Two-Dimensional Correlation Spectroscopy in Analyzing the Concentration-Dependent IR Spectra of Urea Aqueous Solution

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The denaturation of protein induced by urea has been intensively studied.[1-5] More recent studies have suggested that urea denaturates proteins *via* both direct and indirect (water mediated) interaction with moieties of different polarity of the protein. The folding studies conducted over a wide range of water/urea mixtures show that the unfolding effectiveness of urea depends on its concentration. Despite many literature data on the studies of use as a denaturating agent, the detailed mechanism of urea-water interaction is still an unsolved problem. Therefore in this study, I have focused on monitoring of the association process in course of the increase of urea concentration. The concentration dependent IR spectra of urea aqueous solution are analyzed by means of two-dimensional (2D) correlation spectroscopy. Generalized 2D correlation spectroscopy has become one of standard analytical techniques to interpret spectral data sets obtained during the observation of a system under some external perturbation.[6-10]

For IR measurements, deuterated C_{13}-urea solutions with different concentrations (0.5, 1.0, 2.5, 3.0 M, etc.) were prepared in phosphate buffer (pH 6.6) solution prepared with D_2O. The IR spectra were measured at a 2 cm^{-1} resolution with Bomem DA8 FTIR spectrometer equipped with a liquid nitrogen-cooled MCT detector. Spectrum of buffer was first subtracted from spectra of urea's solutions. Then denoising based on wavelets, which is more beneficial than smoothing,[11] was performed before the 2D correlation spectra were constructed. Synchronous and asynchronous 2D correlation spectra were obtained using the same software as those described previously.[10]

Figure 1 shows the concentration-dependent spectra of urea's solutions after final pretreatment process. There are two bands at around 1560 and 1460 cm^{-1} assigned to the $v(CO)$ and the $v_{as}(CN)$ vibrations, respectively.[12] The CO and CN stretching vibrations have small but distinct 2 cm^{-1} shift into a higher and a lower frequency, respectively, with increase of urea concentration. Also the absorption changes of these two bands reveal that above the concentration of 2 M the properties of urea are changed with respect to these at concentration below 2 M. Most probably around the limiting point of 2 M the process of urea self-association reaches appreciable extent. As the strength of the urea-water and
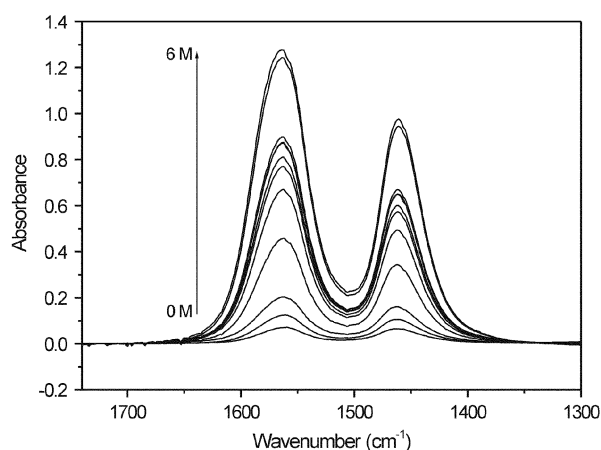
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Figure 1. Concentration-dependent FTIR spectra of urea solutions in region 1740-1300 cm^{-1} after final pretreatment process.

urea-urea complexes is different[13] the shifts of the bands are observed. Therefore, the properties of the urea-water solution are variable with change of urea concentration.

To investigate the actual spectral changes occurring in dependence on the concentration of urea, 2D correlation analysis has been applied to two independent sets of data for the concentration-dependent spectral changes of urea buffer solution. The first set was constructed from spectra from range 0.5-2 M and the second set from all remaining spectra obtained for higher concentration.

Synchronous and asynchronous 2D correlation spectra from a first set of the concentration-dependent spectra (0.5 M-2 M) are given in Figure 2(a) and (b), respectively. In synchronous 2D correlation spectrum, the two prominent peaks at 1562 and 1461 cm^{-1} are strongly positively correlated. Asynchronous 2D correlation spectrum shows the additional peaks in range of the absorption of the $v(CO)$ and the $v_{as}(CN)$ band, e.g., 1585, 1562, 1537, 1469, and 1450 cm^{-1}. It reveals that in the concentration range 0.5 M-2 M the system comprise different kinds of urea species.

As the concentration of urea increase to 2 M in the solution cyclic and/or linear dimers of urea are formed. In range of $v(CO)$ vibration, the peaks at 1562 and 1537 cm^{-1} are assigned to the complexes, while the peak at 1585 cm^{-1} can be assigned to ureas monomers and/or terminal C=O groups of linear dimers that are not directly involved in the hydrogen bonds. In range of the $v_{as}(CN)$ vibration, the peak at ~1469 cm^{-1} is assigned to the dimer form. In cyclic dimer
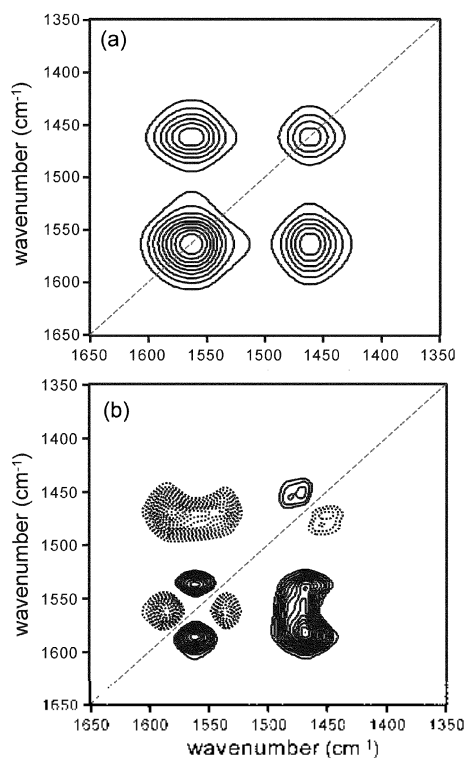
Figure 2. Synchronous (a) and asynchronous (b) 2D IR correlation spectra constructed from the first set (0.5 M-2 M) of concentration-dependent spectral changes. Solid and dashed lines represent positive and negative cross peaks, respectively.
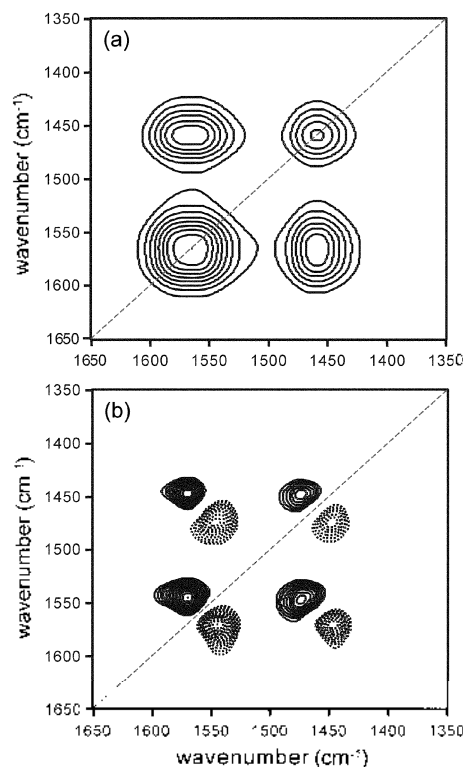

Figure 3. Synchronous (a) and asynchronous (b) 2D IR correlation spectra constructed from the second set (3 M-6 M) of concentration-dependent spectral changes. Solid and dashed lines represent positive and negative cross peaks, respectively.

the resonance assisted hydrogen bonding effect[14] is different than in linear dimmer, therefore the broad and asymmetric asynchronous peak at 1469 cm^{-1} represents the two forms of dimers. It can be supposed that the following couples of frequencies (1562, below 1469) cm^{-1} and (1537, above 1469) cm^{-1} represent linear and cyclic dimers, respectively. The peak at (1585, 1540) cm^{-1} comes from groups unengaged in hydrogen bonds.

Figure 3(a) and (b) display synchronous and asynchronous 2D correlation spectra from the second data set, respectively. Two autopeaks at 1564 and 1458 cm^{-1} are shown in Figure 3(a). Similarly, as it was observed for raw spectra, the peaks correlated with the ν(CO) and the ν_{as}(CN) vibrations are shifted into higher and lower frequency, respectively. In this concentration range the intensity variations of the ν(CO) band are larger than those attributed to the ν_{as}(CN) vibration. It seems that association process, mainly around 2 M, induced higher changes in polarity of the C=O bonds than the C-N bonds. For concentrations of urea higher than 3 M open linear polymers, longer than dimmers, are formed. Such complexes have two kinds of C=O groups, *i.e.* these from ends and from inside of the chains of associated molecules of urea. The asynchronous cross peaks in Figure 3(b) suggest the following couples of frequencies in range of the C=O and the CN vibration: 1570, 1545 cm^{-1}, and 1474, 1446 cm^{-1}. It suggests that the peak at (1570, 1446) cm^{-1} vibrations is little affected by the association process where the C=O and C-N fragments are not directly engaged in the

hydrogen bonds of the oligomers as their terminals. The peak at (1545, 1474) cm^{-1} can be assigned to vibrations hardly participating in the association process.

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