Effect of Temperature and Concentration on the Structure of *N*-Methylacetamide−Water Complexes: Near-Infrared Spectroscopic Study

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Generalized two-dimensional (2D) FT-NIR correlation spectroscopy and chemometric methods have been used to study temperature-dependent spectral changes in pure *N*-methylacetamide (NMA) and NMA−water mixtures. We also examined the effect of varying water content on the structure of the mixture. It has been found that the extent of self-association of NMA in CCl_4 is very high; the association occurs even at concentration of 0.001 M. In the pure liquid NMA, the population of the monomers is negligible and the structure is dominated by the linear associates. An increase in temperature reduces the number of hydrogen bonds, but in contrast to alcohols their strength remains nearly the same. This reflects a difference in the mechanism of thermal breaking of the associates of NMA and alcohols. The present results reveal that the interaction between NMA and water in the NMA-rich region ($X_{H_2O} < 0.1$) does not have a significant effect on the intrinsic structure of NMA. The structure of NMA is dominant, and the molecules of water do not form separate clusters but are dispersed and incorporated into the structure of NMA. We did not observe the presence of the free OH groups in the mixture. This led to the suggestion that each molecule of water forms two hydrogen bonds to two different molecules of NMA. An analysis of the asynchronous spectra reveals that most of the peaks observed in the asynchronous spectra, constructed from the temperature-dependent data, simply result from the frequency shift. This assumption is supported by the simulation studies.

Introduction

The molecule of *N*-methylacetamide (NMA) is the simplest model of the peptide linkage in proteins, and it has therefore been extensively studied by a variety of experimental and theoretical methods.[1−16] Yet, the conflicting results are obtained even in the recent examinations of pure liquid NMA. Liu et al.[4] and Noda et al.[5,6] studied temperature-dependent spectral variations of liquid NMA using 2D correlation vibrational spectroscopy. They found numerous new spectral features in the asynchronous spectra, and these features were assigned to the hydrogen-bonded associates of various sizes. On the other hand, Huang et al. reported that certain new bands revealed in previous studies[4−6] correspond to the difference bands that are a result of temperature-induced frequency shift of the main bands of NMA.[7] These frequencies are not fundamental normal modes of vibration but artifacts only.

The quantitative information concerning the length and population of the associates is not directly accessible from the correlation spectra. A conventional quantitative analysis in NIR region is difficult due to the high complexity of the spectra. There have been a number of quantitative results on self-association of NMA obtained from the fundamental region.[7−9,12−14] Yet, these papers do not represent a uniform picture of the self-association of NMA. Ludwig et al. reported that in neat NMA at low temperatures, the linear pentamers dominate.[9] When the temperature increases, these species are replaced by the linear dimers and monomers. Furer et al. claim that the infrared data on solutions of NMA in CCl_4 are well reproduced by assumption of the presence of monomers, dimers, and trimers,[13] while in the pure liquid NMA the higher associates should be present.[14] On the basis of previous work on polyamides, Huang et al. have estimated an average length of 7 amide units in liquid NMA at 30 °C.[7] Monte Carlo simulations show that the majority of NMA molecules in the pure liquid phase (61%) participate in two hydrogen bonds.[11] Thus, hydrogen-bonded chains dominate the liquid structure. Both the theory and the experimental results show that the methyl groups are not involved in the hydrogen bonding.[8,9,16] During the hydrogen-bonding creation, a redistribution of the electron density occurs mainly within the amide group, whereas the CH_3 groups are hardly influenced.[16]

There have been a number of papers published on NMA−water interaction.[16−28] The theoretical calculations demonstrate that the molecules of NMA in the water-rich region form hydrogen bonds with three molecules of water.[15,17,23] Two molecules of water are bonded to the lone electron pairs of the CO group, and the third molecule is bonded to the NH group. Yet, Guo et al. demonstrated that the most favorable hydrogen bonds include one bond to the CO group and one bond to the NH group.[26] The formation of the second hydrogen bond to the carbonyl group increases the length of this bond and reduces the bond energy. Chen et al. presented the experimental and theoretical evidences that NMA and its hydrogen-bonded water molecules are vibrationally coupled.[20] This coupling results in the increasing complexity of the system because the amide bands become very sensitive to the hydrogen-bonding geometry and the degree of the coupling.[20] The general order of hydrogen-bonded strengths obtained by statistical mechanics calculations is as follows: amide−amide > amide−water > water−water.[17] However, Mitchell and Price have shown that the amide−amide and amide−water interactions should be approximately isoenergetic.[21] The results of ab initio calculations indicate that the hydrogen bonding to the C=O group is stronger than that to

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the NH group.[17,21,27,28] Indeed, a new FT-IR study of NMA−water complexes in CCl_4 reveals that water prefers to act as a proton donor by attaching to the carbonyl group of NMA.[27]

Most of the previous studies on NMA−water interactions have been performed in the water-rich region, whereas the experimental data obtained in the NMA-rich region are limited. This paper provides new experimental results on the effects of temperature and water content on the NIR spectra of the NMA−water mixture in the NMA-rich region. Special attention was paid to the examination of the changes in the structure of NMA resulting from the presence of small and moderate amounts of water. The structure of pure liquid NMA was elucidated, and the conflicting results obtained from previous 2D correlation studies[4−7] were discussed. The interpretation of 2D correlation spectra was supported by the simulation studies.

Experimental Method

NMA and CCl_4 of high purity were purchased from Aldrich Chemical Co. (Germany) and were used without further purification. High-purity water (resistivity 18.2 MΩ cm) was obtained via the Simplicity 185 Ultrapure Water System (Milliphore Corp.). Solutions were prepared gravimetrically from these materials. FT-NIR spectra were recorded at a resolution of 4 cm^{-1} on a Nicolet Magna 860 spectrometer equipped with a DTGS detector, and 512 scans were accumulated. The sample chamber was purged with dry nitrogen. The temperature-dependent FT-NIR spectra were measured in a variable-temperature quartz cell (Hellma) of 5 mm thickness from 30 to 80 °C with a step of 5 °C. The temperature of the sample was monitored with a digital thermometer dipped into the cell.

Computational Methods

Data Pretreatment. The temperature-dependent data set was composed of 11 spectra, while the concentration-dependent series included 10 spectra of the mixture and the spectrum of pure NMA recorded at 30 °C. The spectra were ordered in the direction of increasing temperature and X_{H_2O}. At first, the baseline fluctuations were minimized by an offset at 9100 cm^{-1}. Next, the spectra were corrected for the density change with temperature and the integrated intensity of the second overtone of the CH stretching band (8000−8600 cm^{-1}) was used as a reference.[29]

2D Correlation Analysis. The dynamic spectrum was obtained by subtraction of a reference spectrum from each of the original spectra. The spectrum of pure NMA at 30 °C and the mean spectrum were used as the references for the concentration- and temperature-dependent data sets, respectively. The synchronous intensity was calculated as a cross-product of the dynamic intensity at two different wavenumbers, whereas the asynchronous intensity was computed as a cross-product of the dynamic intensity at ν_1 and Hilbert transform of the dynamic intensity at ν_2.[30,31] A positive synchronous peak at (ν_1, ν_2) means that the intensity changes at these two wavenumbers are in the same direction, while a negative synchronous peak indicates the opposite. The asynchronous intensities were multiplied by a sign of the corresponding synchronous intensities. This way, a positive asynchronous cross-peak at (ν_1, ν_2) means that the spectral change at ν_1 occurs faster (earlier) than that at ν_2, whereas the negative asynchronous peak indicates the opposite. The presence of a synchronous cross-peak at (ν_1, ν_2) suggests the possibility that the peaks at ν_1 and ν_2 originate from the same fragment of molecule or two different fragments strongly interacting. The asynchronous spectrum develops a peak at (ν_1, ν_2) if the spectral changes at ν_1 and ν_2 vary out of phase for some values of the perturbation. The 2D correlation analysis was performed with the MATLAB 6.5 (The Math Works Inc.) program (synasyn.m) written in our laboratory.

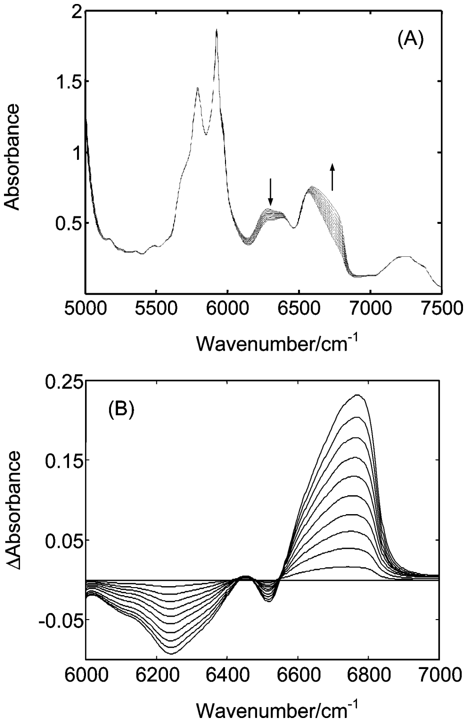

Figure 1. FT-NIR (A) and difference spectra [$D(\nu,T) - D(\nu,T = 30$ °C)] (B) spectra of pure NMA ($X_{H_2O} = 0$) from 30 to 80 °C. Arrows indicate the direction of intensity changes as the temperature is raised.

Chemometric Methods. If all species present in the system contribute to the total absorbance in an additive manner, the data matrix D [$D(\nu,T,X_{H_2O} =$ const.) or $D(\nu,T =$ const., $X_{H_2O})$] can be decomposed as a product of pure concentration profiles matrix (C) and the companion matrix of pure component spectra (S^T):

$$D = CS^T + E$$

where E matrix includes the residual error. At first, the number of species present in the system was determined by principal component analysis (PCA).[32] The number of principal components was also examined by cross-validation procedure,[33] and evolving factor analysis (EFA).[34] The results of EFA provide a starting point for determination of the concentration profiles and the pure component spectra. The concentration profiles and the pure component spectra were obtained with multivariate curve resolution (MCR) with constraints (nonnegativity on concentration and spectra).[35,36] The chemometric analysis was performed by PLS-Toolbox 3.0 (Eigenvector Research Inc.) for use with MATLAB.

Results and Discussion

2D FT-NIR Correlation Study of Pure NMA as a Function of Temperature. Figure 1A displays FT-NIR spectra of liquid NMA from 30 to 80 °C [$D(\nu,T)$]. As can be seen, the most prominent spectral variations take place in the 6000−7000 cm^{-1} region. The changes are better seen in the difference spectra [$D(\nu,T) - D(\nu,T = 30$ °C)] (Figure 1B). The high-frequency band increases in intensity, whereas the other bands show an opposite trend with increasing temperature. The band assignments are collected in Table 1. The corresponding synchronous and asynchronous spectra in the 5000−7500 cm^{-1} range are

TABLE 1: Frequencies and Assignments of Selected IR and NIR Bands of NMA/Water Mixture

vibration	species	position [cm^{-1}]	molecule
ν(NH)	bonded	3310[a]	NMA
ν(NH)	bonded	3365[b]	NMA
ν(NH)	*cis*-monomer	3433[b]	NMA
ν(NH)	*trans*-monomer	3471[a]	NMA
$\nu_2 + \nu_3$	bonded	5142	water
$\nu_2 + \nu_3 + \mathrm{L}^c$	bonded	5610	water
2ν(CH)		5791, 5924	NMA
2ν(NH)	higher multimers	6242	NMA
$2\nu_2 + \nu_3$	bonded	6460	water
$\nu_1 + \nu_3$	bonded	6755	water
2ν(NH)	free terminal NH	6770	NMA
2ν(NH)	*trans*-monomer	6808[b]	NMA
2ν(CH) + δ(CH)		~7270	NMA
3ν(CH)		8470	NMA

[a] NMA in CCl_4, 0.006 M. [b] NMA in CCl_4, 0.001 M. [c] L, librational mode.

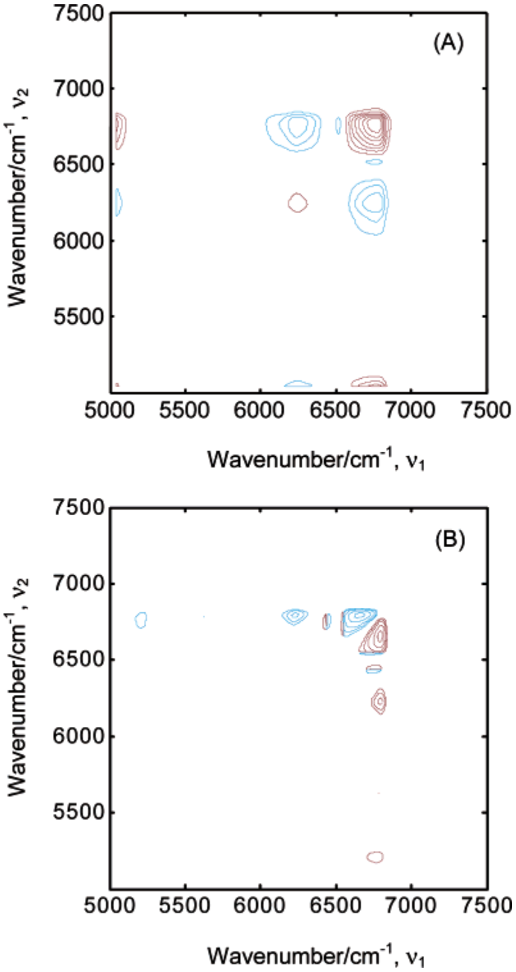

Figure 2. Synchronous (A) and asynchronous (B) spectra of pure NMA from 30 to 80 °C. Red lines represent positive correlation peaks, whereas blue lines represent negative peaks.

shown in Figure 2. The spectra reveal some important differences as compared to the corresponding spectra reported by Liu et al. (Figure 4).[4] In the synchronous spectrum (Figure 2A) dominates the peak at 6770 cm^{-1} being negatively correlated with the peaks at 6242 and 6512 cm^{-1}. This means that the intensity at 6770 cm^{-1} increases, whereas the other bands decrease in intensity as the temperature is raised. In contrast to Liu et al.,[4] we did not observe a synchronous peak at 6440 cm^{-1}. The asynchronous spectrum (Figure 2B) develops a series of peaks at 6794 cm^{-1}. As may be seen from the difference spectra

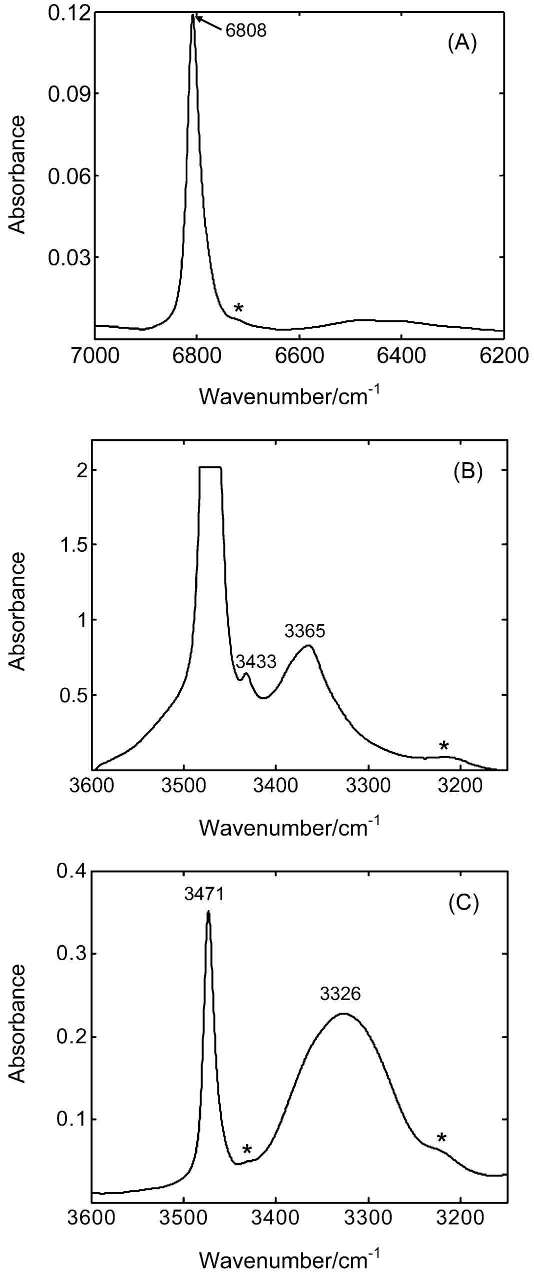

Figure 3. FT-NIR (A) and FT-IR (B) spectra of NMA in CCl_4 (0.001 M) at 30 °C; FT-IR spectrum of NMA in CCl_4 (0.006 M) at 30 °C (C).

(Figure 1B), the higher frequency band shifts by more than 30 cm^{-1}. The simulation studies have shown that the shift in frequency may generate new features in the asynchronous spectra.[37–39] Thus, the origin of the asynchronicity observed in Figure 2B was examined by computer simulations. It has been demonstrated that the positions and relative intensities of the peaks observed in Figure 2B are well reproduced in the synthetic asynchronous spectrum (not shown). Therefore, one can conclude that the correlation peaks at 6794 cm^{-1} do not correspond to true bands but are a result of frequency shift. A similar conclusion was achieved by Huang et al.[7] The authors evidenced that the structure of the ν(NH) mode, observed in the asynchronous spectra, simply results from the frequency shift. Liu et al. have found an asynchronous peak near 6900 cm^{-1} that was tentatively assigned to the baseline fluctuations.[4] This suggestion is reasonable because we did not observe any meaningful asynchronous intensity in this region (Figure 2B). The same authors observed two other correlation peaks at 6440

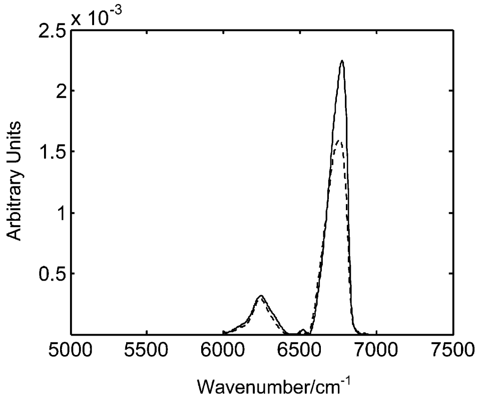

Figure 4. Power spectra constructed from FT-NIR spectra of pure NMA in the temperature ranges of 30−55 °C (- - - -) and 55−80 °C (−).

and 6510 cm^{-1}. As can be seen (Figure 1B), these peaks are located between two prominent bands changing intensity in the opposite direction. Subtracting two single bands that are shifted in frequency results in positive and negative difference bands.[7] The second derivative and Fourier self-deconvolution of the parent spectra did not reveal any peaks near 6440 and 6510 cm^{-1}. Therefore, these peaks were not discussed in this paper.

Liu et al. assigned the peak at 6770 cm^{-1} to the 2ν(NH) mode of the monomeric species.[4] However, Huang et al. demonstrated that the monomers do not exist in liquid NMA and the high-frequency component of ν(NH) mode should be assigned to the end free NH groups in the linear associates.[7] In the NIR spectrum of NMA in diluted CCl_4 (Figure 3A) appears the prominent monomer band at 6808 cm^{-1} and a broad feature ranging from 6200 to 6600 cm^{-1}. The latter feature results from the presence of associates and is better seen in the fundamental region (Figure 3B) near 3365 cm^{-1}. Clearly, the extent of self-association of NMA is very high; the association occurs even at a concentration of 0.001 M. For comparison, the alcohols in inert solvents appear exclusively as monomers up to 0.02 M.[40,41] The band at 6770 cm^{-1} is significantly red-shifted ($\Delta\nu = 38$ cm^{-1}) as compared to the monomer band. The analogous shift for alcohols is appreciable smaller (10 cm^{-1}) and results mainly from the rotational isomerism.[41] Near the melting point (26−28 °C), the population of the monomers is expected to be negligible, and, hence, the absorbance at 6770 cm^{-1} should be weak. This contrasts with the results shown in Figure 1A. The monomer band of alcohols shifts less than 10 cm^{-1} with increasing temperature from 30 to 80 °C, while the analogous shift for NMA is around 30 cm^{-1}. All of these arguments led to the conclusion that the 6770 cm^{-1} band is due to the free terminal NH groups in the linear associates.

Figure 3A shows a minor feature on the low-frequency wing of the monomer band (marked with an asterisk). In the fundamental region (Figure 3B), this feature appears as a clear band at 3433 cm^{-1}. It is known that the molecules of NMA may take two different conformations (cis and trans). The *cis*-NMA is estimated to have a higher energy than the trans form by about 10.5 kJ/mol.[42−44] Thus, at room temperature (25 °C), the population of *cis*-NMA is around 1.5%.[44] Recently, the population of *cis*-NMA, evaluated from FT-IR spectra of NMA in CCl_4, was found to be 2.7% at 20 °C.[27] The monomer frequencies observed by Köddermann and Ludwig for the *trans*-(3475 cm^{-1}) and *cis*-NMA (3431 cm^{-1}) in CCl_4 are close to the values shown in Figure 3 (3471 and 3433 cm^{-1}, respectively). Therefore, the band at 3433 cm^{-1} was assigned to *cis*-NMA. This band could not be assigned to the free terminal NH group because the anharmonicity constant for this vibration ((6770/2) − 3433 = −48 cm^{-1}) would be smaller than that of the monomer band ((6808/2) − 3471 = −67 cm^{-1}).

A negative synchronous peak at 6242/6770 cm^{-1} (Figure 2A) indicates that the absorbance at 6242 cm^{-1} decreases with increasing temperature. Apparently, this band is due to the NH groups involved in the hydrogen bonding.[3,4] Comparison of the power spectra obtained at lower (30−55 °C) and higher (55−80 °C) temperatures (Figure 4) reveals that the 6242 cm^{-1} band slightly decreases in intensity, while its position is almost constant. This implies that an increase in the temperature reduces the number of hydrogen bonds but their strength remains similar. In contrast, the band due to the higher associates of alcohols shows a blue-shift larger than 50 cm^{-1} when temperature increases from 20 to 80 °C. This suggests a different mechanism of the thermal dissociation of the associates of NMA and alcohols. The difference presumably results from two reasons: first, the associates of NMA are larger than those of alcohols; second, the population of the cyclic associates is significant in liquid alcohols, while these associates are absent in NMA.

2D FT-NIR Correlation Study of NMA−Water System as a Function of Temperature. The spectra of the mixtures with $X_{H_2O} = 0.005$, 0.02, 0.06, 0.1, and 0.12 were recorded from 30 to 80 °C. The spectral pattern of the changes was similar regardless of the water content, and therefore the results for selected data set are presented. Figure 5 displays FT-NIR, synchronous, and asynchronous spectra of NMA−water mixture with $X_{H_2O} = 0.06$. As compared to the synchronous spectrum of pure NMA (Figure 2A), one can observe two new auto-peaks at 5126 and 5268 cm^{-1} together with a series of cross-peaks at these coordinates. The pattern of the asynchronous peaks in the 5100−5300 cm^{-1} region (Figure 5C) is similar to that observed for a single band shifted in frequency.[37−39] The origin of the spectral changes in this region was confirmed by the simulation studies. The parameters of the synthetic bands were estimated from the difference spectra [$D(\nu,T,X_{H_2O} = 0.06) - D(\nu,T,X_{H_2O} = 0)$]. A striking similarity between 2D correlation spectra obtained from the simulated (Figure 6) and the experimental data (Figure 5B and C) evidences that the spectral pattern in the 5100−5300 cm^{-1} region arises from a single band that shifts in frequency. This band was assigned to the $\nu_2 + \nu_3$ vibration of hydrogen-bonded water (Table 1).[45] An average position of this band in the studied temperature range is about 5140 cm^{-1}, whereas the corresponding band in pure water shifts from 5183 cm^{-1} at 30 °C to 5224 cm^{-1} at 80 °C. Obviously, the $\nu_2 + \nu_3$ band in the spectrum of the mixture is located at lower frequencies as compared to the spectrum of pure water. This suggests that the molecules of water prefer to interact with the molecules of NMA. First, the interaction of NMA−water is stronger than that of water−water,[11,17] and, second, the molecules of NMA are in majority ($X_{H_2O} < 0.1$). Numerous studies have shown that the hydrogen bonding to the carbonyl group is stronger than that to the NH group.[11,17,21,25] Thus, the 5140 cm^{-1} band can be assigned to water interacting with the free terminal CO group of NMA. The temperature-induced red-shift of this band may simply result from weakening of the hydrogen bonding.[7]

The statistical mechanics simulations of aqueous solutions of NMA show the caging effect around the hydrophobic methyl groups of NMA.[17] As a result, the molecules of water in these regions would show little interaction with the other molecules. The existence of one-bonded water in the complexes with some organic molecules possessing an ether or carbonyl group was

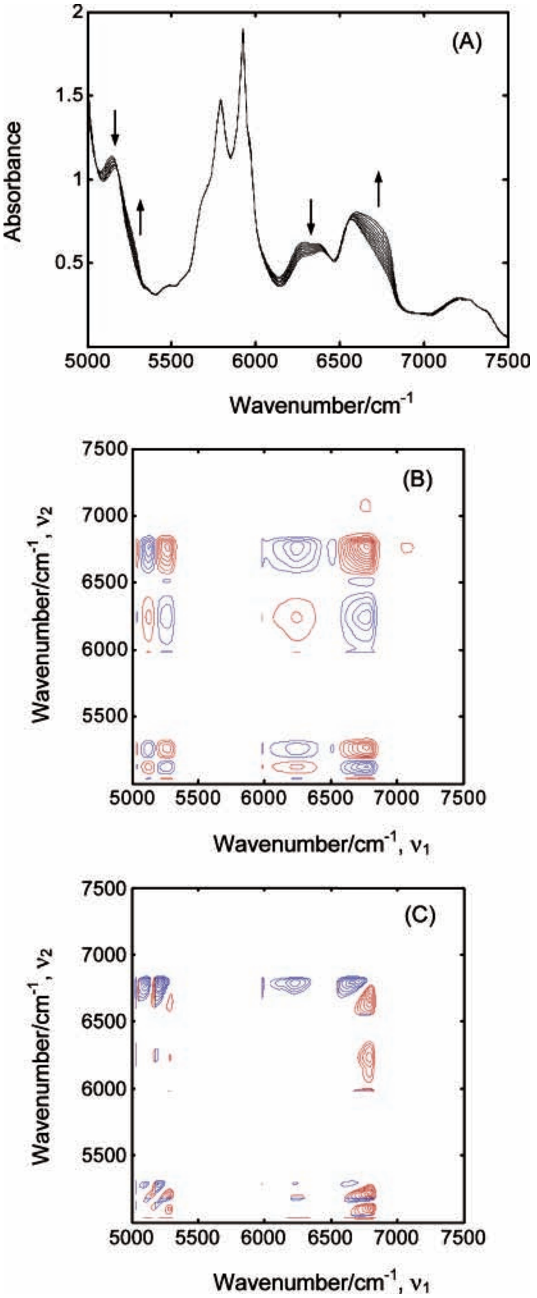

Figure 5. FT-NIR (A), synchronous (B), and asynchronous (C) spectra of NMA/water mixture ($X_{H_2O} = 0.06$) from 30 to 80 °C. Arrows indicate the direction of intensity changes as the temperature is raised. Red lines represent positive correlation peaks, whereas blue lines represent negative peaks.

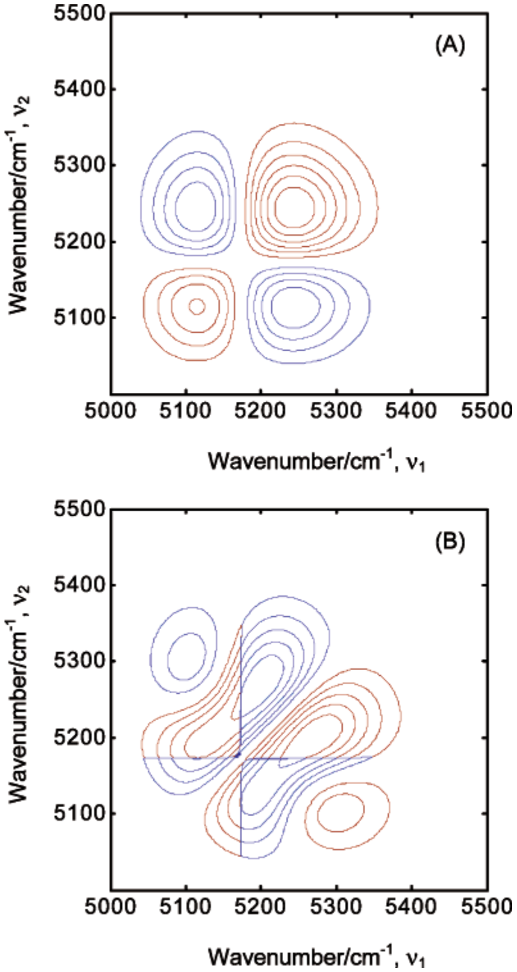

Figure 6. Synchronous (A) and asynchronous (B) spectra constructed from the simulated data. Red lines represent positive correlation peaks, whereas blue lines represent negative peaks.

suggested by Iwamoto et al.[46] Luck has shown the presence of an overtone band due to nonbonded OH groups (>7100 cm^{-1}) both in the pure water and in the alcohol−water mixtures.[47] Recently, nonbonded water was found in a butanol-1-ol/water mixture in the alcohol-rich region.[48] Nevertheless, the present results do not provide any evidence for the existence of the "free" or one-bonded water in the mixture ($X_{H_2O} < 0.1$) and suggest that each molecule of water forms two hydrogen bonds to NMA. The chains of NMA, dominating in the liquid state, possess only one free end carbonyl group. This implies that the molecules of water form two hydrogen bonds with two different molecules of NMA giving rise to the existence of the mixed chains.

2D FT-NIR Correlation Study of NMA−Water System as a Function of X_{H_2O}. Four series of concentration-dependent spectra (X_{H_2O}: 0.001−0.01, 0.002−0.02, 0.005−0.05, and 0.01−0.1) were recorded in a 5 mm cell at 30 °C. The general tendencies for all data sets are similar; therefore, for the sake of clarity the data with the highest content of water are shown. Figure 7A displays FT-NIR spectra of the NMA−water mixture (X_{H_2O} from 0.01 to 0.1) at 30 °C. The dynamic spectrum (Figure 7B) was calculated by subtraction of the spectrum of pure NMA at 30 °C from each of the concentration-dependent spectra [$D(\nu,X_{H_2O}) - D(\nu,X_{H_2O} = 0)$]. This procedure allows for significant elimination of the contribution from NMA. As expected, the most prominent changes appear in the regions of water absorption: at 5145 cm^{-1} ($\nu_2 + \nu_3$) and at 6755 cm^{-1} ($\nu_1 + \nu_3$). Two other bands are located near 5610 and 6460 cm^{-1}. The feature at 6460 cm^{-1} is due to the $2\nu_2 + \nu_3$ mode of the bonded OH group, whereas the 5610 cm^{-1} band can be assigned to the $\nu_2 + \nu_3$ + L (L, librational) vibration of associated water. Figure 7C displays the synchronous spectrum calculated from the dynamic spectra shown in Figure 7B. The asynchronous spectrum (not shown) includes a few peaks of low intensity, yet these peaks cannot be interpreted because the dynamic spectra were not normalized with respect to X_{H_2O}.[29] The asynchronous spectrum constructed from the normalized spectra does not include any meaningful peaks. This indicates that the variation in X_{H_2O} does not vary the relative populations of the NMA species.

A creation of new species of NMA on addition of water would lead to reduction in the population of the other species. As a result, in the synchronous spectrum would appear negative

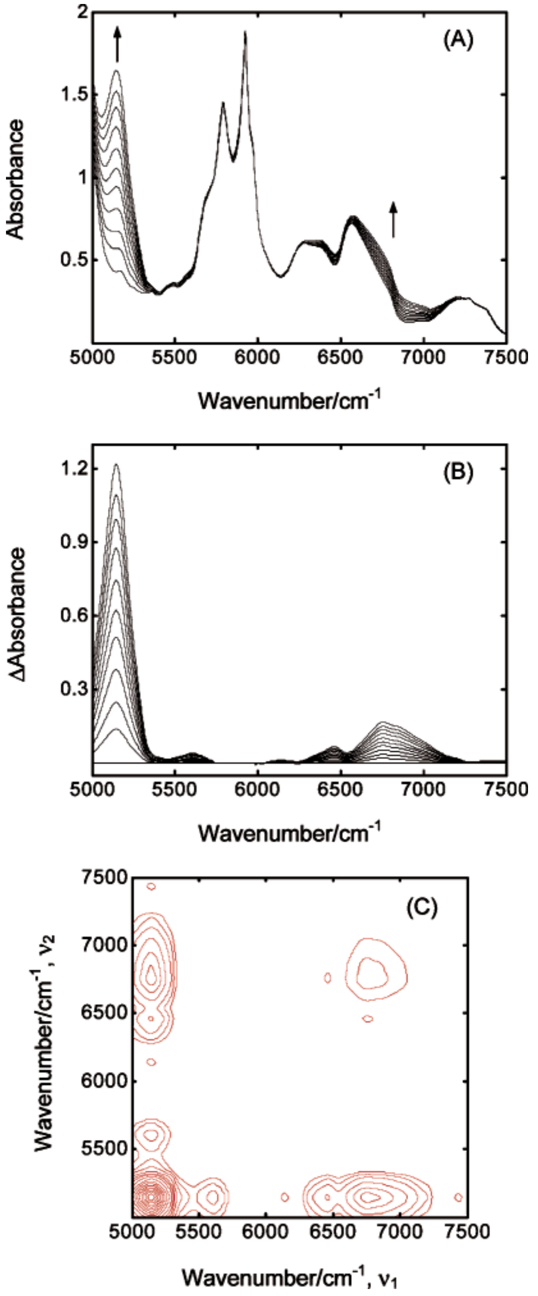

Figure 7. FT-NIR spectra of NMA–water mixture with X_{H_2O} from 0.01 to 0.1 every 0.01 at 30 °C (A), the dynamic spectrum $[D(\nu,X_{H_2O}) - D(\nu,X_{H_2O} = 0)]$ (B), and the corresponding synchronous spectrum (C). Arrows indicate the direction of intensity changes as X_{H_2O} increases. Red lines represent positive synchronous peaks.

peaks due to NMA. As may be seen (Figure 7C), the synchronous peaks are positive, indicating that all species gain in intensity when X_{H_2O} increases. Besides, no synchronous correlation was observed between the bands attributed to water and NMA. This led to the conclusion that the presence of water in the NMA-rich region has a small effect on the self-association of NMA itself. The intrinsic structure of NMA is dominant, and the molecules of water are involved in this structure by hydrogen bonding. A similar conclusion was obtained by Takamuku et al. from IR and X-ray studies of aqueous mixtures of *N,N*-dimethylacetamide (DMA).[49] The authors concluded that the inherent structure of DMA is dominant in the DMA–water mixtures for $X_{DMA} > 0.6$. It has to be noted that an increase in X_{H_2O} for a butan-1-ol/water mixture reduces the population of the polymeric species.[48]

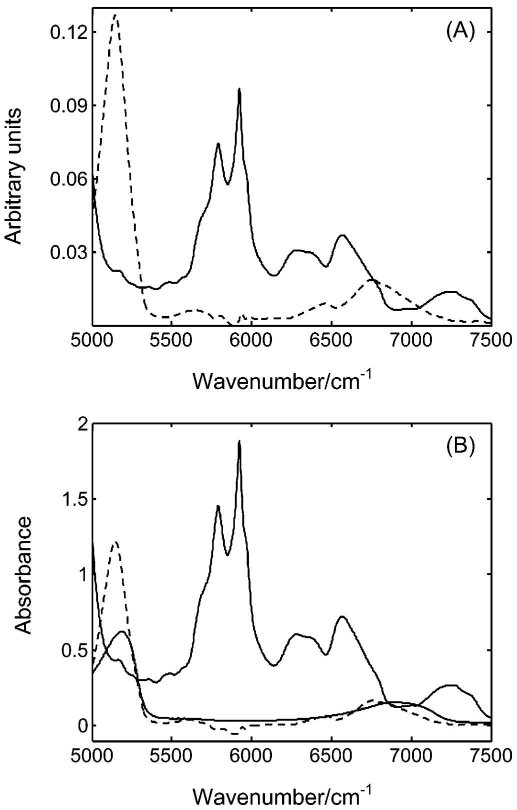

Figure 8. The spectral profiles of NMA–water mixture (X_{H_2O} from 0.001 to 0.1) resolved by MCR (A). The spectra of pure NMA (upper solid line), pure water (lower solid line), and the difference spectrum (dotted line) $[D(\nu,X_{H_2O} = 0.1) - D(\nu,X_{H_2O} = 0)]$ at 30 °C (B).

Chemometric Analysis of NMA–Water System. Both PCA and EFA results suggest the presence of two species responsible for the total intensity variations. Figure 8A shows the spectral profiles of these species. The profile drawn by the solid line is similar to the spectrum of pure NMA, and the population of the corresponding species decreases when X_{H_2O} increases (not shown). The other spectral profile (dotted line) was assigned to water. As expected, the concentration of water shows a constant increase (not shown). For comparison, in Figure 8B are displayed the spectra of pure NMA, pure water, and the difference spectrum $[D(\nu,X_{H_2O} = 0.1) - D(\nu,X_{H_2O} = 0)]$ at 30 °C. The resolved spectral profile of water (Figure 8A) is very similar to the difference spectrum. In contrast, this profile is quite different from the spectrum of pure water (Figure 8B) obtained at the same temperature. The whole profile of water is dramatically shifted to lower frequencies. This implies that the molecules of water dissolved in NMA are involved in stronger hydrogen bonding than that in the pure water. As discussed earlier, the NMA–water interaction is stronger than that of water–water. Interestingly, the spectral profile of NMA is the same as that of the pure NMA. This observation supports our earlier conclusion that an addition of small and moderate amounts of water has a minor effect on the self-association of NMA itself.

Conclusions

An analysis of the experimental spectra measured at different temperatures, confirmed by the simulation studies, leads to the conclusion that most of the asynchronous peaks in the spectra of pure NMA and NMA–water mixtures result from the temperature-induced frequency shift. These peaks do not correspond to true bands but are artifacts. The population of the monomers in the pure liquid NMA is negligible, and the band at 6770 cm^{-1} was assigned to the free terminal NH groups in the linear associates. The monomer band, observed in the diluted solutions of NMA in CCl_4 at 6808 cm^{-1}, has a low-frequency component of weak intensity. This feature was assigned to *cis*-NMA. The extent of self-association of NMA is very high; this process occurs even at a concentration of 0.001 M (in CCl_4). In contrast to the alcohols, an increase in temperature reduces an average number of hydrogen bonds in liquid NMA, but their relative strength remains nearly the same. This indicates that the temperature-induced dissociation of the associates of NMA and alcohols occurs in a different way. No evidence was found for the presence of nonbonded or one-bonded water in the mixture, suggesting that each molecule of water forms two hydrogen bonds to two different molecules of NMA. The results of 2D correlation and chemometric analysis prove that the small and moderate amounts of water in the mixture ($X_{H_2O} < 0.1$) have a minor effect on the structure and population of NMA associates. The inherent structure of NMA dominates, and the molecules of water are involved in this structure by hydrogen bonding to the free terminal C═O groups.

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