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Interactions between hydration spheres of two different solutes in solution: The least squares fitting with constraints as a tool to determine water properties in ternary systems

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ABSTRACT

Biological systems are complex and the problem of their description lies in mutual interactions between their components. This paper is focused on model experiment-based studies which can reduce these difficulties. The ternary aqueous *N*-methylacetamide (NMA)–Na_2HPO_4 system has been studied by means of the FTIR spectroscopy. A novel difference spectra method aimed to extract the spectral contribution of water affected simultaneously by two solutes is proposed. The key improvement is the additional least squares curve fitting procedure with carefully selected constraints which allow obtaining information on the composition of water affected in complex solutions. The fitting parameters are optimized with the Monte Carlo method and the human influence on parameter selection is minimized. The simple method allows determining individual contributions originating in hydration of multiple solution components. FTIR spectra of water in the NMA–Na_2HPO_4 system gave an insight into the interactions and susceptibility for interactions between solvent and co-solutes or hydrated co-solutes.

1. Introduction

Water in the presence of two solutes has been studied before, both theoretically [1–6] and experimentally [7–11]. Computational methods, mainly molecular dynamics, have some superiority over experiments because they allow, practically at the atomic level, to observe changes in the properties of the studied molecules. Radial distribution functions, hydrogen bond life times and many other parameters obtained on the basis of dynamic trajectories give insight into the interactions between the components of solutions. Experimental methods are very limited in this respect. Moreover, they usually do not indicate the contribution of individual solutes on water structure and its properties in complex solutions. With our FTIR-based method we are able to divide solvent into bulk and so-called affected populations. FTIR spectroscopy is a perfect tool for investigation of solute hydration. Relative contributions of each affected population gives information on how and if the hydration layer of a solute is disturbed by interactions in a complex solution.

N-methylacetamide used in our studies is often considered as a single peptide bond model. Many experimental FT Raman spectroscopy [12], NMR [13] and theoretical (ab initio) [12,14] studies, focused on *cis*-*trans* NMA equilibrium, were conducted and it has been shown that *trans* form of NMA is more stable in aqueous solutions [13]. Peptide bonds in proteins have the same configuration. Possibility of dimers, trimers and oligomers of NMA molecule has been confirmed [15–24]. Hydration of NMA has been investigated with many methods, such as: DFT calculation [25,26], neutron diffraction [27], ab initio [28], FTIR spectroscopy [25,29,30], molecular dynamics [24,31–33], 2D IR spectroscopy [34], Kirkwood-Buff analysis [35], measurement of density [36,37], speed of sound [36,37] and viscosity [37].

Most protein and peptide research involves a variety of buffers and the phosphate buffer is one of the most popular. Their presence should not have any effect on other components in solution, however, it has been proven that they can affect co-solutes [38,39]. Studies show that ions in buffers even in low concentrations (typical concentration is 10–100 mM) can exert various effects on buffered molecules [40–42].

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Several studies proved that buffering ions can directly interact with macromolecules through electrostatic and dispersive forces [43–47]. This can affect thermal stability and other properties of biomolecules [38,40,42]. Such interaction can be either beneficial [42,48] or unfavorable [47]. The selected aqueous NMA–Na_2HPO_4 system will serve not only to test the proposed method, but also to investigate possible interactions in much complex solutions of proteins in phosphate buffers. The system has been selected on the basis of our previous results [25,49,50].

In this paper our newly developed method is applied to isolate the contribution of these water molecules that are under the influence of two solutes: NMA and Na_2HPO_4. Difference spectra method allows acquiring solute-affected HDO spectra which serve as a probe of structural and energetical state of the solvent. The novelty of the method is expressed in the fitting procedure which involves carefully selected constraints and the Monte Carlo optimization of fitting parameters.

2. Explanation of terms used in the text

In the following section some names of various water population will be explained. In many cases, a single word cannot reflect the full meaning of the term, thus we encourage to read how we understand them.

- “Affected” water – water under the influence of the solute, different from bulk water.
- “*N* number” – the number of moles (or molecules) of water affected by 1 mol (or molecule) of a solute; it can relate also to a hydrated “pseudo”-complex, i.e. a mixture of two solutes.
- “Experimental affected water” – water affected by two solutes in a mixture; contains the share of water affected in the manner characteristic to each of them separately and the share of water changed by the simultaneous presence of these two solutes.
- “Theoretical affected water” – water affected by these two solutes in the absence of interactions between hydration spheres of both solutes, i.e. the sum of affected water in the hydration layers of both solutes when no interactions occur (see Section 5.1.1). The corresponding theoretical spectrum and R_{OO} reflect a hypothetical situation in solution when both solutes and their hydration spheres do not interact in any way. Such a spectrum or R_{OO} can serve as a reference for analysis of experimental spectra. Any discrepancies between experimental and theoretical spectra or R_{OO} values are in fact evidences that both solutes influence each other.
- “Shared affected water” – water affected simultaneously by both solutes devoid of the contributions of affected water corresponding to pure solutes, created in the overlap of hydration spheres of both solutes.
- “Excess affected water” – water affected simultaneously by hydration spheres of both solutes (cross-linking or bridging these hydration spheres), formed by a slightly affected molecules originating from the bulk solvent.
- “Pseudo-complex” – a hydrated mixture of two solutes treated as one compound, but the real complex does not have to be necessary formed in the solution; this “pseudo-complex” serves only for calculations of *N* numbers corresponding to mixed solutions.

No quotation marks will be used throughout the text.

3. Materials and methods

3.1. Chemicals and solutions

N-methylacetamide (99+%, Sigma-Aldrich), dibasic sodium phosphate dihydrate (99%, Sigma-Aldrich), deuterium oxide (Sigma-Aldrich 99.96%) and deionized water were used to prepare solutions.

Stock solutions were obtained by dissolving solute (NMA or Na_2HPO_4) or both solutes in deionized water. Each stock solution contained different mole fraction of NMA ($x_{NMA} = 0, 0.2, 0.4, 0.5, 0.6, 0.8, 0.85, 0.9, 0.95, 1$). Less concentrated solutions were prepared by dilution of weighed amounts of stock solutions using deionized water. Then, for each concentration the solutions were divided into two parts. Sample solution was prepared by adding D_2O to one of the parts in weight ratio of 4% relative to the amount of H_2O in the sample. This amount of deuterium oxide is sufficient to obtain quantitative amount of HDO (according to equilibrium constant of the reaction $H_2O + D_2O = 2HDO$, $K \approx 4$). Reference solution was obtained by addition of an equivalent amount of H_2O to the second part of solution. All solutions were prepared by weight and their densities were measured by means the Anton Paar DMA 5000 densitometer at 25.000 ± 0.001 °C.

3.2. FTIR measurements

FTIR spectra of aqueous solutions were recorded on Thermo electron Co. Nicolet 8700 spectrometer (resolution of 4 cm^{-1}, 128 scans for each spectrum). Spectrometer's interior was purged with dry nitrogen to eliminate the influence of water vapor and carbon dioxide on recorded spectra shape. FTIR measurements were carried out in a liquid cell (model A145, Bruker Optics) with CaF_2 windows separated by PTFE spacers. The temperature was maintained at 25 °C ± 0.1 °C. A thermocouple was used to monitor a temperature. The path length was approximately 0.029 mm, and was determined interferometrically.

3.3. Spectral data analysis

All spectra have been handled and analyzed using the commercial computer software: OMNIC (Thermo Electron Corporation), GRAMS/AI version 9.3 (Thermo Fisher Scientific Inc.) and RazorTools/8 (Spectrum Square Associates, Inc., Ithaca, NY) run under GRAMS/AI.

The difference spectra method is an excellent method to isolate spectrum of water affected by solute. According to this method, water can be divided in a solution into two additive populations: the bulk water (identical to pure water) and affected water (by interactions with the solute). Spectrum of affected water, extrapolated to the very diluted solution limit (see Section 4.1.3 for discussion of the infinite dilution limit), can be isolated by means of following Eq. (1):

$$\varepsilon_a = \frac{1}{NM}\left(\frac{\delta\varepsilon}{\delta m}\right)_{m=0} + \varepsilon_b \tag{1}$$

where ε_a and ε_b are, accordingly, the molar absorption coefficients of affected water and bulk water, *N* is the number of moles of water affected by 1 mol of solute, *M* is the mean molar mass of water (including mass of D_2O in HDO spectra) ($kg \cdot mol^{-1}$) and *m* is the molality of the solute ($mol \cdot kg^{-1}$). The derivative, $(\frac{\delta\varepsilon}{\delta m})_{m=0}$, is received by an approximation of the molar absorption coefficient value as a function of molality at each wavenumber at the very diluted solution limit. The method was described in details in refs. [51–53].

4. Water affected simultaneously by two components of a solution – development of the method

The idea of the method used to determine the properties of water affected simultaneously by two components, here named *A* and *B*, of a solution is based on the described method of affected spectra. Both compounds A and B in a mixed solution can be regarded as a pseudo-complex compound A_xB_y for which affected water spectrum and a corresponding *N* number are calculated. Principles of the methods are the same, but it requires more experimental effort than the affected water method described above.

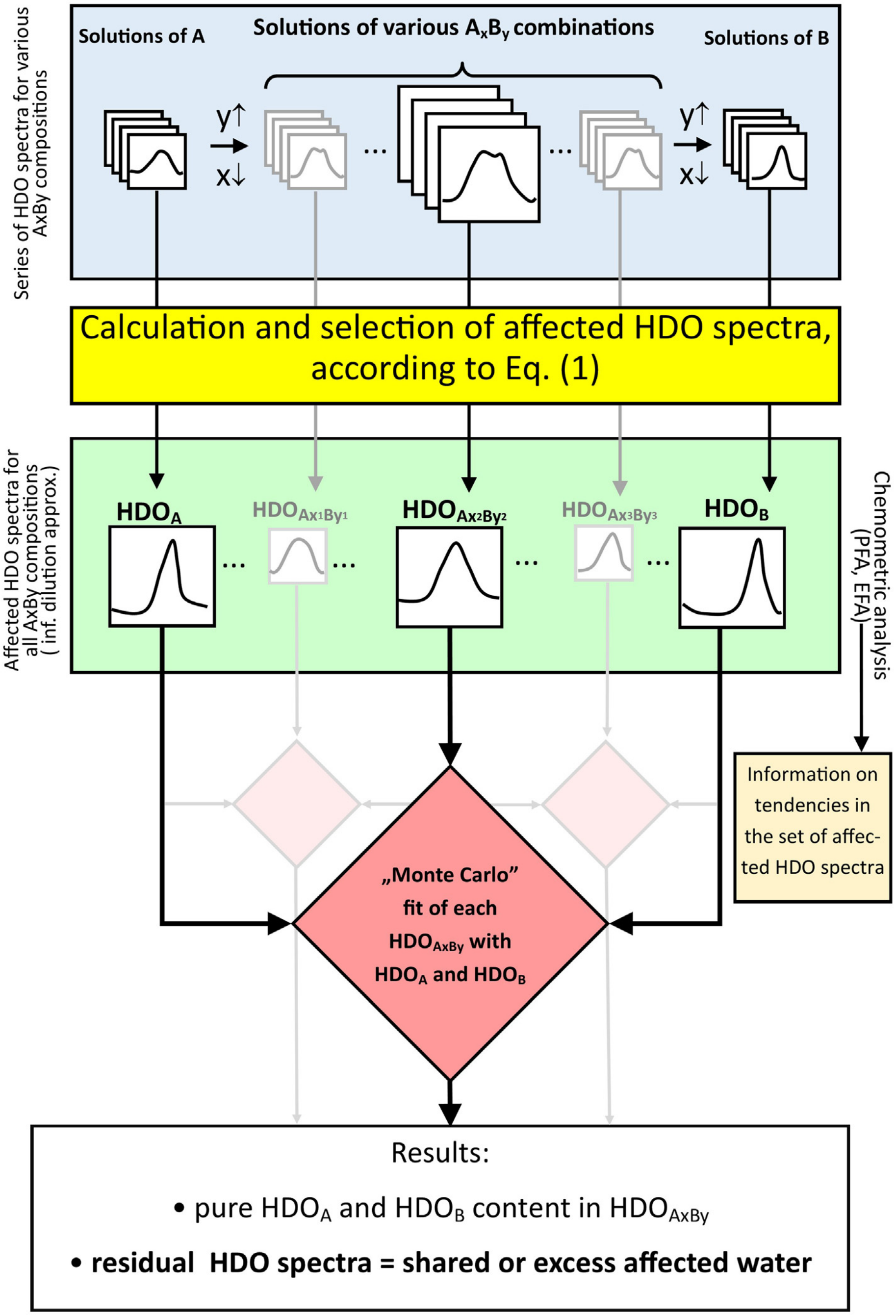

Fig. 1. A diagram of the method of isolation of affected HDO spectra in ternary solutions of A and B. Blue panel: Each stack of spectra represents HDO affected spectra corresponding to the same A to B ratio in the complex (i.e. the composition of the virtual A_xB_y complex is constant), but with different molar concentrations of this complex. The left- and rightmost stacks correspond to solutions in which only one of the components, A (left) or B (right), is present. Yellow panel: A single HDO affected spectrum corresponding to this particular A_xB_y concentration ratio can be obtained from each stack series. Green panel: Affected HDO spectra extrapolated to zero concentration of the A_xB_y complex (or pure A and B) form a series of affected spectra. Each affected spectrum in the series contains the contribution of water affected only by pure A and pure B spectra and the shared or excess affected water (the goal of the method). Before the next analysis step, this series is checked for consistency using chemometric methods of choice. Red panels: each affected spectrum of the series is next subjected to the Monte Carlo fitting procedure using affected HDO spectra in pure A and B. The result is a sought-after shared or excess water spectrum. Shaded elements correspond to the same sequence of operation but for different A:B ratio.

In simple terms, the newly developed method consists of measurements of many series of spectra (a single series is represented as a stack of spectra in blue panel of Fig. 1). In each series of the blue panel, concentrations of both solutes, C_A and C_B, vary, but the ratio of solutes' concentrations, C_A:C_B, remains constant. Thus, such a series can be regarded as a spectral series from which a single spectrum of water affected by the pseudo-complex compound A_xB_y (and a corresponding affected number N_{AB}) can be obtained (x and y are determined by the ratio of concentrations C_A:C_B). Affected HDO spectra corresponding to pure A or B solutions are also isolated at this stage.

When a series of many affected water spectra is obtained (green panel) possible mistakes and errors must be identified and possible

Table 1
Number of affected water molecules (N) for NMA–Na_2HPO_4 system—theoretical and experimental—and their use as constraints for curve fitting procedure.

System composition			Experimental N numbers		Theoretical N numbers		Fitting constraints		ΔN[g]
x_{NMA} (x_{Ph})[a]	n_{Ph}/n_{NMA}[b]	n_{NMA}/n_{Ph}[c]	$N_{exp.\ NMA}$[d]	$N_{exp.\ Ph}$[d]	$N_{theor.\ per\ NMA}$[e]	$N_{theor.\ per\ Ph}$[e]	Max. N_{NMA}[f]	Max. N_{Ph}[f]	
1.00 (0.00)	0.00	–	3.0	–	3.0	–	–	–	–
0.95 (0.05)	0.05	–	2.6	–	3.8	–	3.0	0.8	−1.2
0.90 (0.10)	0.11	–	3.9	–	4.6	–	3.0	1.6	−0.7
0.84 (0.16)	0.19	–	4.5	–	5.7	–	3.0	2.7	−1.2
0.80 (0.20)	0.24	–	6.4	–	6.5	–	3.0	3.5	−0.1
0.60 (0.40)	0.65	–	12.9	–	12.4	–	3.0	9.4	+0.5
0.50 (0.50)	1.00	1.00	19.7	19.7	17.4	17.4	3.0	14.4	+2.3
0.40 (0.60)	–	0.67	–	16.7	–	16.4	2.0	14.4	+0.3
0.20 (0.80)	–	0.25	–	13.3	–	15.2	0.8	14.4	−1.8
0.00 (1.00)	–	0.00	–	14.4	–	14.4	–	–	–

[a] Molar fractions of NMA (Na_2HPO_4) in ternary systems excluding water.
[b] The number of Na_2HPO_4 molecules per one NMA molecule.
[c] The number of NMA molecules per one Na_2HPO_4 molecule.
[d] Experimental values of N number in ternary solutions. The parameter has been counted when either NMA or Na_2HPO_4 are in excess.
[e] The theoretical N number if the molecules did not interact with each other in any way. It can be calculated per 1 mol of NMA or Na_2HPO_4.
[f] Maximum theoretical contributions of N_{NMA} or $N_{Na_2HPO_4}$ in the theoretical N number, can also be calculated from the point of view of NMA or Na_2HPO_4. These values serve as maximum constraints in the fitting procedure.
[g] The difference between experimental and theoretical N numbers for ternary systems ($N_{exp.}$-$N_{theor.}$).

outliers must be rejected.[1] After the series of affected water spectra is checked for outliers, the proper determination of shared or excess affected water spectra can begin. The process is represented symbolically in Fig. 1 as the red panel and described in the following subsection. Each spectrum of the affected water series (except those corresponding to pure A—leftmost in the green panel—and pure B—rightmost in the panel—which, by definition, comprises only of single populations of affected water molecules) corresponds to a mixture of water affected by components A, B and simultaneously by A and B. The goal is to isolate the latter one.

4.1. Fitting procedure

We propose a method of curve fitting procedure with two know spectra and a non-zero residual factor which is the sought after shared or excess HDO spectrum affected simultaneously by two solution components. This non-standard problem needs a properly chosen set of constraints, described in details in one of the following subsections. To set those constraints and to perform the fitting we must first set a reference point which will allow setting these parameters in a relation to the fitted experimental spectrum. Each affected spectrum corresponds to 1 mol of affected water molecules (or simply to one affected water molecule) and it can be multiplied by a corresponding affected number N. This results in a spectrum corresponding to N affected moles of water or alternatively water molecules. Such a procedure does not change any properties of those spectra yet it helps to set and understand the meaning of some fitting parameters (see Section 4.1.2). At any moment, any given affected spectrum corresponding to N affected water molecules can be again re-calculated to the molar spectrum simply by dividing it by the proper N value.

4.1.1. Monte Carlo curve fitting with constraints

Each affected HDO_{AB} spectrum of the series from the green panel is fitted only with two spectra of water populations affected by pure A and pure B (HDO_A and HDO_B in green panel of Fig. 1, respectively) with constraints described in the next paragraph. The number of fitting parameters is limited only to the intensities of contributing spectra.

The result of fitting procedure can vary depending on the set of initial fitting parameters. To avoid any manual selection of these parameters, which can be biased by personal presumptions, we propose a random selection.

A large number (few thousands is usually sufficient) of random parameters sets is selected, and for each of these sets the least squares fitting is performed. In this way a large set of fitting parameters and corresponding measures of goodness-of-fit are obtained. The optimal set of resultant parameters must meet the following conditions: (1) have a physical meaning, give the best fit of HDO_{AB} with HDO_A and HDO_B, and (2) the same fitting result must be obtained for a few of the best goodness-of-fit parameters (i.e. the fitting results must converge to a common optimal set of parameters). The way the optimized parameters are obtained, i.e. a large number of randomly selected sets of parameters converging to a single solution, is actually in accordance with the idea of the Monte Carlo method.

4.1.2. Fit constraints and N numbers

However, some very important remarks must be made at this stage of spectra analysis. Such a fitting of a given spectrum with two other spectra would give meaningless result on its own because it is assumed that additional residual third factor (i.e. spectrum) is present in the A_xB_y affected spectrum (except the scenario where no interactions occur) for which no shape or parameters are known. The lest squares method tries to minimize the difference between HDO_{AB} and a sum of HDO_A and HDO_B and will inevitably minimize the contribution of residual factor and overestimate contributions of pure spectra. Thus, some specific constraints must be imposed. First, maximum possible contributions of HDO_A and HDO_B must be set. Second, the sum of HDO_A and HDO_B at any given wavenumber cannot exceed the analyzed $HDO_{A_xB_y}$ spectrum.

The first constraint origins in the maximum possible contribution of HDO_A or HDO_B in the experimental affected $HDO_{A_xB_y}$ spectrum. It is easy to apply and understand if we take into account the N number. In general, these numbers correspond to the average number of water molecules affected by one molecule of solute. The corresponding affected water spectra are calculated per one mole of such water molecules (and in this context can be considered as spectra of one affected molecule of water). Thus, if these spectra are multiplied by corresponding N numbers, the resultant spectrum corresponds to N moles of affected water molecules (or, similarly, N affected water molecules). In a solution of A and B, the number of water molecules affected solely by A or B cannot exceed these numbers determined for pure A or B solutions yet there can be some population of shared or excess water.

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[1] Chemometric methods are applied mainly to verify if affected water spectra for various A_xB_y and pure solutes systems create a coherent series (yellow panel in Fig. 1). Principal Factor Analysis (PFA) or principal Component Analysis (PCA) allow to determine how many spectral individuals contribute to the series (i.e. different water populations), and any variants of the Evolving Factor Analysis (EFA) or similar methods can be employed to determine how these individuals change in the function of C_A:C_B (x:y) ratio. The information on how many spectral forms are present in the series can be helpful for further analysis of results' correctness. In the case of our results, it was apparent that only two forms of shared or excess affected water were present in solutions and a simple PCF analysis confirmed that conclusion.

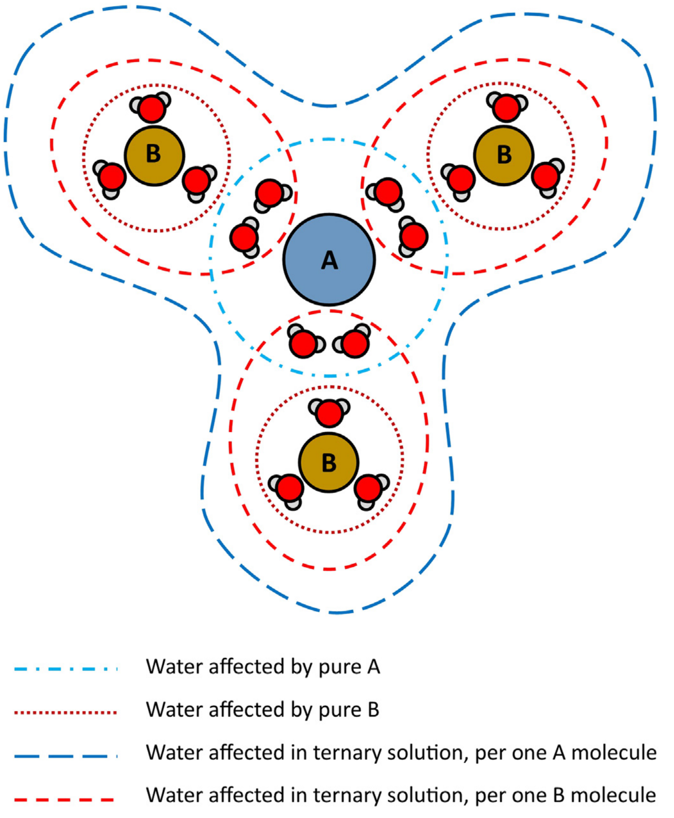

Fig. 2. An example of the apparent differences in the number of affected water molecules in a ternary solution of A and B in 1:3 proportion. Assume that A and B can affect 6 and 3 water molecules in their pure solutions, respectively (circles surrounding A and B). If A is taken as the reference point for calculations, 15 water molecules are affected in total per one A molecule (dark blue dashed line encompassing the whole system). On the other hand, if B is the reference, only 5 water molecules are affected per one B molecule (red ellipse), but the total concentration of such a water population is 3 times larger. Thus, the choice of the reference is free and does not change the total number of affected molecules in the solution, and their spectroscopic picture, or spectral band shape, is still the same.

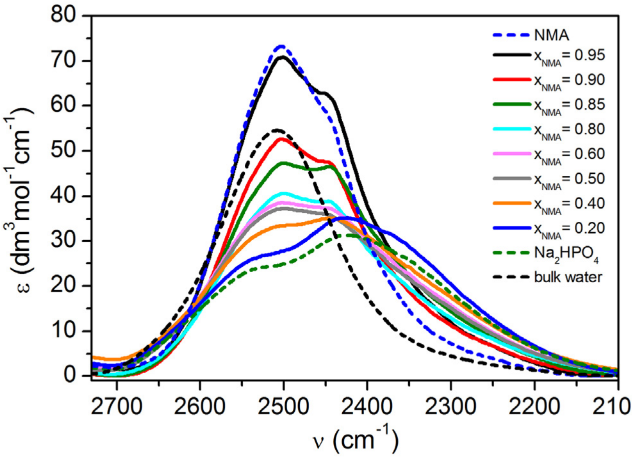

Fig. 3. Solute-affected $\nu(OD)_{aff}$ spectra at the very diluted solution limit (see Section 4.1.3) for NMA, Na_2HPO_4, NMA–Na_2HPO_4 mixtures with corresponding mole fraction of NMA (experimental affected spectra), and the bulk water spectrum.

For example, lets analyze the 1:1 mixture of NMA and Na_2HPO_4 from Table 1 (i.e. $x_{NMA} = 0.50$, where $x = n_{NMA}/(n_{NMA} + n_{Na2HPO4})$). Theoretically, in their pure solutions NMA and Na_2HPO_4 affect natively 3.0 and 14.4 water molecules, respectively. Together, if no interactions occur in their mixed solution, they can affect 17.7 water molecules. In real solution the experimental N number is higher (19.7) and indicate that additional 2.3 excess water molecules are engaged in interactions in such a solution. Yet the maximum contribution of pure components (which must be present in diluted solutions, as in our case) cannot exceed their native values. Thus, in our example the experimental spectrum multiplied by 19.7 will be fitted with HDO_{NMA} and $HDO_{Na2HPO4}$ multiplied by a maximum of 3.0 and 14.4, respectively.

The 1:1 example is simple and intuitive, but a new problem arises if other compositions are considered. The total concentration of A_xB_y complex, necessary for calculations of N_{AB}, can be determined from both sides: A and B, and each of them is correct. Consider NMA–Na_2HPO_4 complex with $x_{NMA} = 0.90$ composition (i.e. 1/9 of Na_2HPO_4 per 1 NMA molecule). From the point of view of NMA, such a complex can theoretically affect $3 + (1/9) \cdot 14.4 = 4.6$ water molecules (if no interactions are assumed), but the experimental $N = 3.9$ (see Table 1). Thus, in this example the experimental spectrum multiplied by 3.9 will be fitted with HDO_{NMA} and $HDO_{Na2HPO4}$ multiplied by a maximum of 3.0 and 1.6, respectively (these are maximum constraints, the real parameters can be lower). Yet if Na_2HPO_4 is taken as the reference for A_xB_y concentration in the mixed solution, than the composition should be described as 9:1, and such a complex should theoretically affect $9 \cdot 3.0 + 1 \cdot 14.4 = 41.4$ water molecules. This may be confusing, however, the selection of reference has no influence on the shape of affected water spectrum. It is only a mathematical transformation and the only difference is the resultant N_{AB} number, not the shape of all kinds of affected spectra. This number, however, is in fact always referenced to the compound of choice. A simple example of how the number of affected water number changes with the reference point is given in Fig. 2.

In our work, we tend to use the reference compound with higher molar ratio for calculations of the excess water molecules in the complex mixture. The reason is that N value is always burdened with error which is proportional to its value and the number gets significantly higher, i.e. unfavorable, when the molar fraction of the reference molecule decreases, as in the case of $N_{Na_2HPO_4}$ in the example of 9:1 NMA–Na_2HPO_4 solution. The higher N number, the higher and more unpredictable error of its derivatives. Thus, the choice of the reference is connected to the minimization of the overall error.

The second constraint results from the nature of fitting procedure. As stated above, fitting of any spectrum with other two spectra will provide a set of parameters which will minimize the sum of squares of intensity differences for all given wavenumbers or wavelengths. However, we assume the presence of the third unknown factor which is to be determined as the residual between the fitted spectrum of complex HDO band and HDO bands corresponding to pure NMA and Na_2HPO_4. Because standard least squares methods try to minimize the sum of squares, the information on the sign of the difference between intensities of fitted spectra at a given wavenumber is irrelevant, and such a fitting procedure will give a residual which can have also negative regions. Yet the sought after residual HDO band cannot have any negative sub-bands. We propose a workaround to the problem – an additional "penalty" fitting factor (>1). At each iteration of the fitting procedure intensity at a given wavenumber is multiplied by the factor if the difference between fitted spectrum and the sum of component spectra at this wavenumber is negative.[2] This way, if the sum exceeds the fitted spectrum, instantly a large contribution to the sum of residual squares appears yet the algorithm is forced to avoid such a situation. High "penalty" factors (>1000) give strict results where the sum is almost impossible to exceed the fitted spectrum, while small (<50) allow to obtain more "realistic" ones where the sum can fluctuate over the fitted spectrum within the value of instrumental error for a given spectrometer. In our work the factor is set to 10.

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[2] In reality, an intrinsic instrumental error must be taken into consideration, and in our script the "penalty" is given if the sum exceeds fitted curve by more than the standard deviation of instrumental noise in the given spectral region.

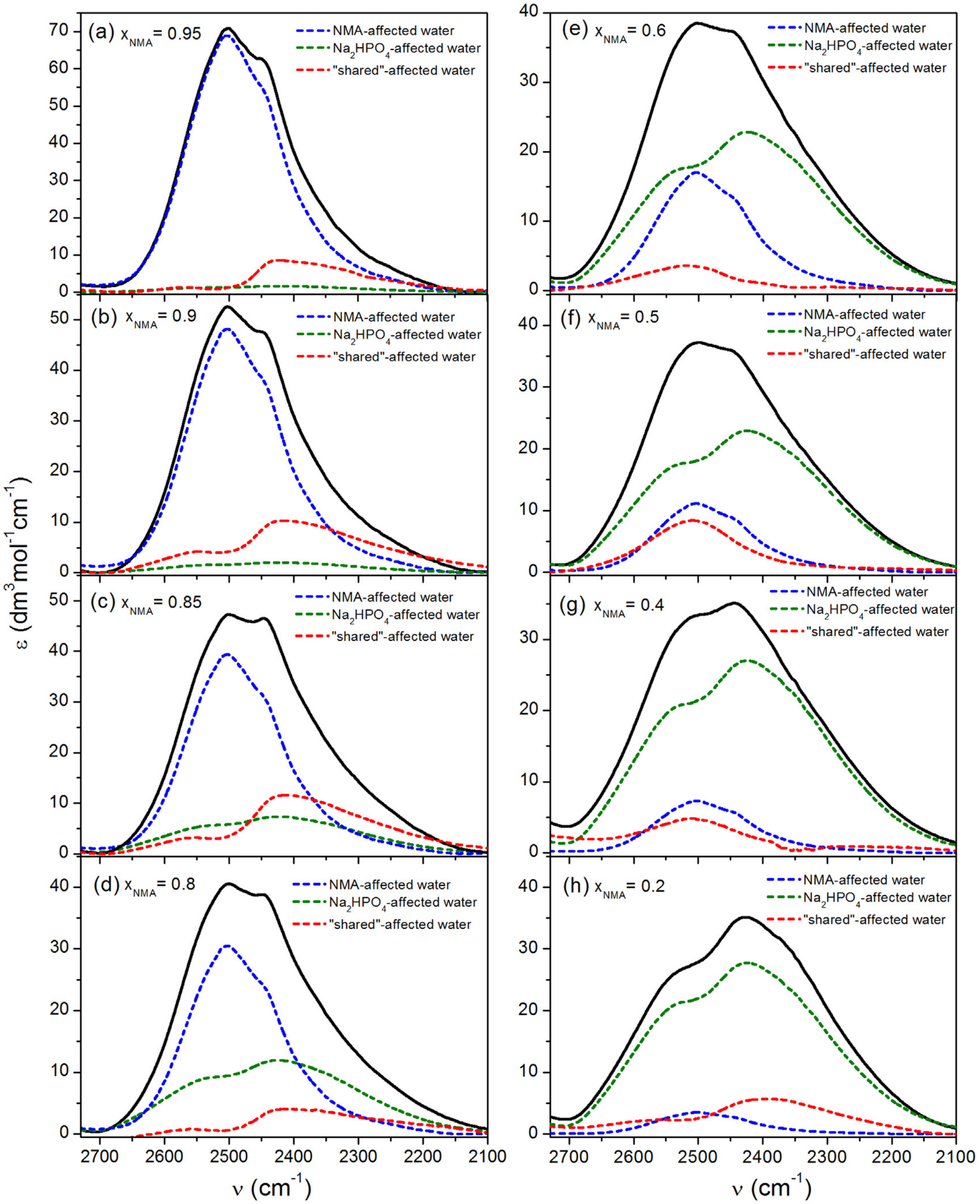

Fig. 4. The spectra of water affected in the NMA–Na_2HPO_4 system for corresponding mole fraction of NMA (from Fig. 3) with separated contribution of shared or excess affected water, NMA-affected water and Na_2HPO_4-affected water.

4.1.3. Limitations of the infinite dilution approximation

In the presented method all affected HDO spectra are calculated with the infinite dilution approximation and a reasonable separation between solutes can be assumed. The actual infinite dilution spectra, however, cannot be obtained in this situation.[3] In the infinitely diluted solution there is no possibility for two solute molecules to meet and interact. Our HDO affected spectra can be treated rather as those corresponding to a very diluted solution. On one hand, some information about the concentration dependent changes can be lost, as in our case. On the other hand, some spontaneous contacts can occur and we can observe the most probable way of interaction. Thus we treat current version of the presented method as a qualitative one, i.e. different populations of water can be ascribed to solution components yet the information on how these populations change in concentrated solution is lost. Currently, a method employing a different approach is under development. It can be puzzling why properties of water are virtually the same in solutions with low x_{NMA} or $x_{Na2HPO4}$. We suspect that it is due to the above mentioned loss of information connected to the extrapolation of spectra. Interactions in such diluted solutions can be regarded as rare whether NMA or Na_2HPO_4 is in excess. Apparently, the significant excess of one of solutes cannot be achieved with the infinite dilution approximation.

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[3] The extrapolation of molar absorption vs. molality for each wavenumber, which is performed during the calculation of HDO spectrum step, employs a set of relatively concentrated solutions due to the relatively low sensitivity of FTIR spectrometers. Thus, the extrapolated spectrum for concentration equal to zero can be rather considered as a very diluted solution approximation.

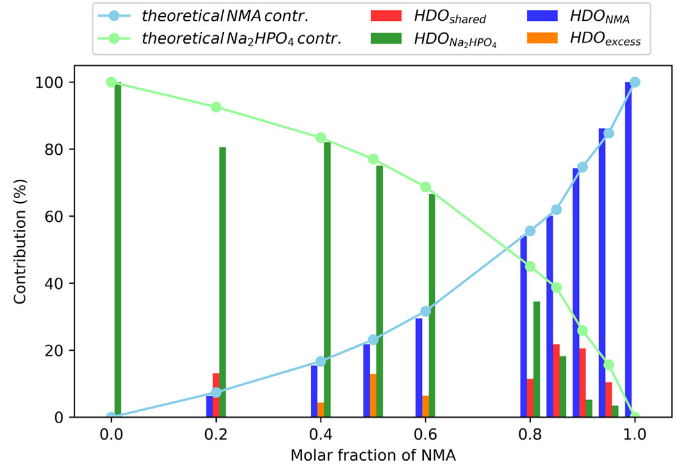

Fig. 5. Percentage contributions of various water sub-populations in the total population of affected water in the NMA–Na_2HPO_4 system. Blue and green bars correspond to contributions of water identical to the one affected in pure NMA or Na_2HPO_4 solutions, respectively. The red one denotes contribution of shared or excess affected water. Lines with dots correspond to the theoretical contribution if no interactions between hydration layers of both solutes occur in the system.

In the next part of the paper the phrase "infinite dilution limit" will be replaced with "very diluted solution limit".

5. Results and discussion

5.1. FTIR investigation of water structure

5.1.1. Characteristic of spectra of water affected by solutes

Fig. 3 shows the solute-affected HDO spectra at very diluted solution limit for NMA, $Na_{Na_2HPO_4}$, NMA–Na_2HPO_4 mixtures with corresponding mole fraction of NMA (experimental affected spectra), and bulk water spectrum. The procedure of spectral data analysis towards extraction of the solute-affected water spectra has been described in section S1 of Supplementary Material.

Spectra of water affected in the NMA–Na_2HPO_4 system were subjected to the fitting procedure (described in Section 4.1). The experimental affected HDO spectra with separated contribution of the shared and excess affected water and the spectral shares of pure components are presented in Fig. 4. Percentage shares of water affected by NMA and Na_2HPO_4 in the experimental affected spectra as a function of the NMA molar fraction are based on the integral intensities of the spectra (see Fig. 5). In addition, the shares of pure solutes in the so-called theoretical affected spectra (i.e. when no interactions between hydration spheres of both solutes are assumed) are also plotted. Theoretical spectrum of affected water (ε_{theor})[4] was constructed from the spectra of affected water by pure components (ε_{NMA} and $\varepsilon_{Na_2HPO_4}$), including the theoretical N number ($N_{Na_2HPO_4}$ and $N_{Na_2HPO_4}$, see Table 1):

$$\varepsilon_{theor} = \frac{N_{NMA}\cdot\varepsilon_{NMA} + N_{Na_2HPO_4}\cdot\varepsilon_{Na_2HPO_4}}{N_{NMA}+N_{Na_2HPO_4}} \qquad (2)$$

The shares of water affected by pure NMA in the experimental and theoretical affected spectrum are comparable and only small differences between them can be seen in the entire composition range. This means that the presence of Na_2HPO_4 has only a slight effect on the water molecules surrounding NMA. However, the share of pure Na_2HPO_4 in the experimental affected spectrum is smaller than in the theoretical one, which indicates a significant influence of NMA on the hydration sphere of the solute. Based on the above observations, it can be concluded that the shared or excess affected water origins mainly in the water from the phosphate hydration sphere. It must be stressed that these findings are in a good accordance with our previous DFT studies of the same system [49].

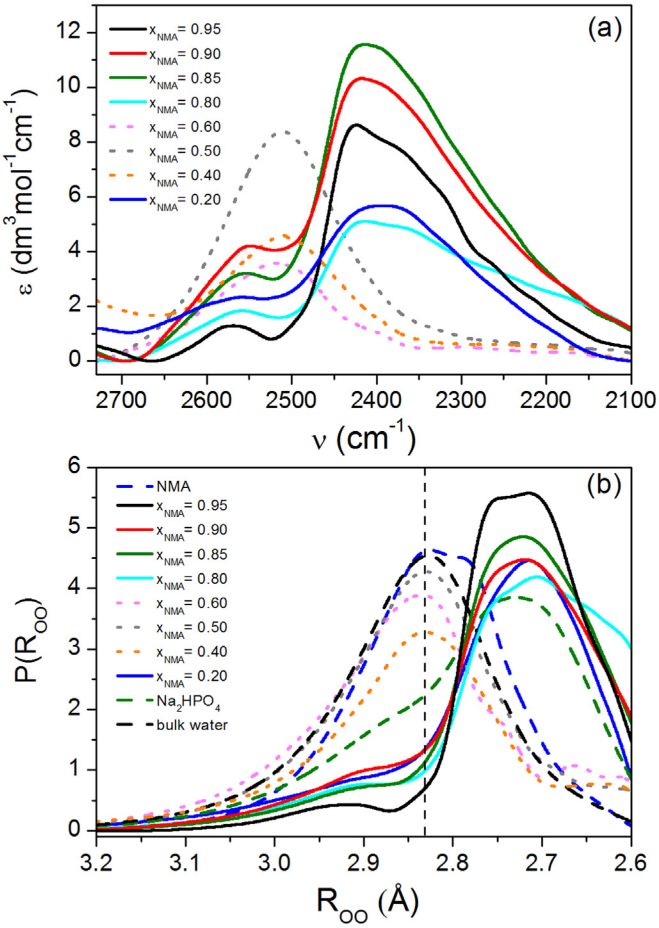

Fig. 6. (a) Contours of spectra of shared and excess affected water with corresponding mole fraction of NMA (from Fig. 4). (b) Interatomic oxygen–oxygen distance distributions function derived from the affected water spectra in NMA–Na_2HPO_4 mixtures with corresponding mole fraction of NMA (from panel a, dot line – excess affected water, solid line – shared affected water); dashed lines represent functions corresponding to NMA-affected, Na_2HPO_4-affected, and bulk water spectra (Fig. 3).

The shapes of shared and excess affected water spectra (from Fig. 4) in various NMA–Na_2HPO_4 mixtures are presented in Fig. 6a. These spectra, NMA- and Na_2HPO_4-affected HDO spectra and bulk water spectrum, were transformed into the oxygen–oxygen distance distribution function, P(R_{OO}) according to the Eq. (3) [54,55]:

$$P(R_{OO}) = [16.01 - \ln(2727 - \nu_{OD})]/3.73 \qquad (3)$$

The obtained distance probability distributions are shown in Fig. 6b. All spectral parameters of shared and excess affected water, water affected by pure solutes, bulk water and theoretical affected water spectra, together with the intermolecular oxygen–oxygen distances, R_{OO}, are summarized in Table 2.

A comparison of the values of the mean oxygen–oxygen distances, R_{OO}^{g}, and the gravity center of OD bands, ν_g, (related to the mean energy of water hydrogen bonds) in shared affected water for $x_{NMA} = 0.20$, 0.80, 0.85, 0.90 and 0.95 in water affected by pure solutes and pure water indicates that shared affected water molecules form on average

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[4] This theoretical spectrum is actually a simple weighted average with weights being the numbers of water molecules affected by components of the pseudo-complex *NMA* − *Na_2HPO_4*. Depending on the solution composition and which component is taken as the reference for further calculations, values of *N* for each component in this equation can vary (see Table 1).

shorter and stronger hydrogen bonds than water affected by pure solutes, and also than pure water itself.

Shared affected water for $x_{NMA} = 0.20$, 0.80, 0.85, 0.90 and 0.95 is characterized by stronger hydrogen bonds than in the absence of contact of hydration spheres, i.e. the theoretical water. This proves that the mutual contact of hydration spheres of both solutes promotes shorter and stronger hydrogen bonds of water. Taking into account that the shared affected water is mainly water in the phosphate hydration sphere, it can be stated that the presence of NMA in the solution strengthens the hydrogen bonding network of water around the phosphate. This is also confirmed with stronger hydrogen bonds between water molecules in experimental affected spectra (Table S1, Supplementary Material) than in theoretical affected spectra. Negative N values (Table 1), calculated as the difference between the experimental and theoretical number of affected molecules mean that for these molar fractions some affected water molecules are under the influence of both solutes simultaneously, i.e. they are shared water molecules in the hydration spheres of both solutes.

A different situation takes place in the case of excess affected water spectra corresponding to x_{NMA} mole fractions: 0.40, 0.50 and 0.60, i.e. with equal or practically equal molar ratio of both components in the solution. Positive ΔN values obtained from the difference between the number of affected water molecules in the experimental and theoretical spectra for these molar fractions (Table 1) indicate that additional water molecules are affected. The excess water creates hydrogen bonds weaker than water affected by pure solutes. The structural and energetic state of these water molecules is very similar to that in the bulk water. For these solutions, hydrogen bonds of water molecules in excess affected water are much weaker than hydrogen bonds in the theoretical affected spectrum. In addition, in theoretical affected spectra hydrogen bonds are stronger than in experimental affected spectra (Table S1, Supplementary Material). In the NMA–Na_2HPO_4 system for 1:1 M ratio there are two water molecules that are very similar to pure water in terms of energy and structure. We can suspect that the contact of the hydration spheres of both solutes occurs at the surface of the hydration shells. Here, two possible scenarios can be considered: 1) additional water molecules originate from the unaffected water molecules in the hydration shells of solutes (i.e. these water molecules which were not considered affected before the contact); 2) the contact of hydration spheres of both solutes occurs through additional water molecules connecting the hydration spheres of both solutes, the so-called bridging water molecules, which come from the bulk surrounding solvent.

The analysis of interatomic oxygen–oxygen distance probability distributions, $P(R_{OO})$ (Fig. 6b), indicates that the hydration sphere of pure NMA consists of hydrogen bonded water molecules similar to those in pure water (most probable R_{OO} for bulk water is 2.823 Å). An analogous probability distribution function for water molecules in the presence of pure phosphate (Fig. 6b) shows that two types of water molecule populations can be distinguished in the hydration sphere: the first one refers to shorter distance values (ca. 2.73 Å) and its share is greater, whereas the second one refers to higher distance values (ca. 2.90 Å). These populations are also present in the contour of Na_2HPO_4-affected HDO spectrum (Fig. 3: green dashed line). In the case of shared affected water spectra for x_{NMA} mole fractions: 0.20, 0.80, 0.85, 0.90 and 0.95 we can also distinguish these two populations of water molecules. The population corresponding to strong hydrogen bonds ($R_{OO} \approx 2.73$ Å) is more pronounced and its contribution is greater than in the hydration sphere of pure phosphate. The contribution of the population of very weak hydrogen bonds (distances ca. 2.90 Å) in the shared affected water is insignificant and smaller than in the presence of pure phosphate.

The excess affected water for $x_{NMA} = 0.40$, 0.50 and 0.60 is characterized by the population of hydrogen bonds equal and only slightly longer than to the most probable distance in pure water ($R_{OO} \approx 2.82$ Å).

6. Conclusions

In this work, we presented a new method of analyzing affected water spectra in the NMA–phosphate system. By using the modified fitting procedure from the experimental spectra of affected water, we

Table 2
Parameters of HDO band of shared and excess affected water in NMA–Na_2HPO_4 system for various solution compositions, theoretical affected water, NMA-affected water, Na_2HPO_4-affected water, the bulk water, and the respective intermolecular oxygen–oxygen distances. R_{OO} errors have been estimated on the basis of the HDO bands position errors.

Solute	ν^o_{OD} [a]	ν^g_{OD} [b]	fwhh [c]	R^o_{OO} [d]	R^g_{OO} [e]
Shared or excess affected water					
$x_{NMA} = 0.95$	2424 ± 2	2374 ± 2	168 ± 4	2.716 ± 0.003	2.721 ± 0.003
$x_{NMA} = 0.90$	2418 ± 2	2396 ± 2	201 ± 4	2.726 ± 0.003	2.729 ± 0.003
$x_{NMA} = 0.85$	2414 ± 2	2385 ± 2	197 ± 4	2.726 ± 0.003	2.724 ± 0.003
$x_{NMA} = 0.80$	2415 ± 2	2378 ± 2	221 ± 4	2.703 ± 0.003	2.711 ± 0.003
$x_{NMA} = 0.60$	2519 ± 2	2491 ± 2	164 ± 4	2.836 ± 0.003	2.851 ± 0.003
$x_{NMA} = 0.50$	2510 ± 2	2494 ± 2	158 ± 4	2.828 ± 0.003	2.838 ± 0.003
$x_{NMA} = 0.40$	2509 ± 2	2491 ± 2	136 ± 4	2.836 ± 0.003	2.849 ± 0.003
$x_{NMA} = 0.20$	2393 ± 2	2391 ± 2	208 ± 4	2.716 ± 0.003	2.744 ± 0.003
Theoretical affected water					
$x_{NMA} = 0.95$	2501 ± 2	2471 ± 2	174 ± 4	2.810 ± 0.003	2.818 ± 0.003
$x_{NMA} = 0.90$	2501 ± 2	2466 ± 2	184 ± 4	2.785 ± 0.003	2.813 ± 0.003
$x_{NMA} = 0.85$	2501 ± 2	2460 ± 2	201 ± 4	2.777 ± 0.003	2.808 ± 0.003
$x_{NMA} = 0.80$	2501 ± 2	2457 ± 2	213 ± 4	2.777 ± 0.003	2.805 ± 0.003
$x_{NMA} = 0.60$	2447 ± 2	2445 ± 2	268 ± 4	2.767 ± 0.003	2.790 ± 0.003
$x_{NMA} = 0.50$	2445 ± 2	2440 ± 2	283 ± 4	2.767 ± 0.003	2.782 ± 0.003
$x_{NMA} = 0.40$	2440 ± 2	2437 ± 2	290 ± 4	2.757 ± 0.003	2.780 ± 0.003
$x_{NMA} = 0.20$	2432 ± 2	2432 ± 2	300 ± 4	2.741 ± 0.003	2.772 ± 0.003
Pure solutions and bulk water					
NMA	2501 ± 2	2478 ± 2	164 ± 4	2.821 ± 0.003	2.826 ± 0.003
Na_2HPO_4	2424 ± 2	2431 ± 2	311 ± 4	2.726 ± 0.003	2.764 ± 0.003
Bulk water	2509 ± 2	2496 ± 2	162 ± 4	2.823 ± 0.003	2.844 ± 0.003

[a] Band position at maximum (cm^{-1}).
[b] Band position at gravity center (cm^{-1}).
[c] Full width at half-height (cm^{-1}).
[d] The most probable O···O distance (Å).
[e] Mean O···O distance (Å).

isolated the share of those water molecules that were affected simultaneously by both solutes, the so-called shared or excess affected water. Analysis of individual spectral contributions in the experimental affected spectra (in the entire composition range) indicates that the presence of NMA in the solution exerts an influence on the hydration sphere of the phosphate, while the effect of phosphate on the NMA hydration sphere is marginal. The shared affected water in the case of a large excess of one of the components of the solution (for $x_{NMA} = 0.20$, 0.80, 0.85, 0.90 and 0.95) exhibits different properties than with equal or practically equal molar ratios of both components in the solution.

Spectra of water affected in ternary solutions of NMA and Na_2HPO_4 for $x_{NMA} = 0.20$, 0.80, 0.85, 0.90 and 0.95 are qualitatively very similar although the molecular surroundings of solutes are different. It suggests that interactions are of the 1:1 kind. Interactions in such solutions are unlikely due to the high dilution of solutes and the compositions of solutions. Yet the effect of interactions is observed, thus such complexes must be relatively probable and favorable. The shared affected water at the extremes of the solution composition range creates on average shorter and stronger hydrogen bonds than water affected by pure solutes as well as pure water. In such a case, the mutual contact of the hydration spheres of both solutes makes common some of affected water molecules, i.e. some affected water molecules in the hydration sphere of one solute simultaneously belong to the hydration sphere of the other solute.

The excess affected water molecules for $x_{NMA} = 0.40$, 0.50 and 0.60 in terms of structure and energy are very similar to pure water. Furthermore, hydrogen bonds in such a population of water are much weaker than hydrogen bonds in the absence of interactions between the hydration spheres of both solutes. In this composition range interactions between hydration spheres and solutes are much more probable and the tendency to create more extended complexes (involving more solute or water molecules) increases. However, the formation of such complexes by sharing two hydration spheres is not favorable but can be realized through weak hydrogen bonds engaging additional water molecules of the bulk solvent and creating bridges or nets between neighboring hydrated molecules. It can be expected that those last interactions are more "symmetrical" due to the relative arrangement of Na^+ and HPO_4^{2-} ion in the solution. Then the cation in the nearest surrounding of the anion neutralizes its charge and the electrolyte can be treated, to some extent, as a neutral solute relative to NMA. At the extremes of the composition range the HPO_4^{2-} ion apparently cannot be symmetrically surrounded by the counter-ion and the hydrated cation–anion system resembles a highly polar compound which promotes interactions between hydration spheres of both solutes. It must be stressed, that the explanation is based on our results concerning neutral and ionic compounds and cannot be directly generalized to other systems.

CRediT authorship contribution statement

Aneta Panuszko: Formal analysis, Investigation, Visualization, Writing - original draft, Writing - review & editing. **Janusz Stangret:** Conceptualization, Supervision. **Bartosz Nowosielski:** Investigation, Writing - original draft. **Piotr Bruździak:** Conceptualization, Methodology, Software, Formal analysis, Project administration, Funding acquisition, Writing - original draft, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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References

[1] A. Chand, S. Chowdhuri, A comparative study of hydrogen bonding structure and dynamics in aqueous urea solution of amides with varying hydrophobicity: effect of addition of trimethylamine N-oxide (TMAO), J. Mol. Liq. 242 (2017) 70–81.

[2] A. Chand, S. Chowdhuri, Effects of dimethyl sulfoxide on the hydrogen bonding structure and dynamics of aqueous N-methylacetamide solution, J. Chem. Sci. 128 (2016) 991–1001.

[3] A. Chand, P. Chettiyankandy, S.K. Pattanayak, S. Chowdhuri, Effects of trimethylamine-N-oxide (TMAO) on aqueous N-methylacetamide solution: a comparison of different force fields of TMAO, J. Mol. Liq. 225 (2017) 926–935.

[4] I. Yu, M. Nagaoka, Slowdown of water diffusion around protein in aqueous solution with ectoine, Chem. Phys. Lett. 388 (2004) 316–321.

[5] S. Paul, S. Paul, Exploring the counteracting mechanism of trehalose on urea conferred protein denaturation: a molecular dynamics simulation study, J. Phys. Chem. B 119 (2015) 9820–9834.

[6] E.A. Oprzeska-Zingrebe, M. Kohagen, J. Kästner, J. Smiatek, Unfolding of DNA by cosolutes: insights from Kirkwood–Buff integrals and transfer free energies, Eur. Phys. J. Special Topics 227 (2019) 1665–1679.

[7] J. Hunger, N. Ottosson, K. Mazur, M. Bonn, H.J. Bakker, Water-mediated interactions between trimethylamine-N-oxide and urea, Phys. Chem. Chem. Phys. 17 (2015) 298–306.

[8] K.I. Oh, C.R. Baiz, Crowding stabilizes DMSO-water hydrogen-bonding interactions, J. Phys. Chem. B 122 (2018) 5984–5990.

[9] V.P. Korolev, O.A. Antonova, N.L. Smirnova, Thermodynamic characteristics, structure, and interactions of L-proline in aqueous solutions of alcohols and urea, J. Struct. Chem. 55 (2014) 353–359.

[10] C. Malardier-Jugroot, D.T. Bowron, A.K. Soper, M.E. Johnson, T. Head-Gordon, Structure and water dynamics of aqueous peptide solutions in the presence of cosolvents, Phys. Chem. Chem. Phys. 12 (2010) 382–392.

[11] D. Russo, The impact of kosmotropes and chaotropes on bulk and hydration shell water dynamics in a model peptide solution, Chem. Phys. 345 (2008) 200–211 (Neutrons in Biology).

[12] G. Nandini, D.N. Sathyanarayana, Ab initio studies on geometry and vibrational spectra of N-methyl formamide and N-methylacetamide, J. Mol. Struct. THEOCHEM 579 (2002) 1–9.

[13] A. Radzicka, L. Pedersen, R. Wolfenden, Influences of solvent water on protein folding: free energies of solvation of cis and trans peptides are nearly identical, Biochemistry 27 (1988) 4538–4541.

[14] H. Guo, M. Karplus, Ab initio studies of hydrogen bonding of N-methylacetamide: structure, cooperativity, and internal rotational barriers, J. Phys. Chem. 96 (1992) 7273–7287.

[15] T.W. Whitfield, G.J. Martyna, S. Allison, S.P. Bates, H. Vass, J. Crain, Structure and hydrogen bonding in neat N-methylacetamide: classical molecular dynamics and Raman spectroscopy studies of a liquid of peptidic fragments, J. Phys. Chem. B 110 (2006) 3624–3637.

[16] U. Adhikari, S. Scheiner, Preferred configurations of peptide-peptide interactions, J. Phys. Chem. A 117 (2013) 489–496.

[17] M. Albrecht, C.A. Rice, M.A. Suhm, Elementary peptide motifs in the gas phase: FTIR aggregation study of formamide, acetamide, N-methylformamide, and N-methylacetamide, J. Phys. Chem. A 112 (2008) 7530–7542.

[18] R. Vargas, J. Garza, R.A. Friesner, H. Stern, B.P. Hay, D.A. Dixon, Strength of the $N-H \cdots O=C$ and $C\text{-}H \cdots O{=}C$ bonds in formamide and N-methylacetamide dimers, J. Phys. Chem. A 105 (2001) 4963–4968.

[19] M. Albertí, N. Faginas Lago, A. Laganà, F. Pirani, A portable intermolecular potential for molecular dynamics studies of NMA-NMA and NMA-H2O aggregates, Phys. Chem. Chem. Phys. 13 (2011) 8422–8432.

[20] X. Jiang, C. Wang, Evaluation of the individual hydrogen bonding energies in N-methylacetamide chains, SCIENCE CHINA Chem. 53 (2010) 1754–1761.

[21] Y. Zhou, G. Deng, Y.Z. Zheng, J. Xu, H. Ashraf, Z.W. Yu, Evidences for cooperative resonance-assisted hydrogen bonds in protein secondary structure analogs, Sci. Rep. 6 (2016) 36932.

[22] T. Forsting, H.C. Gottschalk, B. Hartwig, M. Mons, M.A. Suhm, Correcting the record: the dimers and trimers of: trans-N-methylacetamide, Phys. Chem. Chem. Phys. 19 (2017) 10727–10737.

[23] H. Torii, T. Tatsumi, T. Kanazawa, M. Tasumi, Effects of intermolecular hydrogen-bonding interactions on the amide I mode of N-methylacetamide: matrix-isolation infrared studies and ab initio molecular orbital calculations, J. Phys. Chem. B 102 (1998) 309–314.

[24] N. Faginas-Lago, A. Lombardi, M. Albertí, Aqueous N-methylacetamide: new analytic potentials and a molecular dynamics study, J. Mol. Liq. 224 (2016) 792–800.

[25] A. Panuszko, M.G. Nowak, P. Bruździak, M. Stasiulewicz, J. Stangret, Amides as models to study the hydration of proteins and peptides spectroscopic and theoretical approach on hydration in various temperatures, J. Mol. Liq. 278 (2019) 706–715.

[26] N.S. Kang, Y.K. Kang, Assessment of CCSD(T), MP2, and DFT methods for the calculations of structures and interaction energies of the peptide backbone with water molecules, Chem. Phys. Lett. 687 (2017) 23–30.

[27] M. Di Gioacchino, F. Bruni, M.A. Ricci, N-methylacetamide aqueous solutions: a neutron diffraction study, J. Phys. Chem. B 123 (2019) 1808–1814.

[28] V.K. Yadav, A. Chandra, First-principles simulation study of vibrational spectral diffusion and hydrogen bond fluctuations in aqueous solution of N-methylacetamide, J. Phys. Chem. B 119 (2015) 9858–9867.

[29] L.O. Paulson, D.T. Anderson, Infrared spectroscopy of the amide I mode of N-methylacetamide in solid hydrogen at 2–4 K, J. Phys. Chem. B 115 (2011) 13659–13667.

[30] A. Panuszko, E. Gojło, J. Zielkiewicz, M. Śmiechowski, J. Krakowiak, J. Stangret, Hydration of simple amides. FTIR spectra of HDO and theoretical studies, J. Phys. Chem. B 112 (2008) 2483–2493.

[31] X. Xiao, Y. Tan, L. Zhu, Y. Guo, Z. Wen, M. Li, X. Pu, A. Tian, Effects of the position and manner of hydration on the stability of solvated N-methylacetamides and the strength of binding between N-methylacetamide and water clusters: a computational study, J. Mol. Model. 18 (2011) 1389–1399.

[32] A. Bastida, M.A. Soler, J. Zúñiga, A. Requena, A. Kalstein, S. Fernández-Alberti, Hybrid quantum/classical simulations of the vibrational relaxation of the amide I mode of N-methylacetamide in D2O solution, J. Phys. Chem. B 116 (2012) 2969–2980.

[33] P.A. Cazade, F. Hédin, Z.H. Xu, M. Meuwly, Vibrational relaxation and energy migration of N-methylacetamide in water: the role of nonbonded interactions, J. Phys. Chem. B 119 (2015) 3112–3122.

[34] P.A. Cazade, T. Bereau, M. Meuwly, Computational two-dimensional infrared spectroscopy without maps: N-methylacetamide in water, J. Phys. Chem. B 118 (2014) 8135–8147.

[35] B. Lin, P.E. Lopes, B. Roux, A.D. Mackerell, Kirkwood-Buff analysis of aqueous N-methylacetamide and acetamide solutions modeled by the CHARMM additive and Drude polarizable force fields, J. Chem. Phys. 139 (2013).

[36] E. Kaczkowska, J. Wawer, M. Tyczyńska, M. Jóźwiak, J. Krakowiak, The hydration of selected biologically relevant molecules the temperature effect on apparent molar volume and compression, J. Mol. Liq. 274 (2019) 345–352.

[37] P.P. Patil, V.R. Shaikh, P.D. Patil, A.U. Borse, K.J. Patil, Volumetric, isentropic compressibility and viscosity coefficient studies of binary solutions involving amides as a solute in aqueous and CCl$_4$ solvent systems at 298.15 K, J. Mol. Liq. 264 (2018) 223–232.

[38] X.M. Cao, Y. Tian, Z.Y. Wang, Y.W. Liu, C.X. Wang, Effects of protein and phosphate buffer concentrations on thermal denaturation of lysozyme analyzed by isoconversional method, Bioengineered 7 (2016) 235–240.

[39] A. Blumlein, J.J. McManus, Reversible and non-reversible thermal denaturation of lysozyme with varying pH at low ionic strength, Biochim. Biophys. Acta, Proteins Proteomics 1834 (2013) 2064–2070.

[40] F. Cugia, M. Monduzzi, B.W. Ninham, A. Salis, Interplay of ion specificity, pH and buffers: insights from electrophoretic mobility and pH measurements of lysozyme solutions, RSC Adv. 3 (2013) 5882–5888.

[41] P. Lo Nostro, N. Peruzzi, M. Severi, B.W. Ninham, P. Baglioni, Asymmetric partitioning of anions in lysozyme dispersions, J. Am. Chem. Soc. 132 (2010) 6571–6577.

[42] M.A. Metrick, J.E. Temple, G. Macdonald, The effects of buffers and pH on the thermal stability, unfolding and substrate binding of RecA, Biophys. Chem. 184 (2013) 29–36.

[43] H.I. Okur, J. Hladílková, K.B. Rembert, Y. Cho, J. Heyda, J. Dzubiella, P.S. Cremer, P. Jungwirth, Beyond the Hofmeister series: ion-specific effects on proteins and their biological functions, J. Phys. Chem. B 121 (2017) 1997–2014.

[44] K.D. Collins, G.W. Neilson, J.E. Enderby, Ions in water: characterizing the forces that control chemical processes and biological structure, Biophys. Chem. 128 (2007) 95–104.

[45] X. Tadeo, B. López-Méndez, D. Castaño, T. Trigueros, O. Millet, Protein stabilization and the Hofmeister effect: the role of hydrophobic solvation, Biophys. J. 97 (2009) 2595–2603.

[46] N. Vlachy, B. Jagoda-Cwiklik, R. Vácha, D. Touraud, P. Jungwirth, W. Kunz, Hofmeister series and specific interactions of charged headgroups with aqueous ions, Adv. Colloid Interf. Sci. 146 (2009) 42–47.

[47] A. Salis, M. Monduzzi, Not only pH. Specific buffer effects in biological systems, Curr. Opin. Colloid Interface Sci. 23 (2016) 1–9.

[48] G. Carrea, R. Bovara, P. Pasta, P. Cremonesi, The effect of Hofmeister anions and protein concentration on the activity and stability of some immobilized NADdependent dehydrogenases, Biotechnol. Bioeng. 24 (1982) 1–7.

[49] K.A. Biernacki, E. Kaczkowska, P. Bruździak, Aqueous solutions of NMA, Na$_2$HPO$_4$, and NaH$_2$PO$_4$ as models for interaction studies in phosphate–protein systems, J. Mol. Liq. 265 (2018) 361–371.

[50] M. Śmiechowski, E. Gojto, J. Stangret, Systematic study of hydration patterns of phosphoric(V) acid and its mono-, di-, and tripotassium salts in aqueous solution, J. Phys. Chem. B 113 (2009) 7650–7661.

[51] M. Śmiechowski, J. Stangret, Vibrational spectroscopy of semiheavy water (HDO) as a probe of solute hydration, Pure Appl. Chem. 82 (2010) 1869–1887.

[52] J. Stangret, Solute-affected vibrational spectra of water in Ca(ClO$_4$)$_2$ aqueous solutions, Spectrosc. Lett. 21 (1988) 369–381.

[53] P. Bruździak, P.W. Rakowska, J. Stangret, Chemometric method of spectra analysis leading to isolation of lysozyme and CtDNA spectra affected by osmolytes, Appl. Spectrosc. 66 (2012) 1302–1310.

[54] B. Berglund, J. Lindgren, J. Tegenfeldt, O-H and O-D stretching vibrations in isotopically dilute HDO molecules in some solid hydrates, J. Mol. Struct. 43 (1978) 169–177.

[55] S. Bratos, J.-C. Leicknam, S. Pommeret, Relation between the OH stretching frequency and the OO distance in time-resolved infrared spectroscopy of hydrogen bonding, Chem. Phys. 359 (2009) 53–57.