

ADVANCES IN TWO-DIMENSIONAL CORRELATION SPECTROSCOPY

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Since the initial introduction of the concept in 1986, and subsequent refinement to the more generally applicable form in 1993, two-dimensional (2D) correlation spectroscopy has gained considerable popularity among scientists as a useful tool for extracting pertinent information from spectroscopic data. Steady increase in the practitioners of 2D correlation spectroscopy coupled with the need to exchange new and emerging ideas led to an important milestone in this field, the First International Symposium on Two-Dimensional Correlation Spectroscopy (2DCOS-I) held in Sanda, Japan from August 29 to September 1, 1999. Since then, 2D correlation spectroscopy has experienced remarkable growth in basic theory, instrumental and computational techniques, various scientific and practical applications, and expansion to new areas of analytical methods during the last four years,. An overview is given to capture the significant advances in 2D correlation spectroscopy with several key selected topics.

Some of the noteworthy developments include the greater depth of fundamental understandings for 2D correlation concept, efficient computational algorithms and increased availability of computer software, and better interpretation for complex features of correlation maps based on systematic simulations. The application of 2D correlation concept is now found in numerous molecular spectroscopy methods, such as IR, NIR, Raman, fluorescence, UV-visible, pulsed lasers, and NMR. Even chromatographic analysis can be carried out in the 2D correlation form. The application of hetero-spectral 2D correlation, which brings together data obtained by different analytical probes, is also increasing. In addition to the analysis of dynamic processes, such as polymer deformation and chemical reactions, the effect of static perturbations like temperature, concentration, pressure, spatial distribution, etc. has been extensively utilized for the study of various systems including polymers, biomolecules, liquid crystals, inorganics, and so forth. The combination of 2D correlation with chemometrics techniques and the cross over between 2D correlation concept and traditional pulse-based 2D spectroscopy are also exciting new developments.

Applications of Generalized Correlation Analysis to Nuclear Magnetic Resonance Data

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The concept of generalized two-dimensional spectroscopy was inspired, in part, by the field of NMR. In standard 2D NMR spectroscopy, experiments are designed so that the signals in a collection of one-dimensional spectra are modulated at frequencies that give information about molecular topology, chemical exchange, etc. Processing by double Fourier transformation gives highly informative data maps. Many NMR experiments, however, generate collections of spectra whose signals are not periodically modulated. Nonetheless, the signals do contain information on molecular structure and behavior. Examples include relaxation rate measurements, diffusion coefficient measurements, and studies of time-dependent processes such as microbial fermentation and slow chemical reactions. For these and related types of experiments, generalized correlation analysis provides a powerful approach to interpreting data sets. When applied to diffusion-ordered NMR data, for example, the processing scheme produces two-dimensional output spectra having two frequency axes. Relative diffusion coefficients are encoded in the signs and intensities of the cross peaks. Since the generalized correlation approach does not require all the data to originate from within a single data set, it is possible to use the technique to compare experimental data with reference curves. This allows quantitative determination of key data descriptors such as exponential decay constants. This talk will describe several applications of generalized correlation analysis to gain quantitative information and to visualize the information content of NMR experiments.

PROBING NUCLEOTIDE-BINDING SITE OF ANNEXIN A6

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Annexins belong to a family of homologous Ca²⁺- and phospholipid-binding proteins. At least 12 various subfamilies were identified in different vertebrate tissues. The eight repeat domain annexin A6 is the largest member of the family (M_r 68 KDa). It has been proposed that annexin A6 could participate in nucleotide-dependent processes such as membrane traffic (1) and signal transduction pathways (2). Experimental evidences suggested that annexin A6 may bind in N-terminal region of the protein (3,4). However, the location of nucleotide-binding domain remains uncertain. To determine the location of the nucleotide binding site within annexin A6, infrared spectroscopy, fluorescence spectroscopy and molecular modeling were used. Reaction-induced infrared difference spectra of annexins A6 were obtained after photorelease of GTP- γ -S (a non-hydrolysable nucleotide), ATP or Pi from their respective caged-compounds. Photolytic release of ligands from inactive caged compounds enables investigation of ligand-protein reaction directly in the cuvette, minimizing experimental errors due to sample to sample variation. Recording the infrared spectra of annexins A6 before and after photorelease of ligand and measuring directly their differences revealed time-dependent structural changes caused by the photorelease of ligand and its binding to annexin A6. Interactions between GTP- γ -S and annexin A6 produced similar infrared difference spectra than those observed in the case of interactions between Pi and annexin A6. This finding suggested that nucleotide binding to annexin A6 is mostly dominated by the phosphate groups. Analysis of infrared spectra, by using 2D cross-correlation functions, provided more insight into the nature of molecular interactions. Infrared changes caused by the interactions were relatively small affecting 9-7 amino acid residues and one carboxylate group of either Asp or Glu side chain residue. Combination of infrared results with fluorescence and molecular modeling permitted to propose a putative location of nucleotide binding site in annexin A6.

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GLOBAL PHASE ANGLE FOR GENERALIZED TWO-DIMENSIONAL CORRELATION INFRARED SPECTROSCOPY

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The global phase angle description derived from the generalized two-dimensional correlation infrared spectroscopy (global 2D phase map) has been proposed recently.[1-3] In the presentation, the theoretical development, simulation, and application to the actual experimental data are described.

The basic advantages of the generalized 2D correlation spectroscopy are: 1. the possible use of any kinds of non-periodic perturbations (for instance, temperature, time, concentration, and so on), 2. the enhancement of spectral resolution, and 3. the detection of the different waveforms between two intensity profiles.[4] On the other hand, difference in amplitudes at different spectral positions in dynamic spectra have so far been the serious disadvantage in evaluating the correlation on the 2D synchronous and asynchronous maps quantitatively. To overcome the problem, the global 2D phase map has been introduced.

The intensity profiles as a function of time t at spectral variations ν_1 and ν_2 , $f(\nu_1, t)$ and $f(\nu_2, t)$, are compared with each other in the global 2D phase map. The global 2D phase map can be obtained by taking the arctangent of $\Psi(\nu_1, \nu_2) / \Phi(\nu_1, \nu_2)$, where $\Phi(\nu_1, \nu_2)$ and $\Psi(\nu_1, \nu_2)$ are the synchronous and asynchronous maps calculated in the generalized 2D correlation spectroscopy. The calculation of the global phase angle cancels the amplitude information of the intensity profiles of the one-dimensional spectra. The simulation using the three synthesized bands with exponential decay clearly extracts the difference in the characteristic rate constant of the decay process in the global 2D phase map, in case the three bands have the different initial values of the peak maximum intensity (amplitudes).

The presentation also introduces a new method for pattern recognitions of band shifting and broadening by means of the global 2D phase map. To obtain the distinct patterns for band shifting and broadening, original spectra are subjected to scaling so that the spectra after the scaling do not cross one another. After such pre-treatment the band shifting creates the tilting of a correlation plane, the band overlapping yields the four plane terraces, and the band broadening generates the asymmetric curved plane with respect to the diagonal line in the global 2D phase map.

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SOME CONSIDERATIONS ON THE PRINCIPLES OF THE 2D-COS METHOD

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Absorption spectroscopy is among the first group of techniques used in two-dimensional correlation spectroscopic studies. Considering a targeted component j in a sample, its absorbance at a definite wave number in the entire concentration range can be described by an extended form of the Beer-Lambert Law as shown below:

$$A_j = A_j^i + A_j^e \quad (1)$$

where $A_j^i = \varepsilon_j^0 l c_j$ is the ideal and linear part, and A_j^e is the excess and nonlinear part of the absorbance.

For any concerned component j , if it can satisfy the condition $A_j^e = 0$, normal 2D-COS can be employed to study concentration fluctuations. This is also referred as concentration-oriented analysis. Here concentration fluctuation could be caused by any perturbation-induced chemical reactions or physical changes such as phase transitions. In the latter case, each phase or structure is simply taken as a “component”. Within this category, overlapping of concerned absorption bands could cause problems to the 2D-COS. We found that when overlapping is not very significant, data normalization is unnecessary. On the other hand, when overlapping is very significant, a so-called mean-centered normalization method developed in Ozaki’s group should be used prior to 2D-COS analysis.

By eliminating the linear part of Eq (1), the second term A_j^e can be used to unveil information concerning molecular interactions. This is accomplished by the introduction of the component-normalization method, in which individual absorption bands in a spectrum are normalized by the concentrations of respect components. Further, correlation coefficient as defined in the following equation is used to present relative interaction strength between unlike molecular pairs:

$$\rho_{ij} = \frac{\Phi_{ij}}{\sqrt{\Phi_{ii} \cdot \Phi_{jj}}} \quad (2)$$

Within this category of work, effect of noise on the evaluation of correlation coefficients could be a problem. This has been evaluated to some detail by computer simulations. It was found firstly that higher than 99.99% probability can be reached for $\text{Abs}(\rho) > 0.80$, when signal to noise ratio is better than 5. Secondly, the size of the data sets used in 2D correlation analysis can modulate the effect of noise. In the absence of signal, larger data sets will remove more effectively the artificial peaks and decrease the corresponding ρ more significantly. In the presence of signal, larger size of the data sets will narrow the distribution range of $\text{Abs}(\rho)$. Smoothing treatment of the row data can reduce the effect of noise and improve the appearance of 2D spectrum significantly.

Finally, it is necessary to point out that the above discussions should not be restricted only to absorption spectroscopy. Other techniques such as X-ray diffraction could also apply. Some 2D-COS work on lipid phase transitions using time-resolved XRD technique has been done.

Study on Self-Association of Alcohols by 2D Correlation Spectroscopy

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The effect of temperature and concentration on self-association of selected alcohols has been studied by generalized two-dimensional (2D) Fourier-transform near-infrared (FT-NIR) correlation spectroscopy. Since the temperature and concentration affect the monomers and various associated species to different extent, the corresponding peaks appear as separate features in 2D asynchronous spectra, regardless of the separation in the normal spectrum. This resolution enhancement, in connection with the selective correlation of the peaks in the synchronous and asynchronous spectra, permitted for identification and reliable assignment of a series of bands in the first and second overtone regions. Some of these bands were reported for the first time. From the signs of the correlation peaks it results that the dissociation of the polymeric species in pure liquid alcohols does not occur directly into monomers, but proceeds through the intermediate species. The population of the free OH groups changes faster than that of the associated species with increasing temperature showing that the higher associates at first break into shorter ones and than, at elevated temperatures, the shorted multimers dissociate into monomers. Distinct differences in the mechanism of the thermal dissociation were observed between the primary and branched alcohols. The obtained results prove the presence of at least two kinds of associated species and argue against frequently assumed equilibrium between the monomers and one kind of the associated species from diluted solution to pure liquid alcohol. The combination of 2D FT-NIR spectroscopy with dielectric measurements made possible to gain a deeper insight into the molecular mechanism of self-association of alcohols.

USE OF SPECTRAL SIMULATIONS AND MATHEMATICAL REPRESENTATION OF THE INTENSITY VARIATIONS FOR GENERALIZED TWO-DIMENSIONAL CORRELATION SPECTROSCOPY

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Generalized two-dimensional correlation spectroscopy (2D COS) is a well-established and widely used method to analyze a series of spectra. However, users should take into consideration some particular points when calculating and analyzing 2D maps. First, these maps are generally interpreted in terms of asynchronisms between the band variations. However, it is well known that frequency shifts and changes in bandwidth can also generate asynchronisms [1]. These spectral effects should be taken into account. In this respect, the use of simulations of the experimental data may help in a better understanding of the experimental 2D maps. Second, the reference used for calculating the dynamic spectra must not be zero otherwise wrong results can be obtained. We also showed that any spectrum of the series can be used as a reference. These conclusions have been mathematically demonstrated. This leads to a formalism well suited to clarify the notion of synchronicity. Thus, a criterium for two bands to be synchronous has been found: the ratio of the two dynamic intensities must be constant throughout the experiment. In other words, the dynamic intensity variations must be represented by the same mastercurve. Third, it is demonstrated that, if the variations of the intensities are linear, they will always be recognized as synchronous by the generalized 2D COS method, even if the variations are asynchronous. Therefore, perturbations that vary linearly should be avoided. Finally, caution should be used in interpreting the data since the 2D peak intensities are related to both the correlation intensities (i.e., the synchronicity level) and the amplitudes of the intensity variations. The above formalism naturally leads to a normalization procedure, as it has already been proposed [2], that results in 2D maps that only contains the information relative to the correlation intensity and not to the amplitude of the intensity variations.

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Linearity in Generalized Two-Dimensional Correlation Spectroscopy — a Novel Concept

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The theory and terminology for two-dimensional infrared correlation spectroscopy (2DCOS) were originally developed for the analysis of dynamic infrared signals induced by periodic perturbations. The 2DCOS method defines *synchronous* and *asynchronous* relationships mathematically rigorously, both for periodic and nonperiodic perturbations. However, the concept of synchronicity is difficult to understand, when applying the 2DCOS method to the analysis of nonperiodic perturbations. We have proposed an alternative and novel measure and concept especially useful for nonperiodic perturbations. Our main proposal, when interpreting 2D maps, is to focus on *linearity* between signals instead of synchronicity. We have also proposed a simple plot to reveal, verify and identify linear and nonlinear relationships in the variable space. In addition, we have proposed the alternative use of four terms: 1) *linearity* as an alternative measure and concept to synchronicity, 2) *covariance* maps, peaks etc. as an alternative term to synchronous maps, spectrum, peaks etc., 3) *disvariance* maps, peaks etc. as an alternative term to asynchronous maps, spectrum, peaks etc. and 4) *disrelation coefficient* as an alternative term to normalized asynchronicity.

In an example using FT-IR data from a three-component evaporation experiment, most peaks in the covariance or the traditional synchronous 2D map represented linear relationships, but also peaks representing nonlinear relationships between the involved spectroscopic bands were found. All larger peaks in the disvariance or the traditional asynchronous 2D map were representing nonlinear relationships between the involved spectroscopic variables. Our proposed plot visually revealed the type and the degree of nonlinearity.

QUANTITATIVE 2D IR CORRELATION ANALYSIS OF RATE PROCESSES

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A modified two-dimensional infrared correlation method, called $\beta\nu$ correlation analysis, was recently introduced for quantitatively determining the relative rates of intensity change and the degree of coherence between intensity variations in a discrete set of dynamic spectra [1]. In this method, a mathematical cross correlation is performed between a set of spectra undergoing some dynamic variation against a simple mathematical function. In the present case this is a sine function. Correlation intensities are a function of the phase angle (β) of the sinusoidal function and the spectral frequency (ν). The maximum positive correlation intensity will be observed at one point in the (β, ν) correlation plot. This point is used to define a new parameter, the *effective phase angle*, β_e , of $f(\nu, n)$ – where, for the range $360^\circ \geq \beta \geq 0^\circ$, β_e is simply equal to $\beta + 90^\circ$. We have applied classical 2D IR as well as $\beta\nu$ correlation spectroscopy to *in-situ* IR spectroscopy of protein monolayers at the A/W interface. These techniques enabled us to identify specific protein conformations and followed the reorientation of these protein conformations as a function of increasing surface pressure.

We have recently expanded the scope of this method to encompass an exponential relationship between intensities in the data set. In this method, a mathematical asynchronous cross-correlation is performed between a set of N infrared spectra undergoing a dynamic intensity variation against a set of exponential functions with a range of rate constants. The observed correlation intensities are a function of the rate constant of the exponential function and the spectral frequency. The 2D correlation plots reveal rate relationships between different molecular events in terms of a quantitative and tangible parameter, k , which is the rate constant of the exponential function used in the correlation. Correlation maxima and minima represent the regions of intensity change in the spectral bands. Bands that undergo simultaneous intensity changes in opposite directions can be intuitively distinguished based on the signs of the peaks in the correlation plots, thereby doing away with the necessity to modify the phase parameter, as in the case of the $\beta\nu$ correlation method. The intensity of the correlation peaks gives an indication of the magnitude of change in intensity. This technique is fairly robust in its application to several intensity variation models as well as its application to experimental data. When used in conjunction with conventional 2D IR, it promises to be a useful method for the identification of sequential relationships between associated molecular processes in overlapped peaks.

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Methods for Coherent Multidimensional Vibrational Spectroscopy

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Coherent multidimensional vibrational spectroscopy (CMDVS) is based on driving multiple vibrational transitions with coherent sources. Most work has used nonlinear four wave mixing (FWM) methods to achieve multiple resonances but there has been work on three wave, five wave, and six wave mixing methods. There are two forms of CMDVS-FWM that differ in the nature of the transitions and both will be useful. FWM involves 4 transitions with 3 resonances between different coherences/populations. For vibrational spectroscopy, those 4 transitions can be either all infrared absorptions/emissions or 2 infrared absorptions/emissions and 1 Raman transition. The first is a triply vibrationally enhanced (TRIVE) process and the second is a doubly vibrationally enhanced (DOVE) process. Each provides multidimensional spectra with cross-peaks between vibrational modes that are coupled but they differ in the nature of how the coupling is manifested and the implementation.

A multiresonant nonlinear process is based on coherent excitations creating coherences, i.e. linear combinations of the states connected by photon resonances. Entanglement and quantum level interference are a natural consequence because the coherences can be formed through different equivalent pathways. The multidimensional capabilities arise because each process involves a double vibrational quantum coherence, i.e. a quantum mechanical mixture of two different vibrational states. The double quantum coherence produces cross-peaks in two dimensional spectra that reflect inter- and intra-molecular coupling between the two states in the coherence, just as the two dimensional NMR cross-peaks reflect coupled spins. Modes that are not coupled cannot create cross-peaks. The coupling required for cross-peaks is manifested in the destructive interference between different coherence pathways in TRIVE processes and as multi-quantum transitions in DOVE processes. This difference in coupling makes TRIVE and DOVE methods complementary. In each case though, one can picture the couplings arising because the excitation of mode A causes a modulation in the electron density that in turn modulates the "spring constant" of mode B at the frequency of mode A. In addition, there are important consequences for the quantum level interferences that occur between different coherence pathways and these form the basis for coherent quantum control of the multidimensional spectra.

Coherent two-dimensional vibrational spectroscopies of polypeptides: Fluctuation, dephasing, and delocalization of amide I modes

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Local amide I mode frequencies in a give polypeptide have been found to be strongly affected by the inter-peptide interaction and solvation, because the electronic and molecular structures of the peptide bond change due to interactions with surrounding peptides and solvent molecules. Quantum chemistry calculation methods are used to establish the structure-frequency relationship for model dipeptides, tripeptides, and polypeptides.¹⁻³ Solvation-induced amide I mode frequency shift and fluctuation in liquids water and methanol was studied by using molecular dynamics simulation method in combination with an extrapolation method developed recently.^{1,4} Isotope-exchange effects on the amide I normal modes, site- and 3D-conformation-dependencies of local mode frequencies were elucidated for a variety of tripeptides.³ The inter-peptide vibrational coupling constants and diagonal force constant alterations were fully analyzed by using the Hessian matrix reconstruction method. Both amide I IR band and two-dimensional IR spectra of small polypeptides were numerically calculated and compared with experimental results. The relationship between two-dimensional line shapes of two-color pump-probe spectra and solvation dynamics was investigated and a direct procedure for extracting electronic or vibrational solvation correlation function was proposed.⁵ A novel two-dimensional circularly polarized pump-probe spectroscopy⁶ was theoretically studied and will be briefly discussed in this symposium.

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TWO-DIMENSIONAL INFRARED CORRELATION SPECTROSCOPY FOR BIOMOLECULAR INTERACTIONS INVOLVING NOVEL SOLUBLE CARBON NANOTUBES

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Novel soluble single-walled carbon nanotubes (SWNTs) may have important biological applications. To explore the interactions between the biological molecules and carbon nanotubes, here we choose α anomers of glucose as a model biomolecule for which mutarotation occurs when the glucose anomers dissolve in solvents. By using 2D IR correlation spectroscopy, we have investigated the molecular interactions between the glucose anomers and water-soluble SWNTs functionalized by carboxylic acid and hydroxide groups as well as between fluorinated SWNTs and glucose anomers. 2D IR correlation spectra have been generated based on the IR absorption spectra that are measured with time after the α glucose anomer samples dissolve in pure solvent and SWNTs-contained solvent (H_2O for water-soluble SWNTs and 9:1 $\text{H}_2\text{O}/\text{DMSO}$ (v:v) for fluorinated SWNTs, respectively). Significant changes are observed in the 2D asynchronous spectra of the glucose anomers when the SWNT samples are present in solution, indicating that carbon nanotubes alter the mutarotation process of α - glucose molecules due to molecular interactions. 2D hetero spectra have been used to selectively identify the molecular interactions from different groups of the glucose that interact with soluble SWNTs. Further discussions will be presented in comparison with the results of the α -glucose samples in solutions containing bovine serum albumin and triacetin.

TWO-DIMENSIONAL FLUORESCENCE CORRELATION SPECTROSCOPY: ITS FEATURES AND APPLICATIONS TO BIOCHEMISTRY

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1. Analysis of fluorescence spectra of aromatic hydrocarbons in micellar systems. In order to show the features of two-dimensional (2D) fluorescence correlation spectroscopy, we tried to resolve complicated fluorescence spectra of the mixture of aromatic hydrocarbons. The systems examined were: (1) pyrene (PY) and 1,3,6,8-pyrenetetrasulfonic acid (PYS) in the cationic micellar solutions of cetyltrimethylammonium chloride (CTAC) and (2) PY and 9-anthracencepropionic acid (AP) in anionic micellar solutions of sodium dodecyl sulfate (SDS). Fluorescence quenching was employed as a perturbation mode for causing intensity changes in fluorescence bands (*quenching perturbation*). Iodide ion (I⁻) was used as a quencher in the former system, and cetyl pyridinium chloride (CPC) was used in the latter. Vibronic bands in the complicated fluorescence spectra of the mixture of the analytes were successfully resolved by 2D correlation technique. Furthermore, the information about the order of response of the bands to quenching was obtained by comparing the signs of synchronous and asynchronous peaks.

2. Resolution of fluorescence of two tryptophan residues in horse heart myoglobin. 2D correlation technique was applied to resolve heavily overlapped fluorescence spectra of two tryptophan (Trp) residues in horse heart myoglobin (HM). Fluorescence quenching was employed as a perturbation mode. Two kinds of quenchers, I⁻ and acrylamide (AA), were used for inducing fluorescence intensity change. This technique worked well because the Trp residue located at the 7th position (W7) is known to be easily accessible to the quencher, whereas that located at 14th position (W14) is not. By this technique, the fluorescence spectra of the two Trp residues were clearly resolved. From the signs of synchronous and asynchronous peaks, it was also shown that the quenching of W7 fluorescence is brought about prior to the quenching of W14 fluorescence. This result is consistent with the structure of HM which was proposed earlier.

Dynamic Filtering and 2D-IR Correlation Spectroscopy of Ice Nucleation and Melting

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Vibrational spectroscopy is a very sensitive probe for studying structural composition and conformational changes of complex systems. Unfortunately, for many systems, the infrared spectrum has many overlapping bands and changes in structure will cause only subtle changes in the spectrum. We have applied a two-dimensional correlation analysis to mid IR spectra of NO adsorption on Pt(100), changes in the secondary structures of β -lactoglobulin in D₂O (or H₂O), phase transitions in liquid crystals, and finally to nucleation and melting of ice. A recent infrared study of interfacial melting of thin ice films [V. Sadtschenko and G. E. Ewing, *J. Chem. Phys.* **116**(11), 4686 (2002)] revealed that at temperatures below but near the triple there is a liquid-like layer on the surface of the ice. The infrared spectrum from the ice and liquid-like layer has overlapping peaks that contribute to the entire band profile. Separating the contributions from ice and the liquid-like layer required fitting calculated spectra to the observed band profiles. In contrast, a 2D-IR correlation analysis of the isothermal desorption spectra showed that only two frequencies are strongly correlated. One frequency at 3350 cm⁻¹ is correlated to changes in the liquid-like layer while the other at 3200 cm⁻¹ is tracking the changes in ice. Determination of each component is greatly simplified by plotting the intensity of the band profile at 3350 cm⁻¹ against the intensity at 3200 cm⁻¹. In a continuation of this study, the nucleation and melting of ice has been probed with infrared microscopy and the 2D-IR correlation analysis will be presented.

Moving Window 2D Correlation Spectroscopy And Its Applications

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We report on the two new developments in two-dimensional (2D) correlation spectroscopy; one is the combination of the moving window concept with 2D spectroscopy to facilitate the analysis of complex data sets, and the other is the definition of the noise level in synchronous/asynchronous maps. A graphical criterion for the latter is also proposed. The combination of the moving window concept with correlation spectra allows one to split a large data matrix into smaller and simpler subsets and to analyze them instead of computing overall correlation. A three-component system that mimics a consecutive chemical reaction is used as a model for the illustration of the two ideas. Both types of correlation matrices, variable-variable and sample-sample, are analyzed and a very good agreement between the two is met. The proposed innovations enable one to comprehend the complexity of the data to be analyzed by 2D spectroscopy and thus to avoid the risks of over-interpretation liable to occur whenever improper caution about the number of co-existing species in the system is taken. The method is applied for the determination of phase-transition points of oleic acid and poly-(*N*-isopropylacrylamide) (PNiPA) in an aqueous solution respectively, via their vibrational spectra.

TWO-DIMENSIONAL CORRELATION SPECTROSCOPIC STUDIES OF HYDROGEN-BONDED SUPRAMOLECULAR ASSEMBLIES

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Hydrogen bonds is one of the principal intermolecular forces that often play key roles in the molecular recognition and self-assembly. By choosing an appropriate proton acceptor and a proton donor, stable intermolecular hydrogen bonds can be formed, often thereby providing a novel system with new properties. Recently, we have designed and synthesized a series of supramolecular systems based on hydrogen bonds. The formation of these supramolecular assemblies was promoted by site-specific hydrogen bonds between the complementary hydrogen-bonding groups connected to a functional derivative. The process of hydrogen-bonding directed molecular recognition leading to the generation of the functional assemblies

In the present study, we have employed temperature-dependent IR and Raman spectroscopy to investigate the thermal behavior of different hydrogen-bounded species involved in the supramolecular systems and the heat-induced structural changes. The obtained IR spectra are rather complicated because they contain many overlapping bands involved in the different species of hydrogen bonds. Thus, we have employed generalized two-dimensional (2D) correlation spectroscopy analysis to unravel the complex spectra and to clarify different hydrogen-bounded species in these supramolecular systems.

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NEW APPROACH TO GENERALIZED TWO-DIMENSIONAL CORRELATION SPECTROSCOPY

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This paper introduces the first attempt in developing the fully integrated true combination of chemometrics and two-dimensional (2D) correlation spectroscopy.^[1] The use of reconstructed data matrix based on the significant scores and loading vectors obtained from the principal component analysis (PCA) of raw spectral data is proposed as a method to improve the data quality for 2D correlation analysis. 2D correlation analysis of this reconstructed data matrix, instead of the raw data matrix, can significantly reduce the contribution of noise component to the resulting 2D correlation spectra. We have proposed the concept of a PCA-based 2D (PCA-2D) correlation spectroscopy. In this approach, PCA treatment of spectral data set now becomes an essential and integral part of the subsequent 2D correlation analysis. A new concept of *eigenvalue manipulating transformation* (EMT) for the PCA-2D correlation analysis is also considered.^[2-4] By gradually suppressing the influence of minor PCs, noise is systematically reduced without the abrupt truncation of information. This EMT operation is similar to the simple truncation of noise-dominated minor factors practiced in PCA-2D correlation spectroscopy, but its effect is more gradual with attractive flexibility to continuously fine-tune the balance between the desired noise reduction effect and the retention of pertinent spectral information. By uniformly lowering the power of a set of eigenvalues associated with the original data, the smaller eigenvalues becomes more prominent and the contributions of secondary loadings become amplified. Thus, much more subtle difference of spectral behavior for each component is now highlighted. Further modification of 2D correlation spectra to enhance certain features may also be possible by systematically manipulating the eigenvalues.

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APPLICATIONS OF 2D CORRELATION IN PHASE-, FREQUENCY-, TIME-, AND SPACE-RESOLVED FTIR SPECTROSCOPY

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Two-dimensional infrared (2D-IR) spectroscopy, based on either conventional or generalized formalism,¹⁻² is an effective spectro-mathematical tool to elucidate spectral details and kinetic sequence of a dynamic system.³ The applicable perturbation to the dynamic system can be either sinusoidal or of an arbitrary waveform. This paper reviews applications of 2DIR in phase-, frequency-, time-, and space-resolved FTIR spectroscopy,⁴⁻⁶ particularly, photoacoustic spectral depth profiling of layered materials and rheo-spectral characterization of polymers. Some new applications to spectroscopic imaging characterization of heterogeneous systems are also presented. As an engine to drive most dynamic spectroscopic experiments and imaging data collection, step-scan interferometry is also briefly covered as one of the core technologies behind 2D-IR applications. A new 2D-IR software package, developed by Thermo, with enhanced imaging display is demonstrated in application examples in this paper. The overall advantages, limitations and cautions of using 2D-IR in practical applications are summarized finally.

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2D-Correlation Analysis of Time-Resolved FTIR-Spectroscopic Data of Externally Perturbed Chemical Systems

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Vibrational spectroscopy (mid-infrared, near-infrared and Raman) is an important tool for the compositional analysis and the characterization of physical phenomena of chemical systems. Especially the investigation of time-dependent changes of such a system under the influence of an external perturbation (temperature, concentration gradient, electric and electromagnetic fields, etc.) has become a challenge for time-resolved measurements.

In this respect over the last decade new developments in hard- and software have tremendously expanded the potential of these vibrational spectroscopic techniques and the information content of their data. Thus, the development of light-fiber optics and special in-line and on-line probes, for example, has led to the introduction of reaction monitoring by Raman and NIR as well as IR/ATR (attenuated total reflection) spectroscopy /1/. Furthermore, the increase in time-resolution down to the micro/nanosecond range has opened up novel research areas by offering insights into extremely fast processes, which have not been accessible so far. Thus, in studies of liquid-crystal display cells and photo-addressable polymers, the reorientation of liquid-crystals and liquid-crystalline polymers under the influence of external electric or electromagnetic fields, respectively, has been monitored by time-resolved Fourier-Transform (FT)-IR spectroscopy /2/. FT-IR and FT-NIR spectroscopy have also been successfully combined with sorption measurements to provide a more detailed picture of the diffusion of molecules with different geometries in polymeric materials /3/.

In many cases the discrimination and interpretation power of vibrational spectra can be significantly enhanced with special data treatments such as multivariate chemometric evaluation techniques or 2D-correlation analysis /1/.

With reference to selected examples the lecture is intended to address the potential of vibrational spectroscopy in combination with 2D-correlation analysis and to stimulate its more frequent exploitation for analytical and physical applications.

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TWO-DIMENSIONAL CORRELATION INFRARED ANALYSIS (2D-IR) BASED ON DYNAMIC INFRARED SPECTROSCOPY AS A PROBE OF THE VISCOELASTIC BEHAVIOR OF SIDE CHAIN LIQUID CRYSTALLINE POLYURETHANES

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Abstract

Dynamic infrared dichroism techniques were employed in the study of a complex side chain liquid crystalline segmented polyurethane. The dynamic spectra obtained were further analyzed using two dimensional correlation infrared spectroscopy (2D-IR) that facilitates an easier interpretation of the dynamic response. A new class of materials that allow the coupling of the optical changes brought on by liquid crystal alignment to applied mechanical strains is side chain liquid crystalline polyurethanes. The design and fabrication of thermoplastic elastomers, for which mechanical orientation may be achieved using classic plastic processing techniques to induce changes in liquid crystal orientation, is of high importance to the modern polymer industry. Such thermoplastic elastomers have been designed to exhibit the mechano-optic properties of liquid crystalline cross-linked systems. These materials could be ultimately used in the manufacturing of electro-optic or responsive coatings, sensors and transducers using conformal coatings as the building block. These segmented copolymers have liquid crystals pendant to a low T_g siloxane soft segments. The behavior of the side chain mesogens is monitored by the cyano tag at the end of the molecule while the hard segments response is found in the carbonyl spectral region. This study of the different parts of the macromolecule leads to the understanding of the elastic and the viscous orientation behavior of the polymer under conditions of dynamic strain. In particular, we find that the elastic component of the strain aligns smectic layers parallel and hard domains perpendicular to the direction of strain. The viscous strain component, on the other hand, induces a perpendicular smectic layer and parallel hard domain orientation behavior. The utilization of two-dimensional infrared spectroscopy made possible the discovery of the presence of paracrystalline hard segment “domains” within this material that have slightly different viscoelastic responses. All of these observations are consistent with a model proposed in earlier work that the hard segments and the smectic layers change orientation as the applied strain is increased from low to high strains. Finally, we show conclusive evidence for the coupling of the mechanical deformation behavior of the smectic layers and the hard domains and identified the two primary relaxation times in this system.

TWO-DIMENSIONAL CORRELATION AND PRINCIPAL COMPONENT ANALYSIS OF A POLY(ESTER URETHANE) WITH TEMPERATURE- DEPENDENT DIRLD AND DMA

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Step-scan Fourier transform infrared (S²FTIR) spectroscopy is a powerful tool for probing a sample subjected to modulation at frequencies low with respect to the usual Fourier frequencies of rapid-scan FTIR. This paper will describe the application of dynamic infrared linear dichroism (DIRLD) to the study of a di-block co-polymer, Estane 5703, by the combination of S²FTIR with dynamic mechanical analysis (DMA) in a relatively low-frequency mode. In particular, we will focus on the correlation of the spectroscopic characteristics and the mechanical properties as a function of temperature. By using two external lock-in amplifiers for demodulation of the phase modulation (732 Hz) and sample modulation (20 Hz), the precise and continuous temperature controllability of a research-grade dynamic mechanical analyzer can be fully exploited. The DIRLD spectra of Estane were measured at temperatures ranging from -50 to +30 °C with a maximum resolution of 1 °C. The thermo-mechanical properties were simultaneously recorded by the DMA. Principal component analysis (PCA) and two-dimensional (2D) correlation analysis were employed to analyze the temperature-dependent (TD) DIRLD spectra. A distinct Tan Delta glass transition temperature (T_g) near 11 °C is shown by DMA, which is widely believed to be due to the soft segment transition. PCA reveals that the overall change of the DIRLD as a function of temperature closely correlates to the modulus change of the material. Generally, the intensities of most of the DIRLD peaks decrease with rising temperature. However, the carbonyl stretching peaks show totally different patterns and much more complexity. The strongly overlapped carbonyl components in the static IR spectra are distinctly differentiated in the DIRLD spectra at low temperatures. The 2D correlation of the DIRLD spectra reveals two major and two minor $\nu(\text{CO})$ components associated with different functional groups and/or different hydrogen bonded populations. Both of the major carbonyl bands are blue-shifted at higher temperature and show a transition near the Tan Delta T_g, but the urethane band experiences a much more dramatic shift ($\sim 26 \text{ cm}^{-1}$) than the ester band ($\sim 5 \text{ cm}^{-1}$). These results strongly suggest that the Tan Delta T_g should be attributed to changes in the overall H-bonding network, rather than just to a phase transition of the soft domain.

Key words: two-dimensional correlation; Principal component analysis; Dynamic mechanical analysis; DIRLD; Estane.

**INFRARED SPECTROSCOPIC ANALYSIS OF A SERIES OF BLENDS OF
POLY(LACTIC) ACID AND POLY(3-HYDROXYBUTYRATE-CO-3-
HYDROXYHEXANOATE), A BACTERIAL COPOLYESTER**

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Infrared (IR) spectra were collected on a series of blends of poly(lactic) acid (PLA) and poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (P(3HB-co-3HHx) (3HHx = 13.4 mol%)) using a single-bounce temperature-controlled attenuated-total-reflection (ATR) accessory with a diamond internal reflection element (IRE). Different approaches to the analysis of the raw spectral data will be compared, including peak positions and intensities, principal components, and 2D correlation spectra. Differences in the interactions between the functional groups of the components become apparent as a function of temperature and composition. We hope to learn more about the miscibility of this polymer blend system by understanding the submolecular-level interactions revealed in this study.

Two-Dimensional Correlation Analysis of Visible / Near-Infrared Spectral Intensity Variations of Chicken Breasts with various Chilled and Frozen Storages

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ABSTRACT

Generalized and Hetero two-dimensional (2D) correlation analysis of visible/NIR spectra were performed to characterize the spectral intensity variations of chicken muscles induced by either storage time- / temperature- regime or shear force values. The results showed that intensities of two visible bands at 445 and 560 nm increase with the storage temperature under a 14-day treatment, hinting that frozen storage might enhance the color of meats. The 2D NIR correlation spectra indicated that all NIR bands reduce their spectral intensities, probably due to the water loss and compositional alternations during frozen-thaw process as well as the tenderization development in muscle storage. The heterospectra correlating the spectral bands in both visible and NIR regions exhibited a strong correlation, and suggested the sequential change between color and other meat properties as developed due to storage regime. In addition, NIR spectral intensity variations detected significant differences in spectral feature between tender and tough meats as correlated to shear value.

Keywords: Two-dimensional correlation analysis; visible/NIR spectroscopy; chicken muscles; myoglobin; tenderness

2D-IR AND RELATED ANALYSES OF PROTEIN FOLDING AND MISFOLDING

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2D-IR correlation spectroscopy is a new addition to the IR-based methods employed for the characterization of conformational changes in proteins. We recently reported a 2D correlation analysis performed on the IR spectra of the λ -Cro repressor protein, which provided evidence for sequential events in the unfolding process of the protein [1]. Here, we present our studies of two further proteins, ribonuclease T1 and phosphoglycerate kinase.

Ribonuclease T1 is an α/β protein composed of 104 amino acids. Its secondary structure includes a long α -helix, an antiparallel β -sheet composed of three long and two short β -strands, and a short two-stranded antiparallel β -sheet. The reversibility of the thermal unfolding of ribonuclease T1 permitted a detailed IR analysis of the impact of point mutations on the structure and thermal stability of the protein [2]. Moreover, temperature-jump techniques in combination with time-resolved FTIR spectroscopy provided insights into different phases of β -structure formation and regaining of tertiary structure of the protein [3]. At the same time, no information on refolding of the α -helical structure of ribonuclease T1 was obtained, because of difficulties in the assignment of the corresponding amide I band component(s). A comparative 2D-IR correlation analysis of the spectra of the wild-type protein and two mutants was helpful in identifying amide I features, which can be associated with the α -helix present in ribonuclease T1.

Phosphoglycerate kinase is one of the proteins, although unrelated to proteins involved in known amyloid diseases, which can assemble into amyloid-like structures under certain conditions [4]. The characterization of fibril formation after mixing of acid-unfolded phosphoglycerate kinase with sodium chloride by FTIR spectroscopy (including 2D-IR correlation analysis) and dynamic light scattering revealed that at first a partially folded monomeric intermediate is formed, which has a significant amount of α -helical and some intramolecular β -structure. The next step involves the formation of intermolecular β -sheet structure and of defined oligomers. The growing of the fibrils begins at the level of octamers, which is not associated with further major changes in secondary structure.

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COMPARISON OF TWO-DIMENSIONAL CORRELATION SPECTROSCOPY WITH OTHER METHODS OF ABSORPTION ANALYSIS FOR STUDING NIR SPECTRA OF WATER

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The near-infrared spectra (NIR) of liquid water measured in the range $5600\text{-}4500\text{ cm}^{-1}$ in function of temperature from 20°C to 80°C , have been examined by following approaches: second derivative spectra, difference spectra, curve-fitting procedure, principal component analysis (PCA) and finally by 2D correlation spectroscopy (2DCOS). Because the analyzed spectra of water have been generated by nonperiodic perturbation, we utilized the concept of a linear or nonlinear relationship instead of the synchronous and asynchronous correlations [1]. Keeping in mind the fact that obtained results depend only on changes in absorption values the following pretreatment steps have been done: baseline correction, denoising by means of wavelet analysis, normalization against the concentration changes due to temperature increase. The analysis of the decomposed spectra revealed that not only intensity but also position, width, and shape of individual components have been changed with temperature increase. Moreover, the differences in the positions of the sub-bands are far less than their half-width. All the facts strongly affect the results of the four procedures of analysis. The advantages and disadvantageous of each of these methods will be confronted. The influence of the normalization procedures particularly on PCA i 2DCOS will be also analyzed. The obtained results will be discussed in context of two-component mixture model for the structure of liquid water. This model is extremely successful in providing a quantitative description of water properties.

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Two Dimensional Correlation Infrared Spectroscopy and the Quality Control of Traditional Chinese Medicines

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Abstract: To develop a simple, rapid, computer aided, nondestructive and accurate technique to control the quality of traditional Chinese medicines (TCM), a brand new method, two-dimensional correlation infrared spectroscopy (2D IR), was introduced. According to the finger-print characteristics of the IR spectra, and the auto-peaks and cross-peaks generated by the 2D IR spectroscopy, both raw herbs and products could be classified and identified easily. The producing area and growing environment (wild or implanted) of raw herbs could be identified. Different kinds of formula granules or injections, same granules or injections made by different manufactories or different batches, or different proportion of auxiliary materials in the TCM products could be compared and identified. As to one injection, the deterioration is always a gradual process. When aging time or temperature range for injection is not enough, small changes could be analyzed by 2D-IR technique, which are difficultly examined by IR spectra. For example, the deterioration of the 'Qing Kai Ling' injection in air at room temperature should be due to the oxidation of the flavone compounds mainly, which is associated with conjugated carbonyl and hetero-aromatic ring. When IR spectrum is expanded to 2D correlation spectroscopy, detailed spectral features could be viewed obviously, which could not be examined easily in conventional IR spectrum. 2D IR provides not only an interesting way for the development and research of complicated compounds such as TCM, but also a powerful method for the research of deterioration of TCM as well as the quality control of TCM.

Keyword: Traditional Chinese medicines, two-dimensional correlation infrared spectroscopy, Formula granules, Injections, Quality control,

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TWO-DIMENSIONAL CORRELATION SPECTROSCOPY FOR PROTEIN STUDIES

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Fourier transform infrared spectroscopy (FT-IR) has been used extensively as a powerful technique for exploring the secondary structures of proteins.¹ By using FT-IR one can investigate the secondary structures even for proteins with huge molecular weight. It is also of note that IR spectroscopy allows one compare the secondary structure proteins among aqueous solutions, solids, and crystals. In the IR region, the frequencies of bands due to the amide, $\nu_{\text{C-H}}$, and $\nu_{\text{N-H}}$ vibrations are sensitive to the secondary structure elements of proteins.¹ Particularly, the amide band is useful for the secondary structure studies. In general, the amide bands consist of several component bands assignable to different secondary structures. Therefore, to investigate the secondary structures of proteins by using IR spectroscopy one must deconvolute the amide bands into each component band. The separation of broad amide band into component bands and their assignment to various secondary structures, such as α -helix, β -sheet, and random coil have been the subjects of numerous studies.¹⁻⁵ Usually, second derivative, Fourier self deconvolution (FSD) and curve fitting are used to deconvolute the amide band. However, the results obtained by the above methods are not always conclusive, especially for complex proteins consisting of various secondary structures.

In recent years generalized two-dimensional (2D) correlation spectroscopy² has been introduced as a new technique for analyzing amide, $\nu_{\text{C-H}}$, and $\nu_{\text{N-H}}$ bands of proteins.³⁻⁵ 2D IR correlation spectroscopy is very useful for protein research because of three major reasons. One is that 2D correlation spectroscopy enable one to deconvolute the amide bands into component bands due to different secondary structures. Another is that it allows establish the correlation between secondary structures of protein through selective correlation peaks for given perturbation. Yet another is that it gives information about specific order of secondary structural changes and changes in amino acid residues various environments.

In this conference we report the following our three studies. One is a 2D ATR/IR spectroscopy study of adsorption-induced and concentration-independent spectral variations of β -lactoglobulin (BLG) in aqueous solutions.³ Another is a 2D ATR/IR correlation spectroscopy study of pH-dependent changes in the secondary structures and hydrogen bonding of carboxylic groups of human serum albumin.⁵ Yet another is 2D ATR/IR spectroscopy and principal component analysis studies of the secondary structure and kinetics of hydrogen-deuterium exchange of human serum albumin.⁴

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Two-Dimensional Fluorescence Correlation Spectroscopy for Identification/Classification in Biomedicine and Biotechnology

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Many current problems in biomedicine and biotechnology involve the sensing/identification and subsequent classification of samples based on their physicochemical characteristics. In optical detection of diseases, the diagnosis is based on the spectroscopic signatures of the patient sample. If these signatures resemble those of the library spectra of diseased tissue, the patient sample is classified as non-normal and a positive diagnosis is made. In biotechnology, sensing and identification of multiple genes, pathogens, etc. is of tremendous interest. For example, the identification of bases is central in sequence analysis and gene detection.

Two-dimensional correlation spectroscopy provides a powerful method for species identification and classification, an aspect that has not been thoroughly explored to date. The much-demonstrated utilities of 2D correlation spectroscopy in dynamics and resolution, for example, rely on the similar spectral characteristics of a sample 'class' in the other dimension. For example, resolution of overlapped polymer spectra is based on the similar dynamics of structurally related functional groups. Dynamic studies in chemical reactions are facilitated by the concurrent growth of the product peaks and the reduction in reactant peaks. These similarities in physicochemical characteristics render 2D correlation spectroscopy an excellent tool for species identification and classification. In this talk, we will present applications of two-dimensional fluorescence correlation spectroscopy (2D FCS) in biomedicine and biotechnology, for species identification/classification. Especially, new methodologies for sequence analysis and optical detection of cancerous transformation in tissue have been developed. Both dynamic and statistical methods of 2D FCS will be discussed.

Comparison on the resolution of FTIR of 25- α - and 25- β - Steroids using two-Dimensional correlation analysis and principal component analysis

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Abstract. Two-dimensional infrared correlation was applied on steroidal sapogenin isomers. The asynchronous (disrelational) spectrum of the two-dimensional I. R signals managed to resolve the overlapped peaks of Diosgenin (25 - α -isomers) and Yamogenin (25- β -isomer) at 980 cm^{-1} , and their individual concentrations were directly determined. The result of 2-D correlation were compared with other chemometric techniques such as principal component analysis, PCA. The results obtained by PCA were consistent with 2-D correlation.

Effect of Reference Spectrum on Two-Dimensional Correlation Spectra

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This contribution presents the effect of the reference spectrum on two-dimensional (2D) correlation spectra for noise-free and noisy data. It has been shown that an application of a perturbation-averaged, the first or the last spectrum in the data set as a reference for 2D correlation analysis did not alter the number and signs of the correlation peaks. Therefore, by using any of the three references one can obtain the same sequence of spectral events. On the other hand, selection of the reference spectrum remarkably different from the spectra employed for 2D correlation analysis significantly complicates interpretation of 2D contour plots and in consequence may lead to wrong conclusions. The synchronous and asynchronous spectra computed without the reference spectrum are weakly disturbed by the noise, whereas this effect is more pronounced for the other reference spectra. In all cases, the asynchronous spectra are stronger affected by the noise as compared with the synchronous spectra.

OUTER-PRODUCT ANALYSIS (OPA) USING PLS REGRESSION TO STUDY THE RETROGRADATION OF STARCH

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Outer Product Analysis (OPA) is a method which makes it possible to emphasise co-evolutions of spectral regions in signals obtained from the same domain or from two different domains.

The calculation of the Outer-Product (OP) matrix which links the 2 fields corresponds to a mutual weighting of the two signals.

To highlight these simultaneous variations we can apply various univariate or multivariate statistical techniques to this OP matrix.

Among the multivariate analyses : Principal Components Analysis (PCA) is the simplest. Applying it to the OP and examining the ordering of the samples along the Principal Components (PC) matrix allows to detect those which contain information. The loadings of the interesting PCs reveal the simultaneous variations responsible for the ordering of the samples.

In the same way, PLS regression can be used to direct the analysis so that we can visualise the simultaneous variations associated with a particular evolution of the samples

In this study we characterise the retrogradation of starch by Time-Domain RMN, FT-IR and X-ray diffraction (XRD). During the process of retrogradation, the starch evolves from an amorphous state to a crystalline state and this transformation has an effect on these three signals.

Three OP matrices are calculated corresponding to two-by-two combinations of the three signals. PLS regression is then performed to predict retrogradation time.

The B-coefficients obtained highlight the simultaneous variations in the pairs of signals due to the retrogradation.

OUTER-PRODUCT ANALYSIS (OPA) USING PCA : COMPARISON WITH SAMPLE-SAMPLE 2D CORRELATION SPECTROSCOPY.

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Outer Product Analysis (OPA) is a method which makes it possible to emphasise co-evolutions of spectral regions in signals obtained from two different domains or even the same domain.

The calculation of the Outer-Product (OP) matrix linking the two domains corresponds to a mutual weighting of the two signals.

Various statistical techniques - both univariate and multivariate - can be applied to these matrices to bring out these simultaneous variations.

The simplest multivariate analysis - Principal Components Analysis (PCA) - applied to the OP matrix allows to visualise the distribution of the individuals (scores plots) in order to determine which Principal Components contain information.

The corresponding loadings show the simultaneous variations that are related to the observed distribution of the individuals.

Outer Product Analysis may also be used in way very similar to sample-sample 2D-Correlation Spectroscopy in order to highlight the synchronous and asynchronous evolution of samples.

To do this, the data set is first transposed before calculating the Outer Product (OP) matrix. A Principal Components Analysis (PCA) is then applied to this OP matrix to emphasise the simultaneous variations of the samples.

In this study we applied this sample-sample OPA to a set of data already studied by Ozaki *et al* (*Analytical Chemistry* **2001**, 73, 3153-3161), concerning the evolution of Near Infrared (NIR) spectra of water as a function of the temperature.

We compare the PCA results (loadings and scores) with the sample-sample synchronous and asynchronous spectra of 2D-Correlation Spectroscopy, to determine the resemblances and the differences between these techniques.

OUTER-PRODUCT ANALYSIS (OPA) USING PLS REGRESSION TO STUDY THE EFFECT OF TEMPERATURE ON NIR SPECTRA OF WATER.

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Outer Product Analysis (OPA) is a method which makes it possible to emphasise co-evolutions of spectral regions in signals obtained from two different domains or even the same domain.

The calculation of the Outer-Product (OP) matrix linking the two domains corresponds to a mutual weighting of the two signals.

Various statistical techniques - both univariate and multivariate - can be applied to these matrices to bring out these simultaneous variations.

The simplest multivariate analysis - Principal Components Analysis (PCA) - applied to the OP matrix allows to visualise the distribution of the individuals (scores plots) in order to determine which Principal Components contain information.

The corresponding loadings show the simultaneous variations that are related to the observed distribution of the individuals.

In the case of complex data sets with many sources of variation in the samples, PLS regression can be used to direct the analysis to visualise the simultaneous variations associated with a particular evolution with the samples. In this way, the individuals do not need to be sorted in any particular order.

In this study we applied the OPA to a set of data already studied by Ozaki *et al.* (*Analytical Chemistry* **2001**, 73, 3153-3161), concerning the evolution of Near Infrared (NIR) spectra of water as a function of the temperature.

We compare the different loadings profiles obtained by PLS with the variable-variable synchronous and asynchronous spectra of 2D-Correlation Spectroscopy, to determine the resemblances and the differences between these techniques.

Characterisation and Authentication of Vegetable Oils by Combining Near and Mid Infrared Signals

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Infrared spectroscopy is a powerful analytical technique that is now widely used to provide both qualitative and quantitative information about many kinds of samples. Near Infrared (NIR) and Mid Infrared (MIR) spectroscopy have a wide range of applications. MIR is widely used for molecular structure determination in research laboratories whereas NIR is widely used for quality control in industry, mainly in the agro-food sector. The signals observed in the NIR region correspond to combinations and harmonics of the fundamental molecular vibrations observed directly in the MIR region.

Therefore, a better understanding of the NIR data may be had by conjointly studying the two regions. This comparison should help the analyst to assign NIR bands to particular combinations of the fundamental molecular vibrations observed in the MIR.

The combination of the two signals should also increase the predictive ability of regression or discriminant models created using multivariate chemometric methods. This simultaneous analysis may be done by 2D Correlation Spectroscopy (2DCOS) or by Outer Product Analysis (OPA) which can both reveal how the signals vary simultaneously as a function of physico-chemical properties.

These combinations were done with the two domains matrices, as well as, on matrices of the squared values (for non-linear evaluation), concatenated matrices and 1st and 2nd derivative matrices for a better understanding of the models variation.

In 2DCOS, correlations are calculated between all the frequencies in two different spectral domains as a function of some variation in samples.

The OPA calculates for each sample the product of intensities at all combinations of frequencies in the two domains to produce an Outer Product matrix. The complete set of OP matrices may then be analyzed using chemometric techniques such as PCA, FDA and PLS. Plots of loadings, B coefficient or Discriminant Function are drawn to visualize the simultaneous variations in the two domains as a function of the predicted property or classification criterion.

In this study, these techniques are applied to the characterization and authentication of vegetable oils samples.

It is shown that 2DCOS and OPA facilitate the interpretation of differences between samples. These two techniques are compared in order to determine the advantages and disadvantages of each method.

EUROSPEC THE INTERNATIONAL SPECTROSCOPIC DATA BASE

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EuroSpec is a EU funded project which started in January 2002. It develops an electronic database and archive for spectroscopic and other associated data from peer reviewed publications and other scientific literature.

The project will run initially for three years and develop electronic data submission protocols in close collaboration with the major scientific publishers and directly with authors.

This data base project addresses the massive deficit in the amount of quality reference spectroscopic data currently available to the analytical community when compared to the number of spectra acquired.

The partners in the project are:

- 1- Institut für Spektrochemie und Angewandte Spektrochemie, (D)
- 2- Creon Lab Control AG (D)
- 3- Institut National Agronomique (F)
- 4- Universidade de Aveiro (P)
- 5- Vysoka Skola Chemicko-Technologicka (CZ)
- 6- LGC (North West) Ltd (GB)
- 7- IM Publications (GB)
- 8- Specs and Biospecs BV (NL)

The publishers who have already accepted to participate in the initial phase of the project include Elsevier, Wiley, Springer, RSC, SAS.

After the first three years of work on this project, the rights of the data will be transferred to a non-profit-making body which will use it for the good of the scientific community as a whole.

The existence of such collections of spectroscopic and substance data should stimulate significant progress in chemical research and have wide implications for human health, nutrition science, environmental protection and educational progress.

APPLICATION OF 2D CORRELATION SPECTROSCOPY AND OUTER PRODUCT ANALYSIS TO INFRARED SPECTRA OF SUGAR-BEETS

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The reference method for the measurement of the percentage of sugar in sugar beets is polarimetry. Infrared Spectroscopy is now being more and more used in the food industry. It can be used, in our case to determine the percentage of sugar in sugar beets. Reference values are acquired by polarimetry. Based on these reference values and on Infrared Spectra, regression techniques such as Partial Least Squares (PLS) regression may be used to develop mathematical models to predict the sugar content of beets.

Before establishing the model, it is preferable to eliminate uninformative parts of the Near-Infrared spectra, in order to improve the model performance. Several different methods can be used to determine regions of interest in a spectrum such as Outer Product Analysis (OPA) and 2D Correlation Spectroscopy.

Outer Product Analysis can be used to facilitate the interpretation of Near Infrared (NIR) and Mid Infrared (MIR) signals. In this method, the spectra acquired in the two domains are combined by calculating the Outer Product matrix of the two vectors. It is then possible to perform Principal Components Analysis or Partial Least Squares to the resulting data-set of matrices in order to highlight relations between spectral features in the two domains. This can facilitate the attribution of NIR bands based on their relation with MIR peaks.

Results of the 2D Correlation Spectroscopy will also be presented. This is another method that can be used to allow bands in the NIR spectrum to be resolved and assigned to characteristic absorbances in the MIR spectrum. The principle of this method is to detect regions of both spectra (NIR & MIR) which change simultaneously when sugar content varies.

TWO-DIMENSIONAL CORRELATION SPECTROSCOPIC STUDIES ON THE CATALYTIC MECHANISM OF SELENIUM-CONTAINING GLUTATHIONE PEROXIDASE MIMICS

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Two-dimensional (2D) correlation spectroscopy has been applied to concentration-dependent Uv-vis spectra to reveal the catalytic mechanism of the reduction of hydro-peroxidase catalyzed by selenium-containing glutathione peroxidase mimics, a series of seleno-modified β -cyclodextrin, 2,2'-diseleno-bis- β -CD (2-diSeCD) and 6,6'-diseleno-bis- β -CD (6-diSeCD). Great efforts have been put on revealing why these selenium-containing enzyme models displayed higher GPX-like activity than the previous GPX mimic enzymes as PZ51 *et al.*. Following items have been addressed in the present study; (i) characterization of the intermediates in the catalytic cycling; (ii) clarifying the kinetics of diSeCD-catalyzed reduction of peroxidase; and (iii) making it clear that does the hydrophobic cavities of cyclodextrins (CDs) play an important role for binding substrates.

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NEW FEATURES IN 2D-CORRELATION INFRARED SPECTRA: FACTS OR ARTEFACTS?

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Generalized two-dimensional (2D) correlation spectroscopy has generated considerable interest and has been applied to a range of systems. The technique allows spectra that are obtained as a function of variables such as temperature and concentration to be spread over two frequency dimensions. As a result, it has been claimed that enhanced spectral resolution has been obtained and previously undetected bands revealed. Furthermore, the correlation spectra, most often reported as contour plots, have been interpreted in terms of a sequence of intensity change, which are then, in turn, related to structure or interactions. We have applied correlation infrared spectroscopy to a study of polymer blends, where spectra were obtained as a function of concentration, and materials such as N-Methylacetamide, where spectra were obtained as a function of concentration. We will show that features in the correlation spectra of blends that have been interpreted in terms of the detection of previously hidden bands, then assigned to the effect of interactions or conformational changes, actually correspond to lobes or points of inflection in the difference spectra used to generate the 2D plots. In immiscible blends these features are usually a consequence of small bandwidth changes that are a result of sample preparation problems. In miscible systems, frequency shifts also give rise to characteristic features in the difference spectra that give rise to “new” features in asynchronous plots. However, these features are not fundamental normal modes of vibration. Such frequency shifts also occur in the spectra of materials such as N-Methylacetamide as a function of temperature. Again, subtracting bands that are shifted in frequency from one another results in positive and negative difference bands that result in characteristic features in the asynchronous spectra, but these are not infrared absorption bands.

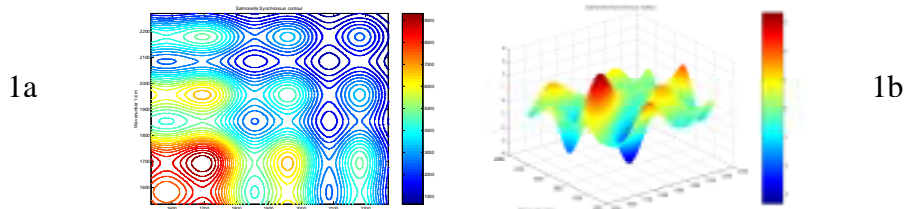
GENERALIZED TWO-DIMENSIONAL CORRELATION RAMAN SPECTROSCOPY APPLICABLE TO IDENTIFICATION AND QUANTIFICATION OF CLINICALLY RELEVANT MICROORGANISMS

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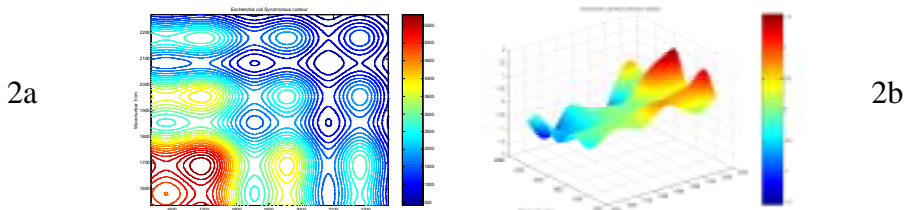
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The identification of pathogenic microorganisms causes of tropical gastroenteritis through classical microbiological methods, is a reasonably simple task and in the great majority of the cases, very efficient. However, the time spent for such identifications is long, about 48h, could generate serious consequences, in cases which the patients are children, senior or adult with low resistance. It means that in these cases, the research for new spectroscopic methods of low cost and new chemometrics methods, which allow the fast identification of these microorganisms, is extremely important. In this work we have used the main microorganisms causes of tropical gastroenteritis: *Escherichia coli*, *Salmonella*, *Shigella*. For each one of the microorganisms it was prepared ten different concentrations, according to MC Farland scale, using physiologic solution as solvent. The Raman spectra of these solutions were recorded using a diode laser at 785 nm. The obtained Spectra were absolutely similar, turning impossible to differentiate the microorganisms. However, applying the 2-D correlation method¹, it was possible to differentiate the evaluated microorganisms, through the asynchronous spectrum and, moreover, it was possible to identify the best regions of the spectrum for a multivariate calibration process. In the Figure 1a and 1b we have showed the synchronous map and the asynchronous surface for *Shigella*.



In the Figure 2a and 2b we have showed the synchronous map and the asynchronous surface for *E. coli*.



We can observe in the synchronous maps the correlations and the best regions for a possible multivariate calibration process. Yet in the asynchronous surfaces it is possible to differentiate microorganisms due to variations out of phase in highly overlapped bands^{2,3}.

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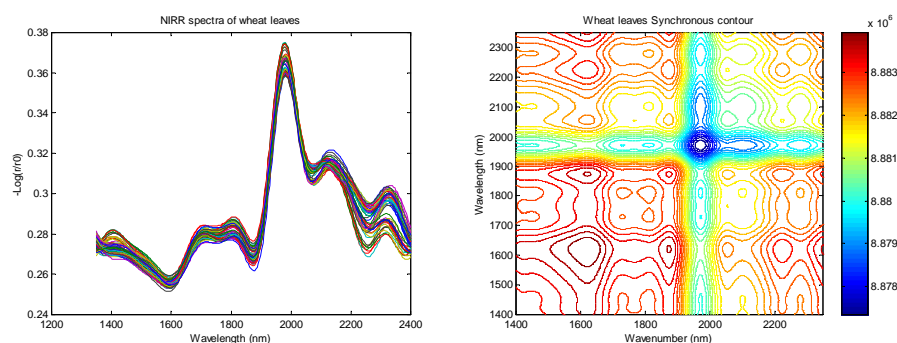
NEURAL NETWORKS AND GENERALIZED TWO-DIMENSIONAL CORRELATION NIRR SPECTROSCOPY APPLICABLE TO DETERMINATION OF THE NITROGEN CONTENT IN WHEAT LEAVES

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The nitrogen content in plants is a parameter extremely important for the prevision of certain plantations' production. Knowing this parameter the farmer can correct the nutrients of the soil and thus optimize the harvest. Traditionally this nitrogen analysis is done through the Kjeldahl method which is laborious, slow, it uses several chemical compounds and it mostly generates a reasonable quantity of residues, highly toxic for the environment. It means that in this case, the development of spectroscopic methods with high sensibility that allow fast and *in situ* determinations is extremely important. A very established method which performs these requisites is the near infrared diffuse reflectance when associated with chemometrics. The diffuse sample spectra were recorded directly over a sample cell with an optical fiber probe, with low hydroxyl content to avoid near-infrared absorption, using a handcraft spectrometer with a monochromator based on the acoustic optic tunable filter approach, set to operate in 1400-2400 nm range. The NIRR spectral data set obtained after preprocessing (FFT Filter, MSC) are showed in the Figure 1a. In the Figure 1b we represented the synchronous map.



Once obtained the correlation map we elaborated a neural network, which the inputs were selected in the regions with larger correlation. The neural networks' results (%SEP=7.6) were compared, through the test F, with the results obtained with models elaborated with PLS (%SEP=8,9). The method of the neural networks with wavelengths selected through the 2-D correlation was superior at 95% confidence level.

GENERALIZED TWO-DIMENSIONAL STUDY OF POLARIZED INFRARED SPECTROSCOPY ON PROTEIN STRUCTURE OF SPIDER'S THREAD

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Spider's thread is a fibrous protein, and has prominent properties on strength and elasticity. The properties are strongly influenced by high-order structure and orientation of protein. Measurement of the conformation and orientation is of particular importance for understanding the mechanisms of the marvelous property on stability. Therefore, we have been studied the protein orientation and structure of spider's thread by micro-ATR infrared spectroscopy. Two-dimensional correlation analyses of polarized infrared spectra of the thread has been applied on the amide I and amide II bands.

The dragline from spider *Araneus Ventricosus* was collected on a wire-reel firsthand. The polarized infrared micro-ATR spectra were measured *in situ*. The bands assigned to amide I and amide II are complexly overlapped with the band components from helical, sheet, and unordered structures of peptide. The intensity of these bands are considerably changed as the polarization angle, which indicates that the main chain of protein in the spider's thread are well oriented. To identify the overlapped band, the generalized two-dimensional correlation analysis has been applied. Of particular note that the band at 1620 cm^{-1} due to sheet conformation of Amide I is strong in the parallel alignment measurement, while the bands near 1680 cm^{-1} due to turn structure of Amide I are intense in the perpendicular alignments. This result suggests that the secondary structure of protein in the thread is highly ordered to parallel direction of the thread. Supposing that the ordered orientation performs the role in the remarkable properties of the spider's thread.

***** STUDY OF CHEESE-MAKING PROCESS BY APPLYING 2DCOS TO
THE NIR AND VISIBLE REGIONS**

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In a previous work by using 2D-IR-Correlation Spectroscopy (2DCOS), we found that every change in the “standard-rennet” coagulation process induced variations in secondary interactions water/water and water/ milk constituents. The aim of this work was to study these process variations by using 2DCOS and Outer Product Analysis (OPA) in the Near Infrared (NIR) and Visible (VIS) regions.

Coagulation tests were carried out at 35°C, using reconstituted skim milk powder as substrate and a calf rennet solution as clotting agent. Different coagulations were carried out changing temperature, pH, and rennet concentration one at a time. NIR spectra were recorded as log (1/R) with a dispersive instrument from 1100 to 2500 nm. VIS spectra were collected with a UV-VIS spectrophotometer from 352 to 800 nm. In both cases, milk samples were injected into the cell at constant temperature, after rennet addition, and spectra were collected every 71 seconds.

2D-NIR-VIS-COS allowed us to identify milk constituents linked with water, and involved in milk system rearrangements, resulting in the variations of hydrogen bonds. OP matrices were analysed using PLSR, and the B coefficient surfaces showed the contribution of free and bonded water during the progress of coagulation.

**THE USE OF TWO-DIMENSIONAL SOFT X-RAY ABSORPTION
SPECTROSCOPY (2D XAS) AND 2D HETERO-SPECTRAL XAS-RAMAN
CORRELATION ANALYSIS IN THE STUDY OF THE ELECTROCHEMICAL
REACTION MECHANISM OF LITHIUM WITH CoO**

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This paper introduces the first application of two-dimensional (2D) correlation spectroscopy to soft X-ray absorption spectroscopy (XAS) and the possibility of 2D hetero-spectral XAS-Raman correlation analysis for nanomaterial research. To investigate the mechanism of the electrochemical insertion of lithium into the CoO electrode in a Li/Li_yCoO cell, we applied a 2D correlation analysis to the spectra of the Li_yCoO system during the first insertion-extraction reaction as obtained by lithium concentration-dependent XAS and Raman spectroscopy. 2D XAS and 2D Raman spectra yield great resolution enhancement and show that the insertion of lithium into the CoO electrode leads to Li₂O formation. Moreover, by analyzing the sign of the asynchronous cross peaks we reveal the sequence of the first insertion process: firstly, the intensity of the band due to CoO decreases and then that of the band due to Li₂O increases. A 2D hetero-spectral XAS-Raman correlation analysis was also undertaken of the same XAS and Raman spectra. This hetero-spectral 2D correlation analysis has elucidated not only the sequence of events between XAS and Raman signals from the same species, but also the correlation between the XAS and Raman bands, confirming their band assignments. The potential of 2D XAS, 2D Raman, and 2D hetero-spectral XAS-Raman correlation analysis in the examination of the mechanism of the electrochemical insertion of lithium into the CoO electrode will be discussed.

CHARACTERIZATION OF ELECTROCHEMICAL REACTION IN THE $\text{Li}_{1+x}\text{V}_3\text{O}_8/\text{Li}$ CELL BY SOFT X-RAY ABSORPTION SPECTROSCOPY AND TWO-DIMENSIONAL CORRELATION ANALYSIS

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We have recently demonstrated that soft X-ray absorption spectroscopy (XAS) and two-dimensional (2D) correlation analysis can be successfully used to study the characteristics of electrode materials.^[1] Determination of the relationship between local structure and the lithium content in metal oxides is very important to the understanding of the electrochemical behavior of these materials. In this study, we applied XAS and 2D XAS correlation analysis to the first lithium insertion-extraction cycle in a $\text{Li}_{1+x}\text{V}_3\text{O}_8/\text{Li}$ cell in order to investigate the electrochemical reactions of lithium with the $\text{Li}_{1+x}\text{V}_3\text{O}_8$ electrode. The V L_{2,3}-edge and O K-edge spectra of the $\text{Li}_{1+x}\text{V}_3\text{O}_8$ electrode were obtained for varying electrode lithium content. The insertion of lithium leads to the reduction of the V^{5+} species present in the pristine $\text{Li}_{1+x}\text{V}_3\text{O}_8$ electrode, and to the red shift and the broadening of the spectral features of the V L_{2,3}-edge compared to those of the pristine electrode. In the extraction process, the main spectral features at the highest value of the extraction of lithium show some differences compared to the features of the pristine electrode spectrum due to the residual lithium ions in the $\text{Li}_{1+x}\text{V}_3\text{O}_8$ structure. The O K-edge spectra revealed that the insertion of lithium mainly affects the V 4sp—O 2p bonds and consequently induces a change in bonding geometry. The 2D correlation analysis of these spectra clearly shows that V—O bonds are significantly perturbed by the insertion-extraction of lithium into the $\text{Li}_{1+x}\text{V}_3\text{O}_8$ electrode.

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**PHOTOREACTION AND MOLECULAR REORIENTATION IN A
NANOSCALED FILM OF POLY(METHYL 4-(METHACRYLOYLOXY)
CINNAMATE) STUDIED BY TWO-DIMENSIONAL FTIR AND UV
CORRELATION SPECTROSCOPY**

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This paper demonstrates photoreaction and photoinduced molecular reorientations in nanoscaled films of poly(methyl 4-(methacryloyloxy)cinnamate) (PMMCi) investigated through two-dimensional (2D) correlation analyses of their infrared (IR) and ultraviolet (UV) absorption spectra[1]. In the PMMCi film the presence of three distinct chromophore types was evident: isolated trans-isomeric cinnamoyl moieties and parallel and end-to-end cinnamoyl aggregates. Their photoinduced reorientations take place in the following sequence: isolated trans isomeric chromophores → parallel chromophore aggregates → end-to-end chromophore aggregates. It turns out that they react photochemically at different rates. The PMMCi polymer molecules were found to undergo both photoisomerization and photodimerization upon UV irradiation, with the trans-cis isomerization of the isolated trans isomeric cinnamoyl moieties occurring more rapidly than the photodimerization of the cinnamoyl aggregates. Of the photodimerization processes, the reorientation of the parallel cinnamoyl aggregates precedes that of the end-to-end cinnamoyl aggregates. The isolated chromophores may partly involve in the photodimerization, but its fraction is expected small because of the film in a confined geometry. The cinnamoyl moiety was found to be present as two rotational conformers, trans-s-trans and trans-s-cis conformers. Upon UV irradiation, the trans-s-trans conformer changes before the trans-s-cis conformer. The cinnamoyl moieties undergo a cooperative reorientation with the methacryloyloxy units during photoreaction. The photoinduced molecular reorientations occur in the following sequence: trans isomer → phenyl ring → trans-s-trans conformer → trans-s-cis conformer → photodimer

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**THE APPLICATION OF TWO-DIMENSIONAL FTIR CORRELATION
SPECTROSCOPY AND PRINCIPAL COMPONENT ANALYSIS IN THE STUDY
OF CONCENTRATION-DEPENDENT SPECTRAL VARIATIONS OF UREA
AQUEOUS SOLUTION**

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In this study it was found from FTIR spectra of urea aqueous solution that the modes of water-urea interaction are controlled by the concentration of urea. The spectral variations of urea solutions were examined by two-dimensional (2D) correlation analysis and principal component analysis (PCA). The frequencies and intensities of the bands assigned to the C=O stretching and N-D in-plane bending vibrations in urea depended upon the concentration of urea. The aggregation of urea molecules starts at 2 M of urea and increases with the concentration of urea. It implies from these observations that the hydrogen bonds between urea and water decrease against the concentration of urea, whereas those between urea molecules increase with that. The simple analysis of the absorption changes at two arbitrarily selected frequencies of C=O and the N-D modes in conventional FTIR spectra can not give the complete picture of the extent of intensity variations occurred at the whole range of the C=O and N-D vibrations. However, PCA gives us comprehensive information of the C=O and N-D vibrations from the whole spectral range of 1740 - 1300 cm⁻¹. The scores plot for PC1 in PCA has confirmed these observations and undoubtedly revealed that two different kinds of urea species were formed in urea aqueous solution. The PC results have also suggested that different interactions between urea and water has been developed below and above 2 M of urea. To supplement our results, we applied 2D correlation analysis to two independent sets of data for the concentration-dependent spectral changes of urea solution. The first set was constructed from spectra from range 0.5 - 2 M of urea and the second set, constructed from spectra for higher concentrations. Results of 2D correlation analysis performed for these two sets of spectra will be discussed in details.

Structure Analysis of Angelica with Different Produce Procedures by 2D IR Spectroscopy

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Abstract: Fourier Transform Infrared spectroscopy (FT-IR) combined with secondary derivative technique and two-dimensional (2D) correlation method was served to study the chemical structure changing rules of angelica with different produce procedures, such as freezing and drying lyophilization (DG1), fumigated by the stalk of horsebean (DG2), fumigated by the straw (DG3), fumigated by the wood (DG4), dried in chamber (DG5) and insolation (DG6). The IR spectra of angelica with different produce procedures are rather similar except that the spectrum of DG1 is distinct from others, especially feature peaks of sucrose, which indicates that a large amount of sucrose was produced. In the secondary derivative spectra of the six angelica, feature peaks of amylase (1078, 985 and 830 cm^{-1}) and grease compounds (1747, 1207 cm^{-1}) are obvious in the fresh angelica by freezing and drying lyophilization, which demonstrates the original property of angelica is retained. Not only grease compounds of low boiling point were maintained, but also condensation of monosaccharose and hydrolysis of amylase were prevented. The other five angelica's secondary derivative spectra are other similar. 2D IR further indicates that the spectra of angelica by the process of DG2, DG3 and DG1 are similar and have strong autopeaks (1200 and 1220 cm^{-1}) while that of DG2, DG3 and DG1 are different and have strong crosspeaks (1200,1220,1280 cm^{-1}), which indicates that the original property of angelica with procedures of DG2 and DG3 was retained more, while that of DG4, DG5 and DG6 was maintained less. To the conclusion, FT-IR combined with secondary derivative technique and 2D correlation method could provide much structural information. Tracing different produce procedures of angelica's influence on chemical ingredient and research of chemical structure changing rules of angelica are beneficial for the overall quality control.

Keywords: Fourier transform infrared spectroscopy, two-dimensional correlation infrared spectroscopy, secondary derivative, angelica, chemical structural analysis

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Identification Astragalus Injections of Different Factories by 2D IR Spectroscopy

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Abstract: The Traditional Chinese Injection, originated from the raw medicinal herbs, is applied widely and has been demonstrated to have excellent clinical effects. However, the absence of the quality criterion causes that some curative effects of the injection are unstable and some malign reactions occur frequently. Infrared (IR) spectroscopy is served as the integrity identifying method; furthermore, two-dimension correlation infrared (2D IR) spectroscopy is introduced in this method as an innovation. Combing IR and 2D IR spectroscopy, the rapid and direct quality criterion can be accessed to control the injections. Astragalus injection is chosen to perform researches on the integrity identification and identifying the specific components. It can be seen from the 2D IR spectroscopy that the samples from different factories shown the great otherness in the region between $900\sim 1200\text{cm}^{-1}$. This revealed the species and constituent differences of glucoside and saccharide in the raw astragalus. Also, the auto-peaks at 1053 and 1077 cm^{-1} are both can be seen in the 2D IR spectra. It can be treated as the characteristic of astragalus injections. In the region between $1200\sim 1800\text{cm}^{-1}$, auto-peaks at 1349 , 1435 , 1579 , 1603 and 1657cm^{-1} demonstrated the difference in procedures of the injections made by different factories. In addition, the mature method, Mass Spectroscopy (MS) and Liquid Chromatography/Mass Spectroscopy (LC-MS), have been chosen to identify the specific components. The information from those methods can validate and reinforce each other's conclusion.

Key words: Traditional Chinese injection, astragalus, infrared spectroscopy, two-dimensional correlation infrared spectroscopy (2D IR)

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Discrimination of American Ginseng According to Geographical Origin with FTIR and Two-Dimensional Correlation IR Spectroscopy

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Abstract: The objective of this study is to develop a nondestructive and accurate method to discriminate American Ginseng of different geographical origins, which is a troublesome work by existing analytical methods. We conducted a systematic study on four kinds of American Ginseng, which are from Beijing, Toronto, Vancouver and Wisconsin, by Fourier transform infrared spectroscopy, secondary derivative spectroscopy, and two-dimensional (2D) correlation infrared spectroscopy under thermal perturbation. Because American Ginseng consist of a large amount of starch in a large scale, the conventional IR spectra of different American Ginseng only have very limited spectral feature differences. Although we can separate different American Ginseng roughly based on these spectral features, this method is not very practical. The second derivative spectra of American Ginseng have obvious fingerprint feature differences especially in the range of $1750\sim 400\text{ cm}^{-1}$. We can distinguish different American Ginseng according to the peaks at 1317 cm^{-1} , 1052 cm^{-1} and 866 cm^{-1} etc. With the 2D correlation spectroscopy of American Ginseng in the range of $940\sim 1110\text{ cm}^{-1}$ and the differences of auto-peaks and cross-peaks, we have not only determined fingerprint features of 4 American Ginseng, but also analyzed material bases which caused these features, and offered structural information of relative compounds. The verification and integration analysis by infrared spectrum and second derivative spectroscopy as well as 2D correlation spectroscopy is a strong method of discriminating different American Ginseng and of material basic research of American Ginseng.

Key words: American Ginseng, infrared spectroscopy, two-dimensional correlation infrared spectroscopy (2D IR), secondary derivative

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THE APPLICATION OF 2-D FTIR SPECTROSCOPY TO THE INVESTIGATION OF DEGRADATIVE PROCESSES IN EPOXY-BASED NETWORKS

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ABSTRACT

Two dimensional infrared (2D IR) correlation spectroscopy has been applied to the study of the thermal-oxidative degradation of two networks based on a tetrafunctional epoxy resin. The use of the 2D IR approach revealed details about the reaction mechanism which remain undetected in the one-dimensional, frequency spectra. In particular, the sites of initiation of the auto-oxidative sequence were identified for the case of the plain epoxy resin, as well as the main competitive pathways through which the degradation reaction proceeds. With respect to the network modified by a thermosetting bismaleimide co-monomer, which displays an IPN-like molecular structure, it was found that the epoxy component undergoes thermal-oxidation via the same mechanism as for the plain epoxy network. A limited degradation was detected also for the bismaleimide component, whose stability represents the underlying reason for the enhanced temperature performances of the ternary system.

**METHIONINE ADENOSYLTRANSFERASE α -HELIX STRUCTURE
UNFOLDS AT LOWER TEMPERATURES THAN β -SHEET. A 2D-IR STUDY.**

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Two-dimensional infrared spectroscopy has been used to characterize rat liver methionine adenosyltransferase (MAT) and the events taking place during its thermal unfolding. Secondary structure data obtained with the native recombinant enzyme are compared to those derived from crystallization and X-ray diffraction, as well as those obtained by circular dichroism. Thermal denaturation studies allow the identification of events associated to individual secondary structure elements during temperature-induced unfolding. They are correlated to the changes observed in enzyme activity and intrinsic fluorescence. In all cases, thermal denaturation proved to be an irreversible process, with a T_m of 47-51 °C. Thermal profiles and two-dimensional infrared spectroscopy show that unfolding starts with α -helical segments and turns, located in the outer part of the protein, while extended structure, associated to subunit contacts, unfolds at higher temperatures. The data indicate a good correlation between the denaturation profiles obtained from activity measurement, fluorescence spectroscopy and the behaviour of the infrared bands. A study of the sequence of events that take place is discussed in the light of the previous knowledge on methionine adenosyltransferase structure and oligomerization pathway.

Informative Variable Selection using Two-Dimensional Correlation Analysis Screening

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A method for the selection of variables in multivariate data sets based on two-dimensional spectroscopy is presented. The method uses random noise equivalent to the level of instrumental noise which is appended to the measured spectra. Two-dimensional correlation spectra of the new matrix are then determined. A series of criteria for variable selection based on the resulting synchronous or asynchronous spectra is shown. Partial-least-square (PLS) analysis is followed on selected variables. Results using both simulated data and measured near infrared spectra show that a lower standard error of the estimate can be achieved as compared to the use of the entire spectra. The variables selected are compared to results using other selection methods, such as uninformative variable elimination-PLS, and stepwise multi-linear regression. The regression results for the methods are shown to be comparable. In addition, the sensitivity of the variables selected to noise in the spectra was determined using random noise added in the measurement. The two-dimensional correlation approach is shown to be less sensitive to noise in the spectra as compared to the other methods. The results suggest that selection based on two-dimensional correlation analysis may provide a useful tool for rapid determination of variables in a calibration.

Two Dimensional Correlation Analyses of N-Way Data

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Methods for applying two dimensional correlation analyses to N-Way data are presented. N-way data is commonly encountered because of the proliferation of computerized, hyphenated instrumentation. Interpreting the large multi-dimensional data sets obtained from these instruments is difficult. We examine several methods of applying two dimensional correlation analyses to N-Way data. First, we compress data along all parameter axes except the axis of interest and perform both synchronous and asynchronous correlation analysis. However, some information is lost with this method. To better obtain insight into the trends with respect to the original parameters, data is stacked lexicographically according to N-1 parameters for synchronous and asynchronous correlation analyses. Trends in the correlation are then visualized using the average synchronous and asynchronous correlation determined along a single original physical parameter. Variance due to other physical parameters is plotted as variations about the average correlation. These visualization methods are applied to both real and simulated three way data. Results show how a small number of figures can illustrate variation in the signal. This provides a rapid means for initial trend-screening of large multi-dimensional data sets. This screening is important for both data acquisition and data analysis.

Two Dimensional Correlation Analysis of Near-Infrared Measurements of Exercising Subjects

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A method for applying two dimensional correlation analyses to spectral measurements of individuals who undergo a physiological perturbation is presented. In particular, we have evaluated the potential of near-infrared spectroscopy (NIRS) in the 1.0-2.5 μm spectral range as a technique for monitor *in vivo* changes of lactate during exercise in humans. Ten healthy adult subjects (six males and four females) were tested during maximal effort made during a 30-s sprint on a modified isokinetic cycle. Diffuse reflectance NIRS measurements and blood samples at four time intervals prior and following exercise were taken. This exercise protocol was used to induce changes within the human physiological ranges for lactate, while minimizing covariance with other species which was verified using the clinical analysis of the blood sample taken. A two dimensional correlation analysis of the resulting spectra ordered according to the physiological perturbation identified lactate as the primary constituent that was monitored by the *in vivo* measurements. To study the parameters that impact the spectra baseline and the correlation between the calculated model and the data, spectra were referenced separately against a baseline spectra for each individual and from a group mean. Specific spectral regions associated with the changing lactate concentration could then more easily be determined. The results suggest that two dimensional correlation analysis applied to spectral measurements, such as NIRS may provide a valuable tool to assess changing *in vivo* physiological status for both research and clinical use.

TWO-DIMENSIONAL CORRELATION ANALYSIS OF MILK PROTEIN IN THE THERMAL DENATURATION STAGE

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Nowadays, the quality control of milk in dairy industry has become very important issue. It has required rapid and precise measurement of milk pasteurization process.

The aim of this study was to develop new method for real-time monitoring of thermal denaturation of milk protein by using two-dimensional correlation spectroscopy, [1][2]. We applied this method to a sample set of milk spectra acquired at every 2 °C in the temperature interval from 60 °C to 80 °C. Synchronous correlation map showed distinguished absorbance pattern of interaction between water bands and amid bands corresponding to the thermal denaturation of milk protein. Asynchronous correlation map indicated the order of dynamical structural changes that occurred under thermal denaturation.

Our results suggest high feasibility for spectral monitoring of milk pasteurization process in real time when using two-dimensional correlation spectroscopy.

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2D COS IR for Heterogeneous Catalysis – Spectroscopy of Adsorbed Species by Pressure Modulation Techniques, and Spectroscopy of the Working Catalyst

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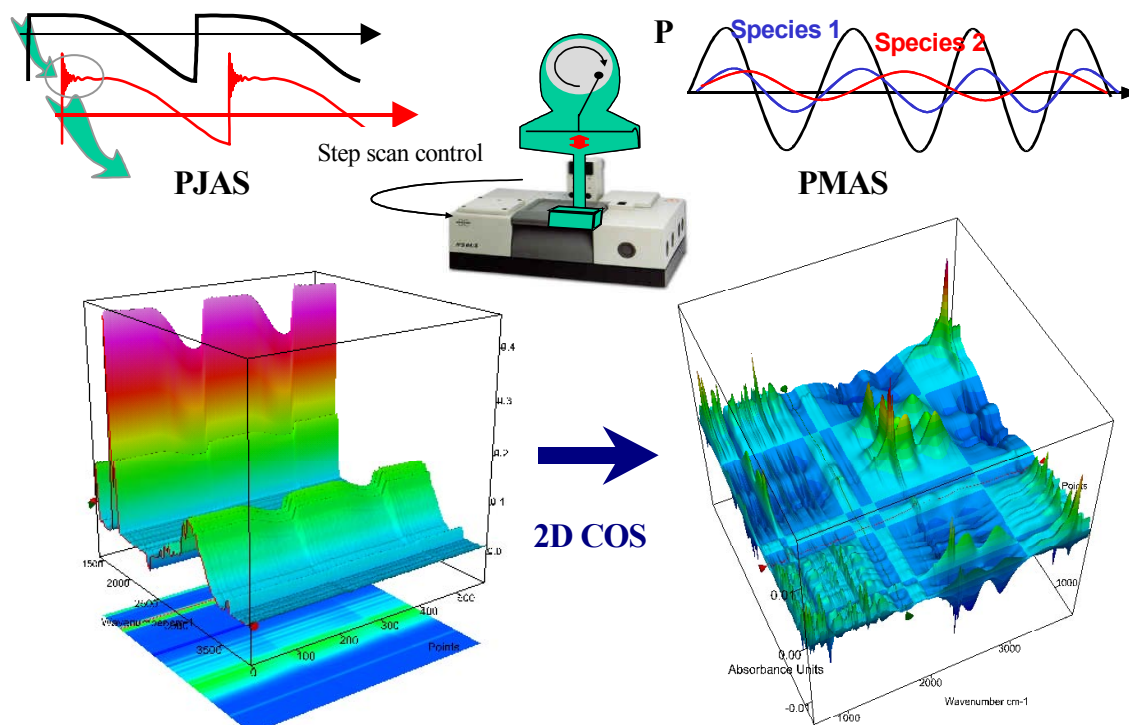
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Two examples of 2D-COS in infrared spectroscopy of heterogeneous catalysis will be presented.

The first is a new method for the study of adsorbed species by step scan and rapid scan techniques based on pressure modulation experiments. Pressure modulation allowed to study complex spectra obtained during adsorption of mixtures on the surface of the catalyst, the monitoring of fast gas reactions was possible. 2D COS was used to extract the information and to identify the individual species in the gas and adsorbed phases. The examples of carboxylic acids on silica and acetonitrile on zeolites will be given.

The second possible use of 2D-COS in catalysis is for the spectroscopic study of a working catalyst. Spectra collected at high temperature on the highly absorbing surface of an oxide catalyst often have a very low signal to noise. The surface species can be unknown or unexpected. The detection of surface reaction intermediates is thus a highly challenging task. 2D COS provided a highly efficient tool for detection of surface species during time resolved experiments, giving indications on the deactivation mechanism.



Schematic drawing of the setup, and principle of 2D PMAS and PJAS experiments. For PJAS, the pressure jumps are used to trigger the infrared acquisition. For PMAS, the pressure modulation is sinusoidal: species 1 follows the modulation, while species 2 does not.

An example of 2D-COS PJAS of acetonitrile on a zeolite is shown in the lower part of the figure.

This study was made possible by support and technical help from Bruker Optics.

PHASE TRANSITION OF POLYHYDROXYALKANOATE/POLYLACTIC ACID BLENDS INVESTIGATED BY TWO-DIMENSIONAL INFRARED CORRELATION SPECTROSCOPY

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The crystalline/amorphous phase transition behavior of polymer blends of a newly developed biodegradable poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (P(HB-co-HHx)) and polylactic acid (PLA) was investigated by using generalized two-dimensional infrared (2D IR) correlation spectroscopy. Three spectral regions, the C-H stretching band (3100-2800 cm⁻¹), C=O stretching band (1780-1680 cm⁻¹), and C-O-C/C-O-H stretching bands (1500-1000 cm⁻¹) regions were selected for monitoring the infrared spectral variations by which the phase transition behavior of the polymer blends were explored. 2D IR spectroscopy was employed for a detailed study of the phase transition mechanism of the polymer blends. Synchronous and asynchronous 2D IR correlation spectra were generated from a set of dynamic infrared spectra. Synchronous 2D spectra are very useful for the determination of the inter- or intramolecular interactions existing in the system of interest, while asynchronous spectra are especially powerful for enhancing the spectral resolution of highly overlapped bands. In addition, the sequential orders of the events occurred during the process of phase transition can be determined by the use of 2D IR spectroscopy.

**Two-Dimensional /Attenuated Total Reflection Infrared Correlation Spectroscopy
Studies on Secondary Structural Changes in Human Serum Albumin in Aqueous
Solutions : pH-Dependent Structural Changes in the Secondary Structures and in the
Hydrogen Bondings of Side Chains**

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Attenuated total reflection (ATR)/infrared (IR) spectra were measured for human serum albumin (HSA) in aqueous solutions over a pH range of 5.0-3.2. Generalized two-dimensional (2D) correlation analysis was applied to the amide I region of the spectra to investigate pH-dependent changes in secondary structures and in hydrogen bondings of side chains of HSA. The synchronous and asynchronous 2D spectra were generated from the pH-dependent spectral variations 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 the 2D spectra is identification of four bands at 1740, 1715, 1705, and 1696 cm^{-1} due to a C=O stretching mode of free and hydrogen bonded (weak, medium, and strong) COOH groups of HSA. The 2D correlation analysis provides the unambiguous evidence for the existence at least the four C=O bands, demonstrating its powerful deconvolution ability. The asynchronous spectrum of the N form is characterized by a rather broad cross peak centered at (1715,1654) cm^{-1} . The sign of the cross peak indicates that protonation of COO⁻ groups of glutamic (Glu) and aspartic (Asp) acid residues occurs at higher pH than structural changes in the α -helices. In the N-F transition, it seems that the formation of free COOH groups and those with the hydrogen bonds of the medium strength occurs is linked with changes in secondary structures of HSA. The asynchronous spectrum indicates that the formation of the strongly hydrogen-bonded COOH groups upon the protonation of COO⁻ groups plays a key role in the initiation of the N-F transition where mainly α -helices undergo the conformational changes. The synchronous and asynchronous spectra of the F form show that β -strands and β -turns of HSA change significantly in this pH region.

Dynamics of a Ferroelectric Liquid Crystal with a Naphthalene Ring during Electric-Field-Induced Switching Studied by Time-Resolved Infrared Spectroscopy combined with Two-Dimensional Correlation Spectroscopy

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The dynamics of a ferroelectric liquid crystal with a naphthalene ring (FLC-3) during the electric-field-induced switching between two surface-stabilized ferroelectric liquid crystal states were investigated by time-resolved infrared (IR) spectroscopy combined with two-dimensional (2D) correlation spectroscopy. Time-resolved IR spectra of FLC-3 in a planar-aligned cell were measured as a function of the polarization angle range from 0 ° to 180 ° under a rectangular electric field of $\pm 40\text{V}$ with a 5kHz frequency in the smectic-*C** (Sm-*C**) phase at 137 °. From these spectra we explore details about the reorientation process of the alkyl chains, the core and the large C=O dipole moments of FLC-3 at all the delay times. The 2D correlation spectroscopy was applied to the polarization angle-dependent spectra for different delay times and to the time-resolved spectra at certain polarization angles to reveal the relative orientation of the C=O groups and the core moiety during the electric-field-induced switching. It was found from the present study that the relative orientation of the C=O groups and the core remains unchanged during the initial period of the reorientation, while it is reversed at a certain moment and then kept unchanged again. Moreover, the alkyl chains, C=O groups, and core moieties possess different dynamics during the fast course of electric-field-induced switching by analyzing time-resolved spectra.

Index Heading: Ferroelectric liquid crystal; Time-resolved infrared spectroscopy; two-dimensional correlation spectroscopy.

**TWO-DIMENSIONAL CORRELATION GEL PERMEATION
CHROMATOGRAPHY (2D GPC). A NOVEL APPLICATION OF 2D
CORRELATION CONCEPT TO CHROMATOGRAPHIC STUDIES**

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The application of the fundamental concept of two-dimensional correlation analysis is not limited to conventional spectroscopic studies. It can be used in many different types of analytical methods which generate a series of data traces, like chromatograms, spectra, thermograms, and so on. We report here the development of a new technique of two-dimensional correlation gel permeation chromatography (2D GPC) method based on the correlation analysis of time-resolved GPC profiles. In 2D GPC, a set of chromatograms collected as functions of *elution time* are acquired sequentially at different *sampling time* corresponding, for example, to reaction stages during a polymerization process. The data set thus collected are converted to 2D GPC maps having two independent elution time axes by following the standard 2D correlation procedure.

As an demonstrative example of the 2D GPC technique, to a set of time-resolved GPC profiles of 1H,1H,2H,2H-perfluorooctyltriethoxysilane (PFOTES) was studied to elucidate intricate details of the polymerization process catalyzed by $1\text{M HCl} \cdot \text{H}_2\text{O}$, over a period. In particular, the splitting behavior of 2D cross peaks and their intensity variation have been examined in detail, leading to the conclusion that the mechanism of the growth process of polymeric aggregates is surprisingly well represented in the patterns of the observed 2D correlation spectra. The 2D correlation GPC method has proved to be a very powerful tool with much future potential in the study of complex reaction mechanisms.

GRAPHICAL REPRESENTATION OF TWO-DIMENSIONAL (2D) CORRELATION IN VECTOR SPACE

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The basic concept of two-dimensional (2D) correlation analysis may be regarded as a form of manipulation and comparison of two vectors, representing the time-dependent signals, temperature variations, spatial positions, concentrations, spectral band positions, *etc.*, measured at two independent spectral variables, such as wavenumbers. The pair-wise comparison of two vectors generates a correlation intensity defined by the given form of a correlation function. The array of correlation intensities plotted as a function of two independent spectral variables then becomes a 2D correlation spectrum. The mode of comparison specifically chosen to accentuate the similarity or difference between the signal vectors determines if the correlation is synchronous, asynchronous, or any other variant form relevant to the analysis.

The manipulation and comparison of two vectors may be represented graphically for easier visualization. Such graphical treatment provides more intuitively understandable way to look at different forms of 2D correlation functions. Standard manipulative operations used in 2D correlation, such as the Hilbert transformation, Gram-Schmidt orthogonalization, and asynchronous correlation can be easily comprehended as the combinations of rotations and projections of signal vectors in the hyperspace. Thus, the graphical representation not only gives a unified tool to compare various forms of current 2D correlation methods but also may potentially assist further evolution of useful correlation methods not yet being explored.