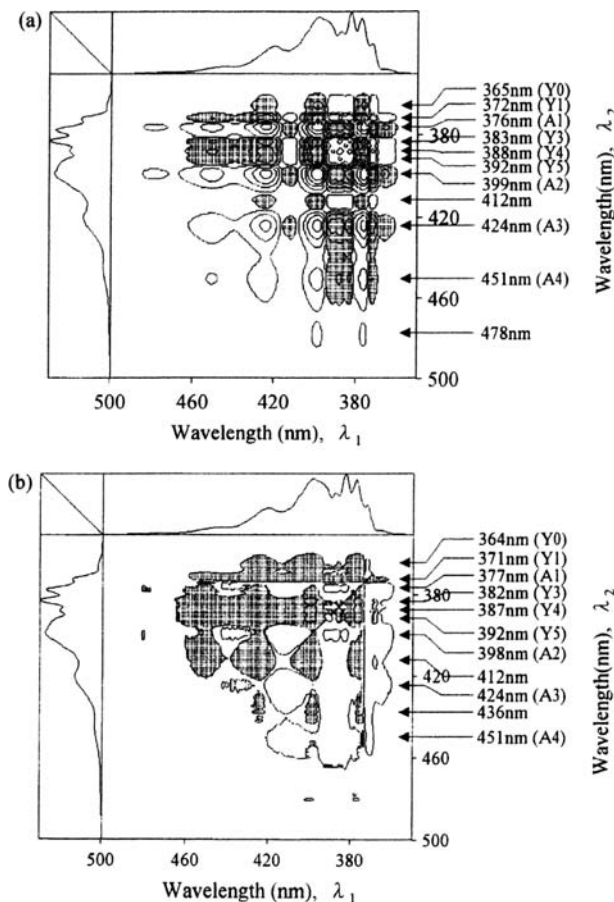


**Figure 9.13** (A) Synchronous and (B) asynchronous 2D fluorescence correlation spectra of the mixtures of AN and PY in cyclohexane solutions, constructed from the spectra of Figure 9.12. (Reproduced with permission from Ref. No. 27. Copyright (2000) American Chemical Society.)

to the band that appears as a shoulder of the 5Y band (Figure 9.11). Although the band at 412 nm is indistinct in the original spectra (Figure 9.12), it appears clearly in the 2D correlation map. This is one of the good examples demonstrating the advantage of the 2D correlation method. The peaks at 376 nm (A1), 399 nm (A2), 424 nm (A3), and 451 nm (A4) share negative correlation with Y1, and thus are attributed to AN. Moreover, it can be seen from the synchronous correlation map that there is positive correlation between the peaks  $Y_i$  and  $Y_j$ , and between the peaks  $A_i$  and  $A_j$ , while there is negative correlation between the peaks  $Y_i$  and  $A_j$ . These results are self-consistent. The asynchronous correlation map (Figure 9.13(B)) develops cross peaks between the AN and PY bands, whereas no cross peak appear between the bands of the same probe. This result

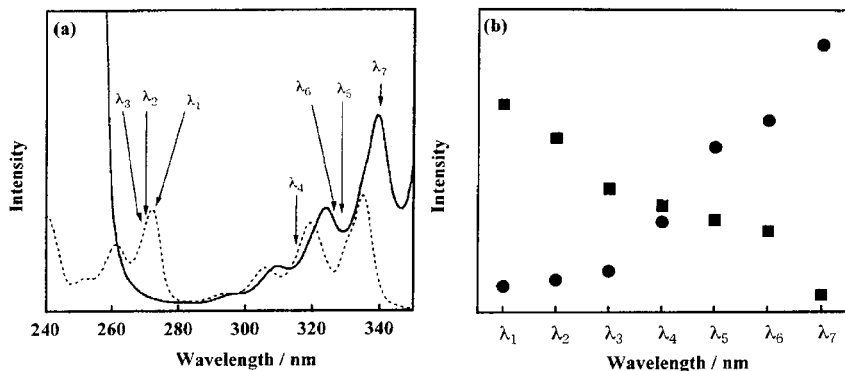


**Figure 9.14** (A) Excitation spectra of AN (—) and PY (···) in cyclohexane solutions. (B) Dependence of the fluorescence intensities of AN (●) and PY (■) on the excitation wavelength. Concentrations: [AN] = 10  $\mu$ M, [PY] = 10  $\mu$ M. The fluorescence is monitored at 398 nm for AN and 382 nm for PY. Excitation and emission band-passes are 1.5 and 5 nm, respectively. Excitation wavelength:  $\lambda_1$  = 272 nm;  $\lambda_2$  = 270 nm;  $\lambda_3$  = 268 nm;  $\lambda_4$  = 315 nm;  $\lambda_5$  = 328 nm;  $\lambda_6$  = 326 nm;  $\lambda_7$  = 343 nm. (Reproduced with permission from Ref. No. 27. Copyright (2000) American Chemical Society.)

provides additional support for the band assignment based on the synchronous correlation map.

Figure 9.14(A) shows excitation spectra of AN and PY obtained for individual solutions.<sup>27</sup> The spectra of AN and PY were obtained by monitoring fluorescence intensities at 398 and 382 nm, respectively. The excitation spectra of both AN and PY are almost identical to their respective absorption spectra. The absorption of PY ranging from 300 to 340 nm is assigned to the  $S_2 \leftarrow S_0$  transition and that from 250 to 280 nm is assigned to the  $S_3 \leftarrow S_0$  transition. The  $S_1 \leftarrow S_0$  transition is located in the region from 350 to 370 nm. However, this transition is forbidden and the bands appear as a shoulder of the strong  $S_2 \leftarrow S_0$  transition. This is the reason for the breakdown of mirror-image symmetry between the absorption and fluorescence spectra of PY.

It is difficult to find a wide spectral region for the AN–PY system where the AN absorption monotonically increases (or decreases), while the PY absorption decreases (or increases). Therefore, Nakashima *et al.* picked the excitation wavelengths ( $\lambda_1$ – $\lambda_2$ ) from several discrete spectral regions as shown in Figure 9.14(A). The fluorescence intensities of AN and PY are plotted against the excitation wavelength in Figure 9.14(B). The intensity of AN fluorescence increases with the change of excitation wavelength from  $\lambda_1$  to  $\lambda_7$ , whereas the intensity of PY fluorescence decreases. Figure 9.15 exhibits a series of fluorescence spectra of a 1 : 1 mixture of AN and PY (5  $\mu$ M for each) in a cyclohexane solution obtained by varying the excitation wavelength from  $\lambda_1$  to  $\lambda_7$ . As expected, the top two spectra are dominated by PY fluorescence, and the bottom two spectra are dominated by AN fluorescence. The middle three spectra are extremely tangled by heavy overlap of the component spectra with comparable intensities.



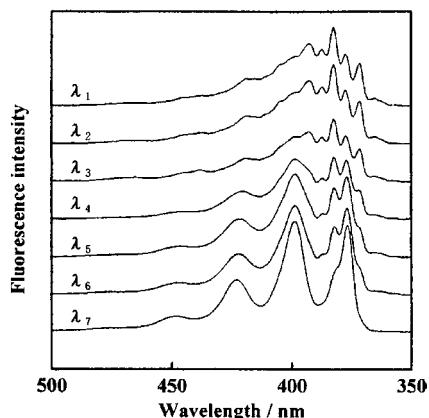


**Figure 9.15** Fluorescence spectra of the mixtures of AN and PY excited at various wavelengths in a cyclohexane solution. Concentrations:  $[AN] = 5 \mu M$ ,  $[PY] = 5 \mu M$ . Excitation and emission band-passes are 1.5 and 5 nm, respectively. Excitation wavelengths are indicated by  $\lambda_i$  ( $i = 1-7$ ), and are the same as those in Figure 9.14. (Reproduced with permission from Ref. No. 27. Copyright (2000) American Chemical Society.)

Figure 9.16(A) and (B) shows synchronous and asynchronous maps generated from the spectra in Figure 9.15.<sup>27</sup> A number of auto- and cross peaks are developed in the synchronous correlation map. This seems to be because the fluorescence intensities of both AN and PY are considerably changed by varying the excitation wavelength from  $\lambda_1$  to  $\lambda_7$ . In the synchronous spectrum, it is obvious that the peak at 365 nm (Y0) arises from the hot band of PY. The band at 371 nm (Y1) is ascribed to PY because this band has positive correlation with Y0. The peaks at 384 nm (Y3) and 388 nm (Y4) have positive correlation with Y1, and thus are assigned to PY. The peaks at 377 nm (A1), 399 nm (A2), 424 nm (A3), and 452 nm (A4) have negative correlation with Y1, and thus are assigned to AN. As in the case of concentration perturbation, there is positive correlation between the peaks  $Y_i$  and  $Y_j$ , and between the peaks  $A_i$  and  $A_j$ , while there is negative correlation between the peaks  $Y_i$  and  $A_j$ .

The asynchronous correlation map (Figure 9.16(B)) yields many cross peaks. As already discussed, the asynchronous cross peaks appear only between the bands for different compounds. Therefore, the asynchronous correlation map is useful for confirming the band assignment based on the synchronous correlation map. Note that there are distinct bands at 409, 438, and 467 nm which have asynchronous correlation with the A2 band. According to the previous discussion, these bands can be assigned to PY. The assignment for the band at 409 nm is also supported by the fact that this band corresponds to the band at 412 nm in Figure 9.13(A). Of note is that we can clearly detect these three PY bands in the 2D correlation maps, although they appear as obscure bands in the original one-dimensional spectrum. This demonstrates the usefulness of 2D correlation method in fluorescence spectroscopy.

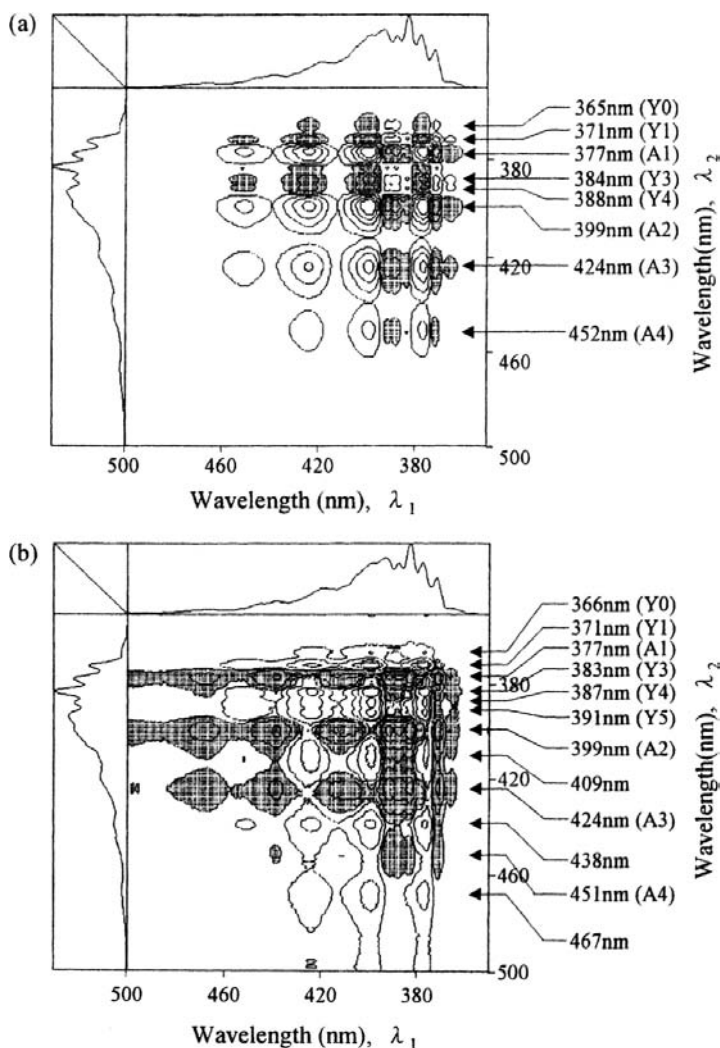


**Figure 9.16** (A) Synchronous and (B) asynchronous 2D fluorescence correlation spectra of the mixtures of AN and PY in a cyclohexane solution, constructed from the spectra of Figure 9.15. (Reproduced with permission from Ref. No. 27. Copyright (2000) American Chemical Society.)

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# 10 Generalized Two-dimensional Correlation Studies of Polymers and Liquid Crystals

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Polymers and liquid crystals (LCs) are extensively studied in 2D correlation spectroscopy.<sup>1–5</sup> In fact, the majority of 2D correlation spectroscopy studies before the introduction of the generalized 2D correlation scheme were focused exclusively on polymers or LCs. The dynamic 2D IR experiment based on the time-resolved measurement of spectral variations of polymers induced by a sinusoidal perturbation marked the beginning of 2D correlation spectroscopy.<sup>1–11</sup> It was soon realized that the reorientation of LCs could be analyzed in a manner similar to the mechanically induced reorientation of polymer segments.<sup>12–14</sup> Thus, the dynamic electro-optical 2D IR spectroscopy studies of LCs reorienting under an AC field became an active area of research. Several pertinent examples of such dynamic 2D IR studies have been discussed in Chapter 8.

2D correlation spectroscopy studies not relying on dynamic repetitive perturbations are also practiced extensively in the investigation of a wide variety of polymer and LC systems.<sup>1,2,4,5,15–28</sup> After the introduction of the generalized 2D correlation scheme, 2D IR studies of LCs have become especially widespread, because the generalized 2D approach provides enormous versatility in terms of the types of perturbation that could be used.<sup>29–35</sup> Nowadays, a number of temperature-, pressure-, position-, time-, phase angle-, and composition-dependent IR spectral variations of polymers and LCs are subjected to 2D correlation analysis. Furthermore, 2D NIR, 2D Raman, and heterospectral correlation have become popular in polymer research. This chapter explores several interesting examples of generalized 2D correlation spectroscopy studies of polymers and LCs.

## 10.1 TEMPERATURE AND PRESSURE EFFECTS ON POLYETHYLENE

We start first with the study of a seemingly simple plastic film made of linear low density polyethylene (LLDPE) as an illustrative example of the application of generalized 2D correlation spectroscopy in polymer analysis.<sup>7,8</sup> LLDPE is a

semicrystalline copolymer of mostly  $\text{CH}_2$  repeating units with a small amount of  $\alpha$ -olefin branches. The presence of short branches disrupts the regularity in the molecular chain structure to lower the crystallinity and impart the desired flexibility.

Samples of semicrystalline polymers such as LLDPE prepared from their melt are believed to possess very complex supermolecular structures consisting of folded-chain crystal lamellae embedded in a liquid-like amorphous matrix.<sup>36</sup> Such crystal lamellae may be oriented in many directions, depending on the growth history, as well as the mechanical deformations imposed on the sample by an external force. Furthermore, the crystal lamellae of polyethylene are sometimes twisted around the  $b$ -axis (i.e., growth direction) to develop even more convoluted structures. A schematic example of a segment of a twisted lamella of semicrystalline LLDPE is shown in Figure 10.1.

In addition to the intrinsic morphological complexities in the static state, LLDPE can undergo highly convoluted transition processes, when temperature and pressure are altered.<sup>36</sup> These transitions include the melting of ordered molecular segments, as well as the glass-to-rubber transition and other relaxation processes of the amorphous component. Physical transitions of polymers usually result in noticeable variations in IR spectra. By studying such spectral changes with generalized 2D correlation analysis, one can often elucidate complex structural and morphological information on semicrystalline polymers.

The IR study of semicrystalline samples is often complicated by the presence of overlapped contributions from coexisting crystalline and amorphous regions. Fortunately, the IR spectrum of the amorphous component is reasonably approximated by the melt spectrum, so the crystalline component can be estimated by subtracting the amorphous component from the room temperature spectrum. Figure 10.2 compares such component-specific spectra of an LLDPE sample

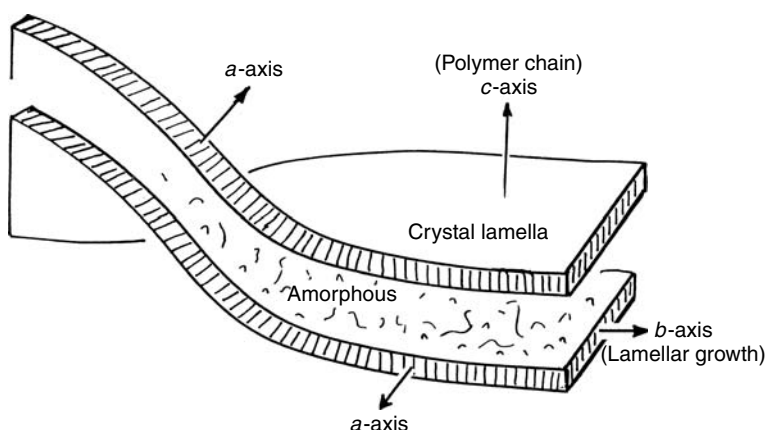


**Figure 10.1** Schematic diagram of twisted lamellae of LLDPE crystals embedded in an amorphous matrix. (Reprinted from *Vibrational Spectroscopy*, **19**, I. Noda, G. M. Story, and C. Marcott, p. 461, Copyright (1999), with permission from Elsevier.)

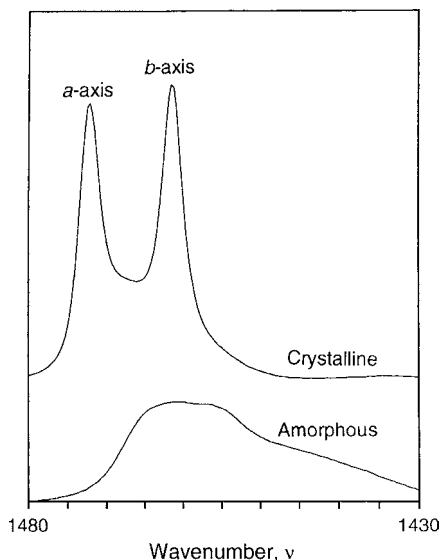


**Figure 10.2** IR spectra representing the purely crystalline and amorphous components of LLDPE in the bending mode region. (Reprinted from *Vibrational Spectroscopy*, **19**, I. Noda, G. M. Story, and C. Marcott, p. 461, Copyright (1999), with permission from Elsevier.)

(Dowlex 2044A, Dow Chemical Company) for the crystalline and amorphous regions. The sharp band around  $1473\text{ cm}^{-1}$  is assignable to the  $\text{CH}_2$  bending vibrations aligned in a direction parallel to the  $a$ -axis of the crystal lamellae.<sup>37,38</sup> The band around  $1463\text{ cm}^{-1}$  is aligned along the crystal  $b$ -axis, i.e., the lamella growth direction. Finally, a much broader peak ranging from  $1470$  to  $1430\text{ cm}^{-1}$  represents the contribution from the liquid-like amorphous component of LLDPE. The detailed experimental conditions for the system studied in this section are reported elsewhere.<sup>7,8</sup>

The LLDPE sample studied here undergoes a reasonably well-defined thermal transition when the sample is heated above the melt temperature ( $T_m$ ) around  $123^\circ\text{C}$ . This melting process is accompanied by a precipitous decrease in the intensity of sharp IR bands around  $1463$  and  $1473\text{ cm}^{-1}$  (Figure 10.3), which are both assignable to the contributions from the crystalline component of LLDPE.<sup>37,38</sup> The decrease in the crystalline band intensities is compensated by the rising intensity of a much broader band ranging from  $1470$  to about  $1440\text{ cm}^{-1}$  associated with the liquid-like amorphous component. The contribution of purely amorphous component is well represented by the spectrum of molten LLDPE measured at  $150^\circ\text{C}$ .

2R IR correlation spectra based on the spectral changes induced by the melting of LLDPE between  $100$  and  $150^\circ\text{C}$  are shown in Figure 10.4. The synchronous 2D IR spectrum (Figure 10.4(A)) shows that the simultaneous decrease in the intensities of two crystalline bands at  $1473$  and  $1463\text{ cm}^{-1}$  is synchronously correlated with

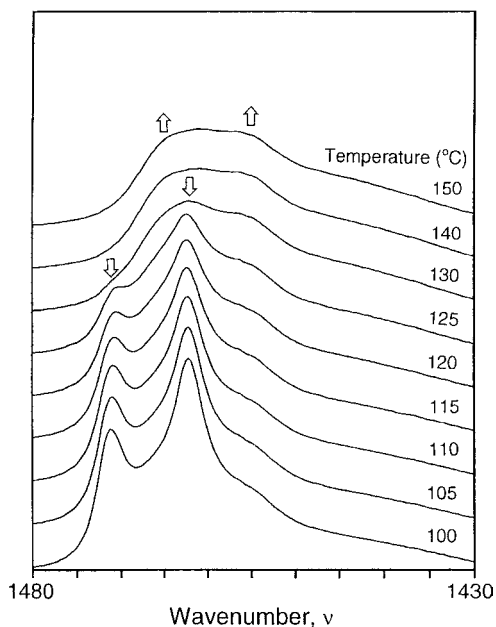


**Figure 10.3** Temperature-dependent IR spectra of LLDPE collected for the melting process between 100 and 150 °C. (Reprinted from *Vibrational Spectroscopy*, **19**, I. Noda, G. M. Story, and C. Marcott, p. 461, Copyright (1999), with permission from Elsevier.)

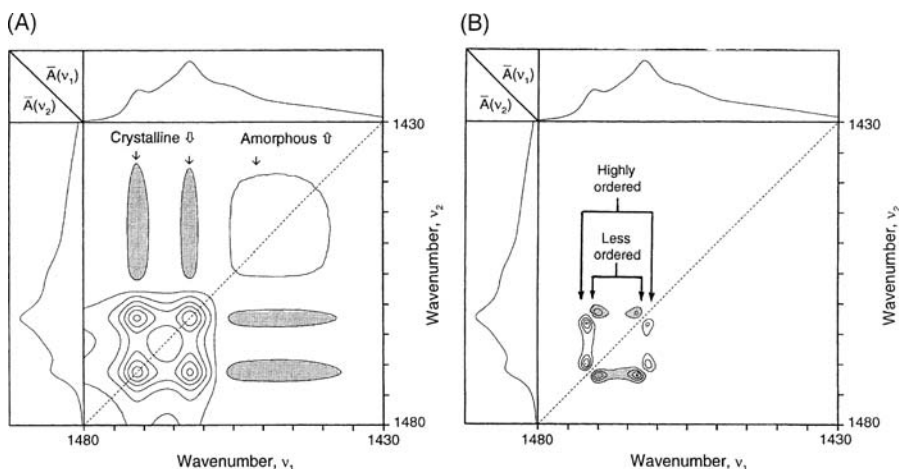


**Figure 10.4** 2R IR correlation spectra based on the spectral changes induced by the melting of LLDPE between 100 and 150 °C: (A) synchronous spectrum and (B) asynchronous spectrum. (Reprinted from *Vibrational Spectroscopy*, **19**, I. Noda, G. M. Story, and C. Marcott, p. 461, Copyright (1999), with permission from Elsevier.)

the presence of a pair of positive cross peaks. Much broader negative (indicated by shading) synchronous cross peaks are also observed between the two crystalline bands, which are decreasing in intensity, and the lower side of the broad amorphous band below  $1455\text{ cm}^{-1}$ , which is increasing in intensity. The observation is consistent with the expected population changes of crystalline and amorphous components during the melting process. Interestingly, the asynchronous 2D IR spectrum (Figure 10.4(B)) shows the presence of several cross peaks. Apparently the decrease in the crystalline component does not result in the simultaneous increase in the amorphous component, when the LLDPE sample is melted. The latter process seems to occur in a somewhat higher temperature range than that for the rapid disappearance of the crystalline component. This result suggests an intriguing possibility of the existence of an intermediate state between the highly ordered crystalline state and totally liquid-like amorphous state.

Even well below the melt temperature ( $T_m$ ), an LLDPE sample upon heating gradually undergoes a partial fusion of less ordered small crystallites, known as *pre-melting*. Intensities of sharp crystalline bands around  $1463$  and  $1473\text{ cm}^{-1}$  gradually decrease during the pre-melting process (Figure 10.5). The decrease of crystalline band intensity is accompanied by the subtle development of shoulders, especially around  $1465$  and  $1455\text{ cm}^{-1}$  arising from the increase in the contribution from the additional amorphous component created by the pre-melting.

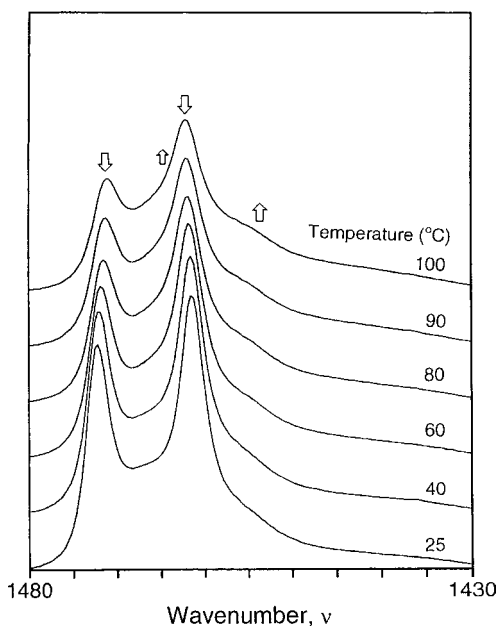


**Figure 10.5** Temperature-dependent IR spectra of LLDPE collected for the pre-melting process between 25 and 100 °C. (Reprinted from *Vibrational Spectroscopy*, **19**, I. Noda, G. M. Story, and C. Marcott, p. 461, Copyright (1999), with permission from Elsevier.)



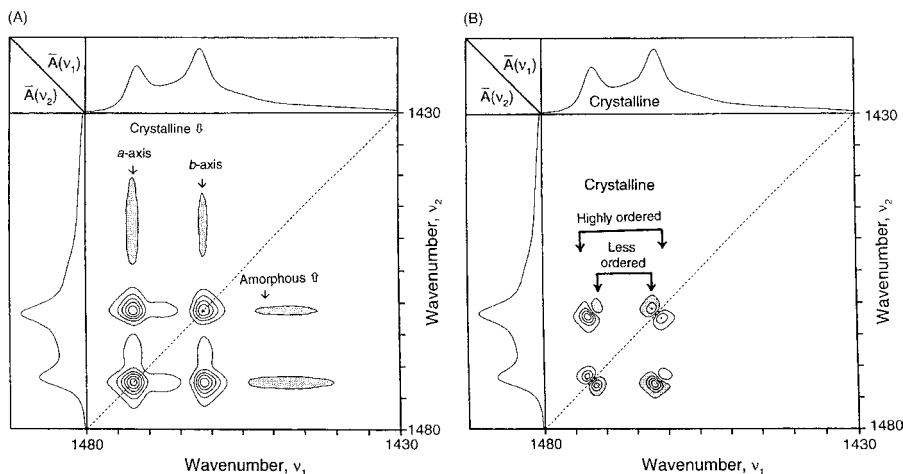


**Figure 10.6** 2R IR correlation spectra based on the small spectral changes induced by the pre-melting of LLDPE between 25 and 100 °C: (A) synchronous spectrum and (B) asynchronous spectrum. (Reprinted from *Vibrational Spectroscopy*, **19**, I. Noda, G. M. Story, and C. Marcott, p. 461, Copyright (1999), with permission from Elsevier.)

The combined effect of the decreasing and increasing intensities of IR bands from crystalline and amorphous contributions gives the appearance of gradual broadening and frequency shifts of the crystalline bands. 2D IR correlation spectra based on the small spectral changes induced by the pre-melting of LLDPE between 25 and 100 °C are shown in Figure 10.6. The results are surprisingly similar to those observed for the regular melting process (Figure 10.4). The synchronous spectrum (Figure 10.6(A)) shows that the decrease in the two crystalline bands are again well correlated to each other by the positive cross peaks, and the increase in the intensity of the lower side of amorphous band is correlated to the decreasing crystalline band intensity by the presence of negative peaks. The asynchronicity, similar to the melting process is again observed between the crystalline and amorphous contributions (Figure 10.6(B)). In short, at the submolecular level probed by IR, the pre-melting phenomenon is fundamentally very similar to the normal melting process, except that it proceed at much lower temperatures.

The IR spectrum of LLDPE is also strongly influenced by the application of compressive stress to the sample. A series of pressure-dependent spectra (Figure 10.7) are obtained at 60 °C under varying levels of mechanical compression using a torque wrench attached to an ATR cell (Graseby Specac Golden Gate diamond ATR accessory). As the compressive pressure normal to the ATR cell is increased, the intensity of the IR band at 1473 cm<sup>-1</sup>, which is attributed to the molecular vibrations parallel to the *a*-axis of the crystalline lamellae, steadily increases. Such an increase in the *a*-axis band intensity may be explained by the flattening of the crystalline superstructure under the compression, involving the untwisting of helical lamellae around the growth direction (*b*-axis). On the other

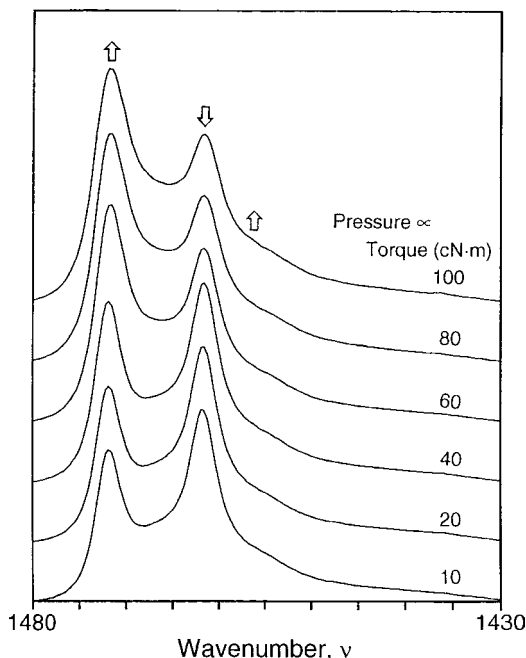


**Figure 10.7** Pressure-dependent IR spectra of LLDPE collected under varying compression pressure at 60 °C. (Reprinted from *Vibrational Spectroscopy*, **19**, I. Noda, G. M. Story, and C. Marcott, p. 461, Copyright (1999), with permission from Elsevier.)

hand, the intensity of the band at  $1463\text{ cm}^{-1}$ , which corresponds to the molecular vibrations parallel to the  $b$ -axis of the crystalline lamellae, slightly decreases probably due to the decreased crystallinity. The subtle development of a small shoulder around  $1455\text{ cm}^{-1}$  also indicates the increase in amorphous component (i.e., melting) of LLDPE under pressure.

2D IR correlation spectra based on the spectral changes induced by the static compression of LLDPE at 60 °C are shown (Figure 10.8). The negative synchronous cross peaks (Figure 10.8(A)) between two crystalline bands at  $1473$  and  $1463\text{ cm}^{-1}$  indicate that the intensity of  $a$ -axis band is increasing while that of the  $b$ -axis band is decreasing. The increase in the intensity of the lower side of the amorphous band below  $1455\text{ cm}^{-1}$  is also positively correlated with the increasing  $a$ -axis band at  $1473\text{ cm}^{-1}$ . The asynchronous cross peaks (Figure 10.8(B)) indicate that the compression-induced melting of crystallites occurs after the flattening of the semicrystalline superstructure via the uncoiling of the crystal lamellae.

Figure 10.9 schematically summarizes the proposed mechanism of deformation based on the 2D IR correlation analysis of compression-induced changes in the IR spectra of LLDPE. Initially, the LLDPE sample consists of a complex superstructure of crystalline lamellae oriented in many different directions embedded within an amorphous matrix. Upon compression, the overall crystalline

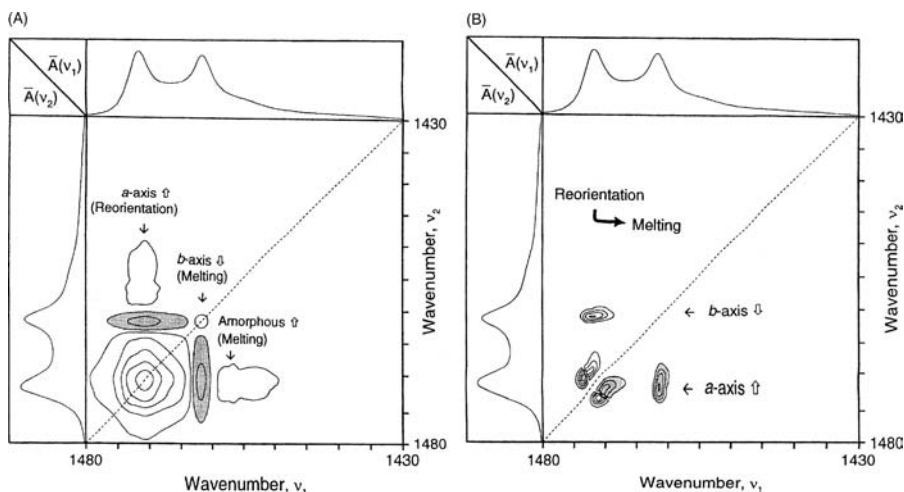


**Figure 10.8** 2R IR correlation spectra based on the spectral changes induced by the static compression of LLDPE at 60 °C: (A) synchronous spectrum and (B) asynchronous spectrum. (Reprinted from *Vibrational Spectroscopy*, **19**, I. Noda, G. M. Story, and C. Marcott, p. 461, Copyright (1999), with permission from Elsevier.)

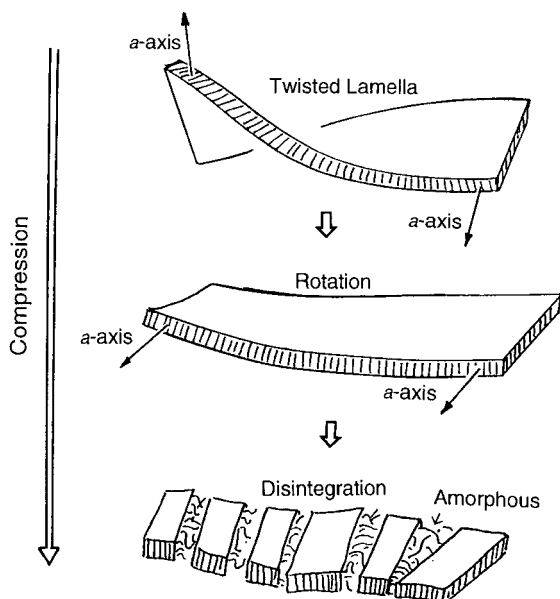


**Figure 10.9** A possible deformation mechanism of a semicrystalline LLDPE film under compression. (Reprinted from *Vibrational Spectroscopy*, **19**, I. Noda, G. M. Story, and C. Marcott, p. 461, Copyright (1999), with permission from Elsevier.)

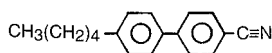
superstructure flattens to accommodate the dimensional change. This flattening is achieved mainly by the local rotation of crystalline lamellae around the growth direction (*b*-axis). Such rotation of lamellae results in the preferred reorientation of the crystalline *a*-axis in the direction normal to the compression. However, the reorientation of lamellae *b*-axis in the direction normal to the compression was not observed. Further compression of the sample results in the disintegration of crystal lamellae and production of more disordered amorphous components.

We thus demonstrated that 2D IR spectroscopy applied to a LLDPE sample under temperature change and compression reveals surprising details of the complex transition mechanisms of this polymer system.

## 10.2 REORIENTATION OF NEMATIC LIQUID CRYSTALS BY AN ELECTRIC FIELD

The study of time-dependent reorientation processes of a liquid crystalline system under external perturbations, such as an electric field, is of great industrial importance in many optical display applications. The applied external field usually varies with time in a nonsinusoidal manner, most often as a step function corresponding to the *switch-on* or *switch-off* process. The subsequent dynamic reorientation responses of liquid crystals are also nonsinusoidal and often exhibit highly nonlinear waveforms with respect to time. Generalized 2D correlation analysis will be ideally suited to the spectroscopic study of such complex transient systems.

We choose here the electric field-induced dynamic reorientation of a simple nematic liquid crystal system of 4-pentyl-4'-cyanobiphenyl (also known as 5CB) as a model. 5CB consists of a rigid cyanobiphenyl head group with a flexible pentyl aliphatic tail (Figure 10.10). The large dipole moment associated with the cyano group makes it possible for initially randomly oriented 5CB to align in a direction under a fixed electric field. Upon the removal of the electric field, the system eventually reverts to the randomly oriented state under the influence of thermal fluctuations of molecules. The process is schematically illustrated in Fig. 10.11, where 5CB molecules are depicted as 'tadpoles' with heads (cyanobiphenyls) and tails (pentyls) responding to the presence or absence of the external electric field. Similar 5CB liquid crystalline systems, typically under the influence of a sinusoidally alternating electric field, have been studied by several research groups using a step-scan FT IR spectrometer.<sup>13,39</sup>



4-pentyl-4'-cyanobiphenyl (5CB)

**Figure 10.10** Molecular structure of 4-pentyl-4'-cyanobiphenyl (also known as 5CB)

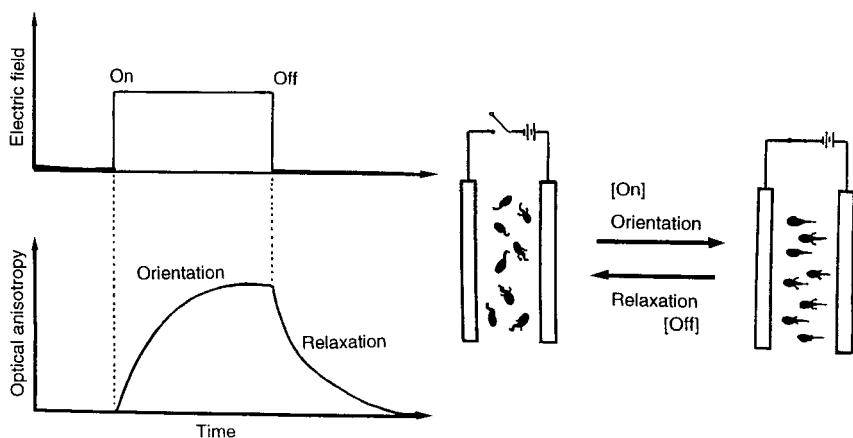


**Figure 10.11** Schematic representation of the electric field induced orientation and relaxation of nematic liquid crystalline 5CB. 5CB is depicted as a molecular tadpole with the cyanobiphenyl head group and a flexible pentyl tail

By using a polarized IR beam, it is possible to monitor the dynamic reorientation processes of 5CB under an electric field at the submolecular level. Because of the complex interactions between constituents, the field-induced molecular reorientation of 5CB does not happen instantaneously, and the observed reorientation responses can often be highly nonlinear with respect to time. Fortunately, generalized 2D correlation analysis can effectively sort out the complex evolutionary process of 5CB reorientation to extract the essential information about the field-induced reorganization of the liquid crystalline system. Figure 10.12 shows the synchronous 2D IR correlation spectrum of 5CB during the orientation process right after the application of an electric field. We observe the development of sharp synchronous cross peaks among bands associated with the cyano groups and biphenyls. This result indicates that they are reorienting together at the same rate under the application of the electric field.

Figure 10.13 shows the 2D IR correlation spectra of the same orientation process of 5CB comparing the dynamics of biphenyl and pentyl groups. The synchronous spectrum (Figure 10.13(A)) indicates that the dynamics of bands associated with the pentyl groups are all synchronized, i.e., they are all reorienting together at the same rate to generate the cross peaks. Likewise, the intensity changes for the two bands from biphenyl are also synchronized, as already observed in Figure 10.12. Interestingly, however, there are no noticeable synchronous cross peaks observed between bands from biphenyl and pentyl groups. The asynchronous 2D correlation spectrum (Figure 10.13(B)) also shows the presence of cross peaks. These results reveal that there is some discrepancy between the reorientation rate of biphenyl groups and that of pentyl groups. According to the sign of the cross peaks, one may conclude that biphenyl head groups (and cyano groups) reorient before the reorientation of pentyl tails, when the electric field is applied to the initially randomly oriented 5CB sample.

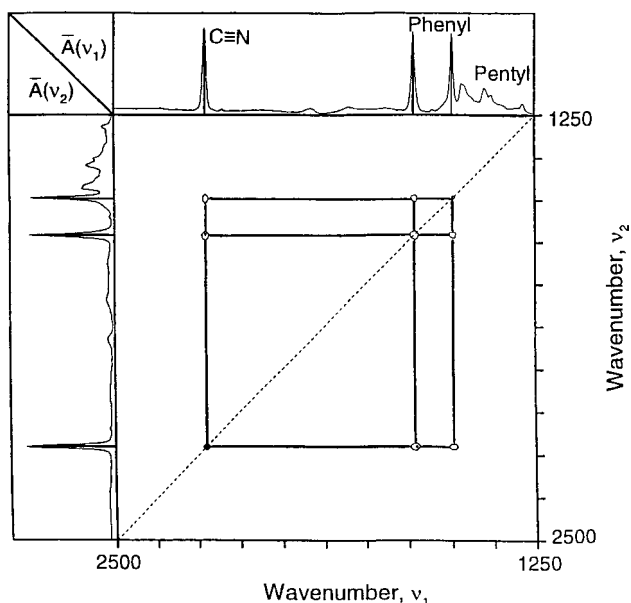


**Figure 10.12** Synchronous 2D IR correlation spectrum of 5CB during the electric field-induced orientation buildup

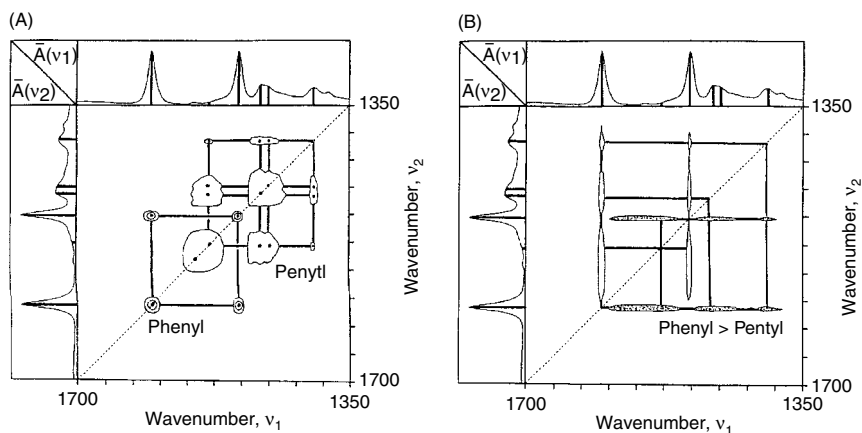


**Figure 10.13** 2D IR correlation spectra of 5CB during the electric field induced orientation: (A) synchronous spectrum; and (B) asynchronous spectrum

In the absence of the field, the orientation of 5CB molecules will gradually relax to the randomly oriented state by thermal fluctuation. The synchronous 2D spectrum for the relaxation process of initially oriented 5CB due to the removal of the electric field is not shown here, as it looks very similar to the case of orientation buildup shown in Figure 10.13(A). Figure 10.14 shows the asynchronous 2D IR

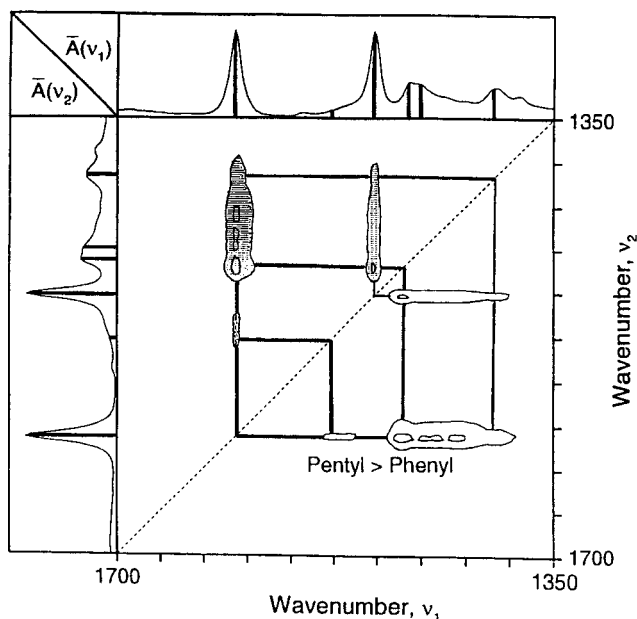


**Figure 10.14** Asynchronous 2D IR correlation spectrum of 5CB during the orientation relaxation upon removal of the electric field

correlation spectrum of 5CB after the electric field is switched off. Again, we observe the presence of asynchronous cross peaks, indicating that the orientation relaxation processes of biphenyl and pentyl groups upon the removal of the electric field are not fully synchronized. Interestingly, however, the sign of these cross peaks is now different from that of the cross peaks previously observed in Figure 10.13(B). They show that, unlike the orientation buildup process under the applied field, the orientation relaxation of the biphenyl head groups after the removal of the electric field actually lags behind that of pentyl tail groups.

By simply combining the observations made for 2D correlation spectra during the orientation buildup and orientation relaxation, one may propose the following mechanisms for the field-induced orientation dynamics of the nematic liquid crystalline system.

- (1) After the prolonged absence of an electric field, the system will be in the more or less isotropic randomly oriented state.
- (2) Upon the application of the electric field, the cyano groups with large dipole moment will immediately start the reorientation process. Since the biphenyl is rigidly attached to the cyano group, these moieties reorient together as integral parts of the whole head group.
- (3) The more flexible pentyl tail groups do not directly feel the effect of the applied electric field at first but are dragged by the head group to eventually

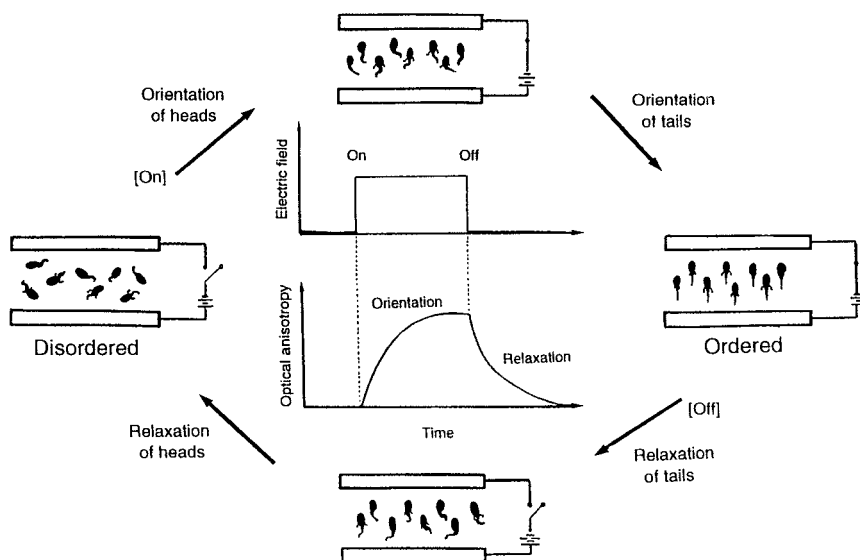


**Figure 10.15** Schematic representation of the proposed orientation and relaxation mechanisms of SCB

align with the rest of molecule. This process will lag behind the orientation of the head group.

- (4) Upon the removal of the electric field, the more flexible and mobile pentyl tail groups will relax the alignment first by the influence of thermal fluctuations. This effect eventually drags the rest of the molecule to a randomly orientated state.

Those steps are schematically represented in Figure 10.15. The above conclusions are drawn strictly from the very simple observations made on the basic features of 2D correlation spectra, i.e., only the presence or absence of cross peaks and their signs. No other information, such as the analytical expression for the actual response curves of spectral intensity signals during the buildup and relaxation of molecular orientations, was required. While the analysis is based strictly on the simple qualitative feature of 2D correlation spectra, the essential component of the information is nicely captured for the complex orientation dynamics of a liquid crystalline system, such as the role of individual submolecular units during the field-induced reorientation process.

### 10.3 TEMPERATURE-DEPENDENT 2D NIR OF AMORPHOUS POLYAMIDE

Temperature-dependent 2D NIR spectroscopy, as in the case of small molecules discussed in Chapter 9, is a powerful tool in the investigation of hydrogen



bonding in associated polymers, such as polyamides. The structures of polyamides have long been studied by using IR, Raman, and NIR spectroscopy, which provide useful information about their conformation and the nature of their hydrogen bonds.<sup>36,37</sup> Wu and Siesler investigated the thermal behavior of a totally amorphous polyamide by means of 2D NIR correlation spectroscopy.<sup>23</sup> In the NIR region, bands due to the stretching modes of free and hydrogen-bonded NH groups are much better resolved. Bands due to free NH groups can be observed much more clearly because of the large anharmonicity constant of the free NH group. Thus, NIR studies of polyamides should provide valuable information about the temperature dependence of hydrogen bonding.

Figure 10.16 shows NIR spectra of an amorphous polyamide in the region  $7000\text{--}5400\text{ cm}^{-1}$  measured over a temperature range of  $25\text{--}200^\circ\text{C}$ .<sup>23</sup> Table 10.1 summarizes assignments for NIR bands of the amorphous polyamide. Bands in the  $6100\text{--}5400\text{ cm}^{-1}$  region due to the overtones and combination bands of CH and  $\text{CH}_2$  groups do not show significant temperature-dependent variations, while the intensities and shapes of bands in the  $6800\text{--}6300\text{ cm}^{-1}$  region due to the amide groups vary with temperature. Of note is that the intensity of a band at  $6765\text{ cm}^{-1}$ , arising from the first overtone of the stretching mode of free NH group, increases markedly as a function of temperature, indicating the increase in the proportion of free NH groups. A band at about  $6535\text{ cm}^{-1}$  decreases in intensity with a small upward shift, whereas the intensities of bands around  $6650\text{ cm}^{-1}$  increase slightly with temperature.

Synchronous and asynchronous 2D NIR spectra in the  $6900\text{--}6200\text{ cm}^{-1}$  region generated from the temperature-dependent spectral variations of the polyamide

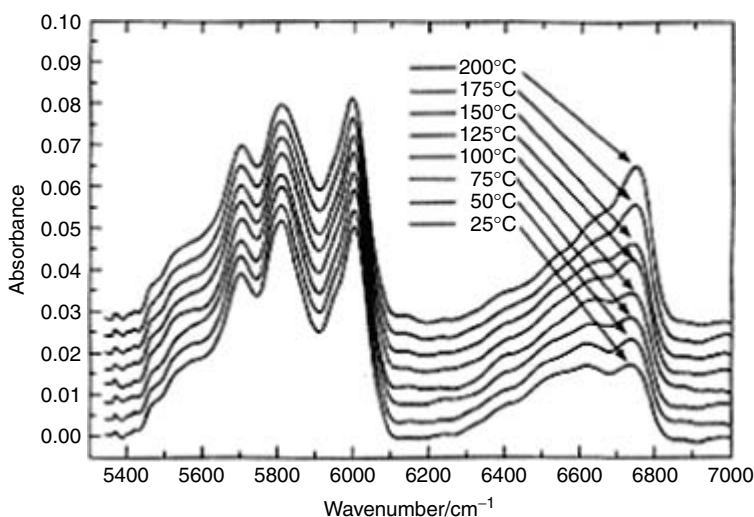


**Figure 10.16** NIR spectra in the  $7000\text{--}5400\text{ cm}^{-1}$  region of the amorphous polyamide measured over a temperature range of  $25\text{--}200^\circ\text{C}$ . (Reproduced with permission from Ref. No. 23. Copyright (2000) American Institute of Physics.)

**Table 10.1** NIR band assignments of the amorphous polyamide (from Ref. No. 23)

| Wavenumber<br>(cm <sup>-1</sup> ) | Intensity | Assignment                                                |
|-----------------------------------|-----------|-----------------------------------------------------------|
| 6765                              | w         | 2 × ν (NH) <sub>f</sub>                                   |
| 6520                              | m         | 2 × ν (NH) <sub>b</sub>                                   |
| 6010                              | m         | 2 × ν (CH) <sub>ar</sub>                                  |
| 5980                              | m         | 2 × ν (CH) <sub>ar</sub>                                  |
| 5900                              | m         | 2 × ν (CH) <sub>ar</sub>                                  |
| 5810                              | m         | 2 × ν <sub>as</sub> (CH <sub>2</sub> )                    |
| 5690                              | m         | 2 × ν <sub>s</sub> (CH <sub>2</sub> )                     |
| 4877                              | m         | ν (NH) <sub>b</sub> + AmideII                             |
| 4659                              | m         | 3 × AmideII                                               |
| 4611                              | m         | ν (NH) <sub>b</sub> + AmideIII                            |
| 4350                              | s         | ν <sub>as</sub> (CH <sub>2</sub> ) + δ (CH <sub>2</sub> ) |

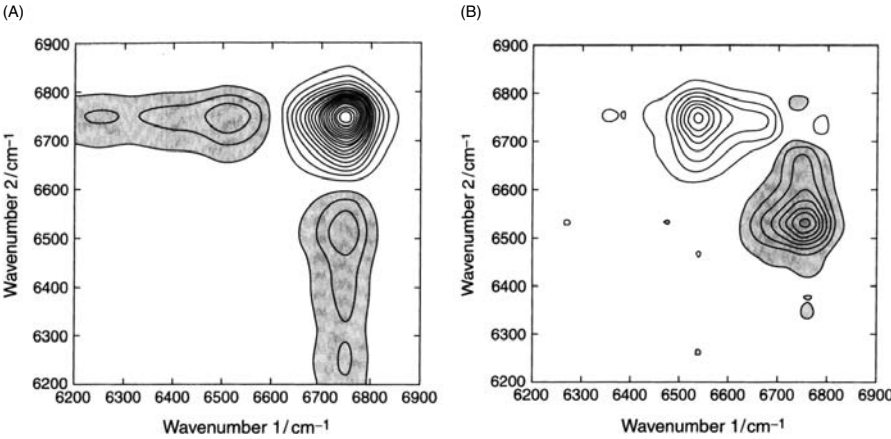


**Figure 10.17** (A) Synchronous and (B) asynchronous 2D NIR spectra in the 6900–6200 cm<sup>-1</sup> region of the polyamide obtained from 25 to 200 °C. (Reproduced with permission from Ref. No. 23. Copyright (2000) American Institute of Physics.)

are shown, respectively, in Figure 10.17(A) and (B). In the synchronous spectrum, a broad autopeak is developed at 6765 cm<sup>-1</sup>, indicating that the intensity of the band due to the first overtone of the stretching mode of free NH groups [ν (NH)<sub>f</sub>] increases significantly during the course of the temperature increase. Two negative cross peaks observed at (6535, 6765) and (6250, 6765 cm<sup>-1</sup>) suggest that the intensities of the bands at 6535 and 6250 cm<sup>-1</sup>, arising, respectively, from the first overtone of the stretching mode of bound NH groups [ν (NH)<sub>b</sub>] and possibly from a combination of NH (bound) and CH stretching modes, decrease with temperature.

The corresponding asynchronous spectrum (Figure 10.17(B)) develops a cross peak at (6765, 6535 cm<sup>-1</sup>). The sign of this cross peak suggests that

the temperature-induced spectral change takes place earlier (i.e., at a lower temperature) at  $6535\text{ cm}^{-1}$  [ $2 \times \nu(\text{NH})_{\text{b}}$ ] than that at  $6765\text{ cm}^{-1}$  [ $2 \times \nu(\text{NH})_{\text{f}}$ ]. The cross peak shows obvious asymmetry, indicating the possible existence of a band near  $6650\text{ cm}^{-1}$ , probably arising from a first overtone of the NH stretching mode of weakly hydrogen-bonded amide groups (Figure 10.18(C)). This observation suggests that the intermolecular hydrogen bonding is gradually weakened during the course of heating. Also of interest in the asynchronous spectrum is the cross peak at  $(6780, 6740\text{ cm}^{-1})$ . This result indicates that the band at  $6765\text{ cm}^{-1}$  consists of two components. Those at  $6780$  and  $6740\text{ cm}^{-1}$  probably originate, respectively, from the totally free NH (Figure 10.18(A)) and the free-end NH (Figure 10.3(B)) groups.

Figure 10.19(A) and (B) shows synchronous and asynchronous 2D NIR correlation spectra in the  $6200\text{--}5400\text{ cm}^{-1}$  region generated from the temperature-dependent spectral variations (from  $25$  to  $200^\circ\text{C}$ ) of the same amorphous polyamide.<sup>23</sup> The first overtones of the aliphatic  $\text{CH}_2$  stretching modes and the aromatic  $\text{CH}$  stretching modes are expected to appear in the  $6100\text{--}5400\text{ cm}^{-1}$  region. Three autopeaks at  $6010$ ,  $5980$ , and  $5900\text{ cm}^{-1}$  are due to the first overtones

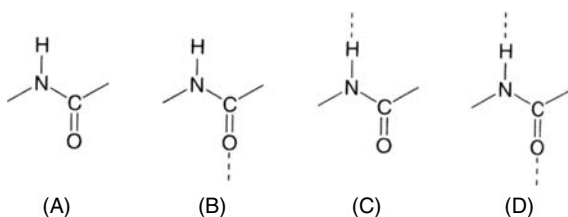


**Figure 10.18** Different hydrogen-bonding structures of amide groups in polyamides. (Reproduced with permission from Ref. No. 23. Copyright (2000) American Institute of Physics.)

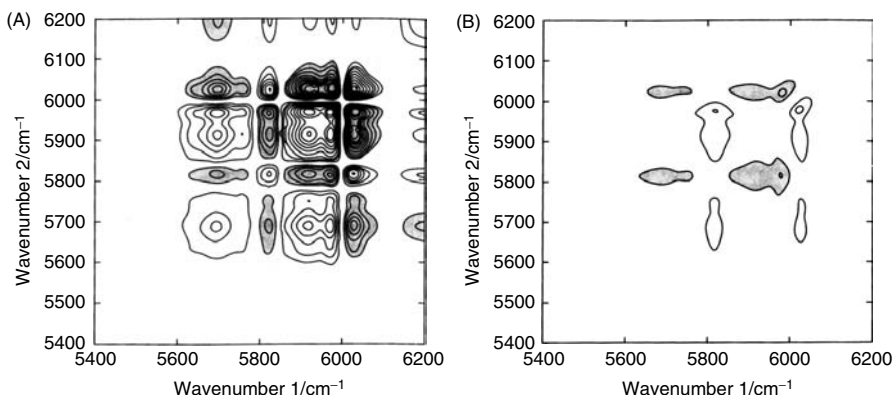


**Figure 10.19** (A) Synchronous and (B) asynchronous 2D NIR spectra in the  $6200\text{--}5400\text{ cm}^{-1}$  region of the polyamide obtained from  $25$  to  $200^\circ\text{C}$ . (Reproduced with permission from Ref. No. 23. Copyright (2000) American Institute of Physics.)

of the aromatic CH stretching modes, while those at  $5810$  and  $5690\text{ cm}^{-1}$  are assignable, respectively, to the first overtones of the antisymmetric and symmetric  $\text{CH}_2$  stretching modes. The intensities of the first overtones of  $\text{CH}_2$  stretching vibrations are very sensitive to the degree of order in polyamides. Wu and Siesler<sup>23</sup> assigned the band at  $5810\text{ cm}^{-1}$  to the first overtone of the  $\text{CH}_2$  stretching mode of a highly associated form of the polyamide. The sign of the asynchronous cross peak at  $(5810, 5690\text{ cm}^{-1})$  reveals that the intensity change at  $5810\text{ cm}^{-1}$  for highly associated groups takes place earlier than that at  $5690\text{ cm}^{-1}$  for less associated groups at a lower temperature. The asynchronicity of intensity changes for bands, due to vibrational modes of free and hydrogen-bonded NH groups, indicates a complicated mechanism of the dissociation and the existence of different hydrogen-bonded NH groups.<sup>23</sup>

#### 10.4 COMPOSITION-BASED 2D RAMAN STUDY OF EVA COPOLYMERS

In the next example, 2D FT-Raman correlation spectroscopy was employed to study the composition-induced structural changes in 11 different ethylene–vinyl acetate (EVA) copolymers with a vinyl acetate (VA) content varying from 6 to 42 wt%.<sup>24</sup> EVA is a commonly used industrial copolymer useful as a major ingredient for adhesives. It is well known that changes in the VA content cause variations in the physical properties such as crystallinity and impact strength.<sup>38,40</sup> The redistribution of phase compositions in EVA with VA increasing from 2.5 to 8.5 wt% was investigated by Strobl and Hagedorn<sup>41</sup> in 1978 using Raman spectroscopy. They proposed a three-phase model for EVA: an orthorhombic crystalline phase, a melt-like amorphous phase, and a disordered phase of anisotropic nature. In the disordered phase, the methylene sequences have all-*trans* conformation, as well as some kind of lateral order, but cannot pack as tightly as those in the crystalline phase. While the previous Raman study was focused on the supramolecular level, the 2D Raman correlation study can also be used to explore the structural changes in EVA copolymers at the molecular and sub-molecular level. This study is aimed at elucidating the cause–effect relationship for structural changes occurring both at the supermolecular phase scale and at the segmental submolecular level.<sup>24</sup>

Figure 10.20 displays FT-Raman spectra of EVA copolymers with VA content ranging from 6 to 42 wt%.<sup>24</sup> Table 10.2 lists the assignments of the pertinent bands in Figure 10.20.<sup>24</sup> Some bands ascribed to the ethylene units are characteristic of amorphous, crystalline, and anisotropic parts. It is noted in Figure 10.20 that bands at  $1739$  and  $629\text{ cm}^{-1}$ , assigned, respectively, to  $\text{C}=\text{O}$  stretching and  $\text{O}-\text{C}=\text{O}$  deformation modes of acetate groups, increase with the increase in the VA content, while bands at  $1438$ ,  $1295$ ,  $1130$ , and  $1060\text{ cm}^{-1}$ , attributed to the ethylene groups, decrease.

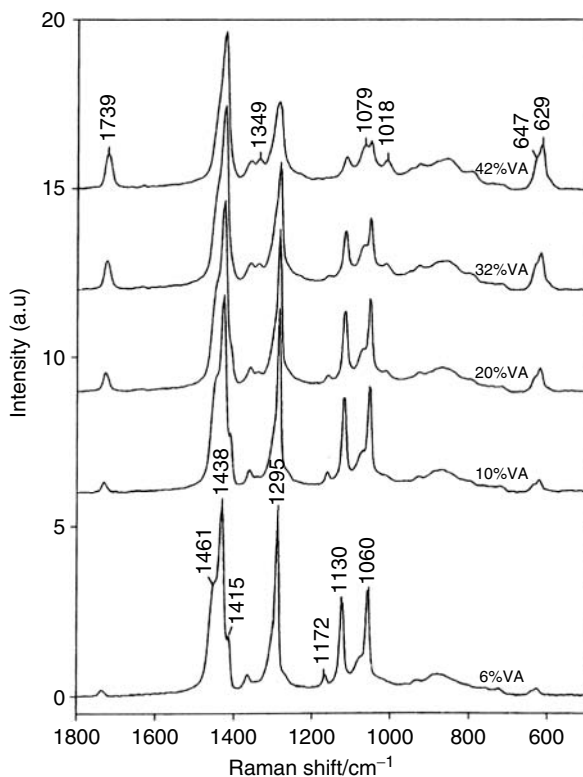


**Figure 10.20** FT-Raman spectra of five representative EVA in pellets. (Reproduced with permission from Ref. No. 24. Copyright (1999) American Chemical Society.)

The composition-dependent spectral variations in Figure 10.20 may be induced by the following factors: (1) compositional change and thus population densities of ethylene and VA contents; (2) configurational and conformational variations in the regularity of methylene sequences due to VA inclusion; (3) disruption of the crystalline packing in a unit cell; and (4) redistribution of methylene groups in different phases. The composition-dependent 2D Raman correlation spectroscopy study of the structural changes in EVA copolymers relies on the fact that a simple composition change alone yields only synchronous correlation peaks but not asynchronous peaks. If the composition change gave rise to a strictly proportional change in band intensities, the corresponding asynchronous correlation intensity would be zero. In fact, a change in the VA content produces irregular and disproportionate structural changes in EVA, resulting in complex spectral changes. Thus, the appearance of any asynchronous peak in this study should indicate the presence of such structural changes. The nonlinear nature of composition-induced spectral variations of EVA is also strongly influenced by the average background level of VA content. To accentuate the effect of the background VA

**Table 10.2** Assignments of the Raman bands of EVA (from Ref. No. 24)

| Frequency<br>(cm <sup>-1</sup> ) | Mode                                  | Features <sup>a</sup>                |
|----------------------------------|---------------------------------------|--------------------------------------|
| 629                              | O=C=O deformation                     | Due to acetate                       |
| 647                              | O=C=O deformation                     | Due to acetate                       |
| 1018                             | C–C stretching of >HC–CH <sub>2</sub> | Due to vinyl                         |
| 1060                             | asymmetric C–C stretching             | Due to all- <i>trans</i> $-(CH_2)_n$ |
| 1079                             | asymmetric C–C stretching             | A ( <i>trans</i> and <i>gauche</i> ) |
| 1110                             | symmetric C–C stretching              | A ( <i>trans</i> and <i>gauche</i> ) |
| 1130                             | symmetric C–C stretching              | Due to all- <i>trans</i> $-(CH_2)_n$ |
| 1172                             | CH <sub>2</sub> rocking               | C                                    |
| 1295                             | CH <sub>2</sub> twisting              | Due to all- <i>trans</i> $-(CH_2)_n$ |
| 1307                             | CH <sub>2</sub> twisting              | A                                    |
| 1330                             | CH <sub>2</sub> wagging               | A                                    |
| 1349                             | CH <sub>2</sub> wagging               | A                                    |
| 1373                             | CH <sub>2</sub> wagging               | A                                    |
| 1380                             | CH <sub>3</sub> symmetric bending     | Due to acetate                       |
| 1415                             | CH <sub>2</sub> bending               | C                                    |
| 1430                             | CH <sub>3</sub> asymmetric bending    | Due to acetate                       |
| 1434                             | CH <sub>2</sub> bending               | N                                    |
| 1438                             | CH <sub>2</sub> bending               | C                                    |
| 1446                             | CH <sub>2</sub> bending               | N                                    |
| 1461                             | 2 × CH <sub>2</sub> rocking           | Due to all- <i>trans</i> $-(CH_2)_n$ |
| 1739                             | C=O stretching                        | Due to acetate                       |

<sup>a</sup> A, amorphous; C, crystalline; N, anisotropic.

level, the spectral data were subdivided into four distinct sets according to their composition range.

Set 1 contained three EVA copolymers with low VA content of 6, 7 and 8 wt%. Synchronous and asynchronous correlation spectra for Set 1 in the region 1500–1200 cm<sup>-1</sup> are shown in Figure 10.21(A) and (B).<sup>24</sup> A band at 1415 cm<sup>-1</sup> is ascribed to the lamellar cores that constitute the orthorhombic crystalline phase, while that at 1307 cm<sup>-1</sup> is attributed to the interlamellar layers that make up the amorphous phase.<sup>41</sup> The negative synchronous cross peak at (1415, 1307 cm<sup>-1</sup>) indicates that the intensity of the crystalline band at 1415 cm<sup>-1</sup> decreases, while that of the amorphous band at 1307 cm<sup>-1</sup> increases. Thus, the crystalline lamellae shrink, and the interlamellar amorphous layers expand with a small increase in VA content.

In the synchronous spectrum, bands at 1446 and 1434 cm<sup>-1</sup> have negative correlation with the crystalline band at 1415 cm<sup>-1</sup> and positive correlation with each other. The band at 1446 cm<sup>-1</sup> has positive correlation with the amorphous band at 1307 cm<sup>-1</sup>. These observations suggest the intensity changes of the bands at 1446 and 1434 cm<sup>-1</sup> have the same direction as the amorphous band at 1307 cm<sup>-1</sup>, and opposite direction with respect to the crystalline band at 1415 cm<sup>-1</sup>. However, it is not enough to conclude that the two bands at 1446 and 1434 cm<sup>-1</sup> arise from the amorphous phase. Note that they share asynchronous cross peaks with the

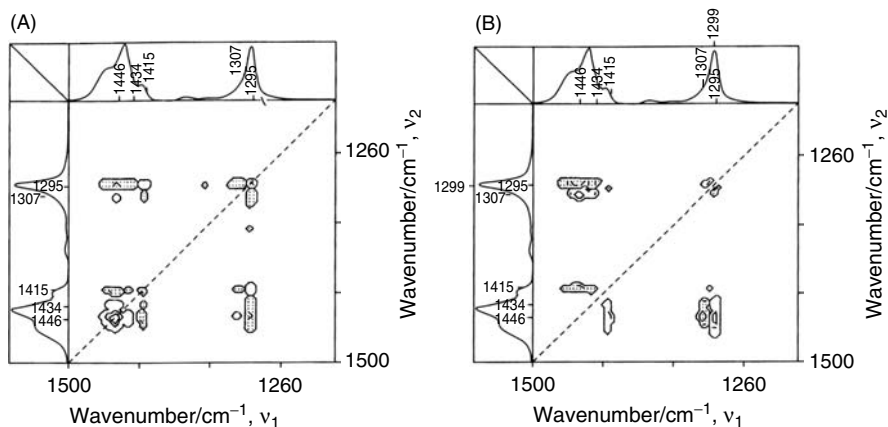


**Figure 10.21** (A) Synchronous 2D Raman correlation spectrum in the 1500–1200  $\text{cm}^{-1}$  region for EVA copolymers with VA from 6 to 8 wt%. (B) The corresponding asynchronous correlation spectrum. (Reproduced with permission from Ref. No. 24. Copyright (1999) American Chemical Society.)

amorphous band at 1307  $\text{cm}^{-1}$  (Figure 10.21(B)). Therefore, it is very likely that these bands arise from an anisotropic phase. This phase is believed to exist as transition layer between the lamella and interlamellar amorphous layers.<sup>41</sup> However, this anisotropic layer is not quite like that originally proposed by Strobl and Hagedorn,<sup>41</sup> which does not have any lateral order. Ren *et al.*<sup>24</sup> concluded that the acetate side groups act as spacers between adjacent chains and reduce the interaction force between their hydrogen atoms, and hence the magnitude of correlation splittings.<sup>42</sup> The bands at 1446 and 1434  $\text{cm}^{-1}$  are thus assigned to the correlation splittings of  $\text{CH}_2$  bending vibrations in the anisotropic phase. The splitting is only 8  $\text{cm}^{-1}$ , considerably smaller than the 23  $\text{cm}^{-1}$  splitting between 1438 and 1415  $\text{cm}^{-1}$  in the crystalline phase.

The order of intensity changes between two bands is listed in Table 10.3.<sup>24</sup> From the first and second rows of Table 10.3, it is concluded that the intensity variations of bands at 1446 and 1434  $\text{cm}^{-1}$  take place before (i.e., at a lower VA content) than that of the band at 1307  $\text{cm}^{-1}$ . Thus, for Set 1 the inclusion of a VA comonomer first causes the expansion of the anisotropic phase, then the expansion of the amorphous phase. The physical implication here is that the acetate side groups act as spacers between adjacent chains, thereby converting the tightly packed orthorhombic phase to the loosely packed anisotropic phase. The anisotropic phase then transforms to the amorphous phase.

Figure 10.22 illustrates a synchronous correlation spectrum for Set 1 in the regions 1500–1200  $\text{cm}^{-1}$  and 1200–1000  $\text{cm}^{-1}$ .<sup>24</sup> Bands at 1130 and 1060  $\text{cm}^{-1}$  are due, respectively, to antisymmetric and symmetric C–C stretching vibrations of all-*trans* chains of  $\text{CH}_3(\text{CH}_2)_n\text{CH}_3$  for  $n \geq 6$ .<sup>43</sup> These bands should be highly overlapped, since they come from both the anisotropic phase and the

**Table 10.3** Synchronous and asynchronous correlation intensities and the order of intensity change between two bands<sup>a</sup> (from Ref. 24)

| Number | $\Phi$                  | $\Psi$                  | Order                                              |
|--------|-------------------------|-------------------------|----------------------------------------------------|
| 1      | $\Phi (1446, 1307) > 0$ | $\Psi (1446, 1307) > 0$ | 1446 cm <sup>-1</sup> before 1307 cm <sup>-1</sup> |
| 2      | $\Phi (1434, 1307) > 0$ | $\Psi (1434, 1307) > 0$ | 1434 cm <sup>-1</sup> before 1307 cm <sup>-1</sup> |
| 3      | $\Phi (1307, 1064) < 0$ | $\Psi (1307, 1064) > 0$ | 1307 cm <sup>-1</sup> after 1064 cm <sup>-1</sup>  |
| 4      | $\Phi (1461, 1415) > 0$ | $\Psi (1461, 1415) > 0$ | 1461 cm <sup>-1</sup> before 1415 cm <sup>-1</sup> |
| 5      | $\Phi (1446, 1430) > 0$ | $\Psi (1446, 1430) < 0$ | 1446 cm <sup>-1</sup> after 1430 cm <sup>-1</sup>  |
| 6      | $\Phi (1446, 1079) > 0$ | $\Psi (1446, 1079) < 0$ | 1446 cm <sup>-1</sup> after 1079 cm <sup>-1</sup>  |
| 7      | $\Phi (1430, 1126) < 0$ | $\Psi (1430, 1126) < 0$ | 1430 cm <sup>-1</sup> before 1126 cm <sup>-1</sup> |
| 8      | $\Phi (1380, 1126) < 0$ | $\Psi (1380, 1126) < 0$ | 1380 cm <sup>-1</sup> before 1126 cm <sup>-1</sup> |
| 9      | $\Phi (1438, 1130) > 0$ | $\Psi (1438, 1130) > 0$ | 1438 cm <sup>-1</sup> before 1130 cm <sup>-1</sup> |
| 10     | $\Phi (1438, 1060) > 0$ | $\Psi (1438, 1060) > 0$ | 1438 cm <sup>-1</sup> before 1060 cm <sup>-1</sup> |
| 11     | $\Phi (1419, 1130) > 0$ | $\Psi (1419, 1130) > 0$ | 1419 cm <sup>-1</sup> before 1130 cm <sup>-1</sup> |
| 12     | $\Phi (1419, 1060) > 0$ | $\Psi (1419, 1060) > 0$ | 1419 cm <sup>-1</sup> before 1060 cm <sup>-1</sup> |
| 13     | $\Phi (1419, 1295) > 0$ | $\Psi (1419, 1295) > 0$ | 1419 cm <sup>-1</sup> before 1295 cm <sup>-1</sup> |
| 14     | $\Phi (1461, 1295) > 0$ | $\Psi (1461, 1295) < 0$ | 1461 cm <sup>-1</sup> after 1295 cm <sup>-1</sup>  |
| 15     | $\Phi (1461, 1130) > 0$ | $\Psi (1461, 1130) < 0$ | 1461 cm <sup>-1</sup> after 1130 cm <sup>-1</sup>  |
| 16     | $\Phi (1461, 1060) > 0$ | $\Psi (1461, 1060) < 0$ | 1461 cm <sup>-1</sup> after 1060 cm <sup>-1</sup>  |
| 17     | $\Phi (1419, 1130) > 0$ | $\Psi (1419, 1130) > 0$ | 1419 cm <sup>-1</sup> before 1130 cm <sup>-1</sup> |
| 18     | $\Phi (1419, 1060) > 0$ | $\Psi (1419, 1060) > 0$ | 1419 cm <sup>-1</sup> before 1060 cm <sup>-1</sup> |

<sup>a</sup>  $v_1$  after (before)  $v_2$  means the intensity change of the band at  $u_1$  occurs at higher (lower) VA contents than that at  $u_2$ .

orthorhombic crystalline phase. The all-*trans* bands at 1130 and 1060 cm<sup>-1</sup> have positive synchronous correlation with the crystalline band at 1415 cm<sup>-1</sup>, negative correlation with the amorphous band at 1307 cm<sup>-1</sup>, and negative correlation with the anisotropic bands at 1446 and 1434 cm<sup>-1</sup>. This observation indicates that the amount of all-*trans* chains in EVA copolymers is reduced with an increase in the VA content. It was also revealed from Figure 10.22 that various *gauche* conformations appear with the incorporation of VA comonomers. One should notice here that the structural variations induced by the change in the VA content of only 2 wt% are clearly detected in the 2D Raman correlation maps.<sup>24</sup>

Set 2 contains three EVA copolymers with somewhat higher VA contents of 10, 15 and 20 wt%. Figure 10.23(A) and (B) shows synchronous and asynchronous correlation spectra for Set 2 in the region 1500–1200 cm<sup>-1</sup>.<sup>24</sup> A band at 1380 cm<sup>-1</sup> is assigned to the CH<sub>3</sub> symmetric bending mode of the acetate groups. It has negative correlation with the crystalline band at 1415 cm<sup>-1</sup> and the all-*trans* band at 1295 cm<sup>-1</sup>, indicating that with the inclusion of VA comonomer the crystallinity of EVA decreases, and the population of all-*trans* conformers reduces. A band at 1461 cm<sup>-1</sup> has been assigned to the first overtone of CH<sub>2</sub> in-phase rocking mode at 730 cm<sup>-1</sup>, which is characteristic of the -(CH<sub>2</sub>)<sub>*n*</sub> structure for *n* ≥ 3 in the crystalline phase.<sup>44</sup> This band shares positive cross peaks with the crystalline band at 1415 cm<sup>-1</sup>, implying the shortening of CH<sub>2</sub> sequences. Moreover, the band at 1461 cm<sup>-1</sup> has an asynchronous relationship



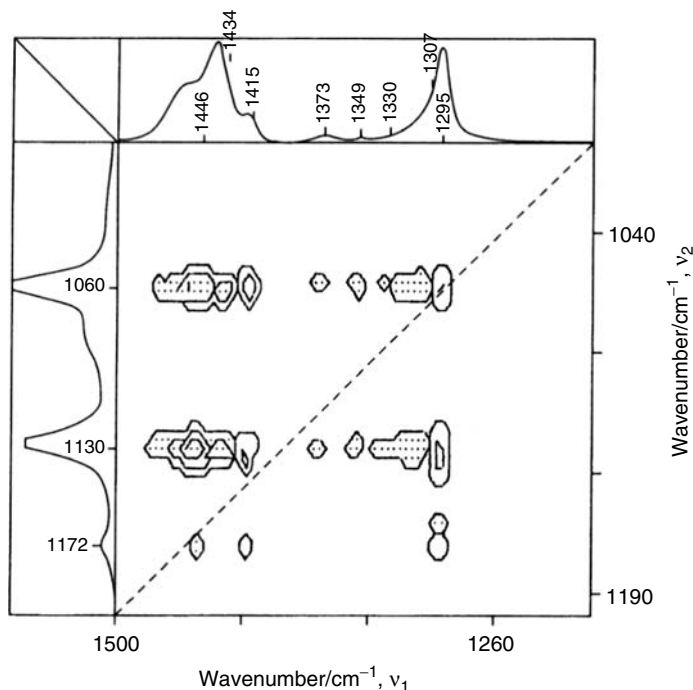


**Figure 10.22** Synchronous 2D Raman correlation spectrum in the region of 1500–1200  $\text{cm}^{-1}$  and 1200–1000  $\text{cm}^{-1}$  for EVA copolymers with VA from 6 to 8 wt%. (Reproduced with permission from Ref. No. 24. Copyright (1999) American Chemical Society.)

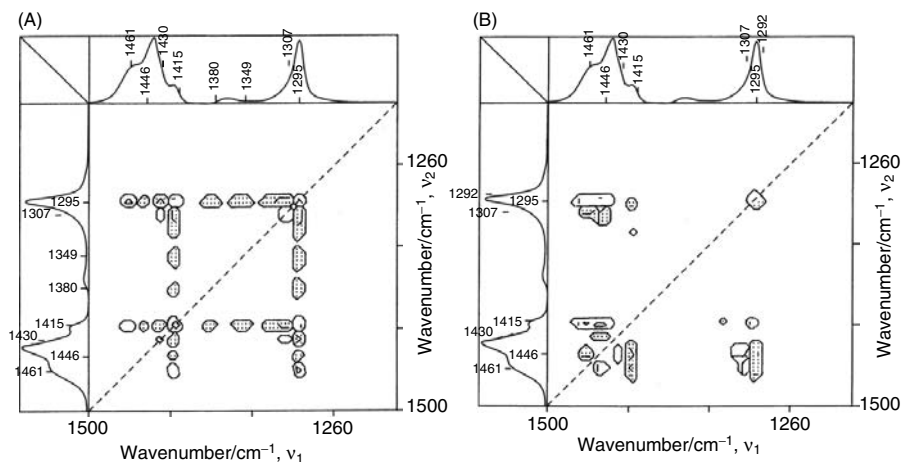


**Figure 10.23** (A) Synchronous 2D Raman correlation spectrum in the 1500–1200  $\text{cm}^{-1}$  region for EVA copolymers with VA from 10 to 20 wt%. (B) The corresponding asynchronous correlation spectrum. (Reproduced with permission from Ref. No. 24. Copyright (1999) American Chemical Society.)

with the band at  $1415\text{ cm}^{-1}$  (Figure 10.23). The fourth row of Table 10.3 shows that the event at  $1461\text{ cm}^{-1}$  occurs before that at  $1415\text{ cm}^{-1}$ , indicating that the shortening of contiguous  $\text{CH}_2$  sequences takes place prior to the shrinking of crystalline lamellae with the increase in the VA content.

Using the same 2D correlation analysis method, Ren *et al.*<sup>24</sup> reached the following conclusions for Set 3 and Set 4. Set 3 contains EVA with middle level VA contents from 25, 26, and 28 wt%. The main event for Set 3 is the shortening of all-*trans*  $\text{CH}_2$  sequences, which reduces the correlation splittings of the crystalline phase. The crystalline packing in the unit cell deforms first, and then the  $\text{CH}_2$  segment in the unit cell loses its all-*trans* conformation. Set 4 contains EVA with high level VA contents from 28, 32, and 42 wt%. The main event for Set 4 is the further shortening of the all-*trans*  $\text{CH}_2$  sequences, resulting in the deformation of the orthorhombic unit cell. The anisotropic phase loses its lateral order for Set 4.

## 10.5 POLARIZATION ANGLE-DEPENDENT 2D IR STUDY OF FERROELECTRIC LIQUID CRYSTALS

The next example deals with an interesting 2D IR correlation study of the spatial or orientational distribution of constituents in a liquid crystal sample. Ferroelectric liquid crystals (FLCs) have received keen interest as the most recent member of the family of ferroelectric materials because of their potential applications in high definition, flat panel displays, and optical processing devices.<sup>45,46</sup> For FLCs in the  $S_m$ - $C^*$  phase, a very thin (only a few  $\mu\text{m}$ ) cell is used to take advantage of the boundary condition imposed by the orientation layers, which is strong enough to suppress the formation of a helix. This effect is called the surface-stabilized ferroelectric liquid crystal (SSFLC) effects. By reversing the polarity of the applied electric field, molecules can be switched back and forth from one state to the other state at a fast speed in the microsecond range.

Despite the great promise of the properties of FLCs, the detailed mechanism of electric field-induced reorientation of different segments of FLCs is not yet fully understood due to the complexity of their structure. During the last decade, time-resolved IR spectroscopy and polarized IR spectroscopy have been used to investigate the dynamics of electric field-induced switching of FLCs. Polarized IR spectroscopy enables one to investigate the molecular structure and alignment of FLCs because it gives useful information about the polarization angle dependence for each functional group. However, the analysis of the polarization angle dependences of IR band intensities is not always straightforward, partly because some bands may overlap with each other, and partly because some bands may show very similar polarization angle dependences. Nagasaki *et al.*<sup>29</sup> applied 2D correlation spectroscopy to analyze the polarization angle dependences of IR band intensities. In this study, the polarization angle is used as the external perturbation variable.

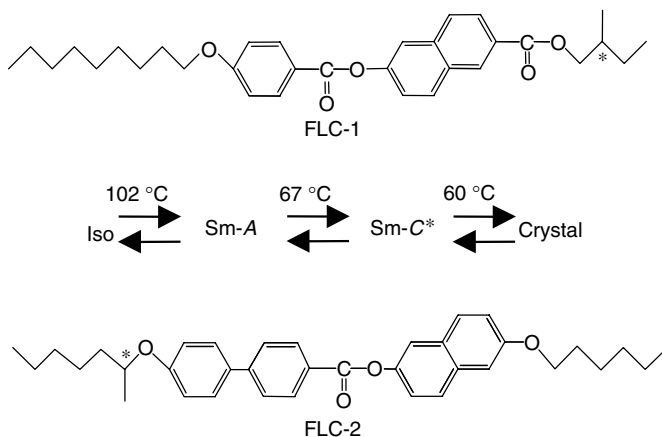


**Figure 10.24** Structure and phase transition sequence of FLC-1. (Reproduced with permission from Ref. No. 29. Copyright (2000) American Chemical Society.)

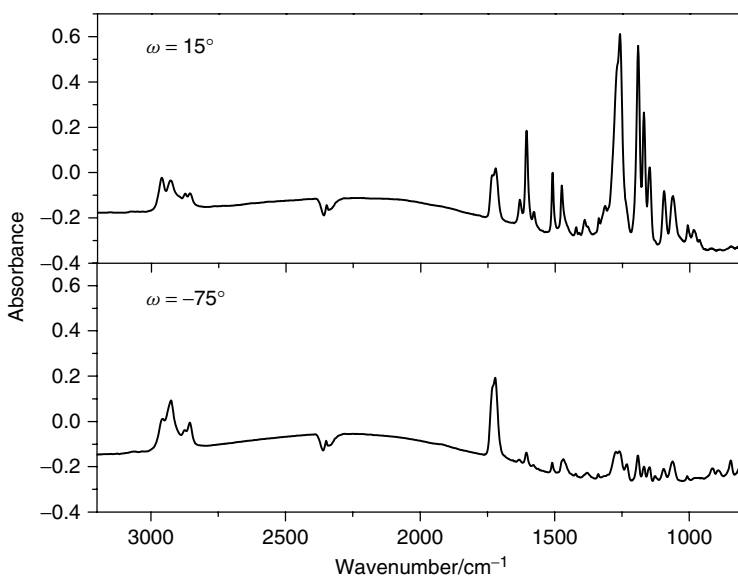


**Figure 10.25** Polarization IR spectra of FLC-1 at 60 °C in the parallel ( $\omega = 15^{\circ}$ ) and perpendicular ( $\omega = -75^{\circ}$ ) polarization geometries. (Reproduced with permission from Ref. No. 29. Copyright (2000) American Chemical Society.)

Figure 10.24 shows the structure of an FLC with a naphthalene ring (FLC-1) that was used for the 2D IR correlation spectroscopy study.<sup>29</sup> The phase transition temperatures of FLC-1 are also shown in Figure 10.24. FLC-1 has a bookshelf layer structure for a particular alignment of the film in the Sm-C\* phase. Figure 10.25 shows polarized IR spectra of FLC-1 at 60 °C in the parallel

( $\omega = 15^\circ$ ) and perpendicular ( $\omega = -75^\circ$ ) polarization geometries.<sup>29</sup> The direction of  $\omega = 15^\circ$  is that of molecular long axis under a dc voltage of +40 V, and the direction of  $\omega = -75^\circ$  is perpendicular to that of  $\omega = 15^\circ$ . From these spectra, the dichroic ratio,  $D$ , defined as the ratio of the absorbances for the parallel and perpendicular polarizations of light, for the absorption bands was calculated. The assignments and dichroic ratios for the isolated and relevant bands are listed in Table 10.4.<sup>29</sup>

Figure 10.26 shows the plot of normalized *absorptivity* (absorptivity =  $1 - \text{transmittance}$ ) versus polarization angle for the bands at 2928, 1736, 1721, 1606, 1192, 1170, and 1150  $\text{cm}^{-1}$ .<sup>29</sup> The two bands at 1736 and 1721  $\text{cm}^{-1}$  are assigned, respectively, to the C=O stretching modes of the carbonyl groups in the core and chiral parts. It is noted that the two C=O stretching bands, particularly the band at 1736  $\text{cm}^{-1}$ , show a polarization angle dependence clearly different from the other bands. The behavior of the two C=O stretching bands is very important because the carbonyl groups have large polarization, and thus are related to the ferroelectricity. The phases in the angle-dependent intensity changes of four bands at 1606, 1192, 1170, and 1150  $\text{cm}^{-1}$  are almost the same, and the change in the band at 2926  $\text{cm}^{-1}$  is antiphase to them. The fact that the phases in the changes of the two C=O bands are different from those of other bands indicates the hindered rotations around the molecular long axis of the carbonyl groups. Since each carbonyl group is attached to the benzene and naphthalene rings separately, there may be significant effects of the hindered rotations of the two carbonyl

**Table 10.4** Dichroic ratio ( $D$ ) and vibrational band assignments for the relevant peaks in the infrared spectra of FLC-1 in the Sm-C\* phase (from Ref. 29)

| Wavenumber ( $\text{cm}^{-1}$ ) <sup>a</sup> | $D$ ( $A_{\parallel}/A_{\perp}$ ) | Assignment <sup>b</sup>      |
|----------------------------------------------|-----------------------------------|------------------------------|
| 2960(m)                                      | 0.8                               | CH <sub>3</sub> asym. st.    |
| 2928(s)                                      | 0.6                               | CH <sub>2</sub> antisym. st. |
| 2874(m)                                      | 0.8                               | CH <sub>3</sub> sym. st.     |
| 2856(m)                                      | 0.5                               | CH <sub>2</sub> sym. st.     |
| 1736(s, sh)                                  | 0.8                               | C=O st. (core)               |
| 1721(s)                                      | 0.6                               | C=O st. (chiral)             |
| 1606(m)                                      | 9.5                               | Ring C=C st.                 |
| 1510(m)                                      | 6.8                               | Ring C=C st.                 |
| 1475(m)                                      | 3.2                               |                              |
| 1274(m)                                      | 6.3                               | C–O–C antisym. st.           |
| 1257(m)                                      | 8.0                               | C–O–C antisym. st.           |
| 1192(m)                                      | 9.0                               |                              |
| 1170(m)                                      | 9.8                               | Ring CH def.                 |
| 1150(m)                                      | 6.5                               |                              |
| 1096(m)                                      | 5.8                               | C–O–C sym. st.               |
| 1065(m)                                      | 2.7                               | C–O–C sym. st.               |

<sup>a</sup>w, weak; m, medium; s, strong; sh, shoulder.

<sup>b</sup>sym, symmetric; asym, asymmetric; antisym, antisymmetric; st, stretching; def, deformation.

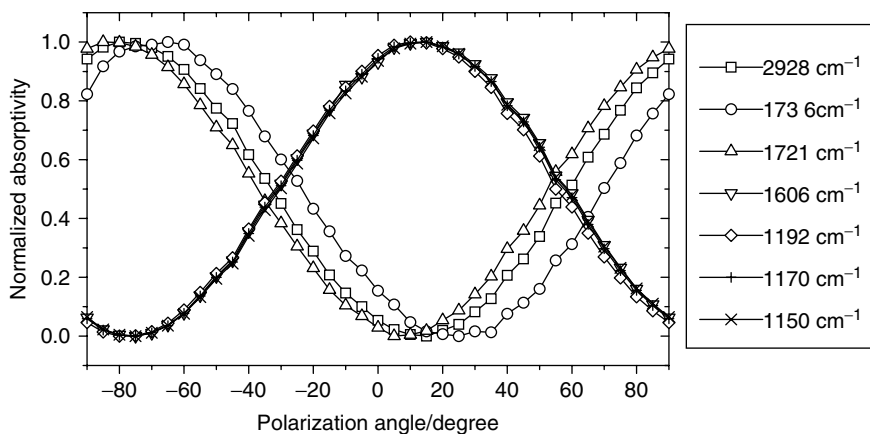


**Figure 10.26** Normalized absorptivity versus polarization angle for some representative bands in the polarized IR spectra of FLC-1 in the Sm-C\* monodomain at 60 °C under a dc electric field of +40 V. (Reproduced with permission from Ref. No. 29. Copyright (2000) American Chemical Society.)

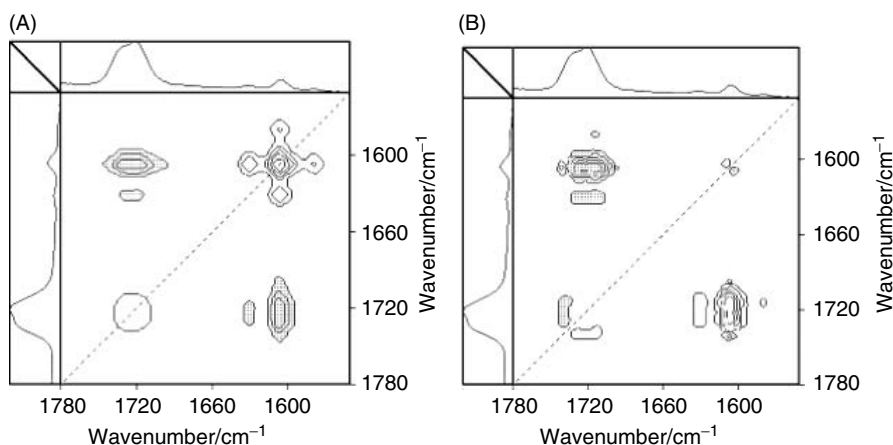


**Figure 10.27** (A) Synchronous and (B) asynchronous 2D IR correlation spectra in the 1780–1550  $\text{cm}^{-1}$  region constructed from the polarization angle-dependent (between  $-90^\circ$  and  $90^\circ$ ) polarized spectral variations of FLC-1 in the Sm-C\* monodomain at 60 °C under a dc electric field of +40 V. (Reproduced with permission from Ref. No. 29. Copyright (2000) American Chemical Society.)

groups on the behavior of the two aromatic rings. The 2D correlation analysis should provide a clear answer for this.

Figure 10.27(A) and (B) shows the synchronous and asynchronous 2D correlation spectrum in the region 1780–1550  $\text{cm}^{-1}$  generated from the polarization angle-dependent IR spectra of FLC-1.<sup>29</sup> An autopeak is developed at 1606  $\text{cm}^{-1}$  in the synchronous map, showing that the intensity of the band due to the

ring stretching modes changes significantly with the polarization angle. Two negative cross peaks at (1736, 1606) and (1721, 1606  $\text{cm}^{-1}$ ) indicate that the two C=O stretching bands and the ring stretching band show the polarization angle-dependent intensity changes in the reverse directions.

The corresponding asynchronous map (Figure 10.27(B)) shows three cross peaks at (1608, 1736), (1608, 1728), and (1608, 1715  $\text{cm}^{-1}$ ). This result is clear evidence for the existence of three C=O stretching bands. The splitting of the band near 1721  $\text{cm}^{-1}$  due to the chiral carbonyl group into two bands at 1728 and 1715  $\text{cm}^{-1}$  may be ascribed to the rotational isomerism around the O–C bonds; there may be two conformers around the O–C bonds. The signs of the cross peaks indicate that the phases of the intensity changes in the two C=O stretching bands at 1728 and 1715  $\text{cm}^{-1}$  are delayed compared with that of the C=O stretching band at 1736  $\text{cm}^{-1}$ . Moreover, the signs of cross peaks between the C=O stretching bands and the ring stretching band suggest that the phases of the band intensity changes are delayed in the order of bands at 1736, 1606, 1728, and 1715  $\text{cm}^{-1}$ . This conclusion is consistent with the observation in Figure 10.26. Another notable observation in the asynchronous spectrum is the appearance of cross peaks near 1600  $\text{cm}^{-1}$ . It seems that the ring stretching bands of the benzene and naphthalene rings are observed separately in the cross peaks. The existence of the cross peaks in the asynchronous spectrum suggests that the directions of the transition moments of the ring stretching modes of two aromatic rings are different, and the rotations of two rings are hindered.

Figure 10.28(A) and (B) shows synchronous and asynchronous maps between the regions 1780–1550  $\text{cm}^{-1}$  and 3000–2800  $\text{cm}^{-1}$  calculated from the

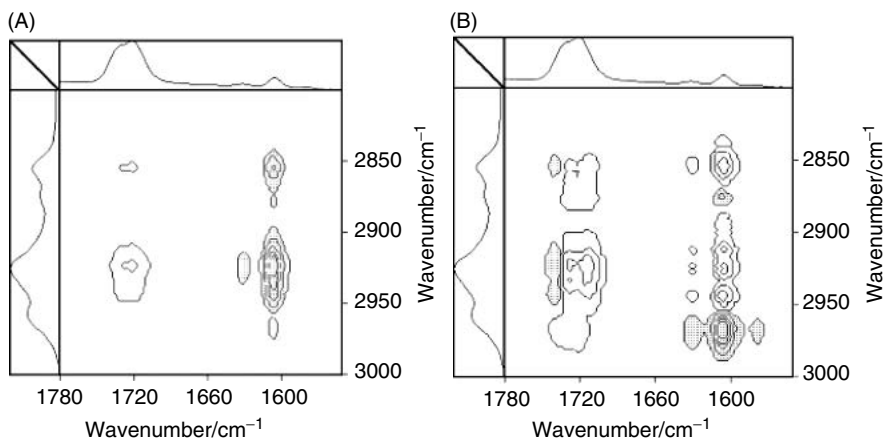


**Figure 10.28** (A) Synchronous and (B) asynchronous 2D IR correlation spectra between the 1780–1550  $\text{cm}^{-1}$  and 3000–2800  $\text{cm}^{-1}$  regions generated from the polarization angle-dependent (between  $-90^\circ$  and  $90^\circ$ ) polarized spectral variations of FLC-1 in the Sm-C\* monodomain at 60  $^\circ\text{C}$  under a dc electric field of +40 V. (Reproduced with permission from Ref. No. 29. Copyright (2000) American Chemical Society.)

polarization angle-dependent spectral variations (between  $-90^\circ$  and  $+90^\circ$ ) of FLC-1 in the Sm- $C^*$  monodomain at  $60^\circ\text{C}$  under a dc electric field of  $+40\text{ V}$ .<sup>29</sup> Of note in the asynchronous spectrum is that the band at  $2945\text{ cm}^{-1}$  has a positive cross peak with the band at  $1606\text{ cm}^{-1}$ , while the band at  $2965\text{ cm}^{-1}$  shares a negative cross peak with the same band. The original spectra show only one band due to the asymmetric  $\text{CH}_3$  stretching mode at  $2960\text{ cm}^{-1}$ . It seems that both bands at  $2965$  and  $2945\text{ cm}^{-1}$  are due to the asymmetric  $\text{CH}_3$  stretching mode of different  $\text{CH}_3$  groups of FLC-1 whose intensities show different polarization angle dependence. The two  $\text{CH}_3$  bands at  $2965$  and  $2945\text{ cm}^{-1}$  show different signs in the cross peaks at  $(2965, 1606)$  and  $(2945, 1606\text{ cm}^{-1})$ , suggesting that the two bands have completely different polarization angle dependences. Nagasaki *et al.*<sup>29</sup> assigned the band at  $2945\text{ cm}^{-1}$  to the chiral  $\text{CH}_3$  groups, because it is likely that only this band, whose transition moment is in the direction of the short molecular axis, appears separately. This study has demonstrated the usefulness of 2D correlation spectroscopy in the detection of slight differences in polarization angle-dependent intensity variations.

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# 11 Two-dimensional Correlation Spectroscopy and Chemical Reactions

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Chemical recreations are also fertile ground for the application of 2D correlation spectroscopy.<sup>1–8</sup> The first reaction-based 2D IR spectra were reported by Nakano *et al.*<sup>1</sup> in 1993, who applied the generalized 2D correlation approach to the spectral changes induced by a photochemical reaction. Other research groups have also studied many different chemical reactions using 2D IR,<sup>2–4,7</sup> 2D NIR,<sup>5</sup> and 2D IR-NIR heterospectral correlation.<sup>5</sup> Types of chemical reactions studied by 2D spectroscopy include H/D exchange reactions to probe the secondary structures of proteins,<sup>2,3</sup> real-time monitoring of the initial oligomerization of bis(hydroxyethyl terephthalate) (BHET),<sup>4–6</sup> and complete and catalyst-modified reaction of the hydrogenation of nitrobenzene.<sup>7</sup> An interesting 2D IR study was also reported on dynamic chemical modulation, where reactant concentrations are periodically varied.<sup>8</sup>

## 11.1 2D ATR/IR STUDY OF BIS(HYDROXYETHYL TEREPHTHALATE) OLIGOMERIZATION

The initial oligomerization of bis(hydroxyethyl terephthalate), BHET, was monitored *in situ* at 220, 250 and 270 °C by attenuated total reflection infrared (ATR/IR) spectroscopy.<sup>4</sup> The sets of IR spectra, which include information about the progress of the reaction, were analyzed by means of 2D correlation spectroscopy. Figure 11.1 depicts the initial oligomerization of BHET, which yields polyethylene terephthalate (PET) as a final product with one molecule of ethylene glycol (EG) released per each condensation step. Figure 11.2 shows ATR/IR spectra collected *in situ* during the oligomerization reaction at 250 °C. The assignments are given for most bands in Figure 11.2. However, the assignments for free EG were not straightforward. Figures 11.3 and 11.4 show, respectively, the 2D correlation spectra in the 1000–800 cm<sup>-1</sup> region calculated from the time-dependent ATR/IR spectra measured at 270 and 220 °C. The synchronous spectrum at 270 °C (Figure 11.3(A)) shows autopeaks at 903 and 870 cm<sup>-1</sup>. The two peaks share a negative cross peak. The peak at 903 cm<sup>-1</sup> is assigned to BHET,

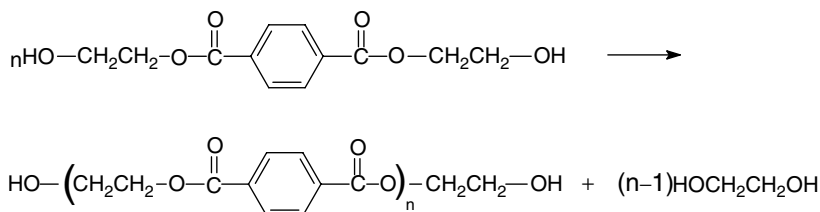


**Figure 11.1** Initial oligomerization of bis(hydroxyethyl terephthalate)

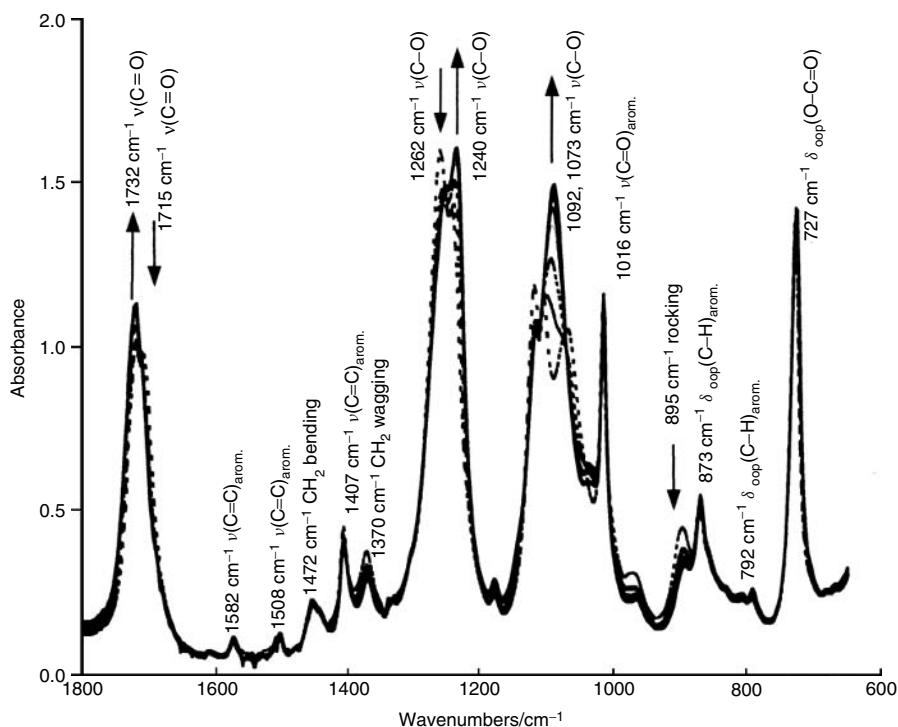


**Figure 11.2** ATR/IR spectra measured *in situ* during the oligomerization reaction shown in Figure 11.1. Arrows show the directions of intensity changes during the course of the reaction. (Reproduced with permission from Ref. No. 4. Copyright (2002) American Chemical Society.)

and its intensity decreases upon oligomerization. Another peak at  $870\text{ cm}^{-1}$ , which is of benzene ring origin, shows a slight increase with time. In the asynchronous spectrum (Figure 11.3(B)), the two peaks also share a cross peak. Since EG is being released from the solution as the reaction proceeds, the concentrations of the species remaining in the solution should increase. This should be the case for the benzene ring, and the intensity change of the corresponding peak may be out of phase with the peak at  $903\text{ cm}^{-1}$ .

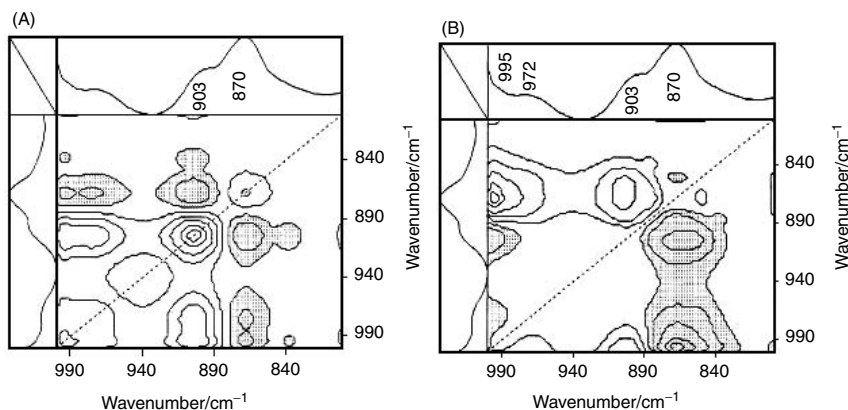


**Figure 11.3** (A) Synchronous and (B) asynchronous 2D IR correlation maps in the 1000–800  $\text{cm}^{-1}$  region generated from the time-dependent spectral variations of the oligomerization reaction at 270 °C. (Reproduced with permission from Ref. No. 4. Copyright (2002) American Chemical Society.)

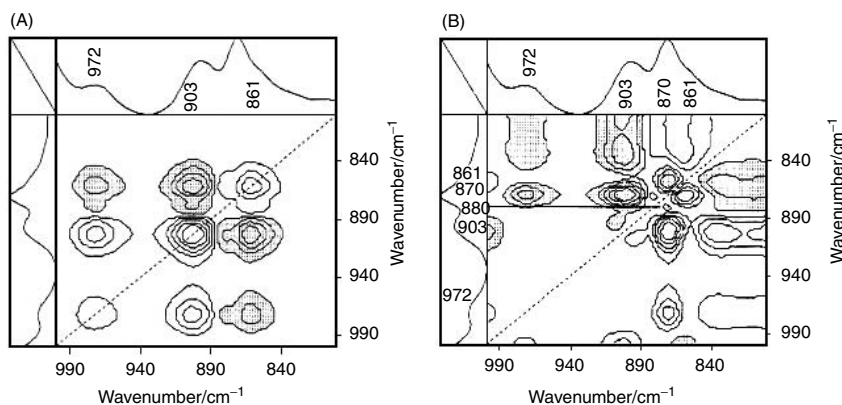


**Figure 11.4** (A) Synchronous and (B) asynchronous 2D IR correlation maps in the 1000–800  $\text{cm}^{-1}$  region of the oligomerization reaction at 220 °C. (Reproduced with permission from Ref. No. 4. Copyright (2002) American Chemical Society.)

The corresponding synchronous spectrum at 220 °C (Figure 11.4(A)) shows autopeaks at 903 and 861  $\text{cm}^{-1}$ . The autopeak at 870  $\text{cm}^{-1}$  has vanished, but in the asynchronous spectrum (Figure 11.4(B)), the peak at 870  $\text{cm}^{-1}$  shares a cross peak with the peak at 861  $\text{cm}^{-1}$ , and also weakly with the 880  $\text{cm}^{-1}$  peak. The two peaks at 861 and 880  $\text{cm}^{-1}$ , which cannot be clearly seen in the 2D spectra at 270 °C, are likely to arise from EG. It was known from a previous study<sup>9</sup> that there is no marked concentration change of EG for the reaction at 270 °C, but a large increase in the EG concentration is seen for the reaction at 220 °C. Thus, EG-relevant peaks may appear in the 2D correlation spectra at 220 °C. To confirm the band assignments of EG, an IR spectrum of neat EG was measured

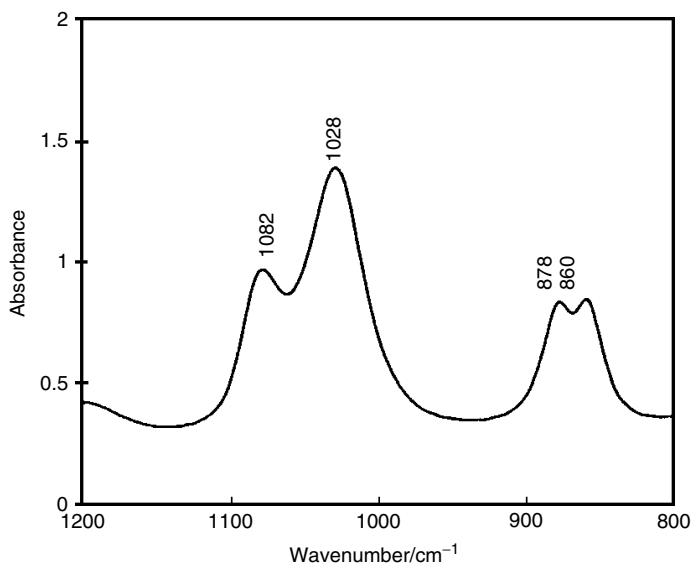


**Figure 11.5** IR spectrum of EG at 200 °C in the 1200–800  $\text{cm}^{-1}$  region (Reproduced with permission from Ref. No. 4. Copyright (2002) American Chemical Society.)

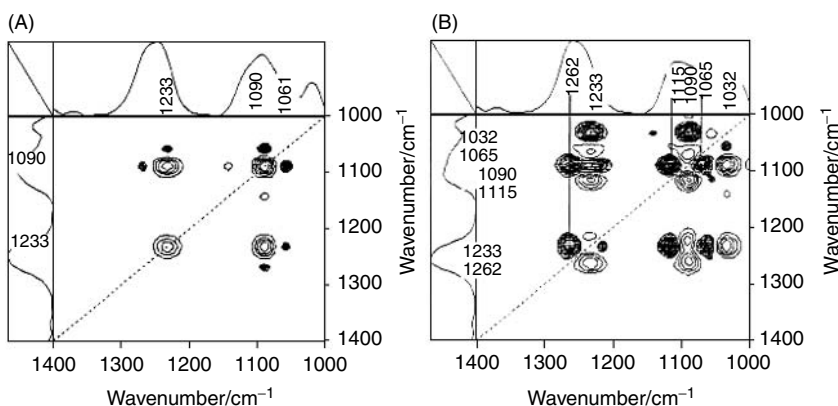


**Figure 11.6** (A) Synchronous and (B) asynchronous 2D IR correlation maps in the 1400–1000  $\text{cm}^{-1}$  region of the oligomerization reaction at 270 °C. (Reproduced with permission from Ref. No. 4. Copyright (2002) American Chemical Society.)

at 200 °C and is shown in Figure 11.5. Note that there are two peaks at 878 and 860  $\text{cm}^{-1}$  assignable to the  $\text{CH}_2$  twisting modes of EG. This observation confirms that the peaks extracted by the 2D correlation analysis come from the free EG being produced during the oligomerization.

Figures 11.6 and 11.7 illustrate synchronous and asynchronous 2D IR correlation maps in the 1400–1000  $\text{cm}^{-1}$  region of the oligomerization reaction at 220

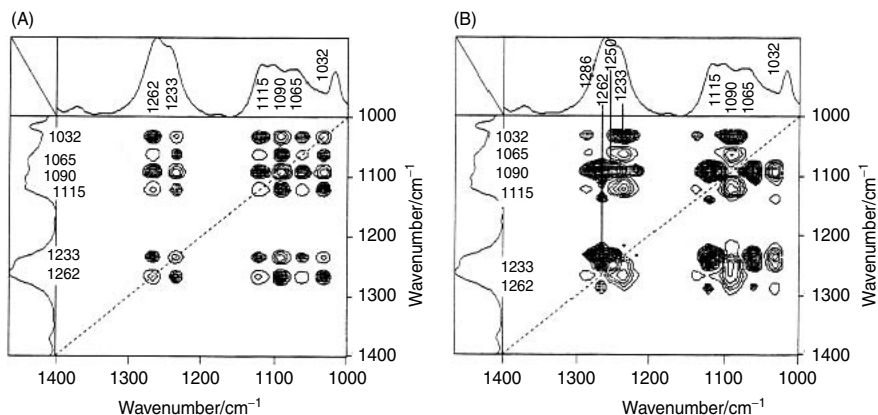


**Figure 11.7** (A) Synchronous and (B) asynchronous 2D IR correlation maps in the 1400–1000  $\text{cm}^{-1}$  region of the oligomerization reaction at 220  $^{\circ}\text{C}$ . (Reproduced with permission from Ref. No. 4. Copyright (2002) American Chemical Society.)

and 270  $^{\circ}\text{C}$ . Compared with the synchronous map at 220  $^{\circ}\text{C}$  (Figure 11.6(A)), the number of autopeaks increases at 270  $^{\circ}\text{C}$  (Figure 11.7(A)), i.e. new peaks emerge at 1262, 1115, and 1065  $\text{cm}^{-1}$ . These new peaks have negative correlation with the first two peaks, and thus are attributed to the bands of BHET. Another peak at 1032  $\text{cm}^{-1}$  appears, and has positive cross peaks with peaks at 1233 and 1090  $\text{cm}^{-1}$ . Amari and Ozaki attributed the band at 1032  $\text{cm}^{-1}$  to the C–O stretching mode of free EG based on the spectrum in Figure 11.5. Figure 11.8 illustrates the slice spectra extracted from the synchronous and asynchronous 2D correlation spectra at 1032  $\text{cm}^{-1}$  shown in Figure 11.7. It can be seen from the synchronous slice spectrum that positive cross peaks develop at (1032, 880) and (1032, 861  $\text{cm}^{-1}$ ). Note that this synchronous slice spectrum is similar to the spectrum of EG (Figure 11.5), and thus it was concluded that the 1032  $\text{cm}^{-1}$  peak is due to EG. The asynchronous slice spectrum shows the development of a cross peak at (1032, 870  $\text{cm}^{-1}$ ). The 870  $\text{cm}^{-1}$  band is assigned to the benzene ring vibration mode.

The asynchronous correlation spectra at 270 and 220  $^{\circ}\text{C}$  are very similar to each other. The 1032  $\text{cm}^{-1}$  band has cross peaks with those at 1233 and 1090  $\text{cm}^{-1}$ , which are assigned to the oligomer C–O stretching modes, but does not show cross peaks with the BHET bands (1262, 1115, and 1065  $\text{cm}^{-1}$ ). This means that the intensity increase of free EG band at 1032  $\text{cm}^{-1}$  and the decrease of BHET bands proceed in phase. Therefore, it is very likely that the intensity decrease of the BHET bands is at least partly due to the relative concentration decrease of BHET upon the formation of free EG. This is probably the reason why the bands from BHET and oligomers have cross peaks, or in other words, change out of phase with each other.

Thus, IR spectra obtained for the *in situ* monitoring of the initial oligomerization of BHET were investigated by using 2D correlation spectroscopy. Careful

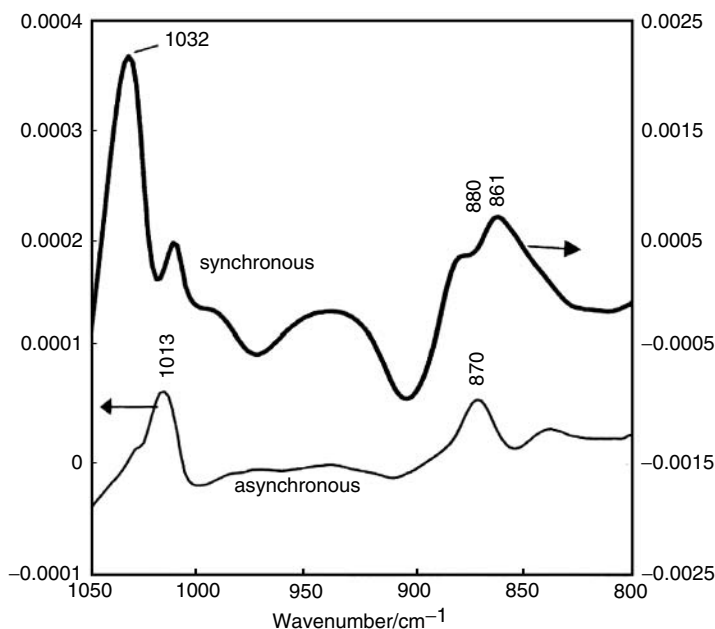


**Figure 11.8** Slice spectra of the synchronous and asynchronous 2D IR correlation spectra at  $1032\text{ cm}^{-1}$  of the oligomerization reaction at  $220^\circ\text{C}$ . (Reproduced with permission from Ref. No. 4. Copyright (2002) American Chemical Society.)

inspection of the 2D IR spectra revealed that the small intensity changes in the  $1000\text{--}800\text{ cm}^{-1}$  region are due to evolving free EG. Amari and Ozaki investigated the same reaction by using 2D NIR and 2D NIR–IR heterocorrelation spectroscopy. The 2D NIR–IR study is outlined in Chapter 14. Šašić *et al.* analyzed the same IR data by means of sample–sample and variable–variable 2D correlation spectroscopy.<sup>6</sup> The sample–sample correlation analysis reveals the correlations among the concentration features of the components, and the variable–variable correlation analysis elucidates the relations among the spectral features.

## 11.2 HYDROGEN–DEUTERIUM EXCHANGE OF HUMAN SERUM ALBUMIN

The hydrogen–deuterium (H/D) exchange of the amide protons is very useful in the investigation of the secondary structure of proteins. Since the amide protons associated with each conformation are not exchanged simultaneously, the contributions from different secondary structures to the amide bands can be separated by using the H/D exchange. Deuterium exchange rates are also a powerful probe for amide structure, providing information about solvent accessibility and hydrogen bond

stability of amide protons. The 2D correlation analysis has provided deeper insight into the mechanism of the H/D exchange in the amide protons of different secondary structures.<sup>2,3</sup> For example, Nabet and Pézolet reported a 2D IR correlation spectroscopy study of the H/D exchange of myoglobin (Mb) earlier.<sup>2</sup> They showed that the use of two different exchange time domains is very efficient to separate the fast kinetics from the slow ones. Wu *et al.* combined 2D correlation spectroscopy and PCA to analyze the kinetics of H/D exchange of human serum albumin (HSA).<sup>3</sup> They measured IR spectra of HSA as a function of time after dissolving it into D<sub>2</sub>O to investigate the secondary structure and kinetics of H/D exchange.<sup>3</sup> 2D IR spectra in the Amide I and Amide II (and II') regions were generated from time-dependent spectral variations in different exposure time domains of HSA in the D<sub>2</sub>O solution. The synchronous and asynchronous spectra in each time domain provided a clear separation of amide bands due to the different secondary structures. The asynchronous spectrum also showed the specific sequence of the secondary structures exposed to the H/D exchange. PCA was used to select the appropriate time domains for the calculation of 2D correlation spectra.

Figure 11.9(A) shows a series of IR spectra measured as a function of time after dissolving HSA in D<sub>2</sub>O (pD 7.0, pD = pD<sub>read</sub> + 0.44).<sup>3</sup> The spectrum shown by a broken line is the spectrum of HSA in H<sub>2</sub>O. The Amide I region shows a broad maximum at 1654 cm<sup>-1</sup> characteristic of a  $\alpha$ -helical structure. It is noted that the

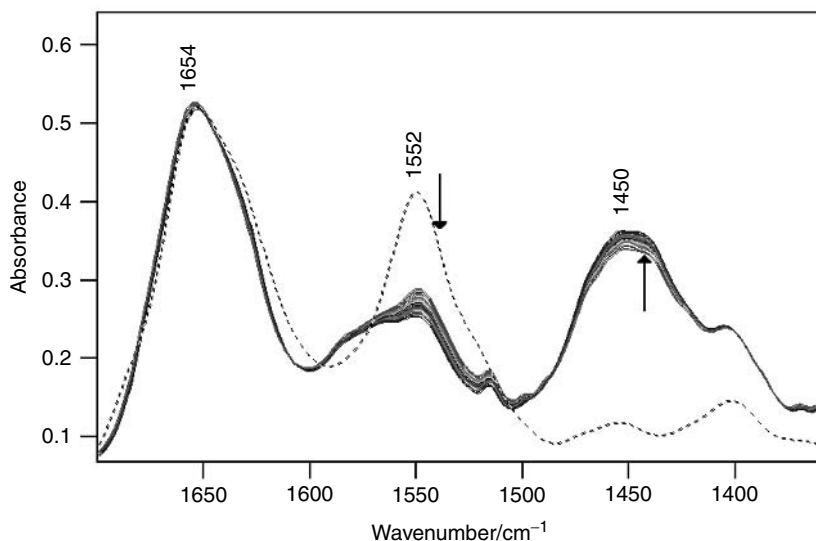


**Figure 11.9** IR spectrum of HSA in an H<sub>2</sub>O solution and those of HSA in a D<sub>2</sub>O solution as a function of exposure time. The spectrum with the dotted line is for the H<sub>2</sub>O solution. The spectra with solid line are for the D<sub>2</sub>O solution and were measured from 1.5 to 260 min after the initiation of the exposure of HSA to D<sub>2</sub>O at 25 °C. In each spectrum the spectrum of H<sub>2</sub>O or D<sub>2</sub>O was subtracted. (Reproduced with permission from Ref. No. 3. Copyright (2001) American Chemical Society.)



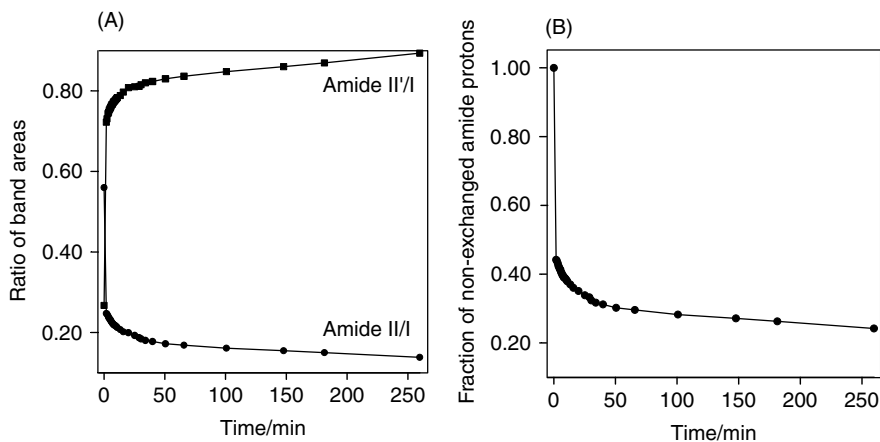


**Figure 11.10** (A) The ratios of the area of Amide II or Amide II' divided by that of Amide I for the spectra shown in Figure 11.9(A) versus time after the initiation of the H/D exchange in HSA in the D<sub>2</sub>O solution. (B) Fraction of the nonexchanged amide protons in HSA in the D<sub>2</sub>O solution (Reproduced with permission from Ref. No. 3. Copyright (2001) American Chemical Society.)

intensity of the Amide II band centered at  $1552\text{ cm}^{-1}$  decreases markedly, while that of a band at  $1450\text{ cm}^{-1}$  (Amide II') increases concomitantly. Wu *et al.* monitored the reaction of  $-\text{CONH}- + \text{D}_2\text{O} \rightarrow -\text{COND}- + \text{HOD}$  by measuring the intensity decrease in the Amide II region. Assuming that during the H/D exchange the intensity of the Amide I band in the  $1700\text{--}1600\text{ cm}^{-1}$  region remains constant, Wu *et al.*<sup>3</sup> took the ratio of the area of the Amide II band (or Amide II') to that of the Amide I band centred at  $1654\text{ cm}^{-1}$  ( $A_{\text{amide II}}/A_{\text{amide I}}$  or  $A_{\text{amide II'}}/A_{\text{amide I}}$ ) to eliminate possible fluctuations in intensity. To estimate the fraction of the nonexchanged amide protons ( $X$ ), the initial value of  $A_{\text{amide II}}/A_{\text{amide I}}$  is expressed by the ratio of the area of the Amide II band to that of the Amide I band of HSA in H<sub>2</sub>O solution. It was found that the initial value of  $A_{\text{amide II}}/A_{\text{amide I}}$  is 0.56.

Figure 11.10(A) plots the ratios  $A_{\text{amide II}}/A_{\text{amide I}}$  and  $A_{\text{amide II'}}/A_{\text{amide I}}$  as functions of time.<sup>3</sup> The decrease in  $A_{\text{amide II}}/A_{\text{amide I}}$  may be described as the sum of the individual exponential decay for each amide proton. It can be seen from Figure 11.10(A) that a large number of amide protons remain unchanged even after a few hours. The fraction,  $X$ , of the nonexchanged amide protons at a time,  $t$ , can be estimated as:

$$X = A_{\text{amide II}}/0.56A_{\text{amide I}} \quad (11.1)$$

Figure 11.10(B) plots the fraction of  $X$  versus time.<sup>3</sup> The plot reveals that roughly 50 % of the amide protons undergo the H/D exchange within 2 min after the exposure of HSA to D<sub>2</sub>O, an additional 12 % over the next 30 min, and a further 10 % over the next a few hours. Roughly 25 % of the amide protons resist being deuterated even 4 h after the exposure of HSA to D<sub>2</sub>O at 25 °C.

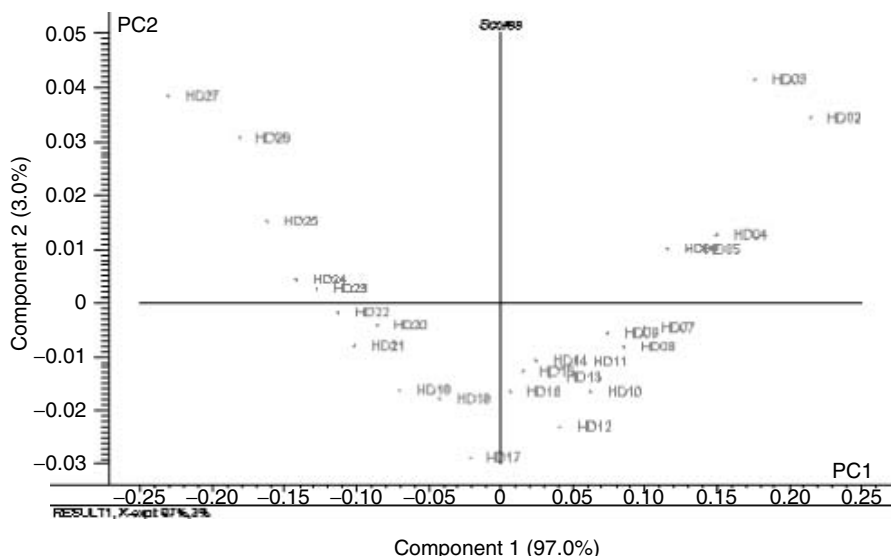


**Figure 11.11** PC1 versus PC2 plot of PCA for the time-dependent IR spectra of HSA shown in Figure 11.9. (Reproduced with permission from Ref. No. 3. Copyright (2001) American Chemical Society.)

Figure 11.11 illustrates a PC1 versus PC2 score plot of the time-dependent IR spectra in the  $1720\text{--}1360\text{ cm}^{-1}$  region. The score plot develops three groups, Groups I, II and III. Groups I, II, and III comprise the spectra measured between 1.8 (HD02) and 5.3 (HD06) min, between 6.4 (HD07) and 13.4 (HD16) min, and between 25.2 (HD19) and 181 (HD27) min after the initiation of the H/D exposure, respectively. The score plot provides a very important basis for the selection of the time domains for the 2D correlation analysis.

Figure 11.12 shows the synchronous and asynchronous correlation spectra of HSA for the rapidly exchanging amide protons, which are constructed from the five spectra in Group I (in Figure 11.11). The power spectrum along the diagonal line of the synchronous spectrum shown in Figure 11.13(I) reveals that the synchronous map is characterized by autopeaks at  $1683$ ,  $1666$ , and  $1625\text{ cm}^{-1}$  in the Amide I region, that at  $1542\text{ cm}^{-1}$  in the Amide II region, and those at  $1452$  and  $1437\text{ cm}^{-1}$  in the Amide II' region. The appearance of these autopeaks indicates that the intensities of these amide bands change significantly with the progress of the H/D exchange. The Amide I bands at  $1683$  and  $1666\text{ cm}^{-1}$  are due to the  $\beta$ -turn structures. Therefore, it seems that the amide groups associated with the  $\beta$ -turn structures are involved in the first H/D exchange during the deuteration process.

A slice spectrum extracted at  $1543\text{ cm}^{-1}$  from the asynchronous spectrum (Figure 11.12(B)) is shown in Figure 11.14(I). The slice spectrum develops four new bands at  $1655$ ,  $1640$ ,  $1613$  and  $1604\text{ cm}^{-1}$  in the Amide I region, assigned, respectively, to  $\alpha$ -helices ( $1655\text{ cm}^{-1}$ ), intramolecular  $\beta$ -strands or random coil

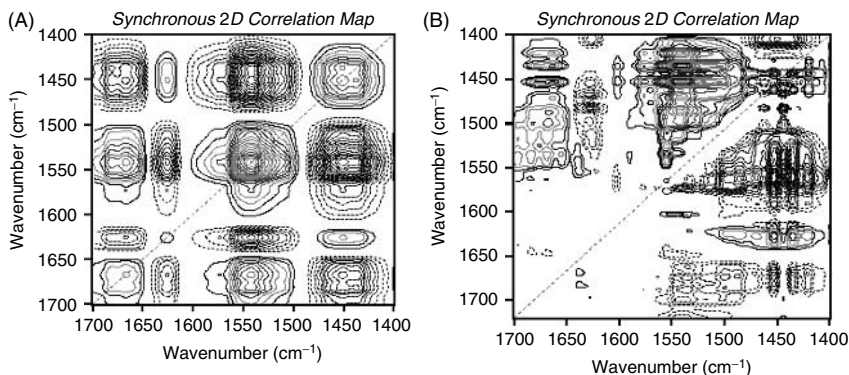


**Figure 11.12** (A) Synchronous and (B) asynchronous 2D IR correlation spectra of HSA calculated from five spectra (Group I), which were measured between 1.83 and 5.30 min after the initiation of the H/D exchange. (Reproduced with permission from Ref. No. 3. Copyright (2001) American Chemical Society.)

( $1640\text{ cm}^{-1}$ ), and side chains ( $1613$  and  $1604\text{ cm}^{-1}$ ). Usually, the band at  $1640\text{ cm}^{-1}$  is attributed to a  $\beta$ -sheet or a random coil structure for proteins.<sup>10–12</sup> For HSA it may be assigned to intramolecular  $\beta$ -strands or random coil.<sup>13,14</sup> Its subtle change due to the H/D exchange cannot be detected in the synchronous 2D contour map (Figure 11.13(I)). In contrast, the asynchronous 2D correlation spectrum demonstrates its strong deconvolution power (Figure 11.14 (I)).

In a 2D asynchronous correlation spectrum, cross peaks between two spectral features are developed only when their intensities vary out of phase with each other. For the H/D exchange process of a protein, the asynchronous map allows one to explore the order in the H/D exchange of secondary structure elements. This may not be achieved by the conventional analysis of intensity changes in IR spectra of proteins. In Figure 11.12(B), asynchronous cross peaks are observed at ( $1640, 1669$ ) and ( $1640, 1685\text{ cm}^{-1}$ ) in the Amide I region. However, no asynchronous cross peak is developed among the bands at  $1685, 1669$ , and  $1625\text{ cm}^{-1}$ , suggesting that the secondary structures giving the peaks at  $1685, 1669$ , and  $1625\text{ cm}^{-1}$  show very similar or almost equal H/D exchange kinetics. In contrast, it seems that the secondary structure that has the band at  $1640\text{ cm}^{-1}$  shows quite different behavior in the H/D exchange from those with the Amide I band at  $1685$  and  $1669\text{ cm}^{-1}$ . The signs of cross peaks at ( $1640, 1669$ ) and ( $1640, 1685\text{ cm}^{-1}$ ) suggest that the H/D exchange occurs in the  $\beta$ -strands ( $1640\text{ cm}^{-1}$ ) earlier than in the  $\beta$ -turn structures ( $1685$  and  $1669\text{ cm}^{-1}$ ), that is

$$\beta\text{-strands } (1640\text{ cm}^{-1}) > \beta\text{-turns } (1685 \text{ and } 1669\text{ cm}^{-1})$$

Figure 11.15 shows the 2D correlation spectra of HSA calculated from nine IR spectra (spectra from HD07 to HD16, Group II in Figure 11.10) measured between 6.4 and 13.4 min after the initiation of the H/D exchange.<sup>3</sup> Figures 11.13(II) and 11.14(II) show, respectively, the power spectrum and the

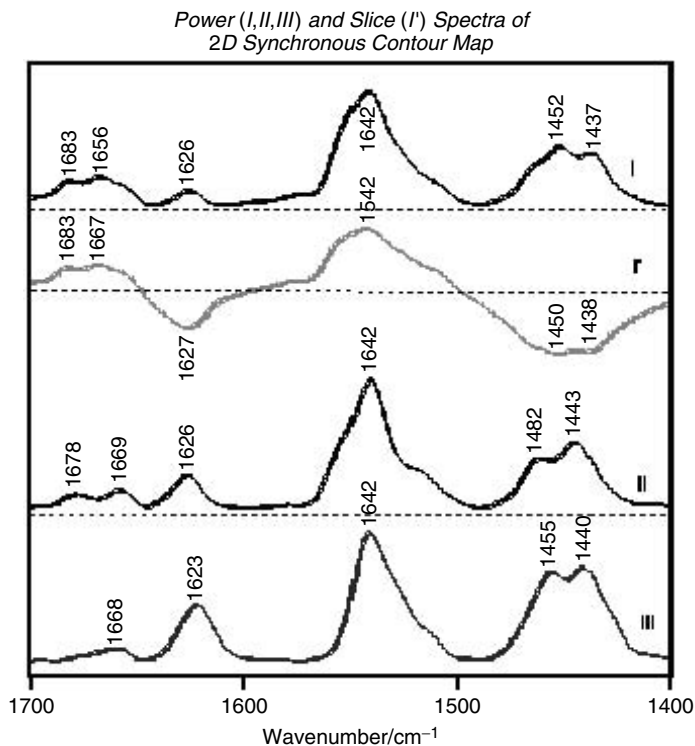


**Figure 11.13** Power spectra extracted from the synchronous 2D contour maps: (I) from Figure 11.12(A), Group I; (II) from Figure 11.15(A), Group II; (III) from Group III. (I') A slice spectrum at  $1667\text{ cm}^{-1}$  extracted from the synchronous 2D contour maps shown in Figure 11.12(A). (Reproduced with permission from Ref. No. 3. Copyright (2001) American Chemical Society.)

slice spectrum extracted from the synchronous and asynchronous spectra shown in Figure 11.15. The synchronous map in Figure 11.15(A) looks similar to that in Figure 11.12(A) generated from the five spectra in Group I. However, close inspection of the two power spectra (Figures 11.13(I) and (II)) calculated from the synchronous maps in Figures 11.12(A) and 11.15(A) shows significant differences between them. The most notable difference is the appearance of an autopeak near  $1658\text{ cm}^{-1}$  in Figure 11.13(II). This band obviously arises from the  $\alpha$ -helix structure. This observation reveals that besides the H/D exchanges in the  $\beta$ -turns ( $1678\text{ cm}^{-1}$ ), the  $\text{D}_2\text{O}$  molecules start penetrating into the accessible parts of  $\alpha$ -helices in the second time domain of the H/D exchange. One more notable change between Figure 11.13(I) and (II) is observed in the Amide II' region; the increasing band at  $1462\text{ cm}^{-1}$  may be assigned to the bending mode of HOD.

The asynchronous contour map in Figure 11.15(B) displays the deconvolution of bands corresponding to different secondary structure elements in more detail.

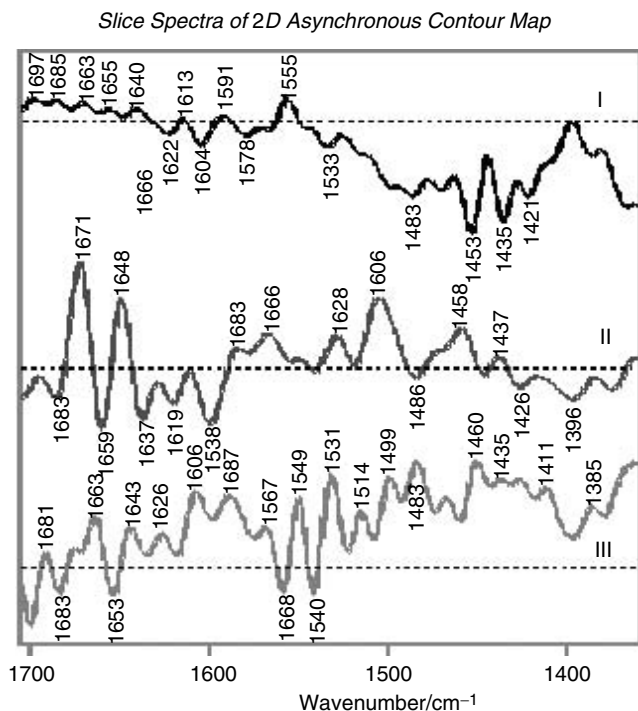


**Figure 11.14** Slice spectra at  $1543\text{ cm}^{-1}$  extracted from the asynchronous 2D contour maps: (I) from Figure 11.12(B), Group I; (II) from Figure 11.15(B), Group II; (III) from Group III. (Reproduced with permission from Ref. No. 3. Copyright (2001) American Chemical Society.)

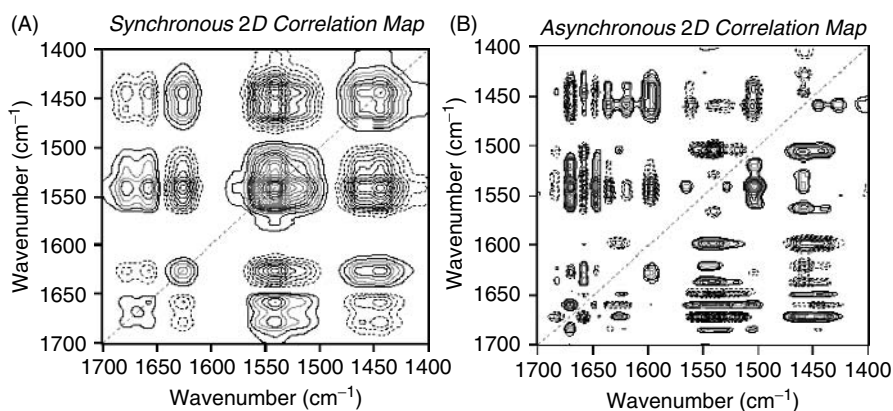


**Figure 11.15** (A) Synchronous and (B) asynchronous 2D IR correlation spectra of HSA calculated from nine spectra (Group II) measured between 6.4 and 13.4 min after the initiation of the H/D exchange. (Reproduced with permission from Ref. No. 3. Copyright (2001) American Chemical Society.)

The slice spectrum at  $1543\text{ cm}^{-1}$  is shown in Figure 11.14(II). A couple of cross peaks are developed at (1671, 1683), (1653, 1683), (1623, 1671), and (1623,  $1653\text{ cm}^{-1}$ ) in the Amide I region of the asynchronous spectrum. The signs of the cross peaks suggest the following sequence of the H/D exchanges in the amide protons.

$\beta$ -turns ( $1683\text{ cm}^{-1}$ ) > aggregated  $\beta$ -strands ( $1623\text{ cm}^{-1}$ ) >  $\beta$ -turns ( $1671\text{ cm}^{-1}$ )  
>  $\alpha$ -helices ( $1653\text{ cm}^{-1}$ )

Therefore, it seems that in the second time domain of the exchange, the amide protons in another kind of  $\beta$ -turns ( $1671\text{ cm}^{-1}$ ) are involved in the H/D exchanges. They are more accessible to the  $\text{D}_2\text{O}$  molecules than the accessible parts of  $\alpha$ -helix structures. Similarly, from the 2D correlation spectra of HSA, constructed from the nine spectra measured between 25.2 and 181 min after the beginning of H/D exchange process (Group III), Wu *et al.* concluded following sequence of spectral changes:

$\beta$ -turns ( $1663\text{ cm}^{-1}$  >  $\beta$ -turns ( $1683\text{ cm}^{-1}$ ), random coil ( $1643, 1540\text{ cm}^{-1}$ )  
>  $\alpha$ -helices ( $1653, 1549\text{ cm}^{-1}$ ) > aggregated  $\beta$ -strand ( $1623\text{ cm}^{-1}$ ).

This study has demonstrated that 2D IR correlation spectroscopy study of the H/D exchanges in protein is a powerful tool to explore the kinetics of the H/D exchanges in HSA and to unravel poorly resolved Amide I, II, and II' regions. Asynchronous spectra are particularly useful to deconvolute Amide I, II, and II' regions and to provide the order in the H/D exchanges. The combination of PCA and 2D correlation analysis turns out to be very efficient in distinguishing the fast kinetics from the slow ones during the H/D exchange process.

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## 12 Protein Research by Two-dimensional Correlation Spectroscopy

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2D correlation spectroscopy is a popular tool in the analysis of IR spectra of proteins.<sup>1–22</sup> For example, it enables the highly overlapped Amide I, II, and III bands of proteins to be resolved into component bands ascribed to various secondary structures. It is also possible to investigate the correlations among Amide I, II, and III bands. For example, Murayama *et al.* demonstrated that 2D correlation spectroscopy is also useful for exploring intensity variations of COOH bands of glutamic and aspartic acid residues of proteins.<sup>18</sup> Wang *et al.* applied 2D NIR correlation spectroscopy to monitor protein–water interaction.<sup>14</sup> One of the earlier examples of the application of 2D IR correlation spectroscopy to protein research was concerned with the secondary structure of myoglobin.<sup>6</sup> Hydrogen–deuterium (H/D) exchange of the amide protons was employed as an external perturbation to generate the 2D spectra. Since that time 2D IR correlation spectroscopy has been used extensively to explore the secondary structure of proteins. Sefara *et al.* studied thermal transitions in  $\beta$ -lactoglobulin (BLG) using both 2D IR and 2D NIR spectroscopy.<sup>7,8</sup> Smeller *et al.*<sup>9,10</sup> and Dzwolak *et al.*<sup>11</sup> reported 2D IR studies of pressure-dependent structural modifications of proteins. Schultz *et al.* carried out 2D IR, 2D NIR, and 2D IR–NIR heterospectral correlation analysis of the thermal unfolding of ribonuclease A (RNase A).<sup>12</sup> It was reported that different structural elements in RNase A respond slightly differently to a temperature increase. Fabian *et al.* also reported 2D IR correlation spectroscopy study on noncooperative unfolding of the Cro-V55C repressor protein.<sup>13</sup> They pointed out that in combination with protein engineering, the 2D IR approach provides insight into the impact of point mutations on the stability of proteins and the presence of folding intermediates as predicted from molecular modeling. Wang *et al.* reported 2D NIR correlation analysis of concentration-dependent spectral changes in ovalbumin solutions at various temperatures.<sup>14</sup> New insight has been gained into the hydration as well as unfolding process of secondary structures of ovalbumin by studying temperature-dependent correlation patterns in 2D NIR spectra. Wu *et al.* used slice spectra of 2D NIR maps generated from the concentration-perturbed spectral variations of HSA solutions



at various levels of hydration and secondary structure.<sup>16</sup> The study of the secondary structure and kinetics of H/D exchange of HSA was outlined in the previous chapter.<sup>17</sup> In this chapter, three additional examples of protein research by 2D correlation spectroscopy are discussed.

## 12.1 ADSORPTION AND CONCENTRATION-DEPENDENT 2D ATR/IR STUDY OF $\beta$ -LACTOGLOBULIN

Czarnik-Matusiewicz *et al.* reported the usefulness of 2D ATR/IR spectroscopy in the studies of adsorption-dependent and concentration-dependent structural variations of  $\beta$ -lactoglobulin (BLG) in buffer solutions.<sup>15</sup> BLG, which is the main component of whey proteins contained in bovine milk, was one of the first proteins studied by X-ray crystallographic analysis.<sup>23</sup> In its mature form, BLG is built from 162 amino acid residues, and in an aqueous solution it exists as a dimer of molecular weight of approximately 36 kDa. Figure 12.1 depicts the structure of BLG elucidated by X-ray crystallography.<sup>23</sup> The secondary structure of BLG consists mainly of antiparallel  $\beta$ -sheets. The core of the BLG molecule is made up of a short  $\alpha$ -helix segment and eight strands of antiparallel  $\beta$ -sheets, labeled  $\beta$ -A to  $\beta$ -H, which wrap round to form a calyx. Three right-handed  $3_{10}$  helices and one  $\alpha$ -helix are attached to this calyx by loops. An extremely flexible loop joins a fourth  $3_{10}$  helix with the final  $\beta$  strand, I, which forms the same  $\beta$ -sheet as the strands E, F, G, H. If one accounts for the total number of amino acids in this protein, the percentages of individual secondary structural components are approximately 35 %  $\beta$ -strands, 18 %  $\alpha$ -helices, 10 %  $3_{10}$  helices, and 48 % nonperiodic fragments of BLG main chain. The two monomers are linked together

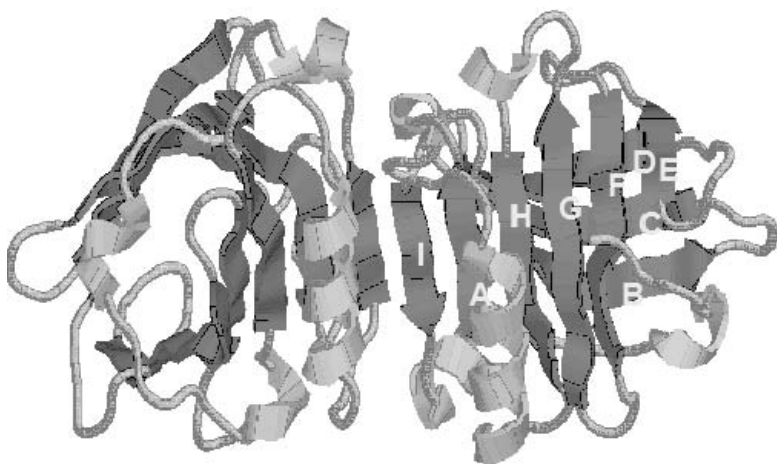


**Figure 12.1** Structure of BLG elucidated by X-ray crystallography. (Reproduced with permission from Ref. No. 15. Copyright (2000) American Chemical Society.)

in the dimer via a short stretch of double-stranded  $\beta$ -sheet comprising one strand from each subunit. It has been reported that the secondary structure of BLG is sensitive to changes in its environment. For example, Qi *et al.* reported in their study on a thermal denaturation of BLG that the denaturation process strongly depends on the concentration of BLG, particularly in a concentration range below 50 mg/ml.<sup>24</sup>

ATR/IR spectra of BLG in buffer solutions with the concentration of 1, 2, 3, 4, and 5 wt% contain contributions from two kinds of perturbations: a concentration change and a change induced by adsorption on the surface of an ATR prism. Both perturbations induce significant spectral changes in the Amide I and II regions of BLG. However, from the conventional, one-dimensional spectra, it is not straightforward to investigate these spectral changes separately. Therefore, Czarnik-Matusewicz *et al.* applied 2D correlation spectroscopy to analyze the intensity variations in the Amide I band region caused by the two perturbations.<sup>15</sup>

Figure 12.2(A) shows ATR/IR spectra in the 4000–650  $\text{cm}^{-1}$  region of the buffer solution and the five kinds of BLG solutions.<sup>15</sup> Before 2D correlation spectra were calculated, a pretreatment procedure consisting of four steps had been applied to the original spectra. At first, each ATR spectrum was normalized over the minimum value of the penetration obtained for the analyzed spectrum, and then the dependence of the penetration depth upon the concentration was eliminated. As the second pretreatment step, the contribution of water was subtracted from the spectra of BLG solutions using the algorithm proposed by Dousseau *et al.*<sup>25</sup> The subtracted spectra were baseline corrected by a two-point level line between 1720 and 1200  $\text{cm}^{-1}$ . The spectra of BLG obtained were smoothed by the maximum entropy method as the third pretreatment step. Figure 12.2(B) displays the spectra of the protein solutions after the four pretreatments. One of the major steps in the preparation of the concentration-dependent spectral data prior to the 2D correlation analysis is normalization. The normalization over the concentration is necessary when the concentration of the molecules studied alters simultaneously with the perturbation. Czarnik-Matusewicz *et al.* examined both adsorption-induced and concentration-dependent spectral variations of BLG using 2D ATR/IR correlation spectroscopy. In this chapter only the concentration-dependent structural changes in BLG are discussed.

Figure 12.3(A) shows the synchronous 2D ATR/IR correlation spectrum obtained from the concentration-dependent spectral changes of the BLG aqueous solutions. Autopeaks at 1652 and 1662  $\text{cm}^{-1}$  are ascribed to hydrophilic secondary structure motives located on the outer surface of BLG that are built from  $\alpha$ -helices and  $3_{10}$ -helices, respectively. These secondary structures are less protected from water penetration than the  $\beta$ -strands forming the calyx. A minimal intensity variation in the band at 1636  $\text{cm}^{-1}$  due to antiparallel ‘buried’  $\beta$ -sheets is consistent with the fact that the C=O groups located inside the hydrophobic core of BLG are more resistant to the interaction with water molecules than those in the hydrophilic, solvent exposed part of BLG.<sup>15</sup>

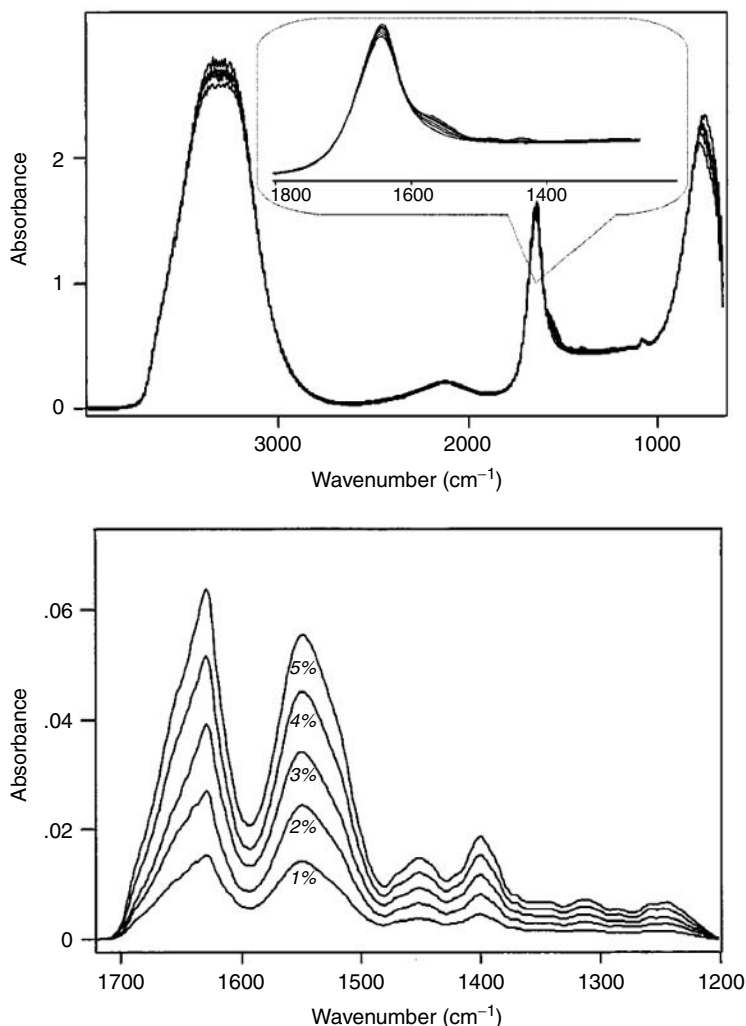


**Figure 12.2** (A) ATR/IR spectra in the 4000–650  $\text{cm}^{-1}$  region of the buffer solution and five different aqueous solutions of BLG with concentrations of 1, 2, 3, 4, and 5 wt%. (B) Concentration-dependent ATR/IR spectral variations in the 1720–1200  $\text{cm}^{-1}$  region of the BLG solutions after the four pretreatments, except for normalization. (Reproduced with permission from Ref. No. 15. Copyright (2000) American Chemical Society.)

Figure 12.3(B) depicts the corresponding asynchronous correlation spectrum and Figure 12.4 shows a slice spectrum extracted at 1665  $\text{cm}^{-1}$  from the asynchronous 2D spectrum in Figure 12.3(B). A slice spectrum at 1640  $\text{cm}^{-1}$  from the asynchronous spectrum calculated from the adsorption-dependent spectral changes is also given in Figure 12.4. Comparison between the two slice spectra shows that the unrelated intensity variations stimulated by concentration change

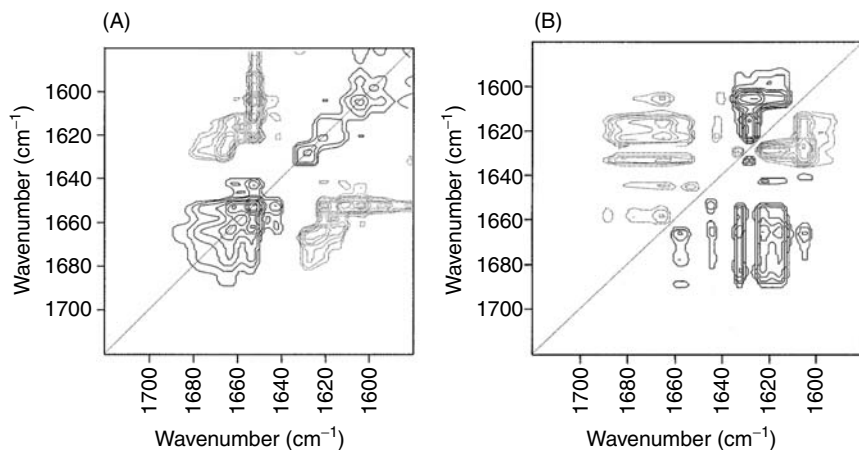


**Figure 12.3** (A) Synchronous 2D ATR/IR correlation spectrum in the 1720–1580  $\text{cm}^{-1}$  region constructed from concentration-dependent spectral changes of BLG. (B) Asynchronous 2D ATR/IR correlation spectrum in the 1720–1580  $\text{cm}^{-1}$  region constructed from concentration-dependent spectral changes of BLG. (Reproduced with permission from Ref. No. 15. Copyright (2000) American Chemical Society.)

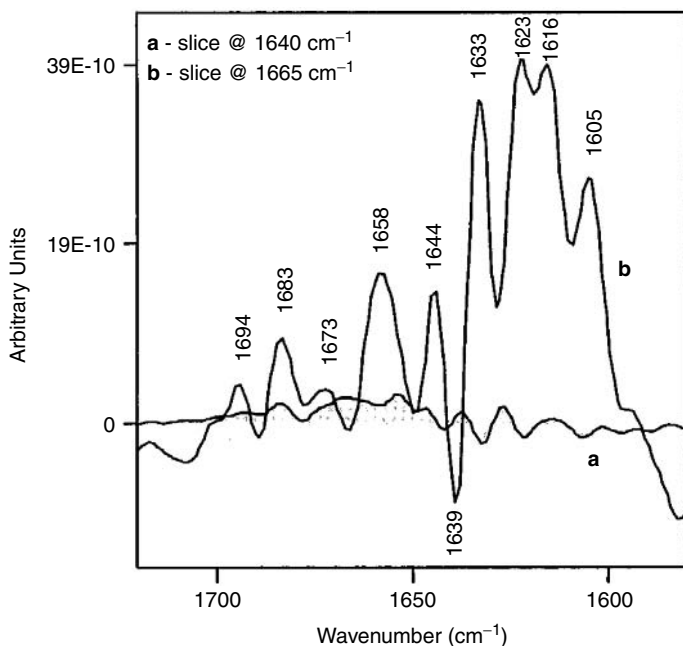
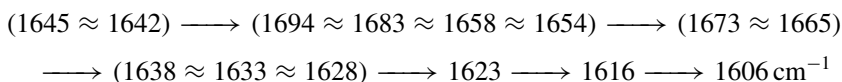


**Figure 12.4** Slice spectra extracted along (A) 1640  $\text{cm}^{-1}$  in the asynchronous spectrum generated from the adsorption-dependent spectral variations and (B) 1665  $\text{cm}^{-1}$  in the asynchronous spectrum presented in Figure 12.3(B). (Reproduced with permission from Ref. No. 15. Copyright (2000) American Chemical Society.)

are at least one order of magnitude higher than those induced by the adsorption process. The asynchronous spectrum in Figure 12.3(B) and the slice spectrum at  $1665\text{ cm}^{-1}$  in Figure 12.4 suggest the following sequence of the spectral events occurring during the concentration increase:



It was found from the above sequence that the first event in the intensity variations occurs for the bands due to the random coil structure ( $1645$  and  $1642\text{ cm}^{-1}$ ), followed by the intensity changes in the bands assigned to the high-wavenumber  $\beta$ -sheet component ( $1694\text{ cm}^{-1}$ ),  $\beta$ -turn ( $1683\text{ cm}^{-1}$ ), and  $\alpha$ -helix ( $1658$  and  $1654\text{ cm}^{-1}$ ). The intensity variations arising from another type of  $\beta$ -turn (probably type III) ( $1673\text{ cm}^{-1}$ ) and  $3_{10}$ -helix ( $1665\text{ cm}^{-1}$ ) take place next. The intensity modifications attributed to both buried ( $1638\text{ cm}^{-1}$ ) and exposed  $\beta$ -strands ( $1633$  and  $1628\text{ cm}^{-1}$ ) are behind those due to the structures described above, but ahead of the changes induced by aggregated components ( $1623\text{ cm}^{-1}$ ). The last event in the sequence is attributed to the side-chain vibrations ( $1616$  and  $1606\text{ cm}^{-1}$ ). The negative sign of the synchronous peak around ( $1652$ ,  $1622\text{ cm}^{-1}$ ) suggests that, at the expense of the intramolecular hydrogen bonds located in the disordered/ $\alpha$ -helix components, the number of intermolecular hydrogen bonds increases. This conclusion is consistent with those reached from thermal denaturation studies of BLG by IR spectroscopy<sup>26</sup> and circular dichroism.<sup>27</sup>

It was found from the adsorption-dependent spectral changes of BLG that the interaction between BLG molecules and an ATR prism is characterized by intensity changes in bands assigned to  $\beta$ -sheet elements buried in the hydrophobic core of the molecule and that the intensity variations induced by the adsorption are about one-tenth of those induced by the concentration changes.

## 12.2 pH-DEPENDENT 2D ATR/IR STUDY OF HUMAN SERUM ALBUMIN

Murayama *et al.* used 2D IR correlation spectroscopy to probe pH-dependent structural variations in the secondary structures and in the hydrogen bondings of side chains of human serum albumin (HSA) in aqueous solutions.<sup>18</sup> The synchronous and asynchronous correlation spectra were calculated from the pH-dependent spectral changes for the three states of HSA: the N isomeric form (pH 5.0–4.4), the N–F transition (pH 4.6–3.8), and the F isomeric form (pH 3.8–3.0). The most interesting finding in this study was identification of four bands at  $1740$ ,  $1715$ ,  $1705$ , and  $1696\text{ cm}^{-1}$  due to the C=O stretching mode of free and hydrogen-bonded (weak, medium, and strong) COOH groups of glutamic (Glu) and aspartic (Asp) acid residues in HSA. The 2D correlation analysis

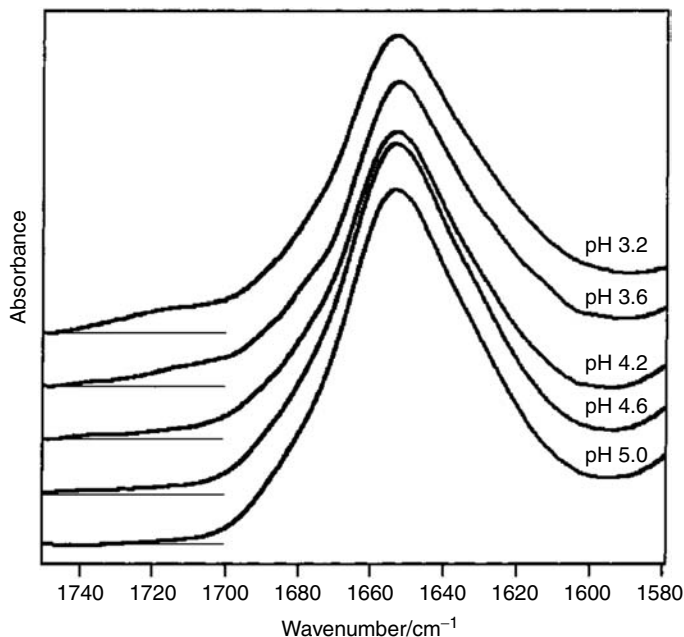


**Figure 12.5** Representative difference IR spectra in the  $1750\text{--}1580\text{ cm}^{-1}$  region of HSA in buffer solutions (2.0 wt%) of pH 3.2, 3.6, 4.2, 4.6 and 5.0. At each pH the spectrum of the buffer solution was subtracted from the corresponding spectrum of the HSA solution. (B) Second derivatives of the spectra shown in (A). (Reproduced with permission from Ref. No. 18. Copyright (2001) American Chemical Society.)

provided unambiguous evidence for the existence of at least four C=O bands, demonstrating its powerful deconvolution ability.

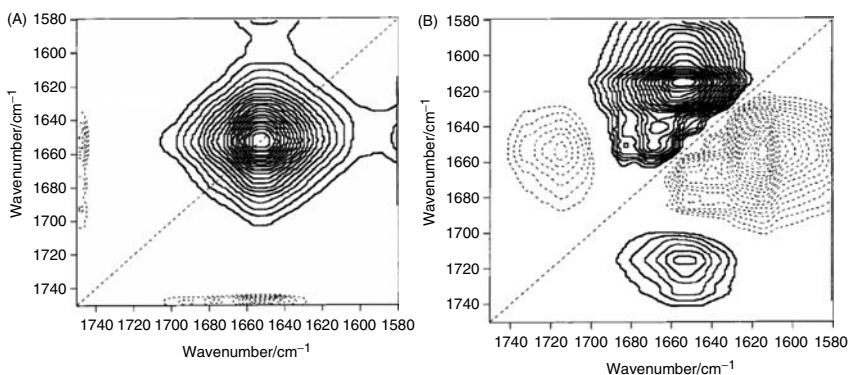
Figure 12.5(A) and (B) shows, respectively, representative ATR/IR spectra in the  $1750\text{--}1580\text{ cm}^{-1}$  region of HSA in aqueous solutions (2.0 wt%) with a pH of 3.2, 3.6, 4.2, 4.6, and 5.0, and their second derivatives. It can be seen from the second derivative spectra that the Amide I band is composed of a strong band at  $1654\text{ cm}^{-1}$  due to the  $\alpha$ -helix and weak satellite components at  $1681$  and  $1628\text{ cm}^{-1}$  assigned to the  $\beta$ -turn and  $\beta$ -strand structures, respectively. The second derivative spectra develop weak features at  $1740$  and  $1598\text{ cm}^{-1}$ . They are ascribed to a C=O stretching mode of COOH groups and a COO<sup>-</sup> antisymmetric stretching mode, respectively, of Glu and Asp acid residues in HSA. The wavenumbers of the bands observed in the second derivative spectra and their assignments are summarized in Table 12.1.

### 12.2.1 N ISOMERIC FORM OF HSA

Figure 12.6 shows 2D IR correlation spectra of the N isomeric form of HSA in a buffer solution, constructed from the pH-dependent (pH 5.0, 4.8, 4.6, and 4.4)

**Table 12.1** Frequencies ( $\text{cm}^{-1}$ ) and assignments of IR bands of HSA solutions observed in the second derivative spectra and 2D correlation maps (from Ref. No. 18)

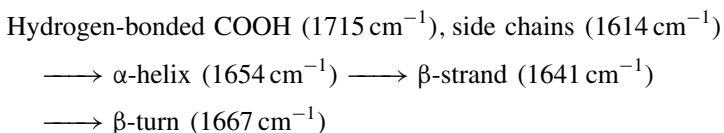
| 2nd derivative | N form (2D) | N-F Transition (2D) | F form (2D) | Assignment                    |
|----------------|-------------|---------------------|-------------|-------------------------------|
| 1740           |             | 1740                |             | Free COOH group               |
| 1715           | 1715        |                     | 1715        | Hydrogen-bonded (weak) COOH   |
|                |             | 1705                |             | Hydrogen-bonded (medium) COOH |
|                |             | 1696                | 1696        | Hydrogen-bonded (strong) COOH |
| 1681           |             |                     | 1678        | $\beta$ -turn                 |
|                | 1667        |                     | 1667        | $\beta$ -turn                 |
| 1654           | 1654        | 1654                |             | $\alpha$ -helix               |
|                |             |                     | 1647        | Random coil                   |
|                | 1640        | 1640                | 1638        | $\beta$ -strand               |
|                |             | 1632                | 1635        | $\beta$ -strand               |
| 1628           |             |                     |             | $\beta$ -strand               |
|                |             | 1620                | 1622        | $\beta$ -sheet                |
| 1614           | 1614        |                     | 1616        | Side chains                   |
| 1598           | 1598        |                     |             | $\text{COO}^-$                |

**Figure 12.6** (A) Synchronous 2D IR correlation spectrum in the  $1750\text{--}1580\text{ cm}^{-1}$  region constructed from the pH-dependent (pH 5.0, 4.8, 4.6 and 4.4) spectral variations of the HSA solutions with a concentration of 2.0 wt%. (B) The corresponding asynchronous correlation spectrum. (Reproduced with permission from Ref. No. 18. Copyright (2001) American Chemical Society.)

spectral variations in the  $1750\text{--}1580\text{ cm}^{-1}$  region, respectively.<sup>18</sup> The synchronous spectrum develops one broad autopeak near  $1654\text{ cm}^{-1}$ , which is characteristic of the  $\alpha$ -helix. The analysis of several cross peaks in the asynchronous spectrum allows one to identify bands at least at 1715, 1667, 1654, 1641, and  $1614\text{ cm}^{-1}$ . The band at  $1715\text{ cm}^{-1}$  is not identified in the second derivative

spectra at pH 5.0 and 4.6, demonstrating that 2D correlation spectroscopy is powerful in detecting subtle spectral changes. Murayama *et al.* ascribed the band at  $1715\text{ cm}^{-1}$  to a C=O stretching mode of the hydrogen-bonded COOH groups of Glu and Asp residues of HSA. About half of the carboxyls of Asp and Glu residues are considered to ionize with an intrinsic  $pK$  of 4.3. The asynchronous spectrum suggests that some carboxylate groups are protonated even in this pH range (pH 4.0–5.0). The broad feature of the cross peak near  $(1715, 1654)\text{ cm}^{-1}$  indicates that the COOH groups with the hydrogen bonds of different strength are formed upon the protonation.

On the basis of Noda's rule, Murayama *et al.* deduced the following sequence of the intensity variations:



The above sequence revealed that the protonation of the  $\text{COO}^-$  groups and micro-environmental changes in the side chains precede the secondary structural changes, which begin with the  $\alpha$ -helices, followed by  $\beta$ -strands, and  $\beta$ -turns. It is very likely that the protonation of some carboxylic groups at relatively low pH (4.5–5.0) is a trigger for the secondary structural changes in the N form. The N-F transition takes place primarily in domain III.<sup>28,29</sup> Thus, probably the 2D correlation maps largely reflect the structural variations in domain III.

### 12.2.2 N-F TRANSITION REGION OF HSA

The synchronous and asynchronous correlation spectra of the N-F transition are shown in Figure 12.7(A) and (B).<sup>18</sup> The synchronous spectrum yields a broad autopeak at  $1654\text{ cm}^{-1}$  and negative cross peaks at  $(1705, 1632)$  and  $(1740, 1640)\text{ cm}^{-1}$ . The bands at  $1740$  and  $1705\text{ cm}^{-1}$  are due to a C=O stretching vibration of free and hydrogen-bonded COOH groups, respectively, of Asp and Glu side chains. In this pH range, many carboxylate groups are protonated. It is very likely that the unfolding or expansion of domain III leads to free COOH groups in the N-F transition. The band at  $1705\text{ cm}^{-1}$  due to the hydrogen-bonded COOH group and that at  $1632\text{ cm}^{-1}$  assignable to  $\beta$ -strand structures share the cross peak, suggesting that the formation of the hydrogen-bonded COOH groups upon the protonation and the secondary structure change in  $\beta$ -strand structures are cooperative in the N-F transition.

In the asynchronous spectrum (Figure 12.7(B)) cross peaks appear at  $(1696, 1654)$  and  $(1620, 1654\text{ cm}^{-1})$ . The bands at  $1696$ ,  $1654$ , and  $1620\text{ cm}^{-1}$  may be due to hydrogen-bonded COOH groups,  $\alpha$ -helices, and  $\beta$ -sheets, respectively. It is noted that the bands due to the C=O stretching modes of COOH groups are observed at  $1705$  and  $1696\text{ cm}^{-1}$  for the N-F transition. It seems that these



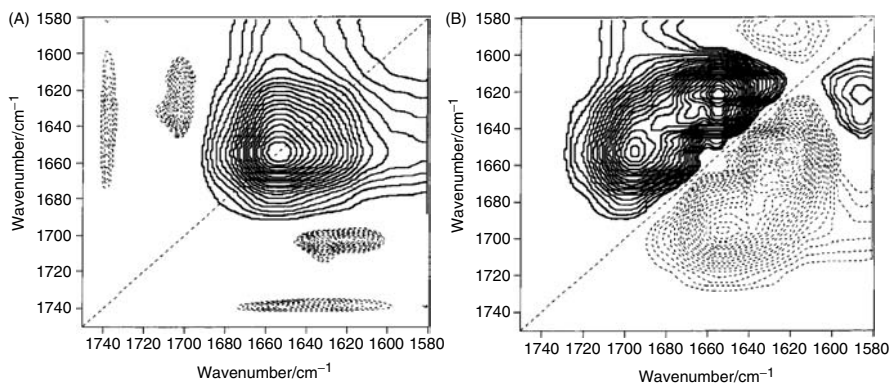


**Figure 12.7** (A) Synchronous 2D IR correlation spectrum in the 1750–1580  $\text{cm}^{-1}$  region constructed from the pH-dependent (pH 4.6, 4.4, 4.2, 4.0 and 3.8) spectral variations of the HSA solutions with a concentration of 2.0 wt%. (B) The corresponding asynchronous correlation spectrum. (Reproduced with permission from Ref. No. 18. Copyright (2001) American Chemical Society.)

COOH groups have hydrogen bonds of different strengths. The C=O groups can form hydrogen bonds with NH groups of the main chains or side chains or with OH groups of side chains or water molecules. The signs of the asynchronous cross peaks indicate the following sequence of structural changes in HSA during the N–F transition:

Hydrogen-bonded COOH ( $1696\text{ cm}^{-1}$ )  $\longrightarrow$   $\alpha$ -helix ( $1654\text{ cm}^{-1}$ )  
 $\longrightarrow$   $\beta$ -sheet ( $1620\text{ cm}^{-1}$ ),  $\beta$ -turn ( $1667\text{ cm}^{-1}$ )  $\longrightarrow$   $\beta$ -strand ( $1632\text{ cm}^{-1}$ ).

The above sequence suggests that the protonation of  $\text{COO}^-$  groups starts the N–F transition, followed by the unfolding of the  $\alpha$ -helices in domain III. In the last step, the  $\beta$ -turns,  $\beta$ -sheets, and  $\beta$ -strands undergo the secondary structure change, leading to the F isomeric form of HSA. Similarly Murayama *et al.* obtained the following sequences for the F form:

- (a)  $\beta$ -strand ( $1638\text{ cm}^{-1}$ )  $\longrightarrow$  side chain ( $1616\text{ cm}^{-1}$ )
- (b)  $\beta$ -turn ( $1667\text{ cm}^{-1}$ )  $\longrightarrow$  hydrogen-bonded COOH ( $1715\text{ cm}^{-1}$ )  
 $\longrightarrow$  side chain ( $1616\text{ cm}^{-1}$ )
- (c)  $\beta$ -turn ( $1667\text{ cm}^{-1}$ )  $\longrightarrow$  random coil ( $1647\text{ cm}^{-1}$ )

It seems very likely that in the F form the conformational changes occur in the  $\beta$ -strands and  $\beta$ -turns first and then they induce changes in the random coil, side chains and hydrogen-bonded COOH groups. This study has demonstrated that 2D correlation analysis enables one to monitor intensity changes even in weak bands such as those due to the COOH and  $\text{COO}^-$  groups of Glu and Asp acid residues and aromatic amino acid residues.

### 12.3 AGGREGATION OF LIPID-BOUND CYTOCHROME *C*

Paquet *et al.* investigated the aggregation of cytochrome *c* in the presence of dimyristoylphosphatidylglycerol (DMPG) by using 2D IR correlation spectroscopy.<sup>19</sup> It is well known that binding to negatively charged lipids destabilizes the structure of cytochrome *c*, since it decreases its denaturation temperature by 25–30 °C, and it increases the structural unfolding process. Paquet *et al.* used a band at 1616 cm<sup>-1</sup>, which is characteristic of aggregated proteins, to monitor the effect of temperature on the aggregation. They found that the final intensity of the 1616 cm<sup>-1</sup> band of the DMPG-bound cytochrome *c* (lipid-to-protein molar ratio = 50:1) is higher with increasing temperature. This result indicates a higher degree of cytochrome *c* aggregation at higher temperature.

Figure 12.8 plots the intensity at 1616 cm<sup>-1</sup> versus temperature increase and then time at 65 °C.<sup>19</sup> The 2D IR correlation analysis was carried out for the time periods shown in Fig. 12.8.<sup>19</sup> Figure 12.9 shows 2D IR correlation spectra of DMPG-bound cytochrome *c* at the beginning of the aggregation period. The one-dimensional spectrum shown in Fig. 12.9 is the average of the difference spectra used for the correlation analysis (see Ref. 19 for details). The synchronous spectrum shows two autopeaks at 1650 and 1616 cm<sup>-1</sup> ascribed, respectively, to  $\alpha$ -helices and hydrogen-bonded extended structures. The two bands share a negative cross peak, indicating that the band at 1650 cm<sup>-1</sup> decreases whereas that at 1616 cm<sup>-1</sup> increases upon aggregation. The asynchronous spectrum in Figure 12.9(B) yields one cross peak at (1650, 1616 cm<sup>-1</sup>). Therefore, it seems

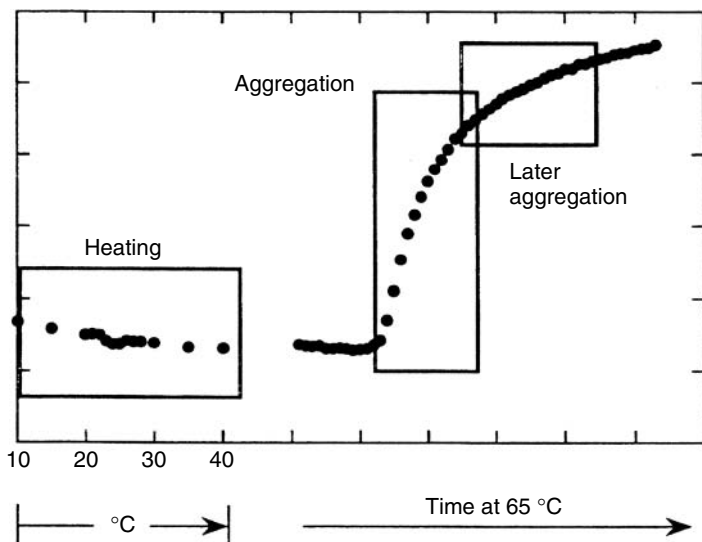


**Figure 12.8** Schematic representation of the time periods used in the 2D IR correlation analyses (Reproduced with permission from Ref. No. 19. Copyright (2000) American Institute of Physics.)

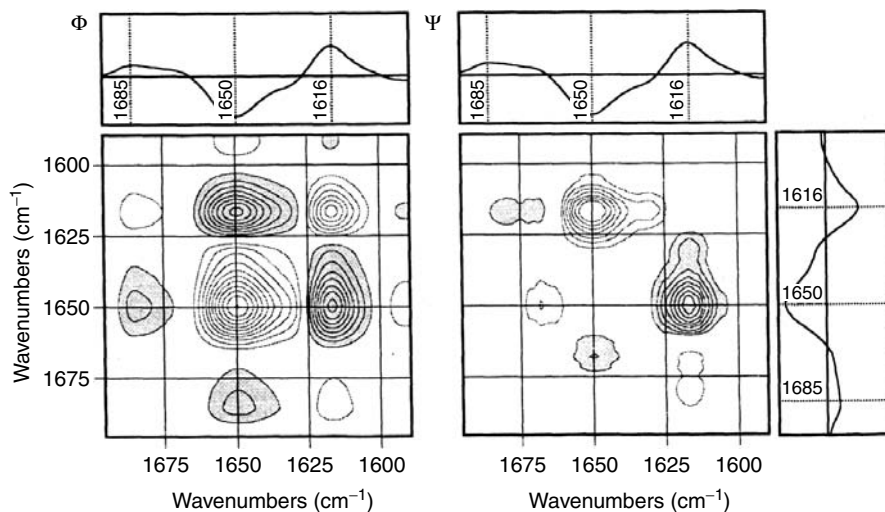


**Figure 12.9** (A) Synchronous and (B) asynchronous 2D IR spectra of DMPG-bound cytochrome *c* at the beginning of the aggregation period. (Reproduced with permission from Ref. No. 19. Copyright (2000) American Institute of Physics.)

that the intermolecular bonds are formed first and then the  $\alpha$ -helices are destabilized, indeed unfolded.

The correlation analysis was made also for the later aggregation (see Figure 12.8) of the DMPG-bound cytochrome *c*. The synchronous spectrum for the later aggregation is very similar to that for the beginning of the aggregation. In contrast, the asynchronous spectrum is quite different and presents only a noisy pattern, showing no evidence of an out-of-phase cross peak. This result indicates a simultaneous formation of intermolecular bonds and unfolding of  $\alpha$ -helices.

To explore how intermolecular bonds are first formed before the secondary structure change, Paquet *et al.* studied the interaction between the protein and DMPG as a function of temperature increase, but below the denaturation point of this complex. It was known that the secondary structure of cytochrome *c* changes little upon binding to DMPG. However, they carried out a 2D correlation analysis on temperature-dependent spectral variations to investigate whether minor changes, which could explain the amide group accessibility necessary for the formation of the first intermolecular bonds, are occurring.

Synchronous and asynchronous correlation spectra generated from the temperature-dependent (10–40 °C) spectral variations of DMPG-bound cytochrome *c* are shown in Figure 12.10. The spectra illustrate the changes associated with both  $\beta$ -turns and  $\beta$ -sheets. Three main cross peaks are observed at (1653, 1660), (1653, 1643), and (1653, 1625)  $\text{cm}^{-1}$  in the asynchronous map. The signs of these peaks reveal that the intensity increase at 1653  $\text{cm}^{-1}$  takes place first, followed by those at 1660 and 1643  $\text{cm}^{-1}$  and finally the intensity at 1625  $\text{cm}^{-1}$  decreases. Based upon these results, Paquet *et al.* concluded that because of the loosening

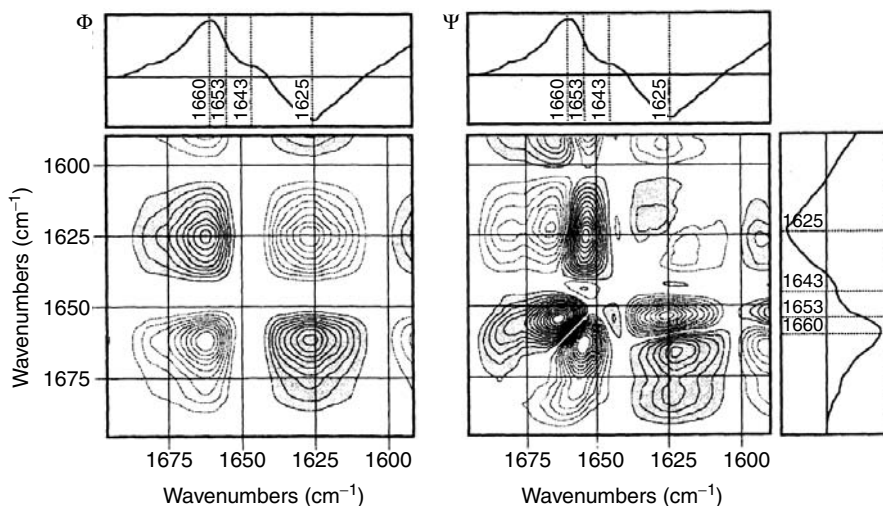


**Figure 12.10** (A) Synchronous and (B) asynchronous 2D IR spectra of DMPG-bound cytochrome *c* generated from the spectra measured at temperatures increasing from 10 to 40 °C. (Reproduced with permission from Ref. No. 19. Copyright (2000) American Institute of Physics.)

of the tertiary structure of cytochrome *c* upon lipid binding a higher frequency  $\alpha$ -helix component appears at 1653  $\text{cm}^{-1}$  and that  $\beta$ -sheets (1625  $\text{cm}^{-1}$ ) are partially unfolded with temperature, inducing an increase of  $\beta$ -turns (1660  $\text{cm}^{-1}$ ) and, to a lesser extent, extended chains (1643  $\text{cm}^{-1}$ ). This study showed that 2D correlation spectroscopy is useful in investigating the structural changes of a protein even in an environment with a lipid-to-protein molar ratio of 50:1.

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## 13 Applications of Two-dimensional Correlation Spectroscopy to Biological and Biomedical Sciences

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In the last chapter, applications of 2D correlation spectroscopy to protein research were outlined. 2D correlation spectroscopy has also been applied to a variety of other biological molecules and materials,<sup>1–3</sup> such as carbohydrates,<sup>4</sup> cellulose,<sup>5</sup> bacteriorhodopsin,<sup>6</sup> polyaminoacid,<sup>7</sup> lipids,<sup>8–10</sup> and biomembranes.<sup>10</sup> It has also been used to investigate the structure, molecular interaction, hydration, and compositional changes of many interesting ‘real world’ biological materials.<sup>11–22</sup> Biological, biomedical, and pharmaceutical sciences are indeed one of the fastest growing areas of applications for generalized 2D correlation spectroscopy.

For example, Gadaleta *et al.* studied the maturation of synthetic and biological hydroxy apatite (HA).<sup>11</sup> Complex IR spectral contours undergoing changes as crystals ripen were successfully analyzed by 2D correlation analysis. Noda *et al.* applied 2D correlation analysis to the rheo-optical dynamic IR data of the stratum corneum of human skin and human hair keratin films.<sup>12,13</sup> These studies have been discussed in Sections 8.24 and 8.25 of Chapter 8. They were probably the first examples of the application of 2D IR correlation spectroscopy to biomedical sciences.

Barton II *et al.* reported 2D correlation spectroscopy studies on agricultural materials.<sup>14,15</sup> They used statistical 2D correlation coefficient mapping described in Chapter 7 for these studies. They proposed a number of band assignments for NIR spectra of agricultural materials based on the correlations between NIR and IR or Raman bands. Liu *et al.* applied 2D Vis/NIR correlation spectroscopy to a study of thermal treatment of chicken meats.<sup>17</sup> They calculated synchronous and asynchronous 2D spectra from time-dependent Vis/NIR reflectance spectra of chicken breast muscles cooked for 3, 6, 9, and 12 min at an air temperature of 150 °C. Tsenkova *et al.* used 2D NIR spectroscopy for the diagnosis of mastitis, which is an intramammary bacterial infectious disease often spread among dairy cattle.<sup>18</sup> They stated that an asynchronous map constructed from the Log SCC (Somatic Cell Counts) level-dependent NIR spectra of ordinary milk is useful for mastitis diagnosis. Czarnik-Matusiewicz *et al.* also employed 2D correlation

analysis to unravel complicated NIR spectra of cows' milk.<sup>19</sup> Sun *et al.* used 2D IR spectroscopy for the identification and quality control of traditional Chinese medicines.<sup>20–22</sup> In this chapter, we discuss some of these interesting applications of 2D correlation spectroscopy to complex life science problems.

### 13.1 2D NIR STUDY OF MILK

In Section 4.2.2 we already briefly mentioned NIR spectra of milk. As shown in Figure 4.5, NIR spectra of milk are featureless and, at first glance, they all look very similar to the NIR spectrum of water. However, NIR spectra actually contain rich and valuable information concerning the quality of milk. Czarnik-Matusewicz *et al.* used 2D correlation spectroscopy to analyze the NIR spectra of this complicated biological fluid, consisting mainly of proteins, fats, and carbohydrates.<sup>19</sup>

A broad band near 1450 nm in the NIR spectra of milk samples is due to the combination of OH symmetric and antisymmetric stretching modes of water, while an intense feature near 1930 nm is assigned to the combination of OH bending and symmetric stretching modes of water (Figure 4.5). The water bands are so strong that it is almost impossible to extract information about fats, proteins and other metabolites of milk directly from the NIR spectra. Multivariate analysis, such as principal component analysis (PCA), is usually used to analyze such complicated spectra. The signal-to-noise ratio of NIR spectra of milk is rather poor, and their baselines change from one spectrum to another (Figure 4.5). Thus, prior to the 2D correlation analysis, the NIR spectra of 165 milk samples with different protein and fat concentrations were subjected to multiplicative scatter correction (MSC) and smoothing (see Section 4.2.2).<sup>19</sup> For the MSC treatment, two data segments were constructed, one from 1100 to 1900 nm and another from 2000 to 2400 nm (Figure 4.6).

To calculate 2D correlation spectra Czarnik-Matusewicz *et al.* used the concentrations of milk proteins and fats as perturbations.<sup>19</sup> Figure 13.1 illustrates correlation plots for the concentrations of fat and proteins in the milk samples. Four sets each of samples from Figure 13.1(A) and (B) were selected for the 2D correlation analysis. In Figure 13.1(A), set 1, set 2, set 3, and set 4, respectively, have the more or less fixed protein concentrations of  $\sim 3.18$ ,  $\sim 3.14$ ,  $\sim 2.90$ , and  $\sim 3.60$  wt%, but their fat concentrations change markedly. So, one can calculate fat concentration-dependent 2D NIR spectra from each set. In Figure 13.1(B), likewise, set 1, set 2, set 3, and set 4, respectively, contain the approximately fixed fat concentrations of  $\sim 3.20$ ,  $\sim 3.30$ ,  $\sim 1.67$ , and  $\sim 5.30$  wt%. From each set in Figure 13.1(B), one can generate protein concentration-based 2D NIR spectra.

Figure 13.2 compares the synchronous 2D correlation spectra in the 2000–2400 nm region generated from the fat and protein concentration-dependent NIR spectra after the data pretreatments.<sup>19</sup> The synchronous spectrum constructed

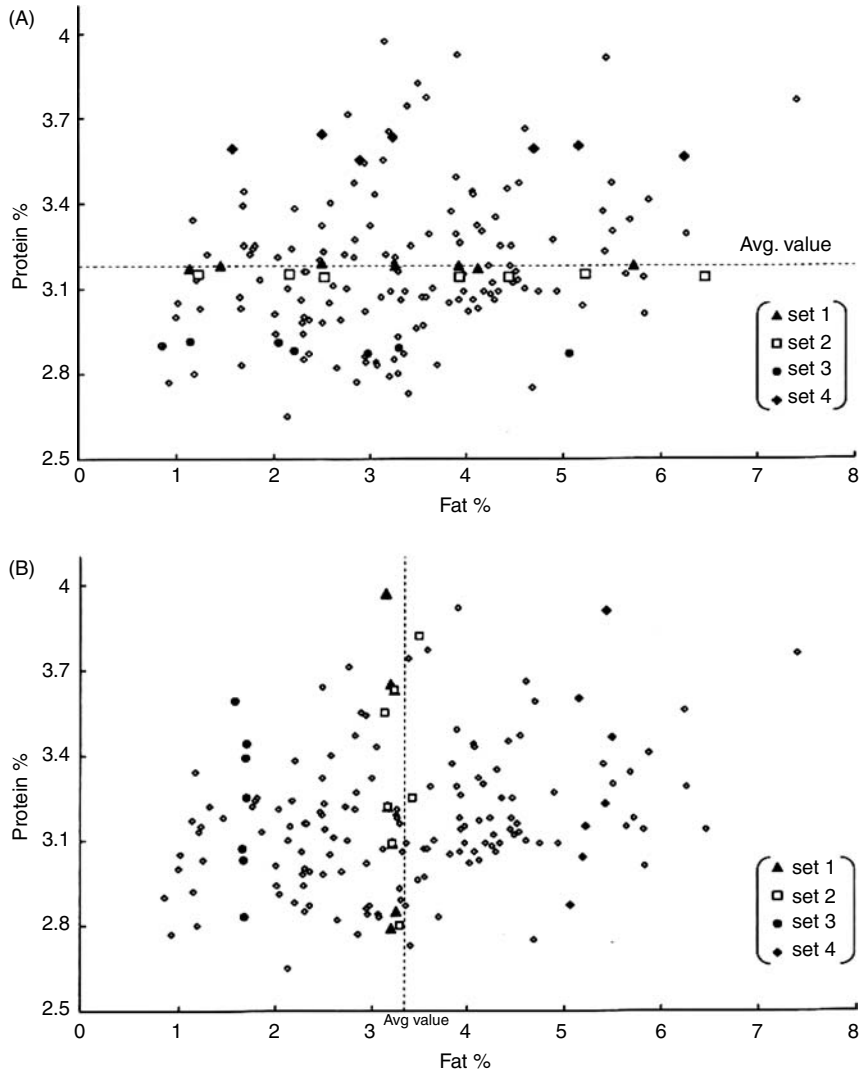


**Figure 13.1** (A) Correlation plot for the concentrations of fat and protein in the milk samples. Four sets of samples were selected for the 2D correlation analysis. (B) The same correlation plot for the concentration of fat and proteins in milk samples as that in (A). Again, four sets of samples were selected for the 2D correlation analysis. (Reproduced with permission from B. Czarnik-Matusewicz *et al.*, *Appl. Spectrosc.*, **53**, 1582 (1999) (Ref. 19). Copyright (1999) Society for Applied Spectroscopy.)

from the fat concentration-dependent NIR spectra (Figure 13.2(A)) develops autopeaks at 2311 and 2346 nm, while the corresponding 2D spectrum from the protein concentration-dependent variations (Figure 13.2(B)) shows autopeaks at 2047, 2075, 2100, 2351, and 2375 nm. The appearance of autopeaks means that



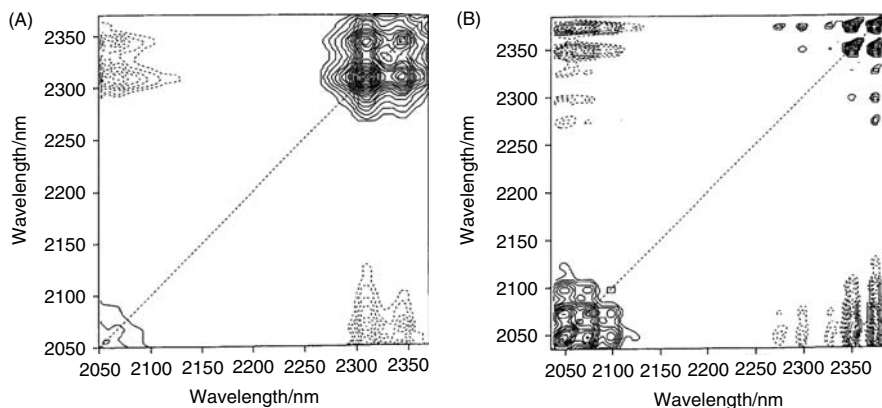


**Figure 13.2** Synchronous 2D NIR correlation spectra in the 2000–2400 nm region constructed from (A) fat and (B) protein concentration-dependent spectral changes of milk after MSC and smoothing. (Reproduced with permission from B. Czarnik-Matusewicz *et al.*, *Appl. Spectrosc.*, **53**, 1582 (1999) (Ref. 19). Copyright (1999) Society for Applied Spectroscopy.)

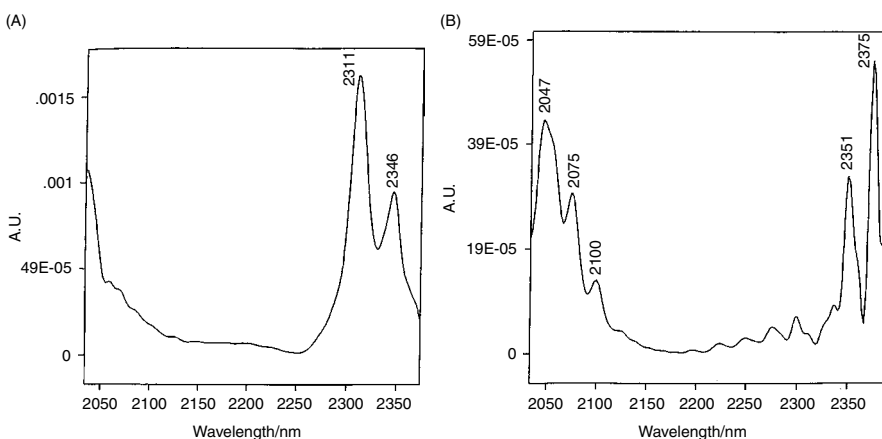


**Figure 13.3** (A) A power spectrum along the diagonal line on the synchronous spectrum shown in Figure 13.2(A). (B) A power spectrum along the diagonal line on the synchronous spectrum shown in Figure 13.2 (B). (Reproduced with permission from B. Czarnik-Matusewicz *et al.*, *Appl. Spectrosc.*, **53**, 1582 (1999) (Ref. 19). Copyright (1999) Society for Applied Spectroscopy.)

the intensities of these bands vary most significantly with the increase in the concentration of fats or proteins in milk.

Figure 13.3 shows power spectra along the diagonal line of the synchronous spectra in Figure 13.2. Of particular note in the power spectra is that most of the peaks appearing in the power spectrum for the fat concentration-dependent

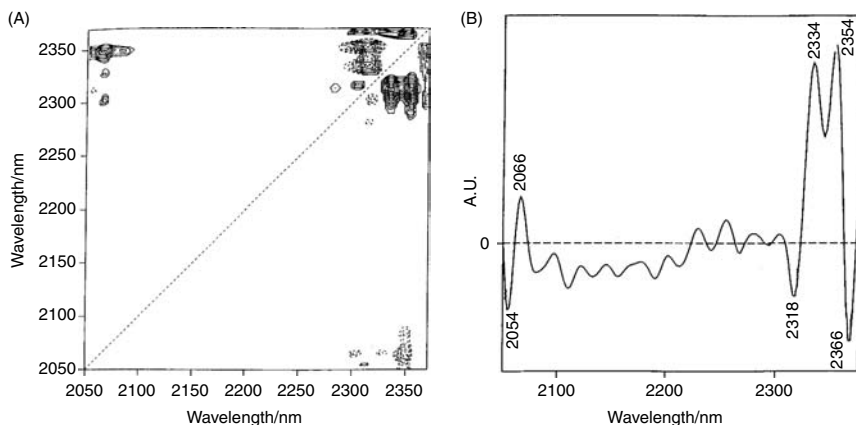


**Figure 13.4** (A) An asynchronous 2D NIR correlation spectrum in the 2000–2400 nm region constructed from fat concentration-dependent spectral changes of milk after the pretreatments. (B) A slice spectrum along the 2311 nm line in the asynchronous spectrum shown in (A). (Reproduced with permission from B. Czarnik-Matusewicz *et al.*, *Appl. Spectrosc.*, **53**, 1582 (1999) (Ref. 19). Copyright (1999) Society for Applied Spectroscopy.)

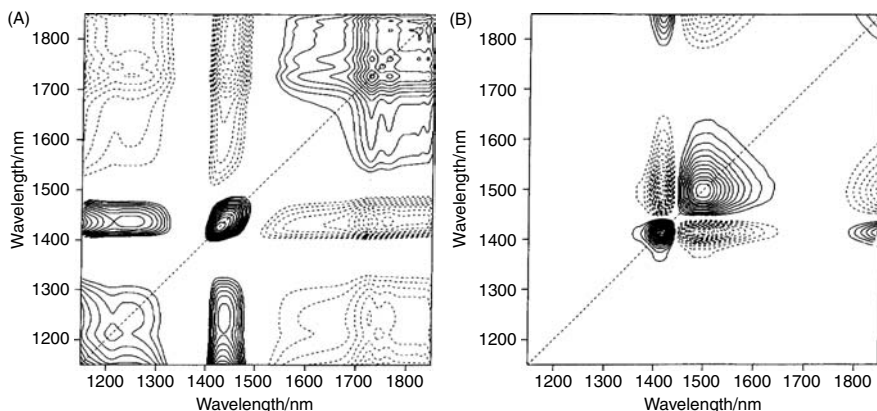
spectra (Figure 13.3(A)) correspond to the bands due to fats, while those in the power spectrum for the protein concentration-dependent spectra (Figure 13.3(B)) are ascribed to proteins. Therefore, 2D correlation spectroscopy can select the bands due to fats and proteins separately from the complicated NIR spectra of milk.

Figure 13.4(A) shows an asynchronous 2D NIR correlation spectrum generated from fat concentration-dependent spectra of milk. Figure 13.4(B) depicts a slice spectrum at 2311 nm, which is a characteristic wavelength for fat species. Peaks are observed at 2054, 2066, 2318, 2334, 2354, and 2366 nm in the slice spectrum. In general, asynchronous correlation spectra have more powerful deconvolution ability for highly overlapped bands. Czarnik-Matusewicz *et al.* proposed band assignment for the 2000–2400 nm region of the NIR spectra of milk based upon the results of 2D correlation analysis (Table 13.1).<sup>19</sup>

Figure 13.5 depicts synchronous 2D correlation spectra in the 1100–1900 nm region constructed from the fat and protein concentration-dependent spectra. The two synchronous spectra show marked differences in the 1400–1500 nm region, where bands due to the combination modes of various species of water are expected to appear. Proteins are hydrophilic, while fats are hydrophobic. Therefore, the protein–water interaction and the fat–water interaction are quite different from each other, giving large spectral differences in the 1400–1500 nm region. It is noted that the bands in the 1400–1500 nm region are separated into two in the synchronous spectrum for the protein concentration-dependent spectra (Figure 13.5(B)). It is very likely that the two autopeaks at 1419 and 1485 nm arise from bulk and hydrated water, respectively.

**Table 13.1** Proposed assignment of bands observed in the 2D synchronous and asynchronous spectra of milk (from Ref. No. 19)

| Band position (nm) | Assignment                                       |
|--------------------|--------------------------------------------------|
| 2382–2366          | $\nu_s(\text{CH}_2) + \nu_s(\text{CH}_2) - psch$ |
| 2355–2351          | $\nu_s(\text{CH}_3) + \nu_s(\text{CH}_3) - psch$ |
| 2346–2345          | $\nu_s(\text{CH}_2) + \nu_s(\text{CH}_2) - fcch$ |
| 2335–2332          | $\nu_s(\text{CH}_2) + \nu_a(\text{CH}_2) - psch$ |
| 2318               | $\nu_a(\text{CH}_2) + \nu_a(\text{CH}_2) - psch$ |
| 2308–2311          | $\nu_s(\text{CH}) + \nu_s(\text{CH}) - fcch$     |
| 2186               | Amide A + Amide III                              |
| 2160               | Amide B + Amide II                               |
| 2124               | Amide B + Amide I                                |
| 2100               | Amide B + Amide I                                |
| 2075–2038          | Amide A + Amide I                                |

**Figure 13.5** Synchronous 2D NIR correlation spectra in the 1100–1900 nm region constructed from (A) fat and (B) protein concentration-dependent spectral changes of milk after the pretreatments. (Reproduced with permission from B. Czarnik-Matusewicz *et al.*, *Appl. Spectrosc.*, **53**, 1582 (1999) (Ref. 19). Copyright (1999) Society for Applied Spectroscopy.)

This study has clearly demonstrated that 2D correlation analysis holds considerable promise in unraveling the NIR spectra of complicated biological materials. By using the power spectra along the diagonal line of synchronous correlation spectra generated from fat and protein concentration-dependent spectra of milk, one can easily pick up bands arising from milk fats and proteins. This ability is obviously quite useful for band assignments of NIR spectra. Moreover, it enables one to understand why particular wavelengths are selected to predict the concentrations of particular components in chemometrics models.

### 13.2 2D IR STUDY OF SYNTHETIC AND BIOLOGICAL APATITES

Structural changes associated with the maturation of poorly crystalline hydroxyapatite (HA) in both synthetic and biological systems have long been investigated by means of various techniques. IR spectroscopy is one of the most powerful techniques for monitoring such changes. However, it is not straightforward to analyze the 1200–900  $\text{cm}^{-1}$  region where the  $\nu_1$  and  $\nu_3$  modes of the phosphate group appear, because the region consists of severely overlapped bands. To unravel the overlapping bands, various methods, such as Fourier self-deconvolution, curve fitting, and second derivative spectroscopy, have been used with varying degree of success. Gadaleta *et al.* employed 2D correlation spectroscopy for the analysis and interpretation of the  $\nu_1$  and  $\nu_3$  phosphate region of the synthetic and biological HA.<sup>11</sup>

Figure 13.6 displays IR spectra in the 1200–900  $\text{cm}^{-1}$  region of HA synthesized at variable pH. Note that a number of bands overlap each other in this region. Synchronous and asynchronous 2D IR correlation spectra of synthetic mineral prepared at variable pH are shown in Figure 13.7.<sup>11</sup> At least seven autopeaks are observed at 1150, 1126, 1095, 1036, 1018, 999 and 961  $\text{cm}^{-1}$  in the synchronous maps. The peaks at 1126, 1036, and 1018  $\text{cm}^{-1}$  correspond to vibrations of  $\text{PO}_4^{3-}$  in a nonstoichiometric/acid phosphate environment of poorly crystalline HA, while those at 1095, 999, and 961  $\text{cm}^{-1}$  correspond to vibrations of  $\text{PO}_4^{3-}$  in an apatitic or stoichiometric environment of poorly crystalline HA. Virtually all peaks due to stoichiometric HA are positively correlated with each other, as are peaks due to nonstoichiometric or acid phosphate HA. Moreover, the former peaks are all negatively correlated with each of the latter peaks. In this way, 2D correlation spectroscopy clearly classified two kinds of peaks. It was concluded that the intensities of the peaks associated with stoichiometric HA simultaneously increase, as the peaks associated with nonstoichiometric or acid phosphate HA decrease.

In this study, interest in the asynchronous spectrum lies in the possibility of peak determination, partly because the positions of the bands can be used to characterize the environment of the phosphate ion, and partly because the peak positions can be employed as input parameters for curve-fitting algorithms. In the asynchronous spectrum, new bands are observed at 1075, 1045, and 1032  $\text{cm}^{-1}$ . The 2D correlation study suggested a new assignment for a band at 1145  $\text{cm}^{-1}$ .<sup>11</sup> This band had been previously attributed to  $\text{HPO}_4^{2-}$  in apatites, but it was reassigned to  $\text{PO}_4^{3-}$  vibrations in an apatitic or stoichiometric environment of poorly crystalline HA.

Figure 13.7 shows the synchronous and asynchronous 2D IR correlation spectrum for a series of IR microscopy spectra obtained from the transitional region of calcified turkey leg tendon.<sup>11</sup> In the synchronous maps five auto-peaks are developed at 1147, 1126, 1075, 1039, and 1019  $\text{cm}^{-1}$ . The peaks at 1126, 1039,

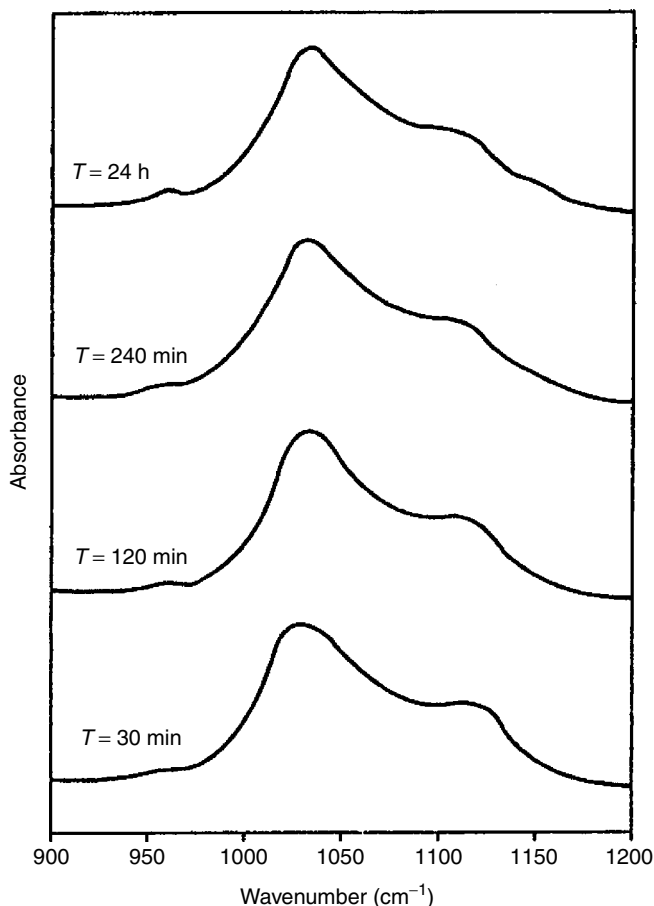


**Figure 13.6** IR spectra of the  $\nu_1, \nu_3$  phosphate region of HA synthesized at variable pH. Times indicated are the times when the aliquots were withdrawn. (Reproduced with permission from Ref. No. 11. Copyright (1996) John Wiley & Sons, Inc.)

and  $1019\text{ cm}^{-1}$  correspond to vibrational modes of  $\text{PO}_4^{3-}$  in a nonstoichiometric or acid phosphate environment of poorly crystalline HA, whereas the peak at  $1075\text{ cm}^{-1}$  represents a mode due to  $\text{PO}_4^{3-}$  in an apatitic or stoichiometric environment of poorly crystalline HA. The mineral from the turkey tendon shows a 2D pattern very similar to that of synthetic HA prepared at variable pH. Gadaleta *et al.* deduced from this comparison of the 2D IR spectra between the synthetic and biological apatites that the spatial variation of HA maturation of mineral along the tendon matches the temporal variation of HA formed at variable pH.<sup>8</sup> This study provided new insight into the subtle changes accompanying the maturation of synthetic and biological apatites, as investigated with IR spectroscopy.

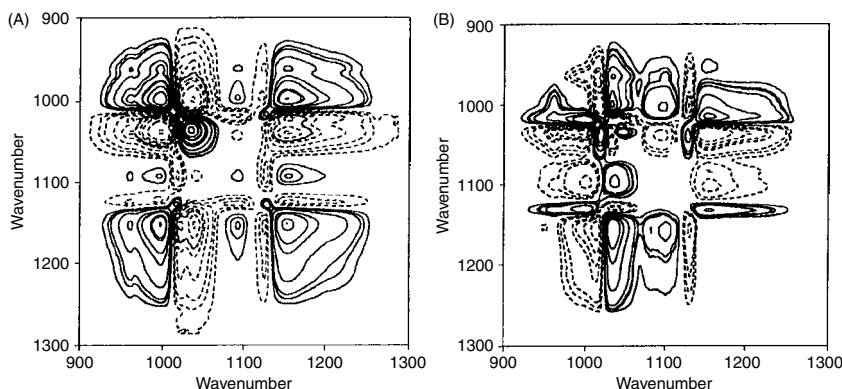


**Figure 13.7** (A) Synchronous and (B) asynchronous 2D IR correlation spectra of synthetic mineral prepared at variable pH. Solid lines represent positive peaks while dashed lines represent negative bands. (Reproduced with permission from Ref. No. 11. Copyright (1996) John Wiley & Sons, Inc.)

### 13.3 IDENTIFICATION AND QUALITY CONTROL OF TRADITIONAL CHINESE MEDICINES

The historical development of *traditional Chinese medicines* (TCMs) is based on the unique underlying theory of holistic Chinese pharmacy, where medicinal materials must take effect in curing diseases as a whole entity. As such, the study of individual components of the medicinal mixture alone is not sufficient for the determination of the efficacy of the medicine, unless the synergistic effect of complex interactions is fully taken into account. Thus, an analytical method which examines the whole mixture of a TCM without separating the individual components is desired.

Sun *et al.* proposed an interesting use of 2D IR spectroscopy to develop a simple, rapid, computer-aided, nondestructive and accurate technique to control the quality of a TCM by taking advantage of the complexity of such mixture.<sup>20–22</sup> In their approach, a TCM sample is subjected to a simple thermal treatment with a fixed rate of temperature rise, which inevitably produces very complex changes in the IR spectrum due to countless transformations and interactions of the mixture ingredients. It is obviously very difficult to figure out the individual mechanisms behind intricate changes in spectral intensities. Interestingly, however, the observed complex spectral changes are very much characteristic of the specific TCM mixture. By transforming the set of such complex IR data into 2D correlation spectra, one can immediately visualize the distinct pattern specific to this TCM. By combining the well-known fingerprint characteristics of IR spectra with distinct visually recognizable patterns of autopeaks and cross peaks generated by 2D IR spectroscopy, both raw material herbs and final TCM products could be readily classified and identified. Even the harvesting region and growing environment (e.g., wild or cultivated) of raw herbs could often be differentiated.

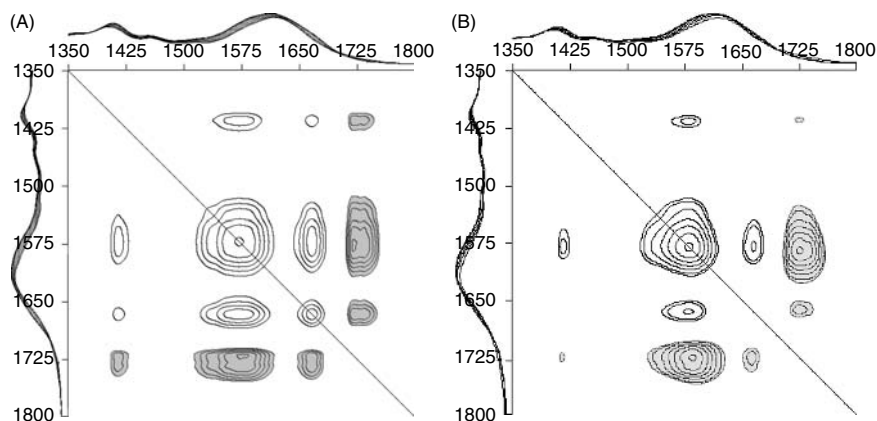


**Figure 13.8** Synchronous 2D IR correlation spectra of (A) fresh and (B) slightly deteriorated Qing Kai Ling injection.

Different kinds of formula granules or injections, similar granules or injections made by different manufacturers or different batches, or different proportions of auxiliary materials in the TCM products could also be compared and identified.

Figure 13.8 shows the example of 2D IR spectra of a common TCM injection formulation called *Qing Kai Ling*.<sup>21</sup> The injection is used for circulatory and phlogistic diseases and virosis and inexplicable fever. It is derived from the broth of a complex mixture of Baikal skullup root, bezoars, honeysuckle flower, isatis root, buffalo horn, and other ingredients. Figure 13.8(A) is the synchronous 2D IR spectrum of fresh Qing Kai Ling, while (B) represents that of slightly deteriorated sample, which was made by exposing it to open air for 48 h. Although the deterioration of the injection formulation is a gradual process, subtle compositional changes caused by the deterioration which are hard to be seen by conventional IR could be readily detected as the visually recognizable pattern difference of 2D IR spectra.

The deterioration of the Qing Kai Ling injection in air at room temperature is believed to be caused mainly by the oxidation of flavone compounds, which are associated with a conjugated carbonyl and hetero-aromatic ring. When a set of IR spectra is expanded to 2D correlation spectra, detailed spectral features could be immediately recognized, which could not be examined easily in conventional IR spectra. The enhancement of spectral resolution by 2D analysis is also a powerful feature here. The peak around  $1611\text{ cm}^{-1}$  is resolved into three separate overlapping bands at 1572, 1667, and  $1729\text{ cm}^{-1}$ . These bands are assigned to

the contributions from alkaloids (heterocyclic compounds), flavone derivatives, and some other carbonyl-containing compounds in Qing Kai Ling.

2D IR spectroscopy provides not only a powerful analytical method to study compounds such as TCM for basic research and development purposes but also an interesting practical tool to determine different TCMs, as well as the quality control of TCMs. The 2D spectral patterns of complex mixture are very diverse and distinct. They are also easily recognized by simple visual inspection. Therefore, heuristic 2D fingerprints of TCMs can be easily established for practical identification purpose. A comprehensive compilation of over several hundred 2D IR spectra of genuine TCMs, as well as some fakes and inferior products, is now available in the form of a convenient atlas.<sup>20</sup>

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# 14 Application of Heterospectral Correlation Analysis

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## 14.1 CORRELATION BETWEEN DIFFERENT SPECTRAL MEASUREMENTS

One of the noteworthy recent trends in the application of 2D correlation spectroscopy is the increased popularity of 2D heterospectral correlation analysis, where separate spectroscopic data obtained by means of two different electromagnetic probes are compared to each other by using the 2D correlation method.<sup>1–4</sup> If there is any commonality between the response patterns of system constituents monitored by two different problems under the same perturbation, one should be able to detect the correlation even between different classes of spectral signals, as described in Section 2.2.4 of Chapter 2. By correlating the less understood spectral features (band assignment, intensity variation, etc.) detected by one electromagnetic probe with the better understood features from another probe, one may resolve the mystery of an uncharted spectral world.

There are two major streams for the applications of 2D heterospectral correlation analysis. The first stream is concerned with heterocorrelation between completely different types of spectroscopic or physical techniques, such as IR and X-ray scattering heterospectral correlation analysis.<sup>5</sup> This type of heterospectral correlation is useful for investigating the structural and physical properties of materials under a particular external perturbation. For examples, Nagai *et al.*<sup>6</sup> reported IR/ESR heterospectral correlation analysis of the Na ion-implanted SiO<sub>2</sub>/Si system, and Choi *et al.*<sup>7</sup> employed X-ray absorption (XAS)/Raman heterospectral analysis for the study of the electrochemical reaction mechanism of lithium with CoO.

The second type of heterospectral correlation is concerned with the comparison between closely related spectroscopic measurements.<sup>8–16</sup> A new generation of 2D correlation spectra, such as IR/NIR, IR/Raman, and UV–Vis/NIR, may be used to enhance the content and quality of spectral information. One can find a number of intriguing examples of such studies. The earliest attempt of this type of 2D correlation can be traced back to the IR/NIR statistical 2D correlation of Barton II *et al.*<sup>9</sup> With the introduction of the formal generalized 2D correlation scheme, a large number of studies have been carried out in the field. For example, Awichi *et al.* carried out 2D IR/NIR heterospectral correlation for the mutarotation of

glucose in water.<sup>10</sup> Czarnecki *et al.* also reported an IR/NIR correlation study of Nylon-11.<sup>11</sup> Jung *et al.* studied concentration-dependent structural variations of  $\beta$ -lactoglobulin (BLG) in phosphate buffer (pH 6.6) using 2D IR/Raman heterospectral correlation spectroscopy.<sup>12</sup> Schultz *et al.* utilized 2D IR, 2D NIR and 2D IR/NIR correlation spectroscopy to explore conformational changes of ribonuclease A (Rnase A) upon thermal unfolding.<sup>13</sup> In this chapter several illustrative examples of representative 2D heterospectral correlation studies are discussed to show the exciting potential of this approach.

## 14.2 SAXS/IR DICHROISM CORRELATION STUDY OF BLOCK COPOLYMER

Noda reported the first heterospectral correlation analysis, which was carried out between the small angle X-ray scattering (SAXS) and IR dichroism spectroscopy measurement of a SBS triblock copolymer sample.<sup>5</sup> This triblock copolymer consisting of two polystyrene (PS) blocks and one polybutadiene (PB) middle block within the same molecular chain was microphase separated to form an ordered submolecular scale domain structure due to the incompatibility between PS and PB blocks. The dynamic rheo-optical 2D SAXS study of the SBS triblock copolymer film under a sinusoidally varying tensile deformation has already been described in Section 8.31 of Chapter 8. The apparent splitting of the local scattering intensity maximum corresponding to the  $d_{100}$  plane of the system into doublet peaks was attributed to the strain-induced change in the interdomain Bragg distance between hexagonally packed microphase domains. A similar dynamic rheo-optical experiment using a polarized IR beam (i.e., DIRLD experiment described in Section 8.1) was also conducted to measure the molecular segmental orientation dynamics of the same triblock copolymer under deformation.

Figure 14.1(A) shows the synchronous heterospectral 2D correlation spectrum obtained from the dynamic small angle X-ray scattering (SAXS) and IR dichroism dual measurements. The dynamic SAXS and IR dichroism results obtained for the same material under the same deformation condition were then compared by using the 2D heterospectral correlation scheme derived in Section 2.2.4. The 2D correlation intensity is plotted on a spectral plane defined by the scattering vector  $q_1$  on one axis and IR wavenumber  $\nu_2$  on the other. The synchronous 2D SAXS spectrum of this block copolymer shows the appearance of doublet peaks at scattering vector coordinates of 0.177 and 0.207 nm<sup>-1</sup> near the  $d_{100}$  peak located around 0.192 nm<sup>-1</sup>. The splitting of the  $d_{100}$  peak into a doublet is attributed to the dynamic position shift of scattering local maximum along the scattering vector axis. The shift is caused by the change in the interdomain Bragg distance of the microphase-separated hexagonally close packed cylindrical PS domains of this block copolymer system. The corresponding asynchronous 2D IR spectrum (not shown) of the same system, carried out by applying the deformation amplitude and frequency identical to the SAXS experiment, indicates

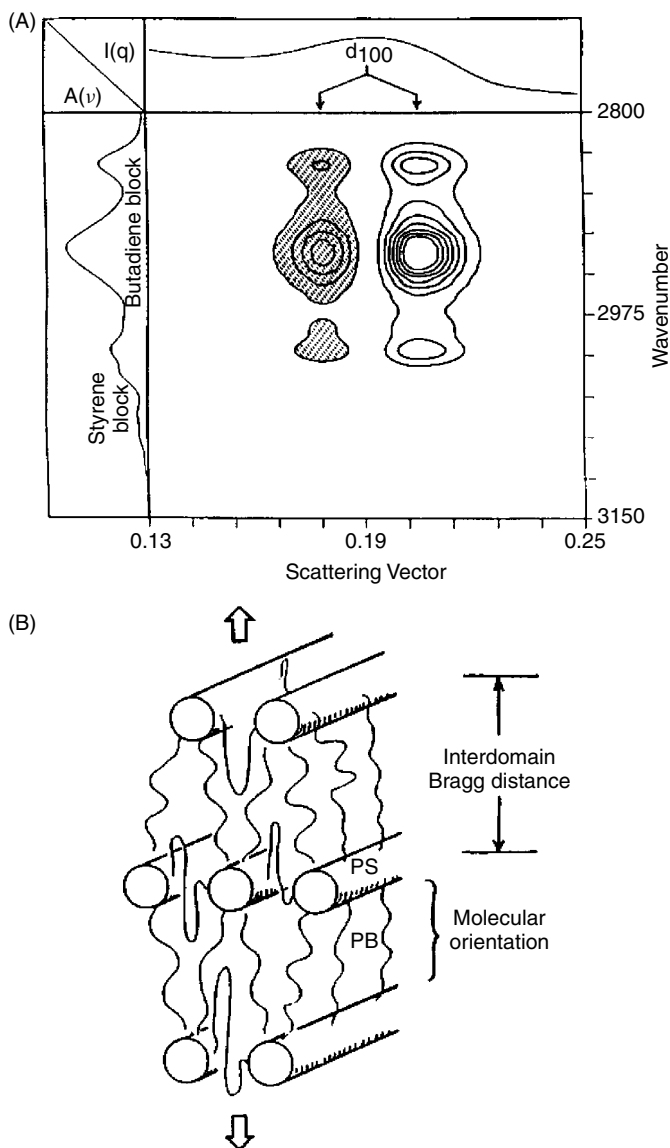


**Figure 14.1** (A) Synchronous 2D SAX/IR heterospectral correlation spectrum of a microphase-separated SBS block copolymer film. (Reproduced with permission from Ref. No. 5. Copyright (1990) Chemtracts.) (B) Coupling between supermolecular scale interdomain Bragg distance change and submolecular scale segmental orientation of matrix domain under a macroscopic deformation. (Copyright © by Chemtracts, originally published in *Chem Tracts: Macromolecular Chemistry*, 1(2): 89–105 and reproduced here with permission.)

the presence of strain-induced reorientational motions of polymer chain segments. The DIRLD signals detected for the reorientation of polymer chain segments are predominantly from the soft continuous matrix PB domain.

The 2D heterospectral correlation spectrum shown in Figure 14.1(A) provides information quite different from that obtained individually from the corresponding 2D IR or 2D SAXS spectra. First of all, autopeaks no longer exist for this 2D correlation spectrum. The two spectral axes are of fundamentally different origin, so a diagonal line of symmetry cannot be assigned to the spectrum. Heterospectral correlation cross peaks are found at the spectral coordinate near the scattering vector position for the  $d_{100}$  Bragg spacing of hexagonally packed cylindrical microdomains consisting of PS segments and the wavenumber ranges for IR absorption arising predominantly from the  $\text{CH}_2$  stretching vibrations of PB segments in the matrix domain. The cross peaks reveal the strong correlation between the dynamic changes in the distance between the dispersed PS microdomains (measured by SAXS) and the molecular orientation of the PB segments in the continuous matrix domain (measured by IR dichroism). When the hard PS domains are spread apart by deformation, the matrix PB molecular segments connecting the PS domains must *simultaneously* be stretched (Figure 14.1(B)). Thus, the supermolecular scale reorganization of PS microdomains and submolecular scale reorientation of rubbery PB matrix material are synchronously correlated under a macroscopic deformation.

### 14.3 RAMAN/NIR CORRELATION STUDY OF PARTIALLY MISCIBLE BLENDS

Ren *et al.* utilized 2D Raman/NIR heterospectral correlation analysis in the investigation of the specific interactions in partially miscible blends of poly(methyl methacrylate) (PMMA) and poly(4-vinylphenol) (PVPh).<sup>14–16</sup> It is well known that PMMA and PVPh are partially miscible through hydrogen bonding between the C=O of PMMA and the OH of PVPh. Ren *et al.* measured FT-Raman and NIR spectra of PMMA/PVPh blends with the PVPh content from 1 to 10 wt% with an increment of 1 wt% and used 2D Raman/NIR heterospectral correlation to analyze the spectra. Figure 14.2(A) and (B), respectively, shows FT-Raman and NIR spectra of PMMA and its blends with PVPh contents of 5 and 10 wt% in the ranges  $1770\text{--}1570\text{ cm}^{-1}$  and  $7500\text{--}6500\text{ cm}^{-1}$ .<sup>14</sup> These regions contain key bands for the specific interactions in the partially miscible blends. A Raman band at  $1729\text{ cm}^{-1}$  is due to the free C=O stretching mode of PMMA, and a band at  $1614\text{ cm}^{-1}$ , whose intensity increases with the increase in the PVPh content, is readily assignable to the aromatic ring stretching mode of PVPh. In the NIR region, a weak feature is observed at  $6764\text{ cm}^{-1}$ . As the intensity of this band increases with the PVPh content, it is assigned to this component.

Figure 14.3(A) and (B) illustrates synchronous and asynchronous 2D Raman/NIR heterospectral correlation spectra between the  $1770\text{--}1570\text{ cm}^{-1}$  and

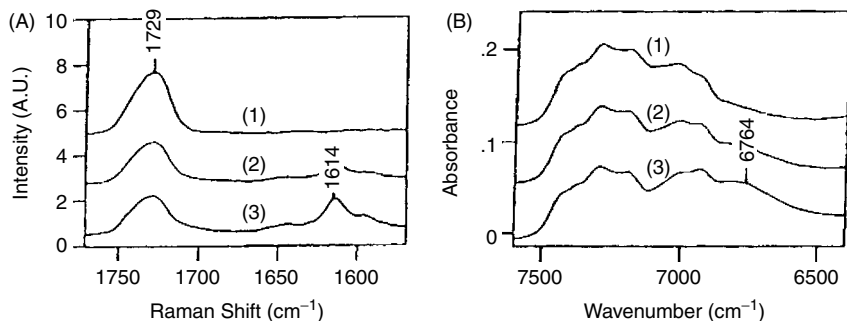


**Figure 14.2** (A) FT-Raman and (B) NIR spectra of PMMA (1) and its blends with PVPh of contents 5 (2) and 10 (3) wt%. (Reproduced with permission from Ref. No. 14. Copyright (2000) American Institute of Physics.)

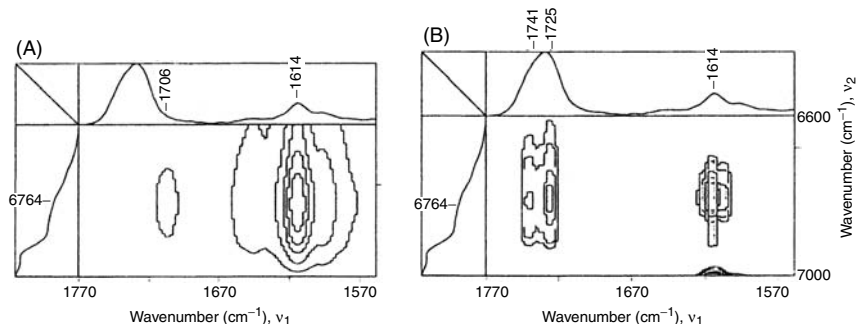


**Figure 14.3** (A) Synchronous and (B) asynchronous 2D Raman/NIR heterospectral correlation spectra generated from FT-Raman and NIR spectra of PMMA/PVPh blends with PVPh contents from 1 to 10 wt%. (Reproduced with permission from Ref. No. 14. Copyright (2000) American Institute of Physics.)

7000–6600 cm<sup>-1</sup> regions, generated from the FT-Raman and NIR spectra of PMMA and partially miscible PMMA/PVPh blends with PVPh content from 1 to 10 wt%. It is noted in Figure 14.3(A) that a cross peak is developed at (6764, 1706 cm<sup>-1</sup>). It had been reported that a Raman band at 1706 cm<sup>-1</sup> due to the C=O stretching mode of the C=O...H-O species is identified in the one-dimensional spectra only for blends containing more than 33 wt% PVPh. The band at 6764 cm<sup>-1</sup> has only synchronous correlation but not asynchronous correlation with the hydrogen-bonded band at 1706 cm<sup>-1</sup>, so that the band at 6764 cm<sup>-1</sup> is also ascribed to the C=O...H-O species. It is most likely due to the first overtone of the hydrogen-bonded OH stretching mode of PVPh. The 2D heterospectral analysis revealed that the two bands at 6764 and 1706 cm<sup>-1</sup> are involved in the specific interaction between PMMA and PVPh that is responsible for the partial miscibility of the blends. Figure 14.3(B) reveals the fact that the band at 6764 cm<sup>-1</sup> has asynchronous correlation with bands at 1741 and

$1725\text{ cm}^{-1}$  due to the free carbonyls of PMMA. The absorption peak at  $1729\text{ cm}^{-1}$  observed in the one-dimensional spectra of Figure 14.2(A) is now seen as two separate bands in the 2D spectra of Figure 14.3(B). The true origin of the band split is not yet clear, but the two bands may well represent different conformers of the C=O groups of PMMA.

#### 14.4 ATR/IR–NIR CORRELATION STUDY OF BIS (HYDROXYETHYL TEREPHTHALATE) OLIGOMERIZATION

In Chapter 11, a 2D ATR/IR correlation study of real-time monitoring of the initial oligomerization of BHET was outlined. In this section the ATR/IR–NIR heterospectral analysis of the same system is described. Before we discuss heterospectral correlation maps of the initial oligomerization of BHET, the corresponding 2D NIR correlation spectra are briefly explained.<sup>17–19</sup>

Figure 14.4 displays time-dependent NIR spectral variations of the reaction mix shown in Figure 11.1. Amari and Ozaki explored the whole region of NIR spectra using 2D correlation analysis.<sup>17</sup> However, here we focus on the  $6400\text{--}5600\text{ cm}^{-1}$  region because this region is very important for the heterospectral

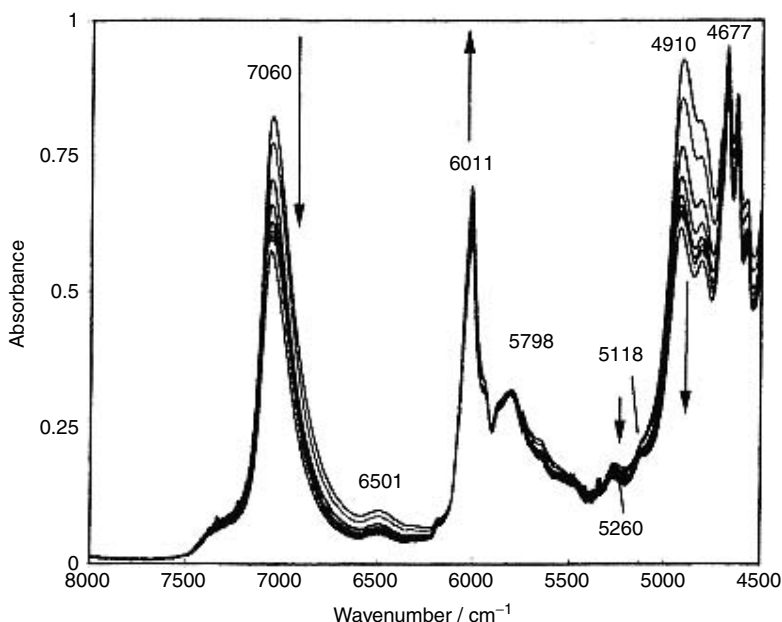


**Figure 14.4** NIR spectra measured *in situ* during the oligomerization reaction in the  $8000\text{--}4500\text{ cm}^{-1}$  region. Arrows show the directions of intensity changes during the course of the reaction. (Reproduced with permission from Ref. No. 19. Copyright (2002) American Chemical Society.)

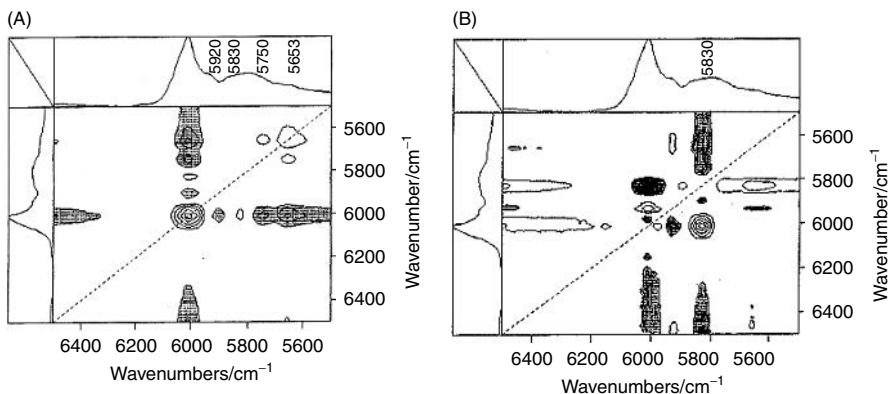


**Figure 14.5** (A) Synchronous and (B) asynchronous 2D NIR correlation maps in the 6500–5500  $\text{cm}^{-1}$  region of the oligomerization at 230 °C. ((A) Reproduced with permission from Ref. No. 19. Copyright (2002) American Chemical Society.) ((B) Reproduced with permission from Ref. No. 16. Copyright (2002) American Chemical Society.)

analysis. An intense band at 6011  $\text{cm}^{-1}$  and a medium band at 5798  $\text{cm}^{-1}$  are assigned to the first overtones of aromatic and aliphatic CH stretching modes, respectively. Figure 14.5(A) and (B) shows synchronous and asynchronous 2D NIR correlation maps in the 6500–5500  $\text{cm}^{-1}$  region of the oligomerization reaction at 230 °C. An intense autopeak is developed at 6005  $\text{cm}^{-1}$ . This peak is due to the first overtone of the aromatic C–H stretching vibration that increases with time. The relative increase in the concentration of the terephthalate aromatic ring with time is the cause of this intensity change. Peaks in the 5900–5500  $\text{cm}^{-1}$  region have negative cross peaks with the aromatic C–H band. These bands originate from the aliphatic C–H stretching vibrations. The relative contributions of the aromatic C–H bonds to the NIR spectra increase, while those of the aliphatic C–H bonds decrease with the oligomerization, if one ignores the contribution from EG that is expelled from the reaction system.

At 230 °C, the synchronous map looks somewhat complex. An increasing peak is identified at 5830  $\text{cm}^{-1}$ . The corresponding asynchronous map at 230 °C is shown in Figure 14.5(B). The two peaks at 6005 and 5830  $\text{cm}^{-1}$  share a cross peak. This shows that the peak at 5830  $\text{cm}^{-1}$  increases out of phase with the aromatic C–H band at 6005  $\text{cm}^{-1}$ . Therefore, it is very likely that this peak corresponds to the aliphatic C–H vibration of free EG, since the amount of free EG increases with time at 230 °C.

The detection of free EG has been investigated in the IR experiments, so that it should be worthwhile to compare the present result with those obtained from the IR spectra.<sup>17</sup> For this purpose, 2D NIR–IR correlation spectroscopy is employed. In the heterocorrelation analysis, taking data of NIR and IR spectra under the same reaction conditions is imperative. Moreover, using data obtained from separate experiments is questionable, because the size and shape of the probes are somewhat different. The change in the probe structure causes slight differences



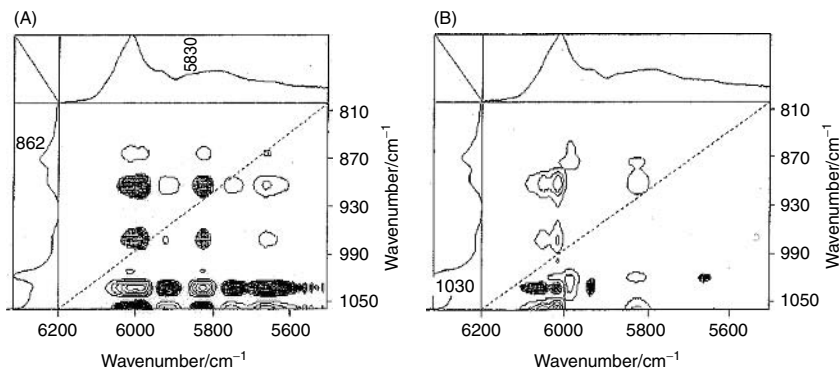


**Figure 14.6** (A) Synchronous and (B) asynchronous 2D NIR/IR heterospectral correlation maps in the  $6500\text{--}5500\text{ cm}^{-1}$  region for NIR and  $1060\text{--}800\text{ cm}^{-1}$  region for IR of the oligomerization at  $235^\circ\text{C}$ . (Reproduced with permission from Ref. No. 19. Copyright (2002) American Chemical Society.)

in the liquid–vapor equilibrium for the separate reactions. To overcome this problem, the reaction vessel was modified so that both ATR/IR and NIR probes could be employed at the same time.

The reaction was carried out at  $235^\circ\text{C}$ . All the data were collected by the same methods as the previous experiments.<sup>17</sup> The first ten spectra (50 min) were used for the 2D correlation analysis. The synchronous and asynchronous 2D hetero-NIR/IR correlation maps are shown in parts (A) and (B) of Figure 14.6. The region used was  $6200\text{--}5500\text{ cm}^{-1}$  for NIR, and  $1060\text{--}800\text{ cm}^{-1}$  for IR. The  $1060\text{--}800\text{ cm}^{-1}$  region contains key peaks that are associated with free EG. In the synchronous spectrum, the peak at  $5830\text{ cm}^{-1}$  in the NIR region has positive cross peaks with a band centered at  $862\text{ cm}^{-1}$  and a peak at  $1030\text{ cm}^{-1}$ . In the asynchronous map, the  $5830\text{ cm}^{-1}$  band does not share any cross peak with either of the two peaks. This strongly suggests that the peak at  $5830\text{ cm}^{-1}$  belongs to free EG, and this is in accord with the finding from the NIR spectral analysis of the  $6500\text{--}5500\text{ cm}^{-1}$  region. This case is a good example that shows a potential of 2D heterospectral correlation in extracting the origin of a particular band, which is overlapped with many other bands, to make unambiguous band assignments. 2D NIR correlation study has extracted the peak at  $5830\text{ cm}^{-1}$  of free EG origin in the aliphatic  $\text{CH}_2$  stretching band region in despite of the severe overlap with other bands. The 2D NIR/IR heterospectral correlation analysis has provided an additional strong supporting evidence for the assignment of the peak at  $5830\text{ cm}^{-1}$ .

#### 14.5 XAS/RAMAN CORRELATION STUDY OF ELECTROCHEMICAL REACTION OF LITHIUM WITH $\text{CoO}$

Choi *et al.* introduced 2D correlation spectroscopy to the field of soft X-ray absorption spectroscopy (XAS) and, furthermore, successfully demonstrated the

utility of 2D heterospectral XAS/Raman correlation analysis in nanomaterials research for electrochemical reactions of batteries.<sup>7,20</sup> To investigate the mechanism of the electrochemical insertion of lithium into the CoO electrode in an  $\text{Li}/\text{Li}_y\text{CoO}$  cell, 2D correlation analysis was applied to two sets of spectra for an  $\text{Li}_y\text{CoO}$  system during the first insertion–extraction reaction as probed by lithium concentration-dependent XAS and Raman spectroscopy. 2D XAS and 2D Raman spectra yielded the expected resolution enhancement and showed that the insertion of lithium into the CoO electrode leads to  $\text{Li}_2\text{O}$  formation.<sup>7,20</sup> In addition, 2D heterospectral XAS/Raman correlation analysis was undertaken for the same XAS and Raman spectra. The heterospectral 2D correlation analysis established the correlation between the XAS and Raman bands and confirmed their band assignments. Furthermore, a sequence of events related to XAS and Raman signals from the same species was detected to show the apparent selectivity difference between two probes.

Figure 14.7 shows the oxygen K-edge XAS spectra of the  $\text{Li}_y\text{CoO}$  electrode in an  $\text{Li}/\text{Li}_y\text{CoO}$  cell for the first cycle of electrochemical insertion (discharging) process. The spectrum of pristine  $\text{Li}_2\text{O}$  is also shown for comparison. The main peak of the XAS spectrum of the pristine CoO is at about 529 eV, which is due to the transition of oxygen 1s electron to the hole states in the oxygen 2p level. The broad absorption features above 533 eV can be assigned to the transition

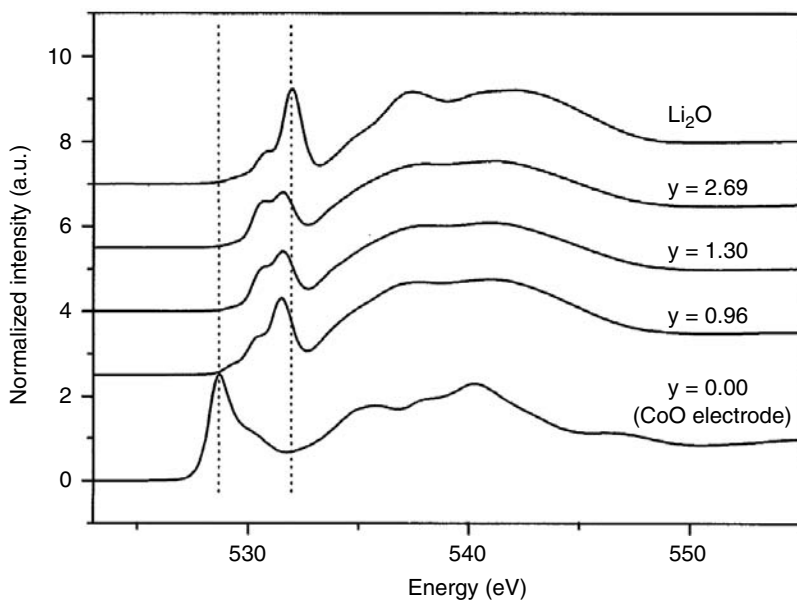


**Figure 14.7** Normalized oxygen K-edge XAS spectra for the electrochemical reaction of lithium with CoO electrode during the first insertion (discharging) process. The lithium content ( $y$  in  $\text{Li}_y\text{CoO}$ ) is as indicated. (Reproduced with permission from Ref. No. 7. Copyright (2003) American Chemical Society.)

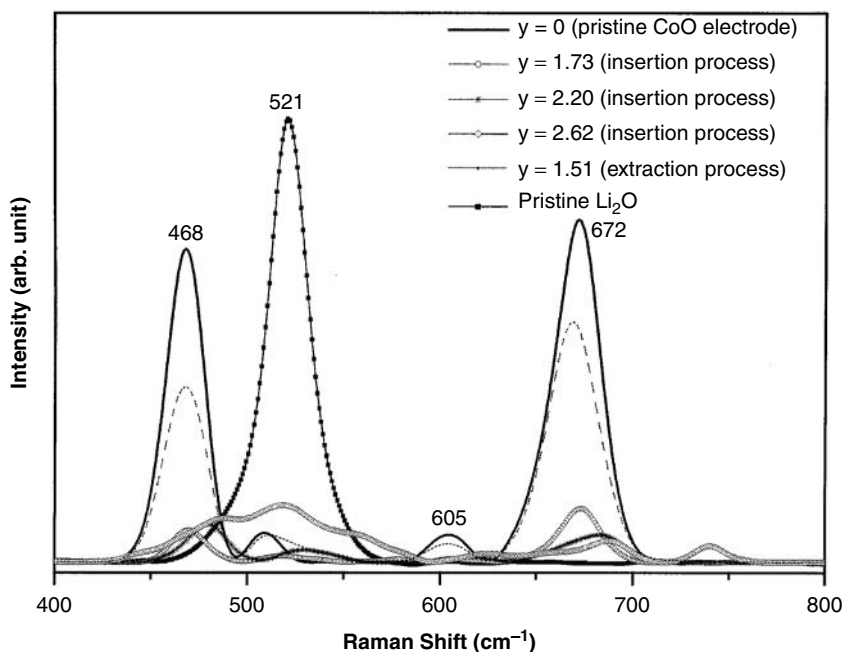
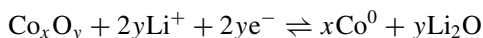


**Figure 14.8** Raman spectra of the  $\text{Li}_y\text{CoO}$  system during the first insertion–extraction process. (Reproduced with permission from Ref. No. 7. Copyright (2003) American Chemical Society.)

to hybridized states of oxygen 2p and Co 4sp orbitals. The XAS spectrum of  $\text{Li}_y\text{CoO}$  changes dramatically when the lithium content ( $y$ ) reaches 0.96 mol. On further insertion of lithium ( $y = 1.30$  and  $2.69$ ), the main changes in the  $\text{Li}_y\text{CoO}$  spectrum compared to  $\text{Li}_{0.96}\text{CoO}$  spectrum are in the relative intensities of two peaks near 531 eV.

Figure 14.8 shows the Raman spectra of  $\text{Li}_y\text{CoO}$  system in the same first insertion–extraction process. The reference spectrum of  $\text{Li}_2\text{O}$  is also shown as a comparison. The band intensities at 672 and  $468\text{ cm}^{-1}$  (assigned to CoO) decrease with the rise in lithium content of the electrode during the first insertion process. The mechanism currently accepted for the reaction of lithium with CoO is given by



The CoO precursor particles in a composite electrode undergo reduction to cobalt nanoparticles dispersed in an  $\text{Li}_2\text{O}$  matrix. Upon the extraction of lithium, cobalt nanoparticles are then reoxidized to nanosized CoO. The observation of less intense Raman bands after the first insertion–extraction process indicates the presence of smaller particles and a lower concentration of CoO, compared with that of pristine CoO electrode.

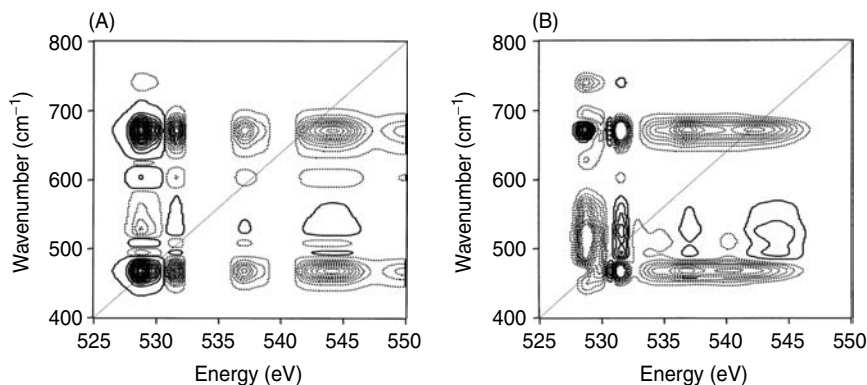
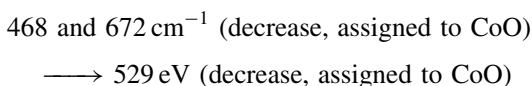


**Figure 14.9** (A) Synchronous and (B) asynchronous 2D heterospectral XAS/Raman correlation spectrum of the  $\text{Li}_y\text{CoO}$  system during the first insertion process. Solid and dotted lines, respectively, represent positive and negative cross peaks. (Reproduced with permission from Ref. No. 7. Copyright (2003) American Chemical Society.)

To examine the detailed mechanism of insertion of lithium into the CoO electrode during the first insertion process and to establish the unambiguous band assignments in the XRS and Raman spectra for this system, 2D heterospectral XAS/Raman correlation analysis was carried out. Figure 14.9(A) shows the synchronous XAS/Raman correlation spectrum. A positive cross peak in the synchronous heterospectral correlation spectrum means that two bands sharing the cross peak may have the same origin, while a negative cross peak means that two bands sharing the cross peak should have different origins. Thus, the negative cross peaks at  $(532 \text{ eV}, 468 \text{ cm}^{-1})$ ,  $(537 \text{ eV}, 468 \text{ cm}^{-1})$ ,  $(544 \text{ eV}, 468 \text{ cm}^{-1})$ ,  $(532 \text{ eV}, 672 \text{ cm}^{-1})$ ,  $(537 \text{ eV}, 672 \text{ cm}^{-1})$ , and  $(544 \text{ eV}, 672 \text{ cm}^{-1})$  show that the bands at 532, 537, and 544 eV in XAS spectra can be assigned to the component different from those giving bands at 468 and  $672 \text{ cm}^{-1}$  assigned to CoO in Raman spectra. The positive cross peaks at  $(532 \text{ eV}, 527 \text{ cm}^{-1})$ ,  $(537 \text{ eV}, 527 \text{ cm}^{-1})$ , and  $(544 \text{ eV}, 527 \text{ cm}^{-1})$  indicate that the Raman band at  $527 \text{ cm}^{-1}$  and the XAS peaks at 532, 537, and 544 eV arise from the same origin, probably related to  $\text{Li}_2\text{O}$  and components of the solid electrode interface. The result suggests that the  $\text{Li}_2\text{O}$  is produced with the insertion of lithium into the CoO electrode.

Figure 14.9(B) shows the asynchronous XAS/Raman correlation spectrum. It can be shown from the straightforward peak sign analysis that the intensities of the Raman bands at 468 and  $672 \text{ cm}^{-1}$  decrease before the decrease of the XAS peak intensity at 529 eV, which in turn occurs before the increase in intensity of the Raman band at  $527 \text{ cm}^{-1}$ . Likewise, the increase in intensity of the Raman band at  $527 \text{ cm}^{-1}$  occurs before that of the XAS peak at 532 eV. By combining the above observations, one can deduce the following sequence of events:



→ 527 cm<sup>-1</sup> (increase, assigned to Li<sub>2</sub>O)

→ 532 eV (increase, assigned to Li<sub>2</sub>O)

The sequential order of the intensity changes of different species are in good agreement with the separate result obtained by 2D XAS and 2D Raman studies. The 2D heterospectral correlation result also shows that both XAS and Raman signal intensities for CoO decrease before those for Li<sub>2</sub>O increase.

Furthermore, the above result gives an additional very interesting insight into the unique feature of 2D heterospectral correlation analysis, i.e., the direct comparison of the specificity of different analytical probes. The simple sign analysis of correlation peaks indicates that even under identical perturbation condition (i.e., changes in the lithium content), some asynchronicity is observed between signals arising from the same species probed by two different techniques. In this particular study, spectral changes detected by the Raman probe always occurred sooner (i.e., at a lower lithium insertion level) than those detected by XAS. The result may seem surprising, at first glance, but actually it really makes sense.

Although XAS and Raman spectra are both affected by the insertion of lithium into the CoO electrode, the specific nature of the effect of lithium insertion on the Raman spectrum is certainly very different from that of XAS. For example, features in the Raman spectrum of a CoO electrode, which are directly related to the molecular vibrations of the system, are easily influenced by the presence of a much lower level of lithium, compared to the corresponding XAS spectrum. Thus, the spectral responses of Raman and XAS under the same perturbation are not expected to be identical to each other. The observed discrepancy must therefore reflect the subtle difference in the selectivity and specificity of these probes toward microscopic or molecular scale changes induced by the applied macroscopic perturbation. Such distinction made for individual analytical probe sensitivities to a given perturbation is a truly unique feature of 2D heterospectral correlation analysis.

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# 15 Extension of Two-dimensional Correlation Analysis to Other Fields

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## 15.1 APPLICATIONS OF 2D CORRELATION BEYOND OPTICAL SPECTROSCOPY

It has been repeatedly shown throughout this book that the generalized 2D correlation scheme is not only a powerful but also a very *versatile* technique applicable to the analysis of many different forms of spectroscopic problems. Up to this point, the discussion on 2D correlation analysis has been focused mostly on optical spectroscopy applications based on measurements made using some sort of electromagnetic probes, such as IR, Raman, X-ray, and UV. However, the possible use of the 2D correlation concept is not really limited to the field of optical spectroscopy. It can be extended further to many forms of measurements aside from conventional spectroscopic studies.

For example, 2D correlation can be effectively combined with other analytical techniques, such as chromatography, thermal and mechanical analyses, mass spectrometry, microscopy, and the like. The 2D correlation method can also be effectively utilized in the theoretical and computational chemistry field. In this final chapter, an exciting possibility will be explored for further extending the application areas of the generalized 2D correlation approach to various fields of scientific studies beyond optical spectroscopy. Specific examples will be given for promising new developments in the application of generalized 2D correlation analysis to techniques such as 2D chromatography and 2D NMR. Readers will immediately notice that the approaches used in these studies are all identical to the formal correlation method described in previous chapters.

## 15.2 2D CORRELATION GEL PERMEATION CHROMATOGRAPHY (GPC)

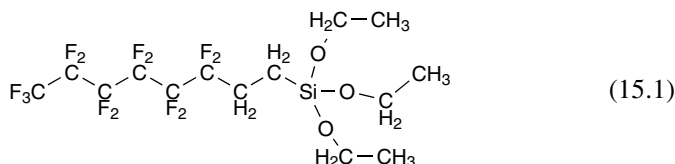
One of the very promising developments in the 2D correlation analysis outside of the spectroscopic field is the use of a correlation scheme in time-resolved chromatographic experiments. In this section, a two-dimensional correlation gel



permeation chromatography (2D GPC) study based on a set of time-resolved chromatograms obtained during a polymerization reaction is used as an illustrative example.<sup>1</sup> The time-resolved GPC trace intensity  $I(E, t)$  can be obtained as a function of the chromatographic elution time  $E$  and reaction sampling time  $t$  for each aliquot, collected during the polymerization reaction process over a time period. A cross-correlation analysis of GPC trace intensities measured at two different elution times,  $E_1$  and  $E_2$ , along the sampling time  $t$  during the reaction yields synchronous and asynchronous 2D GPC correlation maps,  $\Phi(E_1, E_2)$  and  $\Psi(E_1, E_2)$ .

### 15.2.1 TIME-RESOLVED GPC STUDY OF A SOL-GEL POLYMERIZATION PROCESS

As an interesting model system, time-resolved GPC chromatograms obtained during the sol-gel polymerization reaction of 1H,1H,2H,2H-perfluorooctyltriethoxysilane (PFOTES) are examined.



1H,1H,2H,2H-perfluorooctyltriethoxysilane (PFOTES)

The sol-gel polymerization process of tetraalkoxysilane or silane-coupling agents has been extensively studied as a representative example of macroscopic sol-gel transition phenomena.<sup>2,3</sup> As solution processing of silane-coupling agents often involves various intermediates or precursors, better elucidation of the specific nature of these precursors may lead to potential improvements in the preparation of this class of polymers. The time-dependent behavior of randomly polymerizing precursors in the PFOTES-ethanol-1 M HCl-H<sub>2</sub>O system is studied by 2D GPC correlation analysis. Such 2D correlation maps can be used effectively to sort out surprisingly detailed reaction mechanisms of a complex polymerization process.

Time-resolved GPC elution profiles (chromatograms) were obtained by sampling aliquots intermittently during the 30 min of polymerization process of PFOTES catalyzed by 1 M HCl-H<sub>2</sub>O, as shown in Figure 15.1. Such GPC profiles provide information on the time-dependent compositional changes of the system, which in turn directly reflects the details of the polymerization process. The monomer (profile a) has only one elution band at 11.44 min, but after 60 s of the course of reaction (profile b), at least five distinguishable elution bands appear at 10.84 (band E), 11.16 (band D), 11.48 (band C), 12.12 (broad band B) and 13.00 (band A) min. Assignments of these bands are listed in Table 15.1.

Band A, at 13.00 min, is identified as perfluorooctyltriethoxysilane (PFOTHS) produced as a consequence of hydrolysis. Its appearance indicates that, under

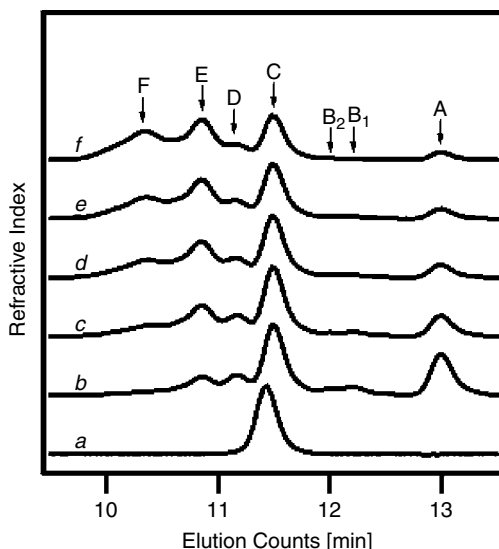


**Figure 15.1** Time-resolved GPC elution profiles of PFOTES (curve a, monomer ( $t = 0$  s); curve b, 60 s; curve c, 300 s; curve d, 600 s; curve e, 1800 s). Assignment of bands A–F is listed in Table 15.1 and discussed in the text. (Reproduced with permission from Ref. No. 1. Copyright (2002) American Chemical Society.)

**Table 15.1** Assignment of GPC peaks

| Elution counts | Band no.       | Tentative assignment             |
|----------------|----------------|----------------------------------|
| 13.0           | A              | Trihydrolyzed – PFOTES (PFOTHS)  |
| 12.2           | B <sub>1</sub> | Dihydrolyzed – PFOTES (PFODHS)   |
| 12.0           | B <sub>2</sub> | Monohydrolyzed – PFOTES (PFOMHS) |
| 11.5           | C              | PFOTES + PFOMHS                  |
| 11.2           | D              | Component I                      |
| 10.8           | E              | Component II                     |
| 10.4           | F              | Component III                    |

the current reaction conditions, the hydrolysis reaction predominantly occurs during the initial 60 s. Bands B<sub>1</sub> and B<sub>2</sub>, at 12.00 and 12.20 min, respectively, are regarded as components which consist of partially hydrolyzed PFOTES monomers, i.e., monohydroxysilane (PFOMHS) and dihydroxysilane (PFODHS). The band C at 11.48 min is assigned to unhydrolyzed PFOTES. The band at 11.16 min and other bands with lower elution counts (bands D, E, and F) come from polymerized precursors (dimer, trimer, and larger oligomers). Thus, the variation in the chromatograms clearly reflects the consumption of monomeric precursors (bands A, B, and C) and production of polymeric precursors (bands D, E, and F).

### 15.2.2 2D GPC CORRELATION MAPS

In order to further understand the details of the polymerization process, 2D correlation analysis is now applied to the set of time-resolved GPC profiles.

#### 15.2.2.1 Stage I

The synchronous 2D correlation map, constructed from time-resolved GPC profiles observed during the period 60–180 s (stage I), is shown in Figure 15.2(A). The synchronous 2D spectrum in this stage has eight cross peaks, showing that every autopeak correlates with at least one other autopeak. A synchronous correlation square (CSq<sub>1</sub>) can be constructed by connecting the two autopeaks ( $E_1 = E_2 = 13.00$ , 10.84) and two cross peaks ( $(E_1, E_2) = (13.00, 10.84)$ ,  $(10.84, 13.00)$ ). The correlation square implies that there exists a coordinated intensity (population) changes between the two elution bands A and E. The very strong intensity of autopeak A at 13.00 min suggests that the PFOTHS monomers are produced following the hydrolysis in the earlier step (0–60 s), and they are rapidly consumed in the condensation reaction. The intensity of band E autopeak is smaller than that of the band A autopeak, indicating a smaller intensity variation in the former peak. The two positive cross peaks, corresponding to bands B<sub>1</sub> and B<sub>2</sub>, indicate that partially hydrolyzed monomers (PFODHS and PFOMHS) are consumed in the subsequent condensation reaction. However, since the intensities of these cross peaks are very weak, these components play only a minor

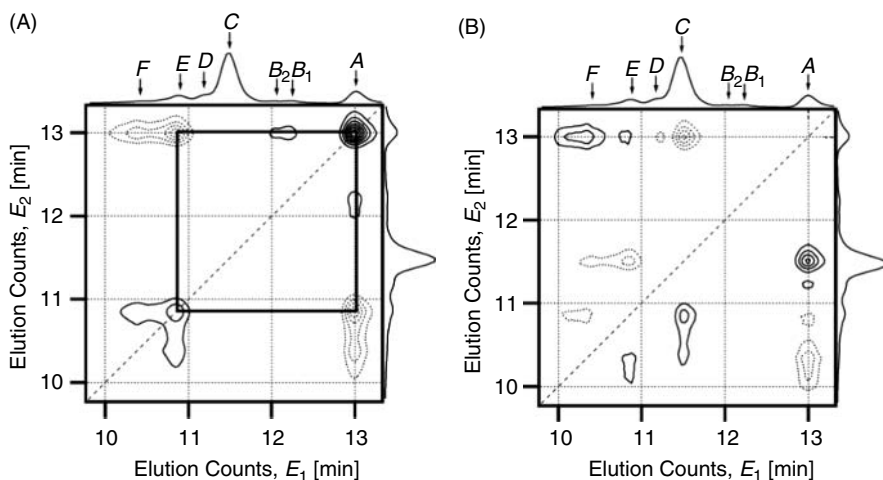


**Figure 15.2** (A) Synchronous and (B) asynchronous correlation maps for stage I, 60–180 s. The solid line indicates positive peaks, and the broken line indicates negative peaks. (Reproduced with permission from Ref. No. 1. Copyright (2002) American Chemical Society.)

role in the overall reduction in intensity. The signs of the two cross peaks arising from bands E and F are negative, reflecting an increase in population of the precursors II and III present among three monomeric species (PFOTHS, PFODHS and PFOMHS).

In the asynchronous 2D correlation map (Figure 15.2(B)) obtained from the time-resolved GPC profiles in stage *I* (60–180 s), we find a relatively strong cross peak at coordinate (13.00, 11.52), which correlates elution band A with band C. The signs of both synchronous and asynchronous cross peaks at the same coordinate are positive. Therefore, the variation in intensity of band A occurs before that of band C. This order of events implies that consumption of PFOTHS monomers, produced by the hydrolysis reaction in the earlier step (0–60 s), occurs during this stage (60–180 s) before further hydrolysis reaction of PFOTES. Since the asynchronous correlation between band E or F and band A provides the negative cross peak at coordinates (13.00, 10.84) or (13.00, 10.34), the variation in intensity of band A occurs after that for bands E or F. Therefore, in stage *I*, consumption of components II and III occurs before that of PFOTHS.

### 15.2.2.2 Stage II

The 2D correlation spectra for stage *II* (240–360 s) are shown in Figure 15.3(A) and (B). In the synchronous correlation map (Figure 15.3(A)), new autopeaks and cross peaks appear, in addition to the auto- and cross peaks found in the synchronous map for stage *I*. The connection of two autopeaks with two cross

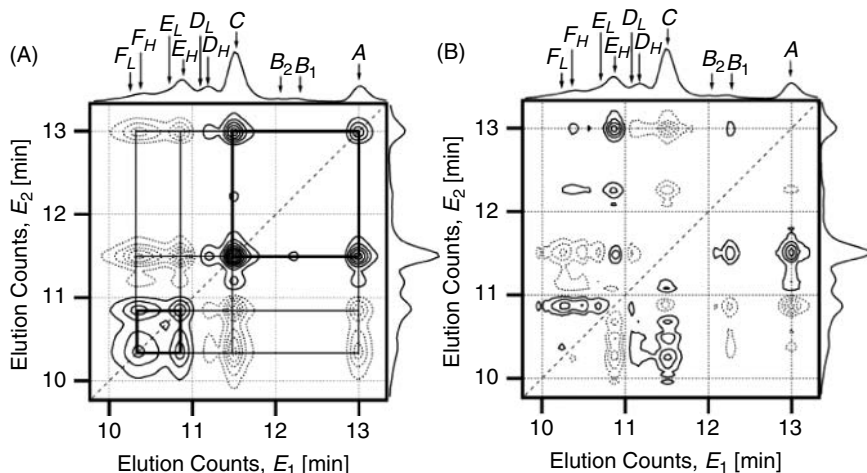


**Figure 15.3** (A) Synchronous and (B) asynchronous correlation maps for stage *II*, 240–360 s. (Reproduced with permission from Ref. No. 1. Copyright (2002) American Chemical Society.)

peaks provides at least six correlation squares. We may classify these correlation squares into three classes: class 1 (CSq<sub>2</sub>, CSq<sub>3</sub>, and CSq<sub>4</sub>), class 2 (CSq<sub>5</sub> and CSq<sub>6</sub>) and class 3 (CSq<sub>7</sub>). In class 1, the correlation square CSq<sub>2</sub> indicates correlation between bands A and C. Furthermore, two correlation squares (CSq<sub>3</sub> and CSq<sub>4</sub>) in class 1 imply correlation between band A and another band, either E or F. In particular, we note that the signs of the two cross peaks, which constitute the CSq<sub>3</sub> and CSq<sub>4</sub> squares, are negative, indicating that the intensity of band A decreases while those of bands E and F increase. Class 2 contains two squares, CSq<sub>5</sub> and CSq<sub>6</sub>, which reflect a reduction in intensity of band C and an increase in intensity of bands E or F. The correlation square CSq<sub>7</sub>, in class 3, reveals that the intensities of bands E and F increase together as a function of sampling time.

The asynchronous 2D correlation spectrum, obtained from the GPC data in stage II, is shown in Figure 15.3(B). When band A on the  $E_1$  axis is compared with bands C and D on the  $E_2$  axis, the signs of both the asynchronous and synchronous cross peaks are all positive. Therefore, in stage II, the decrease in intensity of band A occurs first followed by a decrease in intensity of bands C and D. For the contour maps constructed from band A on the  $E_1$  axis and bands E and F on the  $E_2$  axis, the signs of both the synchronous and asynchronous cross peaks are negative. The increase in intensity of bands E and F occurs first and is followed by the reduction in intensity of band A. The correlation of band C with the polymeric components provides at least five positive cross peaks, demonstrating the resolution-enhancing characteristic of the asynchronous correlation map. Band C decreases in intensity as a consequence of hydrolysis, and the decrease is then followed by the variation in intensity of the polymeric component bands. This resolution-enhancement characteristic of 2D GPC analysis provides further details of subtle changes in GPC profiles in terms of the presence of cross peaks at coordinates (11.12, 10.88) and (11.20, 10.24–10.49). The existence of these cross peaks might be considered to imply the existence of another band between bands D and E and its correlation with the polymeric components. However, the former cross peak can be assigned to the correlation peak between the low-elution component ( $D_L$ ) of band D and the high-elution component ( $E_H$ ) of band E, while assignment of the latter cross peak is to correlation between the high-elution component ( $D_H$ ) of band D and the polymeric component (discussed in stage III). Furthermore, splitting of bands E and F also occurs during this stage, thus providing the high- and low-elution bands (coordinates:  $E_H$  ( $E_1 = 10.92$ ),  $E_L$  ( $E_1 = 10.76$ ),  $F_H$  ( $E_1 = 10.44$ ) and  $F_L$  ( $E_1 = 10.24$ )).

### 15.2.2.3 Stage III

The synchronous and asynchronous correlation maps, derived from the GPC data in 420–600 s (stage III), are shown in Figure 15.4(A) and (B), respectively.

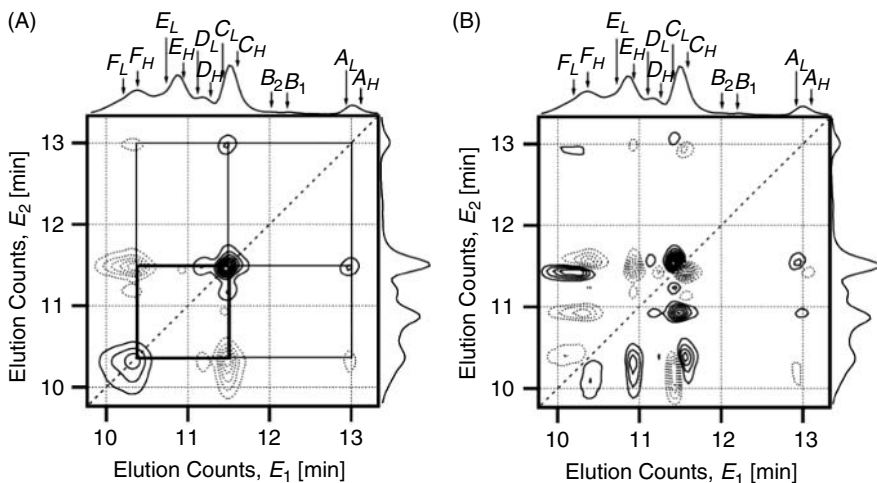


**Figure 15.4** (A) Synchronous and (B) asynchronous correlation maps for stage *III*, 420–600 s. (Reproduced with permission from Ref. No. 1. Copyright (2002) American Chemical Society.)

In the synchronous map, we find the correlation square ( $CSq_{10}$ ), which can be constructed by connecting two autopeaks at 11.44 and 10.24 min and two correlated negative cross peaks. The correlation implies that the strong bands C and F are dominant during this stage, and that the intensity of band C rapidly decreases while that of band F increases. There is no autopeak coming from band A in the synchronous map, probably because of its very weak variation in intensity. However, we may assume the existence of an autopeak at 12.96 min, leading to constitution of two correlation squares,  $CSq_8$  and  $CSq_9$ . Since the four cross peaks at (12.96, 11.44), (11.44, 12.96) and (12.96, 10.24), (10.24, 12.96) are also weak in intensity, we assume that there is little correlation between bands A and C or between bands A and F.

In the asynchronous map (Figure 15.4(B)), obtained from the GPC data during stage *III*, band A consists of two components, arising from enhancement of the spectral resolution. The signs of the coordinates at (12.96, 11.56) and (13.08, 11.44) imply that the intensity decrease of the elution band at 12.96 counts occurs before that of the elution band at 11.56 counts, and that the elution band at 13.08 counts decreases in intensity before the elution band at 11.44 counts. Furthermore, the cross peaks in the region of coordinates (10–12, 10–12) are relatively strong in intensity, reflecting the dominant correlation between band C and the polymeric component bands. Enhancement of the spectral resolution also splits band C into two components ( $C_H$  and  $C_L$ ), which appear at coordinates  $C_H$  ( $E_1 = 11.56$ ) and  $C_L$  ( $E_1 = 11.56$ ) in the asynchronous map. The signs and the order of events indicate that the elution band at 11.56 counts decreases in intensity before that at 11.44 counts. Band D consists of two components ( $D_H$  ( $E_1 = 11.24$ )).

and  $D_L$  ( $E_1 = 11.12$ )), a splitting caused, as before, by enhancement of the spectral resolution. The intense positive cross peaks at (11.56, 10.44) and (11.44, 10.24) are used to interpret the correlation between bands C and F, as well as between the polymeric component bands. The decrease in intensity of the component at 10.44 occurs prior to that of the component at 11.56, and variation in intensity of the elution band on the  $E_2$  axis (10.24) occurs before that of the  $E_1$  elution band (11.44). Moreover, from the resolution-enhancing characteristics of the asynchronous correlation, it is evident that elution band F splits into H and L components.

#### 15.2.2.4 Stages IV and V

The synchronous and asynchronous correlation maps, calculated from the time-resolved GPC data during stage IV (780–1140 s) and stage V (1200–1800 s), are shown in Figures 15.5 and 15.6, respectively. In the synchronous and asynchronous maps, we note that the peaks arising from the correlation between band A and other bands disappear and that the correlation peaks between band C and lower-elution bands are concentrated within the region  $E_1 = E_2 = 10$ –12. This fact implies that most of the PFOTHS monomers which had been hydrolyzed in an earlier step may be consumed by a subsequent condensation reaction in stages IV and V and that the resulting components I and II participate in further reactions to form both polymeric aggregates and PFOTES monomers. In particular, in stage V, autpeak A reappears and a pair of positive and negative cross peaks, arising from the  $E_H$  and  $E_L$  components, respectively, is found, thus reflecting the rapid variation in intensity of the  $E_H$  and  $E_L$  components.

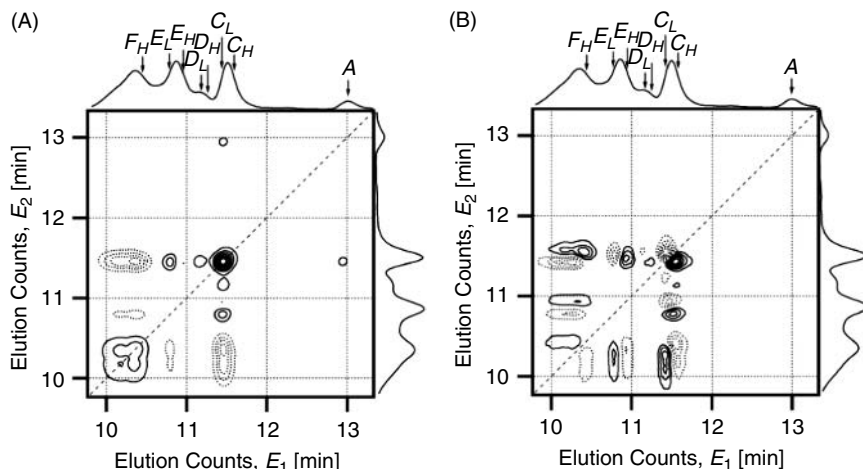


**Figure 15.5** (A) Synchronous and (B) asynchronous correlation maps for stage IV, 780–1140 s. (Reproduced with permission from Ref. No. 1. Copyright (2002) American Chemical Society.)

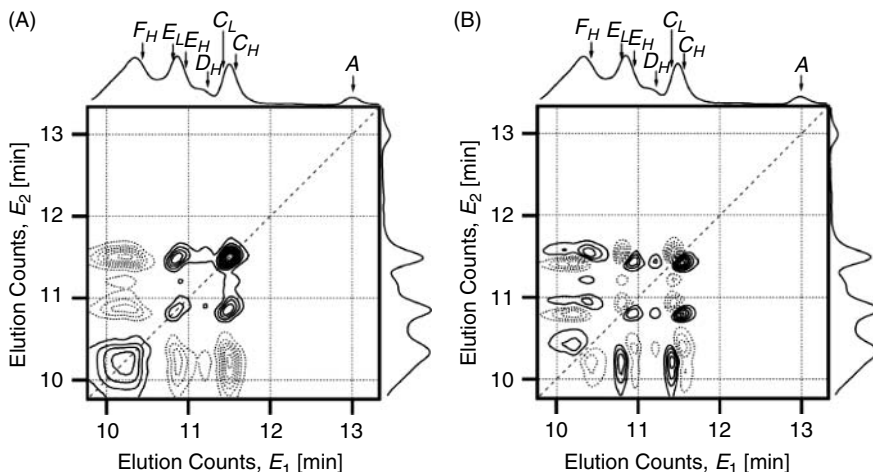


**Figure 15.6** (A) Synchronous and (B) asynchronous correlation maps for stage V, 1200–1800 s. (Reproduced with permission from Ref. No. 1. Copyright (2002) American Chemical Society.)

### 15.2.3 REACTION MECHANISMS DEDUCED FROM THE 2D GPC STUDY

The 2D GPC correlation maps, derived from the time-resolved GPC profiles, reveal the detailed reaction and interaction mechanisms taking place during the polymerization process. In Figure 15.7 a summary of the sequence of these intricate reactions or interactions is presented. The sequence is further discussed below.

*Stage I.* PFOTHS monomers, generated during rapid hydrolysis at the very beginning (0–60 s) of the reaction, produce mainly components II and III, as a consequence of condensation between the PFOTHS monomers. Although there may exist some other reaction-routes (II  $\rightarrow$  III, II or III  $\rightarrow$  highly polymerized component (IV)) during this stage, their total contribution to the whole process is small.

*Stage II.* Following further hydrolysis of PFOTES, the formation of PFOTHS monomers occurs. Furthermore, the trihydroxide (PFOTHS) monomers are consumed to form components II and III. The polymeric components thus produced also contribute to formation of higher components (e.g. II  $\rightarrow$  III and III  $\rightarrow$  IV). In particular, it should be noted that a strong correlation exists between PFOTES and component II (or component III).

*Stage III.* Further hydrolysis of PFOTES results in the formation of PFOTHS. The monomeric molecules thus produced are mostly consumed to form component III, which contributes to the formation of highly polymerized components. During this stage, there exists a strong correlation between PFOTES and component III (or IV).



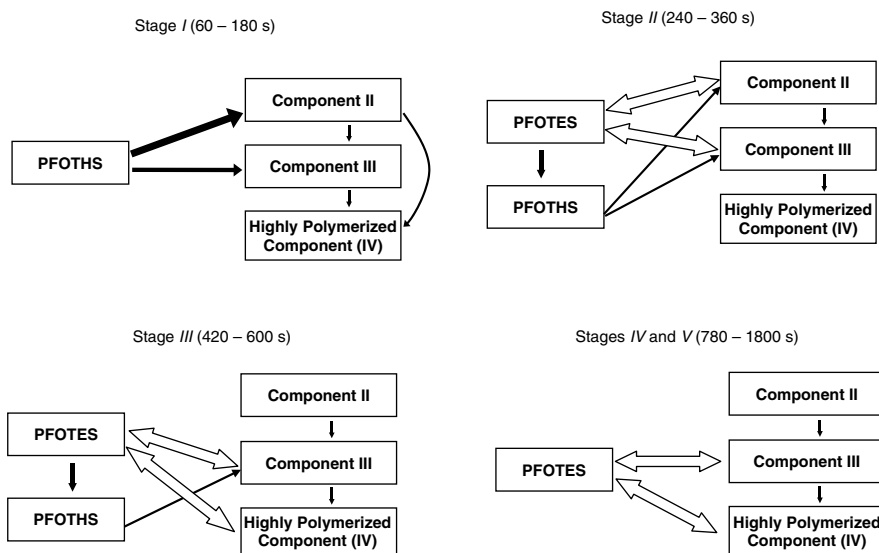


**Figure 15.7** Schematic growth process of PFOTES polymeric aggregates during stages *I*, *II* and *III*. The solid arrows indicate the extent of the reaction (thick arrow great extent; intermediate arrow, intermediate extent; thin arrow, small extent) and the hollow double-headed arrows imply correlation between two components. (Reproduced with permission from Ref. No. 1. Copyright (2002) American Chemical Society.)

*Stages IV and V.* Most of the PFOTHS monomers produced at the very beginning of the reaction finally disappear. The condensation reaction between smaller aggregates progresses further, to form higher-polymeric aggregates.

Thus, two polymerization processes ( $\text{II} \rightarrow \text{III}$ , and  $\text{III} \rightarrow \text{IV}$ ) occur in parallel during the five stages. However, each stage has its own distinct characteristics. In stage *I*, PFOTHS monomers are predominantly consumed to produce lower molecular weight components (II and III). In stage *II*, however, very few PFOTHS monomers are consumed and the reaction is dominated by hydrolysis of PFOTES to PFOTHS. In stage *III*, it is the hydrolysis of PFOTES which is dominant. A few PFOTHS monomers, thus produced, are consumed to form component III. In the later stages (*IV* and *V*), most of the PFOTHS monomers are consumed and the reaction is dominated by condensation of smaller aggregates. A strong correlation peak appears between the bands for PFOTES monomers and a polymeric component (II or III or IV) in the 2D GPC correlation maps for stages *II* and *III*. The perfluorooctyl chains self-assemble during the process of polymerization within the PFOTES–ethanol–1 M HCl–H<sub>2</sub>O system, since the perfluorooctyl portion has both hydrophobic and lipophobic characteristics. Thus, the self-assembling behavior of the perfluorooctylsilane chains should affect the microstructure of the polymerized components. The perfluorooctyl chains of PFOTES monomers are incorporated into the aggregated portions of the perfluorooctyl chains of the polymerized component II or III or IV and of the oligomer–oligomer complexes.

Such complex formation should furnish a strong correlation between PFOTES monomeric and polymeric bands.

The remarkable capability of 2D correlation analysis applied to chromatographic data was thus demonstrated. By simply examining the patterns of cross peaks appearing on 2D GPC correlation maps, one can elucidate surprisingly intricate details of very complex reaction mechanisms of sol–gel polymerization process. Although all pertinent information about the population dynamics of polymerization reaction is embedded in the original set of GPC chromatograms, it becomes much easier to systematically sort out the mechanistic picture of polymerization process with 2D correlation maps.

### 15.3 2D MASS SPECTROMETRY

The field of mass spectrometry had independently developed its own forms of 2D correlation spectral analysis. Earlier examples include photoelectron–photoion–photoion coincidence spectroscopy (PEPIPICO) by Eland *et al.*,<sup>4,5</sup> who produced a 2D contour plot display comprising two axes of flight time for the detection of two photoions in coincidence with a photoelectron. Dynamics of fragmentations of a cation into two charged and one neutral particle is examined to distinguish instantaneous and sequential steps of events. 2D *covariance mapping*, a form of synchronous 2D correlation spectroscopy, has been used extensively in the time-of-flight (TOF) mass spectrometry for the study of dynamics of fragmentation of molecules ionized by an intense short laser pulse.<sup>6–15</sup> For example, the field ionization Coulomb explosion of molecules is studied by correlating fragment atomic ions. Similar concepts in TOF mass spectrometry are sometimes referred to by others as covariance images<sup>16</sup> or coincidence correlation mass spectrometry.<sup>17</sup>

The idea of covariance mapping is based on a relatively straightforward statistical treatment of TOF data.<sup>8,16</sup> Additional standard tools such as confidence interval and hypothesis testing could be readily supplemented with such statistical analyses.<sup>9</sup> The interpretation technique similar to the peak sign analysis of a synchronous 2D spectrum was proposed by Berardi *et al.*<sup>10</sup> In this case, however, two separate covariance maps, one comprising only positive peaks and the other with negative peaks, are created to differentiate the association and anti-association occurring between pairs of TOF spectrum points. Theoretical models for 2D covariance mapping were discussed by Bruce *et al.* for contour features, calculation based on energy and momentum conservation, and Monte Carlo simulation of correlation between first and second arrival ions.<sup>11</sup> Cornaglia showed 2D correlation coefficient maps based on the TOF mass spectra of Coulomb explosion and gave some discussion on the interpretation of the shape of correlation peaks.<sup>12</sup>

With all the activities along the covariance mapping concept in TOF mass spectrometry community, it is curious to note there is an apparent lack of interest in the use of asynchronous correlation. The information obtained in TOF

experiments can be potentially enriched by employing asynchronous correlation analysis, as there are many occasions where sequential events take place in such measurements. Cross *et al.*, for example, considered the distinction of synchronously correlated direct double ionization reaction versus sequential stepwise ionization.<sup>13</sup> Card *et al.* showed the existence of two separate ionization pathways by using positive and negative covariance maps, representing respectively a concerted reaction and a different competitive reaction process.<sup>14,15</sup> Interestingly, these authors do specifically cite the classic paper of generalized 2D correlation,<sup>18</sup> but no asynchronous correlation analysis has been attempted so far in their work.

The use of generalized 2D correlation analysis in mass spectrometry applications is still surprisingly limited, but some promising attempts have recently been made. For example, Okumura *et al.* reported the 2D correlation analysis applied to a set of mass spectra obtained from the thermal desorption spectroscopy (TDS) study.<sup>19</sup> In TDS mass spectrometry, a small amount of gas evolved from a sample material upon heating is analyzed. Mass spectra obtained from a TDS experiment, however, usually contain peaks observed in a very wide range, and each peak is often composed of several fragments with the same mass number that are derived from different species. 2D correlation analysis is then utilized effectively to sort out such complex spectral information.

In 2D TDS mass spectrometry, mass peaks that have significant changes could be readily picked up and the relationship between fragments are examined by the presence of 2D correlation peaks. In the study of Okumura *et al.*,<sup>19</sup> thermal degradation products of a mixture of polyvinylchloride (PVC) and dioctylphthalate (DOP) in ultrahigh vacuum was studied. The 2D TDS mass spectra clearly indicated that the desorption of PVC and degradation of DOP have multiple stages. More volatile DOP desorbed before PVC, as expected. The desorption process of benzene occurred at a temperature slightly below that for dehydrochlorination of PVC. The formation of naphthalene occurred at a temperature just above the release of benzene. It was pointed out that the identification of the detailed sequential order of the desorption process was difficult without the help of 2D correlation, even if the characteristic fragments of the mixture components were known prior to the experiment.

## 15.4 OTHER UNUSUAL APPLICATIONS OF 2D CORRELATION ANALYSIS

The potential application of generalized 2D correlation is not limited to techniques found in analytical chemistry. The concept is so flexible that it can be readily applied to problems in many other scientific disciplines. An interesting example is the application of 2D correlation in computational chemistry, where correlation maps are constructed from a series of calculated data.

For example, Erman and coworkers employed the basic idea of generalized 2D correlation in the area of statistical mechanics.<sup>20</sup> A molecular dynamics calculation of polymer chains was effectively combined with 2D correlation to showcase

synchronously and asynchronously correlated segmental dynamics of polymeric molecules. Lee and Shin used a 2D correlation method for molecular dynamics simulation of temperature-dependent peptide unfolding of  $\beta$ -heparin.<sup>21,22</sup> In their study, essential dynamics (ED) analysis was used for atomic fluctuations in an MD trajectory in conjunction with generalized 2D correlation. Different folding mechanisms, e.g., hydrogen-bond-centric and hydrophobic-centric modes, were successfully analyzed by the 2D correlation spectra.

2D correlation in computational and theoretical chemistry is only one of many other possible applications outside the field of analytical science. These seemingly unusual exploitations of 2D correlation analysis demonstrate the fact that the applicability of this technique is definitely not limited to traditional spectroscopic studies. 2D correlation is indeed a generally applicable universal tool with unlimited potential.

## 15.5 RETURN TO 2D NMR SPECTROSCOPY

### 15.5.1 2D CORRELATION IN NMR

As pointed out earlier, the idea of multidimensional spectroscopy originated in the field of NMR.<sup>23</sup> The initial development of the generalized 2D correlation spectroscopy concept was strongly influenced and motivated by the success of 2D NMR. As the 2D correlation field matured, the apparent similarity of 2D correlation maps with 2D NMR spectra has become much less important to the practitioners of this technique. The 2D correlation has now evolved into a unique and independent tool, which is generally applicable to the analysis of many different types of spectroscopic data.

Interestingly, the generalized 2D correlation scheme has rarely been applied to the analysis of NMR data until recently. There are numerous occasions in the application of NMR, where one finds it desirable to establish correlations among the behavior of signals at different frequencies, in different samples, using other spectroscopic probes, or among samples and models. These are obviously the natural starting points to consider the extension of the generalized 2D correlation concept to NMR. This section examines the emerging field of generalized correlation 2D NMR spectroscopy.

Nearly all implementations of 2D NMR spectroscopy correlate nuclear resonance frequencies in two different experimental time domains, using a standard protocol consisting of a preparation period, an evolution period, a mixing period, and a detection period separated by a sequence of radio frequency pulses.<sup>23,24</sup> Data are converted from the time domain to the frequency domain using double Fourier transformation or a similar technique. In general, the correlations among frequencies present during the evolution and detection periods are established by a coherence transfer process that occurs during the mixing period. While the correlations are usually encoded as different modulation frequencies

for signals in the two time domains, in recent years, 2D NMR spectroscopy has also been extended to include correlations that are not encoded as frequency modulations.<sup>25</sup>

### 15.5.2 GENERALIZED CORRELATION (GECO) NMR

To achieve a more general approach to 2D NMR spectroscopy, a conceptual departure from the conventional NMR mindset may be explored by incorporating the generalized 2D correlation scheme. In the basic framework of generalized 2D correlation spectroscopy depicted in Figure 2.1, most elements of the radio frequency pulse sequences used in 2D NMR can be regarded simply as a set of systematically varied perturbations to the spin systems, while the final pulse and the acquisition period serve to probe the response of the system to the specific perturbation. In the field of NMR, 2D experiments based on generalized correlation scheme is now referred to as GECO NMR.<sup>26</sup>

The method for generating 2D correlation spectra without explicitly requiring coherence transfer follows from the simple observation that the perturbation does not necessarily need to be produced by the same type of process used to probe the response. So long as the response of the system to the perturbation occurs on a time scale that can be probed by spectroscopy, it is possible to generate useful correlation spectra based on the response curves at various frequencies. A similar argument should hold for the possible correlation analysis of nonlinear optical spectroscopy data obtained from measurements using ultrafast laser pulses discussed in Chapter 7.

### 15.5.3 2D CORRELATION IN DIFFUSION-ORDERED NMR

As an illustrative example, the application of generalized 2D correlation to the analysis of diffusion-ordered NMR spectroscopy (DOSY) data is described.<sup>26</sup> DOSY is a technique for separating signals from different molecules within a mixture based on their differing diffusion coefficients.<sup>25,27</sup> DOSY has become a standard tool in the analysis of mixtures by NMR. The DOSY experiment requires the collection of a series of spectra using a pulse sequence for measuring diffusion coefficients. A parameter such as gradient strength is systematically incremented for each member of the series, leading to a decay of the signal intensity according to a known function of the diffusion coefficient and the systematically varied parameter. After collection, the data set must be processed and displayed. A classical DOSY plot consists of a contour plot having chemical shift on one axis and diffusion coefficient on the other. Calculation of the diffusion coefficient or spectrum of diffusion coefficients at each frequency is usually achieved using any of a number of algorithms for curve fitting or deconvolution of the exponential decay functions.<sup>27</sup>

To illustrate the properties for an idealized case, consider a simple example in which the spectra consist of signals which decay exponentially as a function of the external parameter  $p$ .

$$y(\omega_m, p) = a_m e^{-b_m p} \quad (15.2)$$

Response curves of this form often arise in diffusion, relaxation, kinetics, and many other NMR experiments. For most implementations of DOSY,  $p$  corresponds to the square of the gradient strength multiplied by some other constant and known parameters,  $b_m$  corresponds to the diffusion coefficient, and  $a_m$  is the signal intensity.<sup>28</sup> It follows directly from the earlier model calculation for an exponential function carried out in Chapter 2 (Equations 2.24 and 2.25) that the correlation intensities at coordinates  $(\omega_m, \omega_n)$  in the generalized 2D NMR correlation spectrum for DOSY data may be given by the form

$$\Phi(\omega_m, \omega_n) = \frac{\pi a_m a_n}{(b_m + b_n)} \quad (15.3)$$

$$\Psi(\omega_m, \omega_n) = \frac{a_m a_n \ln(b_m/b_n)}{(b_m + b_n)} \quad (15.4)$$

The asynchronous component  $\Psi$  is proportional to the natural logarithm of the ratio of the decay constants. This intensity can be negative, positive, or zero depending on the relative values of the decay constants. Hence, for ideal response curves described by Equation (15.2), the signs and intensities of the cross peaks in the asynchronous 2D spectrum allow one to sort the decay constants for the various molecules. Furthermore, the ratio of the asynchronous and synchronous intensities is a quantitative measure of the logarithm of the ratio of diffusion coefficients.

For many NMR experiments, however, the signal response curves will not always comply with the simple form described by Equation (15.2). For example, the pulsed field gradient for many commercial probes is spatially inhomogeneous, and the resulting response curves are strongly nonexponential.<sup>29</sup> Fortunately, the results of generalized correlation processing of such a data set will still give informative results, because matched response functions will give zero intensity and unmatched response functions will give nonzero intensity in the asynchronous spectrum. Therefore this approach may be particularly useful for analysis of DOSY data generated using many commercially available NMR probes.

In applying pulse field gradient experiments for measuring diffusion coefficients, one has the option of generating either exponential or Gaussian signal response functions by choosing the manner in which the gradient strength is varied.<sup>27</sup> There appears to be certain advantages to the Gaussian form. For example, it is possible to cover a wider range of diffusion coefficients in a single experiment since the gradient strengths do not bunch up at the higher values. A simple quantitative treatment of Gaussian response curves leading to expressions analogous to Equations (15.3) and (15.4) is not possible, because no simple

closed-form analytical expression is available for the Fourier sine transform of the Gaussian function. Nonetheless, it is possible to show numerically that the asynchronous spectrum will have positive, negative, or zero cross peak intensity depending on whether the decay constant of one response curve is greater than, less than, or equal to the decay constant of the response curve to which it is being compared. Therefore, regardless of the functional form of the response functions obtained from diffusion-based experiments, the generalized correlation approach gives spectra that distinguish and rank order signals having different diffusion coefficients.

To demonstrate the use of generalized correlation analysis for the evaluation of diffusion data on mixtures, a solution comprising 32 mM sodium dodecylsulfate (SDS), 32 mM sucrose, 32 mM ethanol and 32 mM methanol in D<sub>2</sub>O was prepared. Data were acquired using the LED pulse sequence<sup>30</sup> modified with bipolar gradients and convection compensation,<sup>31</sup> from which diffusion coefficients and DOSY spectra can be calculated based on the signal response functions. The experiment was carried out using an array of 32 linearly spaced gradient strengths leading to Gaussian response curves. The NMR probe was of the 'ultralinear' design provided by the spectrometer vendor giving a gradient strength of 60 G/cm at the highest setting. Data were processed with MATLAB (The Mathworks, Inc.) using MatNMR<sup>32</sup> to facilitate NMR-specific processing tasks.

Figures 15.8 and 15.9 show the synchronous and asynchronous spectra generated from the data set in the chemical shift range from 3.1 to 4.2 ppm. The synchronous spectrum shows cross peaks among all signals regardless of their

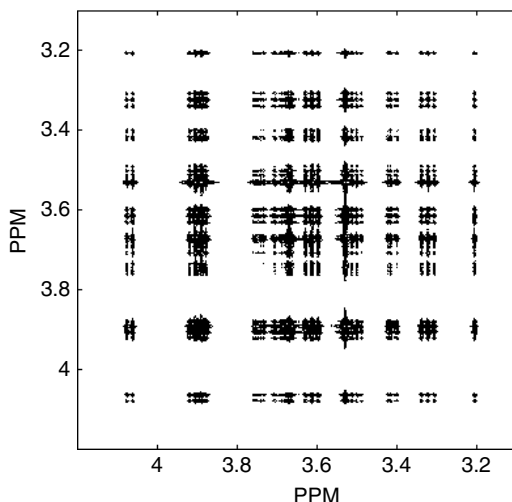


**Figure 15.8** Synchronous generalized correlation spectra of a mixture comprising 32 mM each of sodium dodecyl sulfate, sucrose, ethanol, and methanol in deuterium oxide. (Reproduced with permission from Ref. No. 26. Copyright (2002) American Chemical Society.)

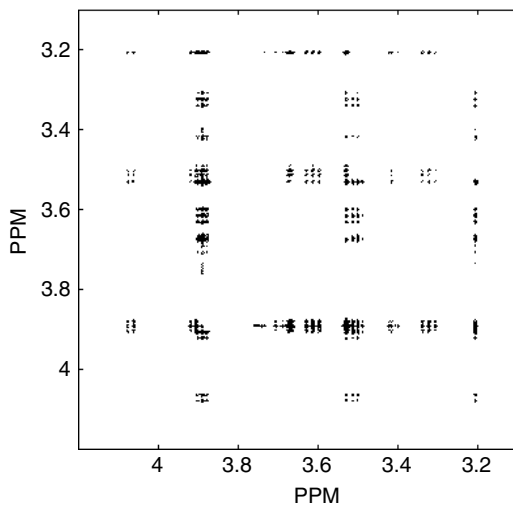


**Figure 15.9** Asynchronous generalized correlation spectra of a mixture comprising 32 mM each of sodium dodecyl sulfate, sucrose, ethanol, and methanol in deuterium oxide. (Reproduced with permission from Ref. No. 26. Copyright (2002) American Chemical Society.)

molecule of origin. As expected, the asynchronous spectrum shows cross peaks only among signals from molecules having different diffusion coefficients. Within this narrow range of chemical shifts, signals from all components except HOD can be observed. Common features of diffusion-resolved experiments processed using generalized correlation analysis are apparent in these plots. The diagonal peak observed in the synchronous spectrum near 4.1 ppm corresponds to sucrose. There is a pattern of signals visible near the 3.9 ppm diagonal position in the asynchronous spectrum. Both SDS and sucrose have signals at this position. Thus, the presence of diagonal peak patterns in the asynchronous spectrum is indicative of near overlap of signals having different response curves. A similar situation is observed near 3.5 ppm, where signals from ethanol and sucrose are partially overlapped. All the remaining signals in the asynchronous spectrum correlate signals from molecules having different diffusion coefficients.

The signs of cross peaks in the asynchronous spectrum, which are not apparent from the contour plot, give information on the relative diffusion coefficients of the components. As an illustration, Figure 15.10 shows one-dimensional horizontal slices through the asynchronous spectrum at chemical shifts corresponding to SDS, sucrose, and ethanol chemical shifts. In each case, signals from molecules having faster or slower diffusion coefficients have positive or negative signs, respectively. Signals arising from atoms in the same molecule have zero intensity in these slices. A mix of positive and negative character can appear at locations for which there is overlap of signals from different molecules, such as near 3.9 ppm in the slice through a sucrose resonance in Figure 15.10.



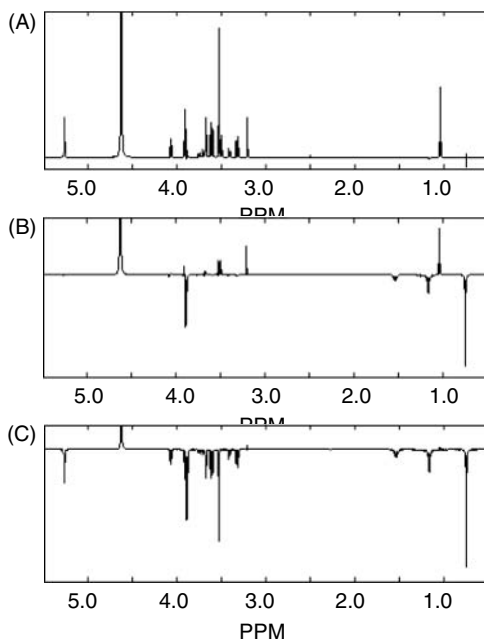


**Figure 15.10** Selected one-dimensional slices of the asynchronous generalized 2D correlation spectrum. Slices through SDS (A), sucrose (B), and ethanol (C) show signals only at chemical shifts corresponding to different molecules. Positive peaks indicate higher diffusion coefficients, negative peaks indicate lower diffusion coefficients. (Reproduced with permission from Ref. No. 26. Copyright (2002) American Chemical Society.)

The above demonstration of diffusion-based 2D NMR mixture analysis is only one of many other possible applications where generalized 2D correlation can play significant role in the NMR field. Many more generalized correlation-based 2D NMR examples are expected in the future. In general, any multiple-pulse, multidimensional experiment which uses a double Fourier transformation scheme to construct 2D spectra could be analyzed in the framework of generalized 2D correlation to produce alternative forms of 2D NMR spectra. Thus, not only NMR but also any spectroscopy experiment with pulsed excitations, e.g., femtosecond laser pulses, may be analyzed by this scheme.

## 15.6 FUTURE DEVELOPMENTS

We have covered in this book numerous topics related to the developing field of generalized 2D correlation spectroscopy. 2D correlation is a powerful tool generally applicable to a very broad range of spectroscopic, analytical, and many other scientific disciplines. The technique is based on the simple analysis of a set of data collected from a system under some form of *perturbation* of any physical

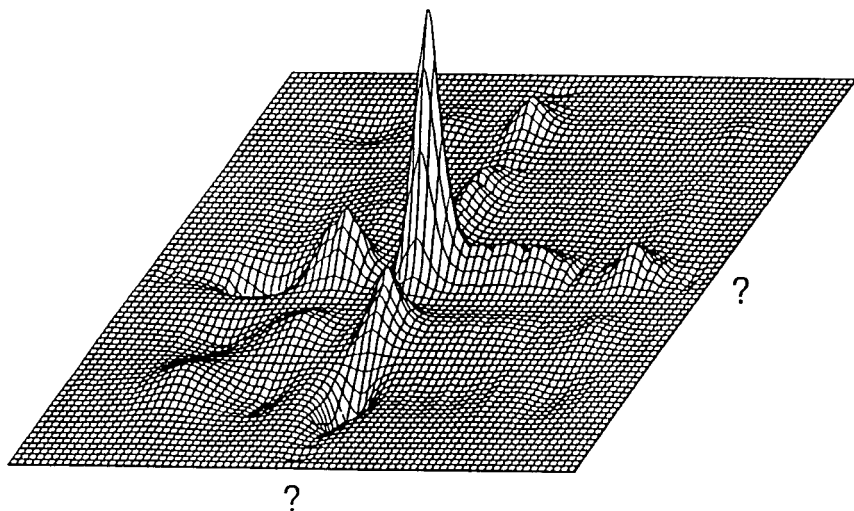


**Figure 15.11** Only one's imagination and creativity should limit the possibility of 2D correlation spectroscopy!

nature and waveform of choice. Either time-dependent or static phenomena may be studied by 2D correlation. Selective development of 2D correlation peaks provides better access to pertinent information, which is not readily observable in the conventional 1D form of data display. Spectral resolution is enhanced by spreading peaks along the second dimension, and signs of cross peaks reveal the relative direction of intensity changes and the sequential order of events associated with the change. It is also possible to combine and compare data from different measurements and samples via a heterospectral correlation scheme.

We would like to conclude the discussion with one more 2D correlation spectrum shown in Fig. 15.11. The spectral variable axes of this ultimate 2D correlation spectrum have not yet been determined. Only one's imagination and creativity should limit the vast possibility of 2D correlation spectroscopy.

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