Determination of Selective Molecular Interactions Using Two-Dimensional Correlation FT-IR Spectroscopy

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A novel method to study competitive molecular interactions in a multicomponent system has been introduced. It is a method based on the framework of a two-dimensional infrared (2D IR) correlation spectroscopy technique with a new data pretreatment strategy, namely, the component-normalization method. In this method, the absorbance of each absorption band is normalized with the concentration of the corresponding component in the system. Competitive interactions are expressed quantitatively by a normalized 2D IR parameter, which is a correlation coefficient of the synchronous 2D IR spectrum. Using the newly proposed method, we examined molecular interactions among dimethyl sulfoxide (DMSO), *n*-decylamine, and water. It was found that the interaction between DMSO and water is the greatest among the three interaction pairs. Furthermore, it was demonstrated that, by a systematic perturbation in the concentration of DMSO of the three-component mixtures, the sequential order of response by individual functional groups in the system could be traced. Thus, the S═O group (DMSO) is affected first, followed by the hydroxyl group (water) and finally the amine group (*n*-decylamine) as DMSO is added to the system. The results have been used to elucidate the possible working mechanism of the dehydration effect of DMSO on hydrated biosystems.

Introduction

Selective or competitive molecular interactions in a multicomponent system lie at the center of understanding the mechanisms of many chemical, physical, or biological processes, including the process of molecular recognition.[1−6] An unsolved question concerning this subject has been how to probe or evaluate such interactions effectively in a complex solution system. In this article, we use the normalized correlation coefficient, which is derived from a two-dimensional infrared (2D IR) correlation spectroscopy method, to tackle the problem. Theoretical considerations in relating these quantities to selective molecular interactions and the proper way to handle data pretreatment are introduced. An example is given to show how competitive interactions between molecular pairs in a ternary system containing dimethyl sulfoxide (DMSO), water, and amine can be evaluated using the new method.

Although only a small molecule, DMSO is often used as an effective cryoprotectant. It can also function as a cell fusogen and a membrane permeation enhancer[7] in competition with polymeric or oligomeric counterparts. Previous studies suggest that DMSO can modulate phase behavior and structural parameters of biomembrane lipids.[8−11] A possible mechanism of the interaction occurs through hydrogen bonding with the amine group of the phosphatidylethanolamines. However, there exists competition between DMSO molecules and, among others, ubiquitous water molecules to form H-bonds with the amine groups. At present, it is not clear which two species can form stronger H-bonds in the presence of the other. Furthermore, high concentrations of DMSO can dehydrate biological systems.[9,12−14] In studies using phospholipids as model membranes, it was found that DMSO could induce shrinkage in the solvent layer of a multilamellar structure, a process opposite to hydration.[9] Dehydration of the cartilage was also recorded with increasing time of DMSO exposure in a study of cartilage proteoglycan synthesis and degradation.[12] Actually, the small organic molecule is sometimes referred to as a dehydrating agent,[13,14] although it is frequently employed as a cryoprotecting agent to prevent intercellular dehydration.[7] What is important to us is that the detailed molecular mechanism of the dehydration process is not yet very clear. The fact that DMSO can work as a supra solvent to so many categories of compounds[15] has also increased the interest in introducing the sulfoxide moiety to new synthetic amphiphiles to improve their compatibility with other materials.[16] In short, a better understanding of the competitive interactions of DMSO with other molecules is of both theoretical and practical importance. In this work, we demonstrate that the suggested new method can be used to study the competitive molecular interactions of DMSO and water with a primary amine. The latter is taken as a model molecule of phospholipids.

Theory

Considering an absorption band of component *j*, let us assume the absorbance in its wavenumber range can be expressed as

$$A_j = A_j^i + A_j^e = \epsilon_j^0 l c_j + A_j^e \tag{1}$$

where A_j^i is the ideal part of the absorbance, which obeys the Beer−Lambert law throughout the entire concentration range of the measurement and can thus be further expressed as the product of the molar absorption coefficient ϵ_j^o, the light path length l, and the concentration c_j. The term A_j^e, however, is the excess part of the absorbance due to molecular interactions or

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other nonideal factors. The identification index $j = 1, 2, ... m$ is assigned individually to the band of interest in a study.

When the second term in eq 1 is zero or negligible, the variation in the absorbance of a spectrum is mainly due to changes in concentration. Two dimensional correlation spectroscopy in this case can be used to monitor variations in concentration. Applications in this category include systems with chemical reactions or physical changes, where the population changes of individual components are the main focus of interest. Most 2D IR applications so far have been in this field.[17−20]

However, if the linear contribution to absorbance by concentration (i.e., Beer−Lambert law) can be removed from dynamic spectra, information obtained from the 2D IR correlation analysis of such excess responses will be a true reflection of specific molecular interactions. It is the nonlinear parts of the spectra that are under investigation.

It is thus clear that the key part of the work in using 2D FT-IR for the investigation of selective molecular interaction lies in the proper data pretreatment to remove the first term of eq 1. We propose in this article a novel normalization method, which can be referred to as the component normalization method. It is the method used to divide each of the absorption bands of interest by the concentration of the corresponding component. The implication of such a normalization method is discussed below.

Assuming the absorption bands of interest are fully resolvable and the cross section of the light path is *s*, the concentration of component *j* can be expressed as $c_j = n_j/(sl)$. Here, n_j is the number of *j* molecules in the light path, in unit of moles. After dividing eq 1 by n_j, we have

$$A_j/n_j = \epsilon_j^0/s + A_j^e/n_j \tag{2}$$

or

$$A_j' = \epsilon_j^0/s + A_j'^e \tag{3}$$

where A_j' is the absorbance per unit of component *j* and $A_j'^e$ is the corresponding unit excess quantity. To perform 2D IR correlation analysis, a set of difference spectra is created by subtracting a reference spectrum from each spectrum. Very often, the averaged spectrum is used as the reference spectrum. The averaged absorbance of band *j* over *n* spectra is

$$\bar{A}_j = \epsilon_j^0/s + \frac{1}{n}\sum_{k=1}^{n} A_{kj}'^e \tag{4}$$

After subtracting eq 4 from eq 3, the absorbance difference of band *j* in spectrum *k* can be expressed as

$$\tilde{A}_{kj} = A_{kj}'^e - \frac{1}{n}\sum_{k=1}^{n} A_{kj}'^e \qquad k = 1, 2, ..., n \tag{5}$$

As can be seen from eq 5, the contribution to the absorbance difference from the fluctuation of concentration has been removed. The series of difference spectra is now ready for correlation analysis.

The absorption bands can also be joined to form a series of artificial spectra with segmented wavenumber ranges:

$$\tilde{A}_k = \sum_{j=1}^{m} \tilde{A}_{kj} \qquad k = 1, 2, ..., n \tag{6}$$

It needs to be pointed out that, in practice, it is not n_j but rather the concentration c_j that is used to divide a measured absorption spectrum as in eq 2. As long as the thickness of the sample, *l*, is a constant, the two methods provide equivalent results.

To further understand the nature of such a 2D IR correlation analysis, the second term in eq 1 is considered to be due to a deviation of the absorption coefficient from the standard state (the pure state, for example) in a mixture; in other words,

$$A_j'^e = \Delta\epsilon_j l c_j \tag{7}$$

After component- or band-based normalization, eq 3 may be recast as

$$A_j' = \epsilon_j^0/s + \Delta\epsilon_j/s \tag{8}$$

and eq 5 is now

$$\tilde{A}_{kj} = \left(\Delta\epsilon_{kj} - \frac{1}{n}\sum_{k=1}^{n}\Delta\epsilon_{kj}\right)/s \qquad k = 1, 2, ..., n \tag{9}$$

Equation 9 suggests that this particular 2D IR correlation analysis is actually the analysis of the deviation of absorption coefficients of the components from the standard state. Thus, we call this specific form of 2D IR study $\Delta\epsilon$ correlation analysis.

The expression employed in the calculation of the synchronous spectrum is shown in eq 10.[17]

$$\Phi_{ij} = \Phi(\nu_i,\nu_j) = \frac{1}{n-1}\sum_{k=1}^{n}\tilde{A}_{ki}\tilde{A}_{kj} \tag{10}$$

Furthermore, the correlation coefficient $\rho_{ij} = \rho(\nu_i, \nu_j)$ between two absorption bands at ν_i and ν_j in a 2D IR spectrum can be calculated using eq 11.[18,21]

$$\rho_{ij} = \frac{\Phi_{ij}}{\sqrt{\Phi_{ii}\Phi_{jj}}} \tag{11}$$

The 2D correlation coefficient gives a much better determination of the extent of synchronous correlation than does the normal synchronous spectrum because the magnitude of the absolute value of the correlation coefficient quantitatively indicates the degree of synchronicity and thus the presence of interactions to coordinate the spectral intensity changes under a given perturbation to the system. Highly coordinated changes of IR spectral intensities will give values of the correlation coefficient near +1 or −1, whereas less-correlated events are characterized by a value near zero. Thus, the normalized 2D correlation coefficient has great potential to be a powerful tool to probe quantitatively the details of highly interacting chemical systems.

Consider two functional groups interacting with each other. They show two characteristic absorbances at ν_i and ν_j, respectively. Suppose variations in one absorbance are induced by variations in another in such a way that $A_{ki}'^e = cA_{kj}'^e$, where *c* is a constant for the whole series of spectra. It can be proved that $\Phi_{ii} = c^2\Phi_{jj}$ and $\Phi_{ij} = c\Phi_{jj}$. Therefore, $\rho_{ij} = \pm 1$. In other words, two highly synchronous absorbances will result in correlation coefficients with values of ±1. In comparison, weaker molecular interactions will result in smaller absolute values of ρ_{ij}.

Unfortunately, however, the 2D correlation coefficient plot has its own weakness, which makes it less suitable for the analysis of real-world samples. The most well recognized

limitation of using the 2D correlation coefficient plot is the susceptibility of such a display method to the influence of noise, especially in the region where the spectral intensity is weak. Thus, fortuitous correlation of noise will create illegible fluctuations of correlation coefficients, and this artificial information will occupy a large portion of the 2D correlation coefficient plot. Furthermore, the diagonal part of the 2D plot has been normalized to a value of 1, thus minimizing the information content around the portion of the 2D plot. Some attempts to minimize the distracting effect of exaggerated noise correlation, such as the artificial masking of noisy weak-signal regions,[22,23] have been reported. To circumvent such limitations while simultaneously retaining the clear advantage of more quantitatively useful information provided by the correlation coefficient plot, we have developed a new way of displaying the correlation coefficient in conjunction with the conventional 2D correlation intensity spectrum.

More specifically, we keep the conventional display method of the 2D correlation spectra by plotting the correlation intensity without the normalization of correlation intensity with the magnitude of spectral intensity fluctuations. In this way, the noise contribution in the weak-signal region is effectively suppressed, and only physically meaningful correlation peaks are displayed. Simultaneously, the autopower spectrum at the diagonal position of the synchronous 2D plot, which gives the magnitude of spectral intensity changes, is preserved. We then display the individual numerical value of the normalized 2D correlation coefficient at each correlation peak maximum. Thus, a quantitative measure of the extent of correlation is now available for individual correlation peaks, which enables a more straightforward interpretation of the 2D spectra.

So far, we have discussed the component-normalization method for absorption bands without overlap. In the case of absorption bands with overlap, this method may have a serious effect on the final results. This effect depends not only on the separation of the bands involved but also on the relative ratio of the two terms in eq 1 (i.e., A^e/A^i) assuming that the same peak parameters are used. When A^i is negligible in comparison with A^e, the component-normalization method can be employed with limited errors in the final results. When the ratio is not very large, the contribution of A^i from one band to another cannot be ignored. As a result, the ideal part of the absorbance cannot be completely removed by using the component-normalization method as discussed above. To solve this problem, one has to apply a band deconvolution treatment prior to the application of component normalization.

Experimental Section

Normal decylamine with purity better than 95% was purchased from Sigma Chemical Co. (St. Louis, MO). Dimethyl sulfoxide, AR grade, was from Yili Fine Chemistry Co. (Beijing, China). *n*-Dodecane was from Shanghai Chemical Reagent Co. (Shanghai, China). A Perkin-Elmer spectrometer (Spectrum GX, England) equipped with a DTGS detector was employed to record infrared spectra (transmission mode) over the range of 4000−900 cm^{-1}.

By using the strategy of systematically changing the concentration of one of the components, such as DMSO in the present study, 2D IR correlation spectra can be acquired for a multicomponent system.[19] In this study, the molar ratio of *n*-decylamine to water was 1:2 throughout the series of measurements. The spectra were collected at different molar ratios of DMSO to H_2O, namely, 0, 0.11, 0.23, 0.35, 0.46, 0.58, 0.69, 0.81, 0.92, and 1.1.

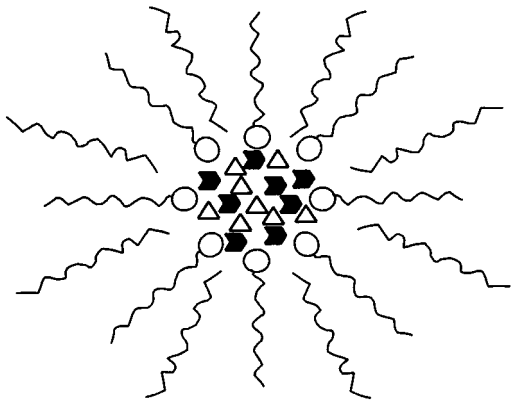


Figure 1. Schematic presentation of a vesicle formed by *n*-decylamine and excess *n*-dodecane . Molecules in the vesicle are H_2O (△) and DMSO .

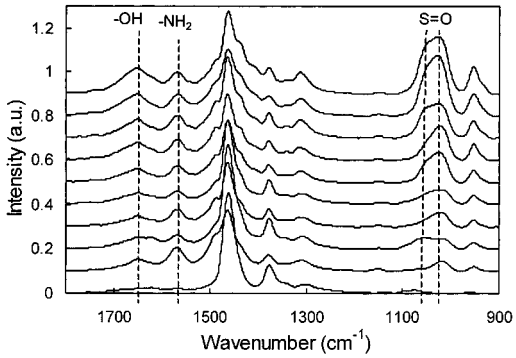


Figure 2. IR spectra of the codispersion of *n*-decylamine−water−DMSO in *n*-dodecane from 1800−900 cm^{-1}. From bottom to top, the molar ratio of DMSO to water increases from 0 to 1.1 (see text for detail), whereas that of decylamine to water remained 1:2.

To further enhance the mutual contacts of the functional groups of interest in the complex system, these molecules were dispersed in the sea of an inert solvent, *n*-dodecane. W/O (water in oil)-type vesicles were then formed, with water and DMSO molecules being restricted only inside the vesicles and the amine groups in the interfacial region as shown in Figure 1. Volume ratio of *n*-dodecane to the mixture of water, DMSO, and amine was 8:3 throughout the series of measurements.

After each spectrum was smoothed and baseline corrected, the bands of interest were divided by the concentrations of the corresponding components. Then the 2D correlation spectra were constructed by using the software developed in Professor G.-Q. Chen's laboratory at Tsinghua University.[24]

Results and Discussion

Depicted in Figure 2 are the 1D spectra of the tertnary system containing *n*-decylamine, water, and DMSO codispersed in *n*-dodecane. The intensity of the strongest absorption band at ca. 1459 cm^{-1} is from the bending vibration mode of CH_2, whereas that at about 952 cm^{-1} is attributed to the bending vibration mode of CH_3 in DMSO molecules. Interestingly, the absorption intensity not only of S═O (1031 cm^{-1}) but also of −OH (1653 cm^{-1}) increases with increasing DMSO concentration (upward direction in Figure 2). The intensity of $-NH_2$ at ca. 1563 cm^{-1} actually showed also the same trend but was less obvious. Although one may expect the absorption strength of both −OH and $-NH_2$ to be reduced because of the decrease in their concentrations in the sequence, we actually observed the opposite trend. Clearly, the simple Beer's law relationship

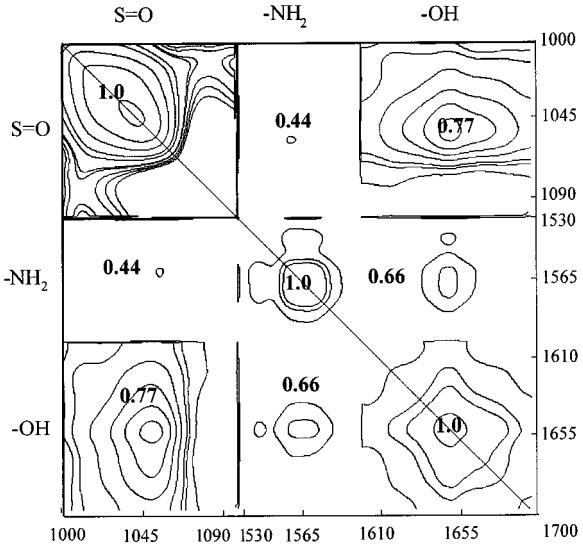
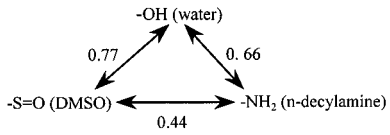


Figure 3. Synchronous 2D correlation spectrum $\Phi(\nu_1, \nu_2)$ in the form of a contour map. The numerical values displayed at the peak maximums correspond to the values of 2D correlation coefficients. The contour map is separated into nine distinct areas because of the discontinuity in the nature of the normalized 1D spectra.

is no longer applicable here. We tentatively attribute the increasing interactions between them and DMSO to this surprising reversed trend. Considering the change in the H-bonding network in the system along with an increase in DMSO concentration, the dipole moments of both the OH and NH_2 groups should change accordingly. Such an effect could compensate and even overpower the effect caused by the decrease in concentration.

The three bands selected for 2D correlation analysis were normalized using the component-normalization method. More specifically, the S═O stretching band over the range of ~1000−1100 cm^{-1} was normalized by the concentration of DMSO, the $-NH_2$ band over ~1530−1600 cm^{-1}, by the concentration of decylamine, and the −OH band over ~1601−1700 cm^{-1}, by the concentration of water. The 1D spectra thus created are discontinuous but contain all the information needed for the correlation analysis.

Two-dimensional correlation spectra can be created on the basis of the set of 1D spectra thus normalized. Shown in Figure 3 is the synchronous 2D correlation spectrum in the region of ~1000−1700 cm^{-1}. Correlation peaks are indicated by the contour map representation. It can be seen that the cross peaks between functional group pairs of (S═O, OH) and (S═O, NH_2) as well as (OH, NH_2) in the synchronous spectrum are all positive under the perturbation of DMSO concentration. The numerical values labeled at those peak maximum locations represent the 2D correlation coefficients, $\rho(\nu_1, \nu_2)$. These coefficients between the functional groups of amine ($-NH_2$, ν = 1533 cm^{-1}), H_2O (−OH, ν = 1668 cm^{-1}), and DMSO (−SO, ν = 1040 cm^{-1}) are shown in the following scheme



which shows clearly that the correlation coefficient between −S═O and −OH is the greatest among the three interaction pairs, implying an unusually strong interaction between DMSO and water.

TABLE 1: Parameters Showing the Order of Events during the Process of Changing the Concentration of Dimethyl Sulfoxide

group pair	$\Phi(\nu_1, \nu_2)$	$\Psi(\nu_1, \nu_2)$	order[a]
$-NH_2$ and S═O	+	−	$-NH_2$ after S═O
−OH and S═O	+	−	−OH after S═O
−OH and $-NH_2$	+	+	−OH before $-NH_2$

[a] After (before) means that the change in band intensity at ν_1 occurs at a higher (lower) concentration of DMSO than does that at ν_2. It is equivalent to a later (earlier) change in intensity at ν_1 than that at ν_2.

With the help of conventional 2D IR correlation analysis combined with the simultaneous 2D correlation coefficient display scheme we have developed, the changing sequence of different groups in response to the perturbation can also be investigated. Table 1 summarizes the signs of the cross peaks of the three representative absorption bands of −OH, S═O, and $-NH_2$. On the basis of the signs of the synchronous and asynchronous correlation spectra under the well-established sequential order rules described before,[25−27] we can deduce the following order of events as the system composition is gradually changed. As expected, the S═O group is found to respond first to the external perturbation (i.e., a change in DMSO concentration). Then comes the −OH group and finally the $-NH_2$ group. Such an observation suggests that when the perturbation is triggered by a change in DMSO concentration, its effect on the energetics of $-NH_2$ is indirect and is mediated by an interaction with water. This explanation is in agreement with the conclusion we draw from the correlation coefficient data.

The above conclusion can also be used to explain the dehydration effect of DMSO on biological systems, where amine-containing phospholipids such as phosphatidylethanolamines are usually essential components in biomembranes.[12−14,28,29] The stronger interaction between water and DMSO molecules makes DMSO better than amine groups in forming H-bonds with water molecules. When considering another major group of lipids, phosphatidylcholines, we find that their interactions with DMSO are even weaker because amine groups are absent. This result implies that DMSO is capable of removing water molecules from biomembranes that are rich in phosphatidylcholines and phosphatidylethanolamines. This process could cause a certain degree of dehydration.

Conclusions

We have demonstrated that the 2D IR method can be used to study selective or competitive molecular interactions. To do this, the newly introduced normalization method, which is the component-normalization method, should be employed. Among water, DMSO, and decylamine, the interaction between the first two is the strongest. When DMSO was added to a mixture of the other two, it would interact first with water molecules. If the concentration of DMSO is high enough, water molecules in the hydration shell of the amine groups can be replaced completely, which could be the mechanism of the dehydration effect of DMSO on biological systems.

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