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Effect of temperature perturbation on hydrogen bonding in aqueous solutions of different urea concentrations†

Noushaba Nusrat Mafy,[a] Tanjina Afrin,[a] M. Muhibur Rahman,[b] M. Yousuf A. Mollah[ab] and Md. Abu Bin Hasan Susan*[a]

Water absorption peaks in near-infrared (NIR) and attenuated total reflectance (ATR)-Fourier transform infrared (FTIR) spectra at different temperatures both in the absence and presence of urea have been analyzed to investigate hydrogen bonding in aqueous solution by perturbing the temperature and concentration of urea. Concentration-dependent basic spectra of aqueous urea solutions represent different clusters in the system originating from the different extent of self-aggregation of urea molecules and water–urea interactions. Derivative and deconvoluted spectra confirm the presence of different structural components or clusters in pure water, but in the presence of urea a new strong water cluster could be identified for the first time above a certain concentration of urea. The degree of perturbation has been evaluated by 2D correlation and difference spectroscopy. The apparent molar volume, free energy change of activation (ΔG), change in enthalpy of activation (ΔH) and change in entropy of activation (ΔS) for the viscous flow of water in the presence and absence of urea have been analyzed. The comprehensive analyses help to infer the different extent of aggregation of the urea molecules and formation of clusters by water–urea interactions in aqueous urea solutions.

1. Introduction

Water is a matter of life and the matrix of all living organisms, and acts as a universal solvent. There has been a surge of interest to unveil the mystery of this simplest but yet possibly most critical and widely studied liquid of the universe. The description of the structure of water beyond any doubt in the pure liquid state, at interfaces, and in solutions has been one of the major goals of current research. The physical and chemical properties[1] and molecular simulations of water[2–5] are important to solve many unresolved issues. Different models have been proposed for describing the structure of water. Continuum and mixture models are extensively used to describe different properties of pure water and aqueous solutions. The continuum model proposed by Pople *et al.*[6] interpreted that liquid water contains a continuous three dimensional network and the hydrogen bonds are never broken, but rather only more or less distorted from their ideal or stable three dimensional structures by any perturbation. On the other hand, the mixture model considers the different degrees of hydrogen bonded species to be in an equilibrium.[7–10] According to this model, water has at least two kinds of species, a bulky species containing some type of H-bonded structure and a dense species such as monomeric or free water molecules. The mixture model is supported by a near infrared (NIR) study[11–14] and Raman spectra of H_2O and HOD studied by Walrafen *et al.*[15,16] Stevenson *et al.*[17] reported that about less than 1% of the free monomer is present in liquid water from 0 to 100 °C, which cannot support the principle of the continuum model. Mid IR (MIR)[7,18–20] and attenuated total reflectance[21,22] studies of liquid water have been widely used to investigate the variation of hydrogen bonding in water. Degrees of hydrogen bonding can vary with pressure, temperature and different additives to bring about consequent changes in the fundamental properties of water.

We have recently reported that fructose can disturb the bond strength and energy of hydrogen bonding and perturb water

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† Electronic supplementary information (ESI) available: Fig. S1: FT-IR spectrum of solid urea. Fig. S2: FTIR-ATR spectra of (a) pure water and (b) aqueous urea solutions at different concentrations. Fig. S3: NIR spectra of pure water at different temperatures. Fig. S4: difference spectra of (a) pure water and (b) a 6.0 M aqueous urea solution obtained with respect to the first spectrum measured at 20 °C. Fig. S5: second derivative spectra of pure water at different temperatures. Fig. S6: second derivative spectra of a 1.5 M aqueous urea solution at different temperatures. Fig. S7: temperature-dependent deconvoluted spectra of pure water. Fig. S8: variation of band position for (a) weak, (b) third or moderate strength, (c) normal or strong, and (d) strong hydrogen-bonded components resolved from the temperature-dependent NIR spectra of pure water and aqueous urea solutions. Fig. S9: power spectra of three distinct groups of aqueous urea solutions. Fig. S10: variation of the apparent molar volume with temperature and concentration. Fig. S11: variation of the entropy with the concentration of urea. See DOI: 10.1039/c5ra10718k

clusters in aqueous solution by different ways. In fructose, the OH groups act as hydrogen bonding sites and a methylene group in the carbon 6 acts as a hydrophobic site to influence the organisation of water. Fructose and also many other sugars are likely to form a complex structure in water due to their muta-rotation into different conformations and configurations.[23-25] Therefore, the comprehensive mechanism of solvation still remains a challenge. Urea is also capable of perturbing the network of water and avoids the complexity arising from fructose or other sugars. In a first model, Frank and Franks (FF)[26] proposed that urea indirectly modify the network of water by forming hydrogen bonds with water. The FF model was supported by many experimental techniques such as NMR,[27] X-ray,[28] Raman[29] and IR[30] spectroscopy. The SKSS model[31-33] proposed by Schellman, Kresheck, Sheraga, and Stokes suggests that urea is present in a dimerized or oligomerized form in aqueous solution by self-aggregation. However, the mechanism of aggregate formation and modification of the network of water are still not clear. Research to date suggests that both the self-aggregated form of urea and water–urea clusters are present in aqueous solution.[33-36] IR,[37] NIR,[38] and Raman spectroscopy,[29] the optical Kerr effect,[39,40] molecular dynamics simulations,[41] and 2D correlation spectroscopy[35] are extensively used to study water–urea interactions. In contrast to the observations stated in most of the literature, few recent studies show that urea, having both hydrophilic (oxygen and hydrogen atoms) and hydrophobic (carbon and nitrogen atoms) sites, acts as a neutral species to a water molecule.[42-44] Urea has been reported in recent years not to significantly perturb the hydrogen bonding of water, but only to influence its dynamics.[45,46] On the basis of such speculations, researchers have illustrated that urea denatures a protein by direct interactions with different groups of the protein,[47] which was supported by NMR spectroscopy,[48] calorimetry,[49] and dielectric spectroscopy.[50] An aqueous urea solution has some remarkable properties such as enhancing the solubility of hydrocarbons, changing the onset concentration of surfactants for micelle formation[51] and the ability to denature a protein. To properly understand such interesting properties, the structure of water in such a system requires systematic studies without or with external stresses.

The present work helps to provide strong evidence for old postulates and new information about pure water and aqueous urea solutions at different temperatures and concentrations. The different sorts of interactions in the system have been analyzed by NIR and ATR-FTIR spectroscopy. The nature and change of hydrogen bonding in water due to the presence of urea at different temperatures have been investigated by 2D correlation spectroscopy. The thermodynamic parameters such as the free energy change of activation (ΔG), change in enthalpy of activation (ΔH) and change in entropy of activation (ΔS) for the viscous flow of aqueous solutions have been determined to explain the thermodynamic state of the solutions with the change of temperature and concentration of urea. The ultimate goal is to confirm the self-aggregation of urea and the water–urea interactions in aqueous solution at different urea concentrations with emphasis on the perturbation with temperature.

2. Experimental

Urea was used as received from Merck. Ultrapure water (specific conductance = 0.055 μS cm^{-1}, BOECO pure, model no-BOE 8082060, Germany) was used for the preparation of aqueous solutions.

ATR-FTIR measurements were performed using a FT-IR 8400S Shimadzu spectrophotometer on a zinc–selenide (Zn–Se) crystal. The absorption spectra were recorded with a resolution of 4.0 cm^{-1} and 100 scans for each sample in the range of 700–4000 cm^{-1}.

NIR and MIR spectra were recorded by a FTIR spectrophotometer (PerkinElmer, USA, Frontier FT-IR/NIR) in absorbance mode with 10 scans for each sample in the range of 4000–12 000 cm^{-1} at 4.0 cm^{-1} resolution. NIR measurements were performed by a highly sensitive heatable liquid sampling cell in which a pair of rectangular CaF$_2$ windows with curved edges (Specac model no: GS20522) of a path length of 0.1 mm were used for each measurement. The path length was controlled by a polytetrafluoroethylene (PTFE) spacer. The temperature was controlled by an electrical heating jacket (Specac model no: GS20730). Cool water was circulated from a chiller through the surrounding of the jacket to control the constant temperature of the sample.

Densities of the solutions were measured with an Anton Paar (model no: DMA 4500) vibrating tube density meter following the oscillating U-tube method. In this method, the sample has been injected into a U-shaped borosilicate glass tube that has been excited to vibrate at a characteristic frequency. The frequency changes depending on the density of the sample. Through the precise determination of this frequency and a mathematical conversion, the density of the sample has been measured.

The viscosities of the solutions were measured with a Lovis 2000 M/ME microviscometer, which measures viscosity by the falling ball principle with an accuracy of ±10^{-6} mPa s. A capillary with a diameter of 1.59 mm and a steel ball with a maximum measurement deviation of 0.2% were used for the measurements. In this method, a ball of known diameter and density rolls through a closed, liquid-filled capillary of known diameter which is inclined at a defined angle. Three inductive sensors determine the rolling time of the ball between defined marks. The viscosity of the sample liquid is related to the time it takes for the ball to pass a distance between two specified lines on the cylindrical tube. Turning the measurement tube results in returning the ball and it is possible to re-measure the time over the same distance. The result is a dynamic viscosity with standard dimension (mPa s).

For 2D correlation analysis, the data were normalized by the density and then a dynamic spectrum was calculated by subtracting the reference spectrum from the ordered series of experimental spectra. The spectrum of pure water was used as a reference for concentration perturbation, while spectra of aqueous urea solutions at 20 °C were used as references for an individual set of measurements for perturbation with temperature. The synchronous map was determined by the cross-

product of the dynamic intensity at two different wavenumbers and the asynchronous map was computed using the Hilbert transform matrix. 2D correlation spectra were computed using MATLAB 13 (The Math Works Inc.) software.

3. Results and discussion

3.1 IR spectra of solid urea

The IR spectrum of solid urea gives the different fundamental bands of urea and helps in association with the NIR results to understand the molecular level interactions in aqueous urea solutions. Fig. S1 (ESI†) shows a band at 3444 cm^{-1} for asymmetric N–H stretching, at 3346 cm^{-1} for symmetric N–H stretching, at 1682 cm^{-1} for C=O stretching, at 1634 cm^{-1} for amide II (NH$_2$ deformation and C–N stretching), at 1606 cm^{-1} for amide I (C=O stretching and C–N stretching), 1466 cm^{-1} for amide III (C–N stretching and N–H deformation) and 1158 for N–H wagging. All these assignments of the different bands are in good agreement with the literature.[52]

3.2 ATR-FTIR spectra of pure water and aqueous urea solutions

The ATR-FTIR spectrum of pure water (Fig. S2a†) shows a narrow band at 1635 cm^{-1} for bending and a wide band at 2110 cm^{-1} for overlapping of bending and libration. A broad band centered at 3362 cm^{-1} with a shoulder at 3242 cm^{-1} could also be identified due to the overlap of symmetric and asymmetric stretching of O–H groups. The ATR-FTIR spectrum of an aqueous urea solution (Fig. S2b†) shows a broad band in the range of 2900–3800 cm^{-1} where two bands (N–H asymmetric stretching (3444 cm^{-1}) and N–H symmetric stretching (3346 cm^{-1})) of urea overlapped with the bands of water. The band of amide III at 1466 cm^{-1} shows a strong concentration dependency. With increasing urea concentration, the band shifts to lower wavenumbers (1468–1460 cm^{-1}), as shown in Fig. 1a. This is due to the fact that intermolecular hydrogen bonding between the urea molecules becomes stronger with increasing concentration in aqueous solution by self-aggregation. This is supported by the SKSS model. The overlapping band at 1635 cm^{-1} for the overlap of water (O–H bending) and urea (amide II) in Fig. 1b also shifts to lower wavenumbers (1642–1622 cm^{-1}), revealing that hydrogen bonding between both water and the urea molecules will be stronger with increasing urea concentration, and the urea molecules strongly perturb the bending of water. The band assignments are in agreement with the literature.[53]

3.3 NIR spectra of pure water and aqueous urea solutions at different temperatures

The NIR spectra give strong evidence of hydrogen bonded species. The major problem in studying the NIR bands is the assignment of the different bands. Fig. S3† shows the temperature-dependent NIR spectra of pure water over the temperature range 20–65 °C in the 4000–6000 cm^{-1} region. In this region, a strong absorption from the combination of asymmetric stretching and bending of OH in water could be

found. The specification of the different bands of pure water in this region is well established.[14,23] With increasing temperature, the band position shifts to higher wavenumbers since the hydrogen bonds between the water molecules become weaker. An isosbestic point is observed at 5161 cm^{-1}, which indicates the presence of different clusters of water in dynamic equilibrium with each other.[14] NIR spectra of aqueous solutions of urea (Fig. 2) were recorded at different temperatures and concentrations of urea. Two new bands appear in the range of 4970–4250 cm^{-1} in the presence of urea, which are absent in the spectra of water. The bands at 4644 and 4550 cm^{-1} correspond to the combination of the symmetric (3346 cm^{-1}) and asymmetric (3444 cm^{-1}) stretching bands coupled with the N–H bending vibration, respectively. We assume that the appearance of these bands is due to self-aggregation of urea in water because both bands are shifted to lower wavenumbers and the intensity of these bands increases with increasing concentration of urea. A band in the region of 4600–4500 cm^{-1} was reported to be present in both an emulsion of urea in nujol oil and an aqueous urea solution at high concentrations. Such observation assists in concluding that some solid-like behavior of urea giving rise to clusters through aggregation is also present in aqueous urea solutions. Earlier, molecular dynamics[54] and Monte Carlo simulation[55] studies established a certain tendency for self-association of urea in aqueous solutions. A new organized or ice-like water cluster is formed around aggregated urea. This is also supported by computational[36] and 2D correlation methods.[35] The number of hydrogen bonds per urea molecule increases with increasing urea concentration, since urea–urea hydrogen bonds formed are larger in number than urea–water hydrogen bonds. With increasing urea concentration, the band centered at 5161 cm^{-1} in water shows two prominent shoulders at 5104 and 5164 cm^{-1}. Two shoulders are also developed at 4952 cm^{-1} and 4831 cm^{-1} in the presence of urea. These correspond to different types of clusters of water and urea and their intensity increases with increasing urea concentration. The hydrogen bond strength of these species is not equal to yield different shoulders at different positions. Fig. 2b shows that the band intensity increases with increasing temperature due to an increase in free OH groups. Moreover, the shifting of the band to higher frequency values indicates that a higher energy is required to vibrate the molecules as the bond length decreases with increasing temperature. The presence of urea makes water more sensitive toward temperature. This is clearly observed from the deconvoluted spectra. The intensity of this shoulder decreases with increasing temperature due to the decrease in strong hydrogen bonded species. Weakly hydrogen bonded species and free water also increase with increasing urea concentration and temperature.

3.4 Difference spectroscopy of an aqueous urea solution

Difference spectroscopy is widely used to investigate the spectral changes induced by temperature or other perturbations. Difference spectroscopy enables to subtract the absorbance of a component from the spectrum which is invariable with perturbation. Moreover, the difference spectra show a direction
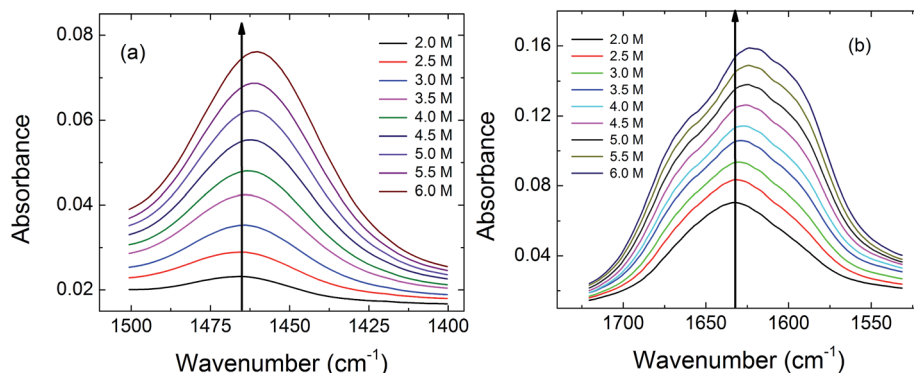
Fig. 1 ATR-FTIR spectra of aqueous urea solutions: (a) amide III band of urea and (b) overlapping band of O−H bending and amide II.

for these changes. The difference spectra of pure water (Fig. S4a†) and an aqueous urea solution (Fig. S4b†) show positive peaks at higher wavenumbers and negative peaks at lower wavenumbers. The intensity of both peaks increases with increasing temperature. This reveals that the number of weak hydrogen bonded species increases and that of strong hydrogen bonded species decreases with increasing temperature. However, the negative peaks of the aqueous urea solution are not smooth like those of pure water. In fact, different types of water–urea complexes are formed and these are susceptible to temperature changes to a different extent. An isosbestic point reveals that weak hydrogen bonded and strong hydrogen bonded species are in equilibrium. Conversion occurs through an intermediate species.

3.5   Derivative spectra of an aqueous urea solution

A linear shift in the center frequency of a broad resonance shows up as the derivative of the line shape, leading to a positive wing on one side and a negative wing on the other in the derivative spectra. This indicates a shift of a single resonance and any discontinuity or irregular pattern in any wing of the derivative spectra can provide indication of other molecular events in the system. The intensity and width of these peaks are affected by the anharmonicity. Second derivative spectra of water in the temperature range of 20–65 °C (Fig. S5†) indicate

that the peaks at 5240 cm^{-1} appear due to the combination of asymmetrical O–H stretching and bending. Fig. 3 shows four overlapping bands for a 6.0 M aqueous urea solution in the region 4700–5700 cm^{-1}, but three bands appeared for a 1.5 M solution in the same region (Fig. S6†). All bands are temperature susceptible to a different extent. The band at 5240 cm^{-1} is responsible for the combination of asymmetric OH stretching and bending, that at 5089 cm^{-1} for the combination of asymmetric NH stretching and amide II, that at 4948 cm^{-1} for the combination of symmetric NH stretching and amide II, and that at 4825 cm^{-1} for the combination of asymmetric NH stretching and amide III. Changes were also observed at 5181 cm^{-1} where a shoulder is developed in the presence or absence of urea and which disappears with increasing temperature. To analyze the spectral dynamics and the presence of different species, deconvolution and 2D correlation spectroscopy have been utilized.

3.6   Deconvolution of the temperature-dependent NIR spectra of pure water and aqueous urea solutions

Deconvolution helps to identify different kinds of components and the quantitative evaluation of their relative contribution at different temperatures or other perturbations. Therefore, the spectra have been deconvoluted into several Gaussian peaks for the analysis of different water species in pure water and
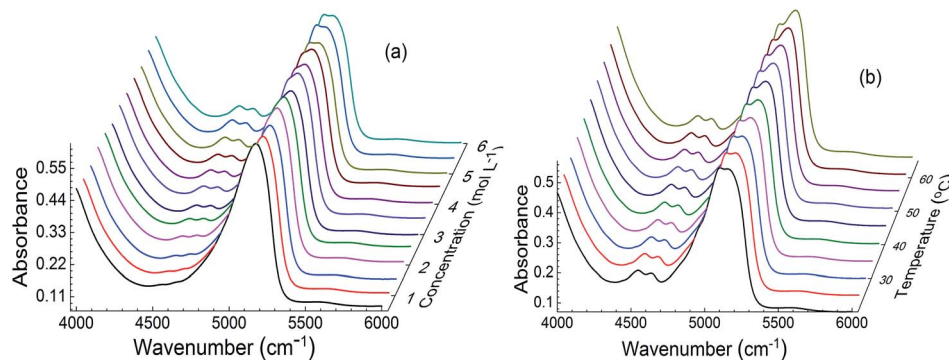

Fig. 2 NIR spectra of aqueous urea solutions as a function of (a) the urea concentration at 25 °C and (b) temperature for a 6.0 M solution, in the range of 6000–4000 cm^{-1}.
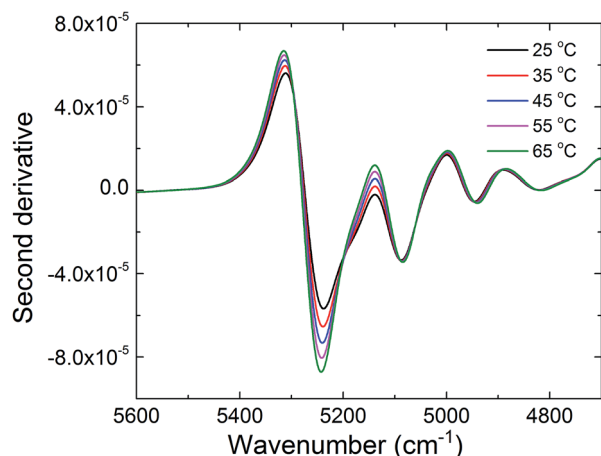
Fig. 3 Second derivative spectra of a 6.0 M aqueous urea solution at different temperatures.

aqueous urea solutions. Laenen et al.[56,57] showed that pure water consists of: (I) ice-like species in the lower frequency region, (II) bifurcated hydrogen bonded species in the higher frequency region, and (III) bridged species in between the above mentioned two species. The dynamic model of liquid water defines that pure water consists of five different hydrogen bonded species: (I) strong, (II) normal, (III) P-bifurcated, (IV) T-bifurcated, and (V) free.[58] Among these, three species (normal, P-bifurcated, T-bifurcated) are susceptible to temperature changes than the other two species. Deconvoluted spectra of pure water (Fig. S7†) consist of three bands centering at 5037 cm^{-1}, 5166 cm^{-1}, and 5236 cm^{-1} and corresponding to the strong, moderate strength and weak hydrogen bonded components of water due to their different geometries. Two components are strongly correlated with the change of temperature. With increasing temperature, the band intensity of the strong hydrogen bonded species at lower wavenumbers decreases and that of the weak hydrogen bonded species at higher wavenumbers increases. The third component, defined as the hydrogen bonded component of moderate strength, is significantly resistant to temperature change in the range of 20–65 °C. A temperature effect can thus be observed by changing the two components.

Fig. 4 shows the deconvoluted spectra of aqueous urea solutions. It is clear that in presence of urea all the species including the third one are sensitive to both the urea concentration and temperature. At urea concentrations above 2.0 M, a new band is developed in the lower wavenumber region and the intensity increases with increasing urea concentration. The intensity of the third component increases significantly with increasing urea concentration. The structural change was reported not to change below and above 2.0 M of an aqueous solution of urea[35] in an identical manner. At concentrations below 2.0 M, a small amount of urea can form a H-bond with water and small aggregates like dimers or trimers are formed by self-association of urea molecules. Therefore, the effect of urea is negligible. At concentrations above 2.0 M, urea molecules tend to form large oligomeric and polymeric chains in the

system. However, the formation of chains does not follow any definite pattern, but they are rather randomly arranged by different intermolecular hydrogen bonding between the urea molecules. Monte Carlo simulations also suggested that dimers of urea are present at a ~1 M concentration, and that above ~5.5 M a network of urea is formed. Nevertheless, at concentrations below 1 M, urea does not affect the network of water.[59]

The new band centering at the lowest wavenumber side of the original band (4810–4820 cm^{-1}) signifies that self-aggregated urea helps to form ice-like water in an aqueous urea system. Stumpe and Grubmüller used theoretical treatments and compared them with neutron scattering data to infer that urea helps to strengthen the water cluster and makes it ice-like with a perfect tetrahedral arrangement.[36]

Fig. 5 shows the variation of the band position of different species. With increasing urea concentration, the absorption bands of the weak, moderate and normal or strong hydrogen-bonded species shift to lower wavenumbers. The absorption band centering at ca. 4810–4820 cm^{-1} for the strong hydrogen-bonded species in the presence of urea is shifted to lower wavenumbers until a 4.5 M urea concentration and then shifts to higher wavenumbers. This nonlinear variation may be attributed to the random aggregation of urea with increasing concentration. The measured spectra can therefore be divided into three groups: one group is considered for an up to 2.0 M urea concentration where ribbon and chain dimers are developed. The second group is considered for 2.5 M to 4.5 M of urea concentration in which oligomeric chains of urea molecules are formed. The third group is considered for concentrations between 5.0 M to 6.0 M in which polymeric chains of urea molecules are formed. Different aggregates perturb the hydrogen bonding of liquid water to a varying extent. Aggregated urea assists in forming more organized ice-like water. With increasing temperature, the bands of all corresponding species shift to higher wavenumbers. However, the bands at 4990 cm^{-1} and 4834 cm^{-1} for two strong hydrogen bonded species for 3.0 and 6.0 M remain unchanged with increasing temperature (Fig. S8†).

3.7 2D FT-NIR correlation study of an aqueous urea solution at different temperatures

Fig. 6a represents the synchronous spectrum of an aqueous urea solution in the range of 4600–5600 cm^{-1} as a function of temperature. For an aqueous urea solution, the diagonal peaks or autopeaks appear at 5254 and 5033 cm^{-1}. The autopeaks are positive and these autopeaks indicate that the changes of these peaks are simultaneous. The negative cross-peaks at 5254 and 5033 cm^{-1} indicate that spectral intensity changes for both bands occur in the opposite direction. In other words, the band intensity at 5033 cm^{-1} decreases and that at 5254 cm^{-1} increases. With increasing temperature, hydrogen bonds break down which results in an increase in intensity of the 5254 cm^{-1} band at the expense of the band at 5033 cm^{-1}. However, the increase in population of monomers is correlated with a decrease in population of the polymers. In the synchronous spectrum, a broad autopeak is developed at 5254 cm^{-1}. So, with
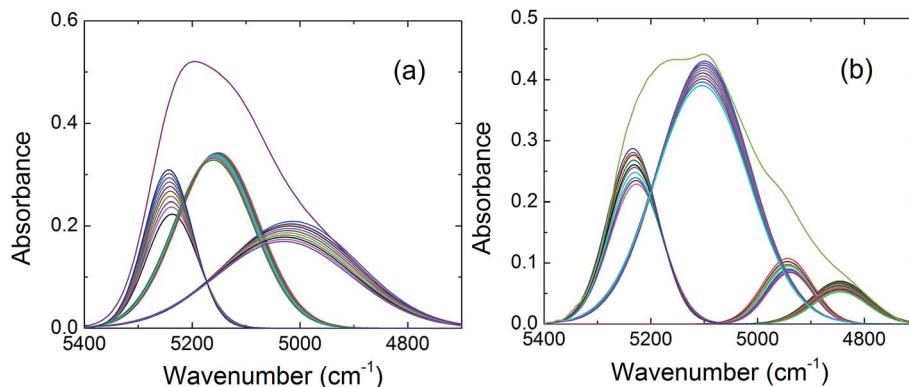
Fig. 4   Temperature-dependent deconvoluted spectra of aqueous urea solutions: (a) 0.5 M and (b) 6.0 M.
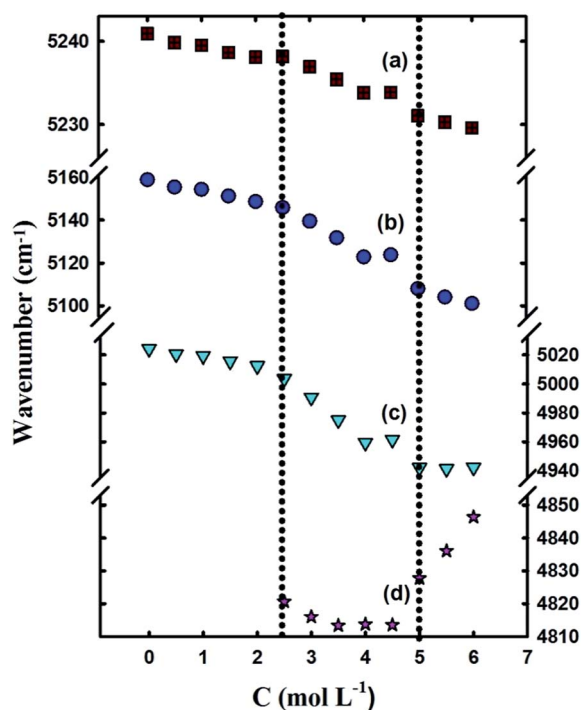

Fig. 5   Variation of the band position for (a) weak, (b) third or moderate strength, (c) normal or strong, and (d) strong hydrogen-bonded components resolved from the concentration-dependent NIR spectra of pure water and aqueous urea solutions at 35 °C.

increasing temperature the number of weak hydrogen bonded species increases. The asynchronous spectrum is antisymmetric with respect to the diagonal line and its intensity represents the sequential or successive change at two different wavenumbers. Fig. 6b shows the two peaks at 5253 cm^{-1} and 5153 cm^{-1} indicating that out-of-phase spectral changes occur at these wavenumbers. It also shows that in the presence of urea a new band at 5153 cm^{-1} also develops in addition to those at 5254 and 5033 cm^{-1} with a temperature perturbation just like pure water. This reveals that in the presence of urea three components of water exist. 2D correlation of pure water also shows that water has three structural components, one is resistant to

temperature perturbation.[14,60] However, the third or moderate strength hydrogen bonded species in an aqueous urea solution which is perturbed by temperature is not clearly identified from the power spectra. The third species may not be significantly perturbed by temperature and the change is negligible than those of the other two species. The asynchronous peak is distorted from the standard butterfly pattern which reveals that spectral intensity changes are coupled with the band position. The direction of the peak shift is determined by the signs of the asynchronous cross-peaks within the butterfly cluster. Above the diagonal, the sign of the cross-peak is negative while below the diagonal the peak is positive. This reveals that the band shifts to higher wavenumbers with increasing temperature.

3.8   2D FT-NIR correlation study of aqueous urea solutions of different concentrations

2D correlation spectra were constructed from the difference spectra to obtain more detailed information about urea in solution (Fig. 7). Difference spectra were calculated by subtracting the spectra of pure water from those of the mixture. In this way, variations of the bands of the different species of water were strongly suppressed and the bands of urea became more distinct. In the synchronous spectrum, a broad autopeak is developed at 5276 cm^{-1}, indicating that the intensity of the band due to the aggregated urea increases with increasing urea concentration. The corresponding asynchronous spectrum develops a cross-peak at 5180 and 5262 cm^{-1}. The cross-peak shows asymmetry, indicating the existence of a band near 5160 cm^{-1}, possibly arising from the weakly hydrogen bonded species. Above the diagonal, the sign of the cross-peak is positive, while it is negative below the diagonal. This observation suggests that with increasing concentration the band shifts to lower wavenumbers. Power spectra of aqueous urea solutions in three different concentration ranges (Fig. S9†) show that the peak position shifts to lower wavenumbers suggesting that intermolecular hydrogen bonding between urea molecules is progressively stronger with increasing urea concentration.
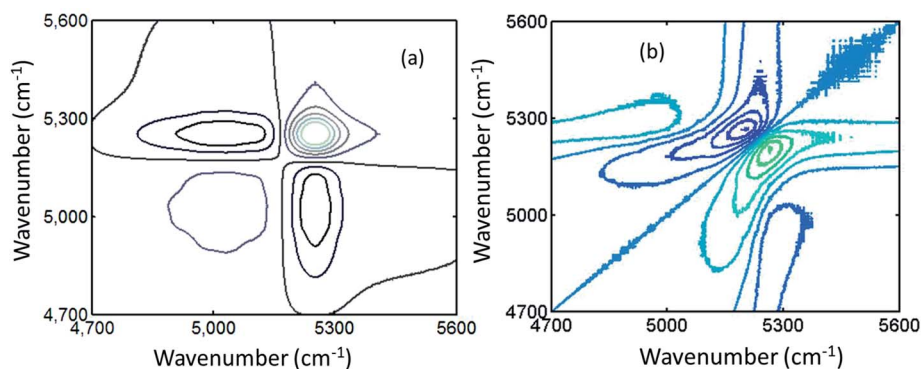
Fig. 6 (a) Synchronous and (b) asynchronous 2D NIR correlation spectra calculated from the temperature-dependent spectral changes (20–65 °C) of an aqueous urea solution.

3.9 Volumetric study of an aqueous urea solution and pure water

The density increases with increasing concentration of urea due to the formation of different types of hydrogen bonds between water and urea. An increase in temperature, on the other hand, lowers the density because of the decrease in the number as well as strength of hydrogen bonds. The apparent molar volume of the water–urea system at different temperatures and concentrations has been calculated from the density data of the solutions to investigate the nature of solute–solvent and solute–solute interactions[61,62] (Table 1, Fig. S10†). The linear increase of the apparent molar volume with increasing concentration indicates that the water–urea interaction increases with increasing urea concentration. In an aqueous solution of urea, hydrogen bonds between water and urea forms to a small degree and the apparent molar volume changes less significantly with the concentration of urea as compared to the corresponding change with temperature (Fig. S10†).

The values of Φ_v^0 are positive, indicating strong water–urea interactions. Φ_v^0 increases with increasing temperature suggesting that the urea–water interaction is lowered with increasing temperature. The positive values of S_v suggest a strong urea–urea interaction. S_v decreases with increasing temperature, in other words, the urea–urea interaction

decreases with increasing temperature. The positive value of the first derivative of Φ_v^0 decreases with increasing temperature since the breaking tendency increases as the temperature rises.

3.10 Viscometric study of aqueous urea solutions and pure water

The viscosity increases with increasing urea concentration due to the increase in intermolecular force or hydrogen bonding which results in flow resistance. On the other hand, the viscosity decreases with increasing temperature due to weaker intermolecular forces in the aqueous solutions of urea. The free energy changes of activation of the viscous flow per mole of solute and solvent at different temperatures (ΔG) have been evaluated by the Eyring viscosity relation and described by the hole theory. The results of the thermodynamic parameters contradict the reported values (Fig. 8).[62] The values of ΔG are positive and increase with increasing temperature and concentration. The positive value of ΔG indicates that the system is not spontaneous. Molecules absorb energy to perform useful work. When urea is added, a small amount of urea forms hydrogen bonds with water, but most of the urea molecules self-aggregate and are surrounded by tetrahedrally arranged water molecules to form an ice-like structure. The sizes of the different clusters are larger after the addition of urea, which cannot easily transform
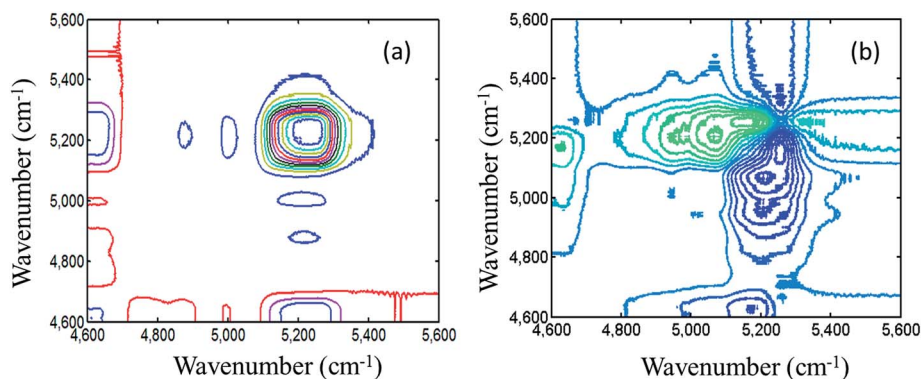

Fig. 7 (a) Synchronous and (b) asynchronous 2D NIR correlation spectra calculated from the concentration-dependent spectral changes (2.0–4.5 M) of aqueous urea solutions.

Table 1 Values of Φ_v^0 and S_v of aqueous solutions of urea at different temperatures

Temperature (K)	S_v	Φ_v^0 cm^3 mol^{-1}	$\partial\Phi_v^0/\partial T$ cm^3 mol^{-1} K^{-2}
293.0	0.2334	42.9572	0.0810
303.0	0.2039	43.7215	0.0685
313.0	0.1859	44.3645	0.0628
323.0	0.1557	45.0374	0.0531
333.0	0.1859	45.3175	0.0225
338.0	0.1672	45.5834	0.0184

into hole or create new holes. More energy is required for the movement of large clusters.

The positive values of ΔH indicate that the solvation of urea in water is endothermic. ΔH decreases with increasing urea concentration and temperature. The dissolution process involves the disruption of the normal network of water to create space for the urea molecules to disperse and enable the formation of a mixture. When urea molecules are confined in the solid lattice they cannot vibrate easily. The molecules dispersed in the solvent, on the other hand, can translate and rotate as well as vibrate. Therefore, energy is required to break intermolecular interactions of urea molecules in the solid state. The values of ΔS are negative and decrease with increasing concentration of urea. This indicates that the system will be more organized with increasing concentration of urea. The less negative value of ΔS at lower concentrations of urea indicates the formation of dimers, trimers or small aggregates of urea in addition to the different water clusters present in the system. With increasing urea concentration, the value of ΔS becomes more negative at all temperatures (Fig. S11†) since the oligomeric and polymeric aggregates of urea tend to organize the water molecules. Jung *et al.* also confirmed the different extent of aggregation at different urea concentrations.[35]

3.11 Structural variation of pure water and aqueous urea solutions

In liquid water, different types of water clusters are formed by hydrogen bonding. Water can form two types of clusters, one is an open structure and another is a close or ring structure. Both types of clusters are formed in a cooperative manner. If one molecule acts as a donor another one serves as an acceptor. The ring structure is more favored over the open structure, which can easily be formed and broken. The hydrogen bond energies of three dimensional structures are high due to their high connectivity to neighbors, which means that the cooperative effect is high. Dimers, trimers or other small clusters have low hydrogen bond energies. With increasing ring size of the cluster, the hydrogen bond energy of the system increases. A strong change can be observed in the system for small cyclic clusters, which have a strong hydrogen-bond energy. These are sensitive to temperature since the small cyclic structure has a high strain. Large cyclic structures such as pentamers or hexamers have long O–H bonds and a low strain; they are not sensitive to temperature, but sensitive to additives.
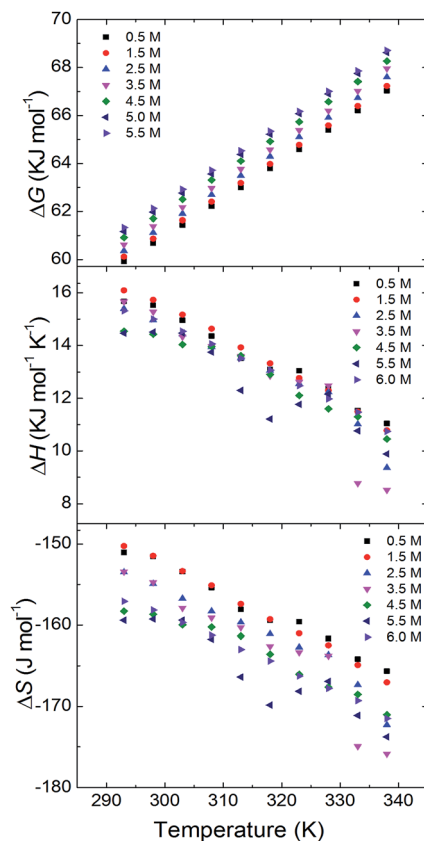
Fig. 8 Free energy changes of activation (ΔG), changes in enthalpy of activation (ΔH) and changes in entropy of activation (ΔS) for the viscous flow as a function of the temperature.

Water molecules form hydrogen bonds with urea by opening the ring structure or *via* side chains. Urea molecule, which is larger in size as compared to a water molecule, distorts the hydrogen bond strength of the whole network. So, the third component will be temperature sensitive. Different degrees of urea–urea aggregates help to strengthen the hydrogen bonding of water.

4.   Conclusions

Water in its pure liquid state and in solutions has three structural components with hydrogen bonding of varying strength. The concentrations of weaker and stronger hydrogen bonds are altered in a cooperative manner with increasing temperature, while the concentration of a third component is much more resistant to temperature changes in the range of 20–65 °C. The hydrogen-bonded species in water are in dynamic equilibrium with each other, and the weak and strong hydrogen-bonded species may undergo interconversion through an intermediate species. In aqueous solutions of urea, a small amount of urea forms hydrogen bonds with water. However, most of the urea molecules self-aggregate: at concentrations below 2.0 M they form small clusters like dimers or trimers, in the range of 2.0–4.5 M concentrations they form oligomers, and above 4.5 M they form polymeric chains in the system. The aggregated urea

species are surrounded by tetrahedrally arranged water molecules forming an ice-like structure. The system becomes more organized with increasing concentration of urea.

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