

# Understanding the interactions between protein stabilizers and the peptide bond through the analysis of the volumetric and compressibility properties for the model systems

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This work confirms our earlier supposition, that volumetric and acoustic parameters for simple model proposed by us are directly related to stabilizing/destabilizing effect of osmolytes on proteins structure. The apparent molar volumes,  $V_{\Phi}$ , and the apparent molar isentropic compressions,  $K_{S,\Phi}$ , of glycine and  $N,N,N$ -trimethylglycine (betaine) were determined from densities and speed of sound measurements in aqueous solution of  $N$ -methylacetamide (as a model of peptide bond of protein). The standard molar parameters for osmolytes were obtained from concentration dependence of the calculated quantities at NMA concentration equal 2, 4, 6 and 8 (mol/kg) and at temperature  $T = (288.15, 298.15 \text{ and } 308.15) \text{ K}$ . The standard values were combined with volumetric and compressibility data for amino acids in pure water to obtain transfer properties from water to aqueous  $N$ -methylacetamide solutions. The standard partial molar volume of transfer of studied osmolytes,  $\Delta_t V_{\Phi}^0$ , seems to be related mainly to hydrophilic/hydrophobic features of molecules. The stabilizing/destabilizing effect of solute on protein structure is reflected in temperature influence on the limiting partial molar quantities.

**KEYWORDS:** apparent molar isentropic compression; apparent molar volume; glycine;  $N,N,N$ -trimethylglycine; transfer functions;  $N$ -methylacetamide.

## 1. Introduction

The number of neutral solutes that exert significant influence on the protein stability is tremendous. The effects corresponding to osmolyte-protein interactions have been examined previously by us with the help of low molecular weight compound *N*-methylacetamide (NMA) which serves as a model of peptide bond of protein[1,2]. This approach has been also applied in the present work. In the earlier works we presented the results for two osmolytes which have different impact on protein stability. *n*-butylurea (BU, denaturant of protein) and *N,N,N*-trimethylamine-*N*-oxide (TMAO, stabilizer of protein) show some distinctly different features in aqueous NMA solutions what allowed us to conclude that these differences are related to stabilizing or destabilizing impact of the solute on protein molecule. Standard compression of transfer from water to aqueous NMA solutions is much bigger for BU[2] than for TMAO[1]. Moreover, the influence of temperature on apparent molar quantities is much more bigger in the case of used denaturant than for the stabilizer of protein. This hypothesis has been verified for next two osmolytes i.e. for glycine and its derivative *N,N,N*-trimethylglycine (betaine). Both of these compounds have visibly different hydrophilic/hydrophobic character, so their hydration phenomenon should be related to these features. The volumetric and acoustic parameters measured for these solutes in pure water should reflect hydrophobic/hydrophilic hydration of the osmolytes. The studied solutes are protein structure stabilizers[3–5], thus the influence of NMA on the discussed parameters should be similar for glycine and betaine if our hypothesis is correct.

The volumetric properties of selected amino acids in ternary systems containing water and amides have been previously reported[6–8] but the available data are scarce. To best of our knowledge, there are no additional papers in which NMA has been studied, using volumetric and acoustic measurements, as a co-solute and protein model.

In this work, from the measured densities and the speed of sound data, the isentropic compressibilities,  $\kappa_S$ , apparent molar isentropic compressions,  $K_{S,\Phi}$ , and the apparent molar volumes,  $V_\Phi$ , were calculated for glycine and betaine in aqueous solutions of *N*-methylacetamide at  $T = (288.15, 298.15 \text{ and } 308.15) \text{ K}$ . The concentration of the NMA ranged from about 2, 4, 6 to 8 mol/(kg of pure water). Additionally, the volumetric and acoustic parameters were determined for betaine in pure water at the same conditions due to lack of such data in literature. The obtained apparent molar quantities have been extrapolated to infinite dilution. In this way the standard partial molar volumes and compressions were estimated. The

standard transfer partial molar compressions and volumes of studied amino acids from water to aqueous solutions of NMA,  $\Delta_t V_{\phi}^0$  and  $\Delta_t K_{S,\phi}^0$ , have been determined. In the next the interaction parameters have been estimated using the McMillan-Mayer theory formalism[9].

The obtained parameters are discussed in terms of intermolecular interactions and the hydration phenomena are analysed. Validity of the proposed criteria of the osmolyte effectiveness to change the protein stability has been checked.

## 2. Experimental

### 2.1. Materials

The source and grade of compounds used in this work are presented in Table 1. The chemicals were used as purchased without further purification. Ultra-pure water Type 1 (MilliporeSigma™ Synergy Ultrapure Water Purification System) degassed by boiling for about 20 minutes at reduced pressure was used for solution preparation.

All of the solutions were prepared by weight with the precision 0.01 mg (analytical balance RADWAG XA 60/220). The uncertainty of the concentration of a solute was estimated to be equal 1% for both *N*-methylacetamide and glycine/betaine. The combined uncertainty of the molality determination was around 0.080 mol·kg<sup>-1</sup> and 0.0035 mol·kg<sup>-1</sup> for the most concentrated solutions of NMA and Gly/Bet, respectively (0.95 level of confidence).

### 2.2. Measurements of densities and speeds of sound

The densities of the solutions and the speeds of sound were measured using the Anton Paar DSA 5000 M densimeter. The accuracy of single measurement of the density was equal 5.0·10<sup>-2</sup> kg·m<sup>-3</sup> and for the speed of sound determination the accuracy was 0.5 m·s<sup>-1</sup>. The speed of sound in studied systems was determined at the frequency of 3 MHz and the all measurements were performed at the pressure of 0.1 MPa. The temperature was kept constant at (288.15, 298.15 and 308.15) K with accuracy of 0.01 K.

Directly before each series of measurements the apparatus was calibrated with degassed ultra-pure water. The reference data for densities[10] and speeds of sound[11] were used for this purpose. The average values of densities and sound speeds for the pure water and pure NMA used for solution preparation are the same as in previous work[2] they are in good

agreement with the literature data as it was shown and discussed. The comparison of the experimental data has been also carried out for aqueous NMA and betaine solutions and it is presented in Figs 1 and 2. As it can be seen for NMA solutions, the good agreement is observed between the measured data and those published by Victor et al.[12] for speed of sound. In the case of the density data, the values obtained by Bøje et al.[13], Makarov et al.[14] are very close to the trend line fitted to the values presented in this work, while the visible differences are observed in comparison with Victor et al.[12] and Kortuem et al.[15] density data. This discrepancy can be explained by different techniques used for density measurement, i.e. employing pycnometer and commercial vibrating U-tube densimeter. Moreover the density of pure water obtained by Victor et al. deviates fairly from the reference value[10]. As a result the differences between reported densities of the solution[12] and our data are noticeable. Both discussed parameters were also measured by Patil et al.[16] and by Swenson et. al[17] for aqueous NMA but for more diluted solutions as compared to our study, however these data fit very well for general relations obtained in this work. Density data published by Swenson were measured at a higher pressure, but not enough high for a liquids to see pressure influence on the density data. Makarov et al.[18] obtained also data for aqueous NMA at pressure between 10 MPa and 100 MPa, but this data are not compared with our due to significant difference in conditions. In the case of betaine solutions, the measured values of density are in a good agreement with literature data or they are a little bigger as compared to published densities[19–22]. Speed of sound data in aqueous betaine are scarce[19,22,23] and only two works with this parameter were found for studied range of concentration[19,22]. Observed small differences can be related to the different techniques and frequencies used for acoustic measurements.

The additional comparison was done for our densities data of aqueous NMA solutions prepared for all studied ternary systems, i.e for glycine, betaine, TMAO and n-butylurea as a solute[1,2]. The differences between densities are very small and cannot be properly presented by plotting. Thus the results of the repeatability analysis are presented in Table 2. Firstly, the trend line of  $d=f(m)$  relation was determined for the data for aqueous NMA solution used for the system with betaine. In the next step the values interpolated from this trend line ( $d_{cal}$ ) for a given composition of the solution for the other systems have been subtracted from the measured densities ( $d$ ). The obtained ( $d - d_{cal}$ ) data were presented in Table 2. The value of deviation was rounded according to the precision of density measurements. As it can be seen, the values of deviation are equal zero or are close to zero except for one solution (with 4.01 mole of NMA in the system with glycine), for which the deviation is visibly bigger but very close to the



accuracy of density measurement. From here, the repeatability and reliability of our density measurement can be considered as high.

### 3. Results

#### 3.1. Density and speed of sound

Tables 3 and 4 present the density ( $d$ ) and the speed of sound ( $u$ ) data obtained for betaine in pure water and for glycine and betaine in *N*-methylacetamide (NMA) aqueous solutions at the temperature ranging from 288.15 to 308.15 K.

The data in Tables 3-4 reveal that the densities of solutions and the speeds of sound increase with concentration of solute. This effect is different for studied solutes. The increase in density of solution is much more bigger for the systems with glycine than with betaine, whereas for the speed of sound data the influence of the solute is opposite i.e. the presence of betaine in aqueous NMA solution alters sound propagation much more than the presence of glycine as a solute. Moreover, the system with glycine shows specific features both for density and speed of sound parameters. In the case of the density concentration dependence, the rise of NMA amount in solutions decreases the influence of the temperature on change of the density with glycine concentration. For the highest NMA concentration,  $(d-d_0)=f(m)$  relation is the same for all measured temperatures. The change of the speed of sound in glycine aqueous NMA solutions is much less pronounced than in betaine solutions. Moreover the studied systems presented specific temperature dependence of the speed of sound for both amino acids. The measured  $u$  data for the solutions with 2 moles of NMA increase visibly with temperature. In the case of the solutions with 4 moles of NMA this effect changed, the temperature influence on speed of sound is weak and  $u$  data have the maximum value at the temperature of 298.15 K. The higher concentration of NMA (6 and 8 moles) resulted in visible decrease in the speed of sound in the solution with temperature elevation. Some additional subtle difference between the systems with studied amino acids is observed. The change of speed of sound with the concentration ( $\Delta u/\Delta m$ ) of betaine in all NMA solutions decreases as the temperature increases, whereas the opposite phenomenon is observed for glycine systems. The change of speed of sound with the concentration ( $\Delta u/\Delta m$ ) of glycine in the solutions with 2 and 4 moles of NMA decreases as the temperature increases, whereas this parameter increases with temperature rise in glycine solutions with 6 and 8 moles of NMA.



### 3.2. Isentropic compressibility

From the measured densities,  $d$ , and speeds of sound,  $u$ , the isentropic compressibilities of solvent,  $\kappa_{S,0}$ , and solution,  $\kappa_S$ , were calculated according to the equation:

$$\kappa_S = 1/(u^2 d) \quad (1)$$

In the studied range of concentration, the compressibility is linear function of concentration:

$$\kappa_S = \kappa_{S,0} + A_\kappa m \quad (2)$$

where  $m$  ( $\text{mol}\cdot\text{kg}^{-1}$ ) is the molality of a solute and  $A_\kappa$  is the empirical coefficient. The parameters  $A_\kappa$  are collected in Table 5. The biggest changes of  $\kappa_S$  with the concentration of the solute are observed for the lowest concentration of NMA. As the concentration of NMA increases, each additional portion of dissolved Gly or Bet exerts smaller influence on the solution compressibility. This effect is more pronounced for glycine solutions.

### 3.3. Apparent molar volumes isentropic compressions

From the obtained density data the apparent molar volumes,  $V_\Phi$  ( $\text{m}^3\cdot\text{mol}^{-1}$ ), were calculated:

$$V_\Phi = M_2/d - (d - d_0)/(m d d_0) \quad (3)$$

where  $d_0$  ( $\text{kg}\cdot\text{m}^{-3}$ ) is the density of solvent i.e. water for binary system and the aqueous solution of NMA for ternary system; and  $M_2$  ( $\text{kg}\cdot\text{mol}^{-1}$ ) is the molar mass of the solute (glycine/betaine).

Within studied concentration range in all systems in study the apparent molar volumes of the solutes are linear function of concentration. The limiting apparent molar volumes of the solutes,  $V_\Phi^0$ , are, by definition, equal to the partial molar volumes in infinitely diluted solution,



$\bar{V}^0$ . In the current work we obtained the  $V_{\Phi}^0$  values, at all temperatures, by the least squares fitting of the simple linear equation to the experimental points:

$$V_{\Phi} = V_{\Phi}^0 + S_v m \quad (4)$$

where  $S_v$  is the empirical interaction coefficient. The calculated parameters of the above equation are presented in Table 6. The obtained data show that the  $S_v$  parameters from Eq. (4) does not change significantly with the *N*-methylacetamide concentration nor with temperature. Moreover, it is close to zero for the solutions with betaine and much bigger for the solutions with glycine. In studied systems the limiting apparent molar volume  $V_{\Phi}^0$  of glycine increases slightly with the concentration of NMA and with temperature, while the limiting apparent molar volume of betaine in NMA solutions has almost similar value (lower than in pure water) for all studied NMA concentrations and increases slightly as temperature rises.

The apparent molar isentropic compressions,  $K_{S,\Phi}$  ( $\text{m}^5 \cdot \text{mol}^{-1} \cdot \text{N}^{-1}$ ) were calculated from the density and ultrasound speed data according to the equation:

$$K_{S,\Phi} = M_2 \kappa_S / d + (\kappa_S d_0 - \kappa_{S,0} d) / (m d d_0) \quad (5)$$

For all studied systems, the obtained apparent molar isentropic compressions are linear function of concentration at all measured temperatures and thus they can be described by the equation:

$$K_{S,\Phi} = K_{S,\Phi}^0 + S_{K,S} m \quad (6)$$

By the analogy to the equation (4) the  $K_{S,\Phi}^0$  parameter from the above relation is the limiting apparent isentropic compression. This value is equal to the standard partial molar compression, the second factor i.e. the  $S_{K,S}$  parameter is the empirical-interaction coefficient. The parameters of the Eq. (6) are presented in Table 5. The positive  $S_{K,S}$  values for glycine solutions results from the fact that  $K_{S,\Phi}$  increases with the concentration of the solute in all investigated systems. Betaine has different influence on the change of the apparent isentropic molar compression, the  $S_{K,S}$  parameter is rather small and takes both negative and positive values. All obtained apparent molar compressions, as well as the calculated values of  $K_{S,\Phi}^0$ , for glycine in studied aqueous NMA solutions are negative. In the case of betaine solutions, the calculated apparent molar

isentropic compressions are positive in most cases. The negative data are obtained in pure water and in the diluted NMA solutions at 288.15 and 298.15 K. The temperature influence on the apparent molar compression of the solute depends on concentration of NMA. The increase in temperature increases the  $K_{S,\Phi}^0$  values. This effect becomes much smaller for higher concentration of NMA. In the case of the systems with glycine, at the higher concentration of NMA the increase in temperature leads to the decreases in the  $K_{S,\Phi}^0$  parameter.

The comparison of the obtained apparent molar quantities with the literature data can be done only for the limiting apparent molar volumes of betaine [19–21]. As it can be seen in Table 6, the obtained  $V_{\Phi}^0$  data are in good agreement with the values presented by the other researchers. In the case of the apparent isentropic molar compression, to the best of our knowledge, there are no literature data available.

### 3.4. Standard partial molar quantities of transfer

Standard partial molar volumes of transfer,  $\Delta_t V_{\Phi}^0$ , of glycine and betaine from water to aqueous solutions of NMA have been determined from the  $V_{\Phi}^0(NMA\ solution)$  data shown in Table 6. The data for glycine in pure water were taken from the literature[24]. The  $\Delta_t V_{\Phi}^0$  values have been calculated according to the relation:

$$\Delta_t V_{\Phi}^0 = V_{\Phi}^0(NMA\ solution) - V_{\Phi}^0(water) \quad (7)$$

The obtained values are presented in Table 7 and in Fig. 3. All of the data for glycine are positive. Moreover, as can be seen, they increase as the concentration of NMA increases. Different effects are observed for the solutions with betaine. For this solute, the values of  $\Delta_t V_{\Phi}^0$  are negative and do not differ markedly with the change of NMA concentration.

The values of standard molar compressions of transfer,  $\Delta_t K_{S,\Phi}^0$ , of studied amino acids from water to aqueous solution of *N*-methylacetamide have been obtained from the equation:

$$\Delta_t K_{S,\Phi}^0 = K_{S,\Phi}^0(NMA\ solution) - K_{S,\Phi}^0(water) \quad (8)$$

The values of  $K_{S,\Phi}^0$  for glycine in pure water were taken from the literature[24]. The parameters  $\Delta_t K_{S,\Phi}^0$  are shown in Table 7 and in Fig. 4. The reported values of  $\Delta_t K_{S,\Phi}^0$  are positive and they



increase with the concentration of *N*-methylacetamide. Additionally, the change of the apparent isentropic molar compression with the rise of NMA concentration is slightly bigger for glycine systems.

The influence of temperature on  $\Delta_t K_{S,\Phi}^0$  is similar for both solutes. The increase in temperature results in the decrease of the absolute values of the transfer parameters. In the case of  $\Delta_t V_{\Phi}^0$  values, the influence of temperature is typical for glycine solutions, i.e. the absolute value of this parameter is smaller for higher temperature, while the solutions with betaine reveal different features.  $\Delta_t V_{\Phi}^0$  for betaine in the solution of 2 and 4 moles of NMA is almost temperature independent. For the higher NMA concentration (6 and 8 moles per 1 kg of water) the absolute value of standard partial molar volume of transfer changes marginally with temperature and increases with the rise of temperature. However, the observed differences are in the range of the uncertainty of the calculated  $\Delta_t V_{\Phi}^0$  data. It can be concluded that, at studied conditions, temperature does not affect the volume effect when betaine is transferred from water to aqueous NMA solutions.

### 3.5. Volumetric and compression interaction parameters

According to the McMillan-Mayer theory for the multicomponent solutions, it is possible to assign the effects of the interactions to specific supramolecular clusters consisting of two (or more) solute-cosolute molecules[9,25,26]. The interaction parameters can be obtained from transfer data:

$$\Delta_t \bar{V}^0 = 2y_{AB}m_B + 3y_{ABB}m^2 + 4y_{ABBB}m^3 + \dots \quad (9)$$

The letters A and B refer to glycine/betaine and *N*-methylacetamide, respectively. In this equation  $y_{AB}$ ,  $y_{ABB}$ ,  $y_{ABBB}$  factors are pair, triplet and quartet interaction properties. For the standard partial molar volumes the Eq. (9) can be written as:

$$\Delta_t \bar{V}^0 = 2v_{AB}m_B + 3v_{ABB}m_B^2 \quad (10)$$

For the standard partial molar isentropic compression the equation is analogous:

$$\Delta_t \bar{K}_S^0 = 2k_{AB}m_B + 3k_{ABB}m_B^2 \quad (11)$$

The parameters of the equations (10-11) have been obtained by using the least squares fitting (see Table 8). The calculated volumetric parameters are positive for glycine in aqueous NMA solutions, whereas for betaine the pair volumetric interaction parameters are negative at all measured temperatures and the triplet volumetric parameters are positive. As far as the absolute values are concerned the  $v_{ABB}$  is smaller than  $v_{AB}$ . Moreover, the absolute value of these parameters for betaine are bigger than for glycine. The compression interaction parameters, for both studied amino acids, show almost the same contribution of the pair and triplet interaction parameters to the standard partial molar isentropic compression of transfer. The pair interaction parameter is positive and the triplet interaction parameter is smaller and negative.

The standard deviations,  $\sigma$ , were calculated for all relations as follows:  

$$\sigma = \left( \sum (Y_{cal} - Y_{exp})^2 / f \right)^{1/2}$$
 where:  $Y_{exp}$  is experimental value; and  $Y_{cal}$  is the value calculated from fitted equation; the  $f$  parameter is the number of degrees of freedom.

#### 4. Discussion

The previously published results show that the interactions between *N*-methylacetamide and water are not strong[27]. However the visible influence of *N*-methylacetamide molecules on the hydration of osmolytes has been already observed and described[1,2]. As it was mentioned above, the hydration properties of the systems with denaturant and stabilizer of protein are to some point similar but also reveal distinctive differences, which can be correlated with their influence on protein stability. The present study is conducted for glycine and betaine, two osmolytes well-known as stabilizers of protein structure. The partial molar compression of transfer from water to aqueous NMA solutions for both amino acids is almost equal and as it can be seen in Fig. 5 these data are close to the values for other protein stabilizer i.e. trimethylamine-N-oxide. The obtained lines are clearly below the trend line for a protein destabilizer *n*-butylurea. The next feature which allows to differentiate between stabilizer and denaturant is the influence of temperature on apparent molar quantities. This phenomenon is illustrated by Figs 6 and 7. The behavior of studied amino acids confirms our previous conclusions about solvation parameters which can reflect the impact of osmolyte on protein stability. The change of the limiting partial molar volume and limiting partial isentropic compression with temperature for glycine and betaine is distinctly smaller than for BU in

aqueous NMA solutions and even close to zero for compression data in 4, 6 and 8 mol solutions of NMA. As it can be seen the data for the other stabilizer (TMAO) are close to the trends for studied amino acids.

The values of the partial molar volumes of osmolytes in aqueous NMA solutions and in pure water, seem to be correlated mainly with the hydrophilic/hydrophobic nature of the solute molecules. The volumetric parameters are sensitive to the change of hydration sphere and allow observation of disorder in hydrophilic or hydrophobic hydration of a solute[7]. According to the co-sphere overlap model, a given single type of interaction between solute and cosolute would lead to positive or negative contribution to the summary volume effect. The values of transfer  $\Delta_t V_{\Phi}^0$  from water to *N*-methylacetamide aqueous solutions for four examined organic molecules (osmolytes), are shown in Fig. 8. For these data three different relations are observed. The positive values, increasing with the increase in NMA concentration, are observed only for glycine, i.e. for hydrophilic solute which forms zwitterions in water. The positive value of  $\Delta_t V_{\Phi}^0$  reflects the reduction of the electrostriction phenomenon due to hydrophilic-hydrophilic interactions between glycine and NMA as dominant. The second type of relation is noticed for betaine and TMAO. Both osmolytes have compact molecules with hydrophilic and hydrophobic residues at their opposite sites. These two solutes form zwitterions and interact strongly with water. The presence of NMA in solutions results in similar effect, i.e.  $\Delta_t V_{\Phi}^0$  is negative and does not change significantly with *N*-methylacetamide concentration. The negative value of  $\Delta_t V_{\Phi}^0$  is a result of predominance of hydrophobic-hydrophilic and hydrophobic-hydrophobic interactions between these osmolytes and NMA. The absolute  $\Delta_t V_{\Phi}^0$  value for betaine is smaller than for TMAO probably due to stronger hydrophilic hydration of the amino acid molecule as compared to TMAO. The third type of relation observed in Fig. 8 was detected for BU, i.e. for the uncharged solute with big hydrophobic residue and hydrophilic group. For this solute, the biggest (negative) change of the limiting partial molar volume (as compared to pure water) is reported for the lowest concentration of *N*-methylacetamide in our study. The increase in NMA concentration results in the decrease in absolute  $\Delta_t V_{\Phi}^0$  value for this system to the value close to zero for the highest studied NMA concentration[2]. The most negative value of  $\Delta_t V_{\Phi}^0$  for the lowest studied NMA concentration can be explained as a result of predominance of hydrophobic-hydrophobic interactions between BU and NMA. However, the increase in NMA concentration in the system leads to an increase in hydrophilic-hydrophilic interactions between solute and cosolute. For the highest NMA concentration the partial molar

volume of transfer of BU from water to NMA aqueous solution approximates zero, so hydrophilic-hydrophilic interactions are balanced by the interactions with hydrophobic nature.

Similar volumetric study was conducted for three amino acids: glycine, L-alanine and L-serine[7]. For these solutes the partial molar volumes of transfer from water to aqueous *N,N*-dimethylacetamide (DMA) solutions were calculated. The general conclusion from this research coincides with our observation. For the hydrophilic molecules (glycine and L-serine) the values  $\Delta_t V_{\Phi}^0$  are positive and increase as the concentration of co-solute (protein model, DMA) increases. In the case of L-alanine, the hydrophobicity is much more pronounced and the  $\Delta_t V_{\Phi}^0$  values are small and negative and change negligibly with DMA concentration. Moreover, the data of the partial molar volume of transfer for glycine from water to aqueous DMA solutions are to some point similar to the data with NMA. The different range of amide concentration used in both studies does not allow to comparison of the behavior of glycine in analogous system, but similar values for the NMA and DMA systems suggest that hydrophilic-hydrophilic interactions dominate in presented solutions with two protein models at least for lower concentration of amides. It is possible that higher concentration of the cosolute allows observation contribution of the hydrophobic interactions in summary volume effect.

The standard partial molar volume of transfer,  $\Delta_t V_{\Phi}^0$ , of betaine from water to *N*-methylacetamide aqueous solutions is negative and does not differ significantly with *N*-methylacetamide concentration. The volume pair interaction parameter is negative and the volume triplet interaction parameter is positive. Similar contributions of  $v_{AB}$  and  $v_{ABB}$  parameters were obtained for the systems with TMAO or BU. In the case of solutions with glycine, the standard partial molar volume,  $\Delta_t V_{\Phi}^0$ , of transfer from water to aqueous solutions of NMA is positive and both obtained volume interaction parameters are positive. The absolute values of  $v_{AB}$  and  $v_{ABB}$  parameters for glycine are relatively small as compared to corresponding data for betaine, TMAO and BU.

The compression interaction parameters obtained from  $\Delta_t K_{S,\Phi}^0$  data are similar for both studied solutes, moreover the pair interaction parameter is always positive and the triplet interaction parameter is negative and absolute value of  $k_{ABB}$  parameter is a few times smaller than the absolute value of  $k_{AB}$  parameter. Similar relations between compression interaction parameters are observed for BU solutions, however for this osmolyte the pair interaction parameter is significantly bigger than for glycine and betaine systems. For the record, both interaction parameters are positive for TMAO in NMA aqueous solutions.

The change of  $V_\Phi$  with the concentration of the solute depends mainly on the solute-solute interactions. The slope of the studied relations does not depend significantly on the cosolute (*N*-methylacetamide) concentration nor on temperature. This phenomenon was observed also for BU and TMAO in aqueous NMA solutions. However, small differences for glycine and betaine are observed, i.e.  $S_v$  parameter is always positive for glycine solutions whereas for betaine  $S_v$  parameter oscillates around zero and takes positive or negative value. Similar effect is observed for variation of the apparent isentropic molar compression with solute concentration.  $K_{S,\Phi}$  increases as glycine concentration rises. This quantity varies to a lesser extent with betaine concentration (as compared to glycine systems) with different tendency, i.e. increases or decreases in studied systems.

## 5. Conclusions

The volumetric and acoustic data for glycine and betaine in aqueous NMA solutions confirm our previous suppositions that some of these quantities can be used as a characteristic features strictly related to the osmolyte abilities to change the stability of the protein. In the case of denaturants of protein, the values of standard molar compressions of transfer,  $\Delta_t K_{S,\Phi}^0$ , are much more bigger than for stabilizers of proteins. Moreover, the influence of temperature on the limiting partial molar volume and isentropic compression is more pronounced for studied denaturant than for stabilizers.

The standard partial molar volume of transfer of studied osmolytes from water to aqueous solutions of NMA,  $\Delta_t V_\Phi^0$ , seems to be mainly related to hydrophilic/hydrophobic features of molecules.

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Table 1. Source and purity of chemicals used in this work.

Chemical	Chemical formula	Molar mass/(g·mol <sup>-1</sup> )	Source	Purity <sup>a</sup> (mass fraction)	CAS number
<i>N</i> -methylacetamide (NMA)	C <sub>3</sub> H <sub>7</sub> NO	73.09	Aldrich Chemicals	≥0.99	79-16-3
glycine (Gly)	C <sub>2</sub> H <sub>5</sub> NO <sub>2</sub>	75.07	Sigma	≥0.99	56-40-6
<i>N,N,N</i> -trimethylglycine (betaine, Bet)	C <sub>5</sub> H <sub>11</sub> NO <sub>2</sub>	117.15	Alfa Aesar	≥0.98	107-43-7

<sup>a</sup> Purities are provided by the suppliers.

Table 2. Comparison of the density data for aqueous NMA solution with literature values [1, 2]. The difference between the measured density of aqueous NMA solution,  $d$ , and the density,  $d_{cal}$ , calculated based on trend line determined for of NMA solutions used for the system with betaine at temperature 308.15 K and at pressure  $p = 0.10$  MPa<sup>a</sup>.

osmolyte	$d - d_{cal} / \text{kg}\cdot\text{m}^{-3}$ (and NMA concentration in mol·kg <sup>-1</sup> of water)			
<i>N,N,N</i> -trimethylglycine	0.00 (2.00)	0.00 (4.06)	0.00 (6.05)	0.00 (8.19)
Glycine	0.01 (2.02)	0.06 (4.01)	0.01 (5.97)	0.01 (8.03)
<i>n</i> -butylurea <sup>b</sup>	0.01 (1.99)	0.00 (4.04)	0.01 (6.01)	0.01 (8.02)
<i>N,N,N</i> - trimethylamine- <i>N</i> -oxide <sup>c</sup>	0.01 (2.00)	0.00 (4.00)	0.01 (6.05)	-0.01 (8.05)

<sup>a</sup> Expanded uncertainties  $U$  calculated at 0.95 level of confidence are:  $U(d) = 0.05 \text{ kg}\cdot\text{m}^{-3}$ ,  $U(T) = 0.01\text{K}$ ,  $U(m) = 0.080 \text{ mol}\cdot\text{kg}^{-1}$  and  $U(p) = 0.002 \text{ MPa}$ ;

<sup>b</sup> Reference [2]

<sup>c</sup> Reference [1]

Table 3. Densities,  $d$ , and speed of sound,  $u$ , of solutions of  $N,N,N$ -trimethylglycine in pure water and in aqueous solution of  $N$ -methylacetamide at different temperatures and at pressure  $p = 0.10 \text{ MPa}^a$ .

$T/\text{K}$	288.15	298.15	308.15	288.15	298.15	308.15
$m^b$ $/(\text{mol}\cdot\text{kg}^{-1})$	$d/(\text{kg}\cdot\text{m}^{-3})$			$u/(\text{m}\cdot\text{s}^{-1})$		
in pure water						
0	999.10	997.04	994.03	1466.4	1497.0	1519.8
0.0782	1000.57	998.46	995.41	1472.9	1502.8	1525.2
0.1085	1001.14	999.00	995.94	1475.4	1505.1	1527.3
0.1483	1001.87	999.70	996.62	1478.6	1508.0	1530.0
0.1988	1002.80	1000.58	997.48	1482.7	1511.8	1533.4
0.2501	1003.74	1001.48	998.38	1486.8	1515.5	1536.8
0.3013	1004.66	1002.40	999.25	1490.9	1519.2	1540.1
in $2.00 \text{ mol}\cdot\text{kg}^{-1}$ of NMA						
0	1000.63	997.68	993.92	1555.9	1569.8	1578.7
0.1427	1003.42	1000.38	996.56	1566.3	1579.4	1587.6
0.1821	1004.18	1001.11	997.27	1569.2	1582.0	1590.1
0.2304	1005.11	1002.01	998.15	1572.7	1585.2	1593.0
0.2717	1005.89	1002.76	998.88	1575.7	1588.0	1595.6
0.3100	1006.61	1003.46	999.56	1578.4	1590.6	1598.0
0.3402	1007.16	1004.00	1000.09	1580.6	1592.5	1599.8
in $4.06 \text{ mol}\cdot\text{kg}^{-1}$ of NMA						
0	1003.73	999.71	995.04	1620.0	1620.8	1618.6
0.1446	1006.56	1002.46	997.75	1629.4	1629.7	1627.0
0.1848	1007.34	1003.22	998.50	1632.0	1632.2	1629.4
0.2338	1008.27	1004.13	999.39	1635.2	1635.2	1632.2
0.2744	1009.05	1004.88	1000.13	1637.9	1637.7	1634.6
0.3136	1009.78	1005.60	1000.84	1640.5	1640.1	1636.8
0.3443	1010.35	1006.16	1001.39	1642.4	1641.9	1638.5
in $6.05 \text{ mol}\cdot\text{kg}^{-1}$ of NMA						
0	1006.43	1001.48	996.04	1660.6	1652.4	1642.2
0.1442	1009.20	1004.21	998.75	1669.1	1660.5	1650.0
0.1841	1009.96	1004.96	999.49	1671.5	1662.8	1652.2
0.2331	1010.88	1005.86	1000.38	1674.3	1665.5	1654.9
0.2729	1011.63	1006.60	1001.11	1676.8	1667.8	1657.1
0.3125	1012.37	1007.32	1001.83	1679.1	1670.1	1659.2
0.3434	1012.93	1007.88	1002.38	1680.9	1671.7	1660.8
in $8.19 \text{ mol}\cdot\text{kg}^{-1}$ of NMA						
0	1008.51	1002.78	996.67	1686.4	1671.5	1655.4
0.1444	1011.23	1005.48	999.36	1694.4	1679.2	1662.8
0.1841	1011.97	1006.21	1000.09	1696.6	1681.3	1664.9
0.2328	1012.85	1007.09	1000.96	1699.1	1683.8	1667.3

0.2736	1013.59	1007.82	1001.70	1701.4	1685.9	1669.4
0.3126	1014.30	1008.53	1002.40	1703.4	1688.0	1671.4
0.3433	1014.83	1009.06	1002.94	1704.9	1689.4	1672.8

<sup>a</sup> Expanded uncertainties  $U$  calculated at 0.95 level of confidence are:  $U(d) = 0.15 \text{ kg}\cdot\text{m}^{-3}$ ,  $U(u) = 0.5 \text{ m}\cdot\text{s}^{-1}$ ,  $U(T) = 0.01\text{K}$ ,  $U(m) = 0.0035 \text{ mol}\cdot\text{kg}^{-1}$  (for betaine concentration),  $U(m) = 0.080 \text{ mol}\cdot\text{kg}^{-1}$  (for NMA concentration) and  $U(p) = 0.002 \text{ MPa}$ ; <sup>b</sup> – the concentration expressed as the number of moles of the solute in 1 kg of solvent (i.e. the aqueous solution of NMA)

Table 4. Densities,  $d$ , and speed of sound,  $u$ , of solutions of glycine in aqueous solution of *N*-methylacetamide at different temperatures and at pressure  $p = 0.10 \text{ MPa}^a$ .

$T/\text{K}$	288.15	298.15	308.15	288.15	298.15	308.15
$m^b$ $/(\text{mol}\cdot\text{kg}^{-1})$	$d/(\text{kg}\cdot\text{m}^{-3})$			$u/(\text{m}\cdot\text{s}^{-1})$		
in $2.02 \text{ mol}\cdot\text{kg}^{-1}$ of NMA						
0	1000.65	997.69	993.92	1556.2	1570.1	1578.9
0.1467	1005.30	1002.25	998.43	1563.1	1576.8	1585.4
0.2368	1008.12	1005.02	1001.16	1567.3	1580.8	1589.3
0.2776	1009.38	1006.26	1002.38	1569.2	1582.6	1591.0
0.3160	1010.55	1007.40	1003.51	1571.0	1584.2	1592.6
0.3466	1011.49	1008.33	1004.43	1572.3	1585.6	1593.9
in $4.01 \text{ mol}\cdot\text{kg}^{-1}$ of NMA						
0	1003.63	999.53	994.96	1618.5	1619.7	1617.7
0.1856	1009.38	1005.26	1000.60	1626.0	1627.1	1625.0
0.2347	1010.88	1006.74	1002.07	1627.9	1629.0	1626.9
0.2745	1012.08	1007.96	1003.25	1629.4	1630.5	1628.3
0.3139	1013.25	1009.12	1004.40	1630.9	1631.9	1629.8
0.3441	1014.15	1010.01	1005.28	1632.1	1633.1	1630.9
in $5.97 \text{ mol}\cdot\text{kg}^{-1}$ of NMA						
0	1006.31	1001.40	996.00	1659.0	1651.1	1641.3
0.1463	1010.70	1005.77	1000.34	1663.8	1656.0	1646.3
0.1860	1011.88	1006.94	1001.50	1665.1	1657.3	1647.6
0.2354	1013.33	1008.38	1002.94	1666.7	1659.0	1649.3
0.2750	1014.50	1009.53	1004.09	1668.0	1660.3	1650.6
0.3138	1015.62	1010.65	1005.21	1669.2	1661.6	1651.9
0.3442	1016.50	1011.52	1006.08	1670.2	1662.6	1653.0
in $8.03 \text{ mol}\cdot\text{kg}^{-1}$ of NMA						
0	1008.38	1002.70	996.64	1685.0	1670.5	1654.7
0.1462	1012.63	1006.95	1000.89	1688.9	1674.6	1659.1
0.1856	1013.76	1008.08	1002.02	1690.0	1675.7	1660.3
0.2340	1015.14	1009.46	1003.41	1691.3	1677.2	1661.7
0.2744	1016.28	1010.60	1004.54	1692.4	1678.3	1662.9
0.3130	1017.38	1011.70	1005.64	1693.5	1679.4	1664.0



0.3435      1018.22      1012.54      1006.48      1694.2      1680.2      1664.9

<sup>a</sup> Expanded uncertainties  $U$  calculated at 0.95 level of confidence are:  $U(d) = 0.15 \text{ kg}\cdot\text{m}^{-3}$ ,  $U(u) = 0.5 \text{ m}\cdot\text{s}^{-1}$ ,  $U(T) = 0.01\text{K}$ ,  $U(m) = 0.0035 \text{ mol}\cdot\text{kg}^{-1}$  (for glycine concentration),  $U(m) = 0.080 \text{ mol}\cdot\text{kg}^{-1}$  (for NMA concentration) and  $U(p) = 0.002 \text{ MPa}$ ; <sup>b</sup> – the concentration expressed as the number of moles of the solute in 1 kg of solvent (i.e. the aqueous solution of NMA)

Table 5. Parameter ( $A_k$ ) of Eq. (2) and parameters of Eq. (6) for of *N,N,N*-trimethylglycine in pure water and in aqueous solution of *N*-methylacetamide and for glycine in aqueous solutions of NMA at different temperatures and at pressure  $p = 0.1\text{MPa}$ . Standard deviation ( $\sigma$ ) is presented for discussed relations.

$T/\text{K}$	$m_{\text{NMA}}/(\text{mol}\cdot\text{kg}^{-1})$	$10^{15}\cdot K^0_{\Phi}/(\text{m}^5\cdot(\text{N}\cdot\text{mol})^{-1})$	$10^{15}\cdot S_{K,S}/(\text{kg}\cdot\text{m}^5\cdot\text{N}^{-1}\cdot\text{mol}^{-2})$	$10^{16}\cdot\sigma/(\text{m}^5\cdot(\text{N}\cdot\text{mol})^{-1})$	$10^{11}\cdot A_k/(\text{kg}\cdot\text{m}^2\cdot(\text{N}\cdot\text{mol})^{-1})$	$10^{14}\sigma/\text{m}^2\cdot\text{N}^{-1}$
<i>N,N,N</i> -trimethylglycine						
288.15	0	-17.2±0.19	8.0±0.93	1.8	-5.95±0.036	18
298.15	0	-8.6±0.14	1.6±0.71	1.4	-5.13±0.021	10
308.15	0	-3.8±0.15	1.6±0.74	1.4	-4.55±0.021	10
288.15	2.00	-6.9±0.12	0.8±0.44	0.8	-4.56±0.015	9.1
298.15	2.00	-2.9±0.14	0.4±0.52	0.9	-4.15±0.012	7.4
308.15	2.00	0.0±0.12	0.5±0.46	0.8	-3.85±0.012	7.0
288.15	4.06	-0.9±0.19	-1.1±0.67	0.9	-3.71±0.010	4.7
298.15	4.06	0.73±0.090	0.4±0.33	0.5	-3.54±0.010	6.2
308.15	4.06	2.3±0.15	1.0±0.51	0.7	-3.40±0.011	7.1
288.15	6.05	2.7±0.16	-1.2±0.60	1.1	-3.19±0.010	4.0
298.15	6.05	4.0±0.16	-1.0±0.60	1.0	-3.13±0.010	4.5
308.15	6.05	4.8±0.14	-1.0±0.54	1.0	-3.11±0.010	4.9
288.15	8.19	4.2±0.18	2.6±0.67	1.2	-2.85±0.015	9.4
298.15	8.19	5.2±0.14	1.8±0.54	1.0	-2.86±0.013	7.9
308.15	8.19	6.1±0.17	0.7±0.65	1.2	-2.89±0.011	6.6
glycine						
288.15	2.02	-20.6±0.13	3.1±0.46	0.8	-3.70±0.013	7.8
298.15	2.02	-19.1±0.14	4.3±0.49	0.8	-3.52±0.015	9.0
308.15	2.02	-18.1±0.10	4.4±0.31	0.5	-3.40±0.015	9.1
288.15	4.01	-14.7±0.11	4.5±0.37	0.5	-2.98±0.013	8.0
298.15	4.01	-14.6±0.14	4.7±0.51	0.7	-2.97±0.015	8.8

308.15	4.01	-14.3±0.14	4.8±0.50	0.7	-2.95±0.014	8.7
288.15	5.97	-8.98±0.056	1.7±0.22	0.4	-2.45±0.010	4.4
298.15	5.97	-9.44±0.052	1.3±0.52	0.4	-2.53±0.010	3.6
308.15	5.97	-10.0±0.11	1.5±0.42	0.7	-2.60±0.010	3.8
288.15	8.03	-5.1±0.12	0.5±0.5	0.8	-2.09±0.010	3.1
298.15	8.03	-6.0±0.15	0.2±0.55	1.0	-2.21±0.010	3.2
308.15	8.03	-7.64±0.043	2.1±0.17	0.3	-2.35±0.010	4.9

Table 6. Parameters ( $S_v$  and  $V^0_\Phi$ ) of Eq. (4) for *N,N,N*-trimethylglycine in pure water (with literature data) and in aqueous solution of *N*-methylacetamide and for glycine in aqueous solutions of NMA at different temperatures. Standard deviation ( $\sigma$ ) is presented for discussed relation.

$T/K$	$m_{\text{NMA}}/(\text{mol}\cdot\text{kg}^{-1})$	$10^6\cdot V^0_\Phi/(\text{m}^3\cdot\text{mol}^{-1})$	$10^6\cdot S_v/(\text{kg}\cdot\text{m}^3\cdot\text{mol}^{-2})$	$10^8\cdot\sigma/(\text{m}^3\cdot\text{mol}^{-1})$
<i>N,N,N</i> -trimethylglycine				
288.15	0	98.28±0.026 97.91 <sup>a</sup> ; 97.26 <sup>b</sup> ; 97.78 <sup>c</sup>	-0.2±0.13 -	2.5 -
298.15	0	99.14±0.075 98.43 <sup>a</sup> ; 99.20 <sup>b</sup> ; 98.59 <sup>c</sup>	0.0±0.38 -	7.3 -
308.15	0	99.91±0.062 98.74 <sup>a</sup> ; 99.52 <sup>b</sup> ; 99.29 <sup>c</sup>	-0.3±0.32 -	6.2 -
288.15	2.00	97.27±0.040	-0.1±0.16	2.6
298.15	2.00	98.16±0.025	-0.1±0.10	1.6
308.15	2.00	98.88±0.029	0.0±0.12	1.9
288.15	4.06	96.98±0.042	0.0±0.16	1.9
298.15	4.06	97.87±0.010	-0.18±0.032	0.9
308.15	4.06	98.51±0.034	-0.1±0.13	1.5
288.15	6.05	97.22±0.026	-0.5±0.10	1.7
298.15	6.05	97.86±0.026	-0.3±0.10	1.7
308.15	6.05	98.42±0.026	-0.1±0.10	1.7
288.15	8.19	97.31±0.040	0.3±0.16	2.5
298.15	8.19	97.93±0.032	0.2±0.13	2.1
308.15	8.19	98.51±0.035	0.1±0.14	2.3
glycine				
288.15	2.02	43.03±0.043	0.8±0.16	2.4
298.15	2.02	43.69±0.063	0.7±0.23	3.6
308.15	2.02	44.07±0.044	0.9±0.16	2.5

288.15	4.01	43.53±0.048	1.3±0.18	2.2
298.15	4.01	43.73±0.090	1.2±0.33	4.1
308.15	4.01	44.25±0.043	1.2±0.16	2.0
288.15	5.97	44.66±0.028	0.7±0.11	1.8
298.15	5.97	44.82±0.010	1.05±0.036	0.6
308.15	5.97	45.17±0.028	0.6±0.11	1.8
288.15	8.03	45.56±0.028	0.7±0.11	1.8
298.15	8.03	45.65±0.028	0.8±0.11	1.9
308.15	8.03	45.74±0.042	0.8±0.17	2.8

<sup>a</sup> Reference [21]

<sup>b</sup> Reference [22]

<sup>c</sup> Reference [20]

Table 7. Standard partial molar volume of transfer,  $\Delta_t V_{\Phi}^0$ , and standard transfer molar compression,  $\Delta_t K_{S,\Phi}^0$ , of *N,N,N*-trimethylglycine and glycine from water to aqueous solutions of NMA at different temperatures.

T/K	288.15	298.15	308.15	288.15	298.15	308.15
$m_{NMA}/$ (mol·kg <sup>-1</sup> )		$10^6 \cdot \Delta_t V_{\Phi}^0 /$ (m <sup>3</sup> ·mol <sup>-1</sup> )		$10^{15} \cdot \Delta_t K_{S,\Phi}^0 /$ (m <sup>5</sup> ·(N·mol) <sup>-1</sup> )		
<i>N,N,N</i> -trimethylglycine						
2.00	-1.01±0.066	-1.0±0.10	-1.03±0.091	10±1.4	6±1.3	4±1.2
4.06	-1.27±0.068	-1.27±0.085	-1.40±0.096	16±1.6	9±1.1	6±1.3
6.05	-1.06±0.052	-1.3±0.11	-1.49±0.088	20±1.5	13±1.3	9±1.3
8.19	-0.97±0.066	-1.2±0.11	-1.40±0.097	21±1.6	14±1.3	10±1.4
glycine						
2.02	0.71±0.063	0.58±0.073	0.39±0.064	11.1±0.22	7.9±0.24	5.3±0.20
4.01	1.21±0.068	0.6±0.10	0.57±0.063	17.0±0.19	12.4±0.24	9.1±0.24
5.97	2.34±0.048	1.71±0.020	1.49±0.048	22.7±0.14	17.6±0.16	13.4±0.21
8.03	3.24±0.048	2.54±0.038	2.06±0.062	26.6±0.20	21.0±0.25	15.8±0.15

Table 8. Interaction parameters for Eqs (10) and (11) at different temperatures.

T/K	$10^6 \cdot v_{AB} /$ (kg·m <sup>3</sup> ·mol <sup>-2</sup> )	$10^6 \cdot v_{ABB} /$ (kg <sup>2</sup> ·m <sup>3</sup> ·mol <sup>-3</sup> )	$10^{15} \cdot k_{AB} /$ (kg·m <sup>2</sup> ·(N·mol) <sup>-1</sup> )	$10^{15} \cdot k_{ABB} /$
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$(\text{kg}^2 \cdot \text{m}^2 \cdot (\text{N} \cdot \text{mol}^2)^{-1})$				
<i>N,N,N</i> -trimethylglycine				
288.15	-0.30±0.031	0.021±0.0037	2.84±0.085	-0.13±0.011
298.15	-0.30±0.028	0.019±0.0033	1.6±0.13	-0.06±0.016
308.15	-0.30±0.022	0.018±0.0026	1.06±0.096	-0.04±0.012
glycine				
288.15	0.15±0.025	0.004±0.0031	3.0±0.21	-0.12±0.025
298.15	0.10±0.050	0.004±0.0061	2.1±0.15	-0.07±0.018
308.15	0.07±0.028	0.005±0.0035	1.40±0.058	-0.035±0.0070

## LEGEND TO THE FIGURES

**FIG. 1.** Plots of: a) the density of aqueous NMA solution,  $d$ , against the molar fraction of NMA,  $X_{\text{NMA}}$ . This study: at 288.15 K ( $\diamond$  - for betaine solution;  $*$  - for glycine solution), at 298.15 K ( $\Delta$  - for betaine solution;  $\dagger$  - for glycine solution) and at 308.15 K ( $\circ$  - for betaine solution;  $\times$  - for glycine solution). The dotted line is the trend line fitted to the data from this study; b) the speed of sound in aqueous NMA solution,  $u$ , against the molar fraction of NMA,  $X_{\text{NMA}}$ . This study at 298.15 K ( $\Delta$  - for betaine solution;  $\dagger$  - for glycine solution) and at 308.15 K ( $\circ$  - for betaine solution;  $\times$  - for glycine solution). The dotted line is the trend line fitted to the data from this study. The references to literature data are listed in graph.

**FIG. 2.** Plots of: a) the density of aqueous betaine solution,  $d$ , against the molar fraction of betaine,  $X_{\text{BET}}$ . This study: at 288.15 K ( $\Delta$ ), at 298.15 K ( $\mathbf{x}$ ) and at 308.15 K ( $\circ$ ). The trend lines are fitted to the each group of data for better visualization. The style of line is related to the temperature of measurement: solid line presents data at 285.15K, dotted line at 298.15 K and dashed lined at 308.15K; b) the speed of sound in aqueous betaine solution,  $u$ , against the molar fraction of betaine,  $X_{\text{BET}}$ . This study at 288.15 K ( $\Delta$ ), at 298.15 K ( $\circ$ ) and at 308.15 K ( $\diamond$ ). The dotted line is the trend line fitted to the data from this study. The references to literature data are listed in graph.

**FIG. 3.** Plot of the apparent molar volume of transfer,  $\Delta_t V_{\Phi}^0$ , from water to aqueous solution of NMA against the concentration  $m_{\text{NMA}}$  of NMA (mol/kg of water) for: glycine (at 288.15 K ( $\bullet$ ), 298.15 K ( $\blacktriangle$ ) and 308.15 K ( $\blacklozenge$ )) and betaine (at 288.15 K ( $\circ$ ), 298.15 K ( $\Delta$ ) and 308.15 K ( $\diamond$ ))

**FIG. 4.** Plot of the apparent molar isentropic compression of transfer,  $\Delta_t K_{S,\Phi}^0$  from water to aqueous solution of NMA against the concentration  $m_{\text{NMA}}$  of NMA (mol/kg of water) for: glycine (at 288.15 K ( $\bullet$ ), 298.15 K ( $\blacktriangle$ ) and 308.15 K ( $\blacklozenge$ )) and betaine (at 288.15 K ( $\circ$ ), 298.15 K ( $\Delta$ ) and 308.15 K ( $\diamond$ ))



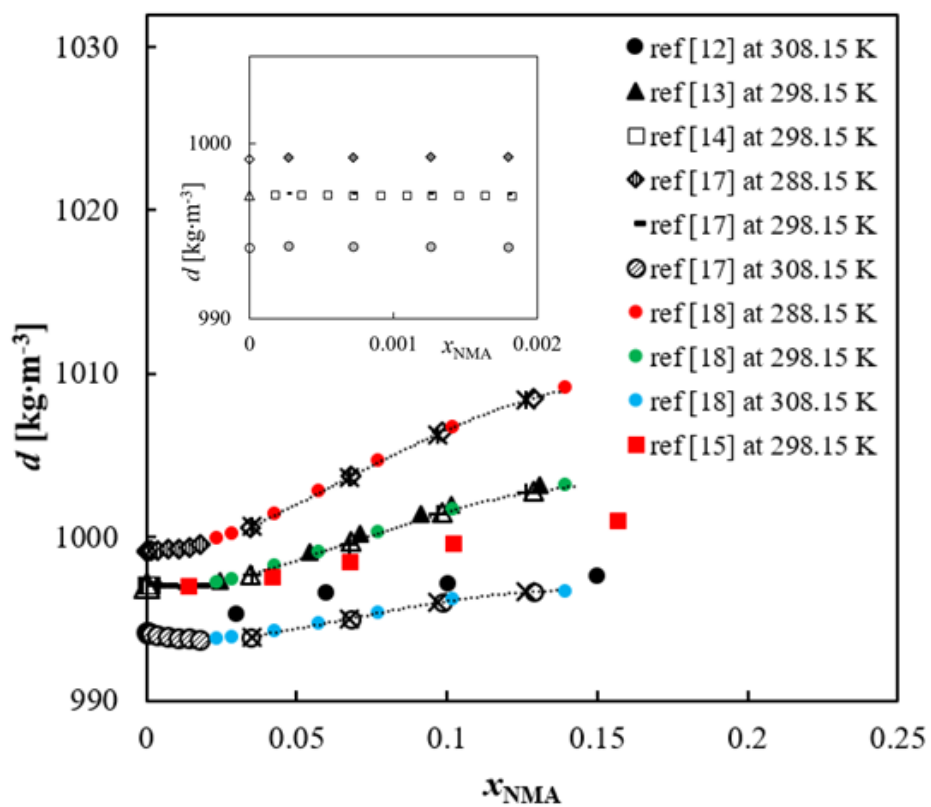
**FIG. 5.** Plot of the apparent molar isentropic compression of transfer,  $\Delta_t K_{S,\Phi}^0$  from water to aqueous solution of NMA against the concentration  $m_{\text{NMA}}$  of NMA (mol/kg of water) at 288.15 K for: glycine ( $\circ$ ), betaine ( $\bullet$ ), TMAO ( $\square$ )[1] and n-butylurea ( $\Delta$ )[2].

**FIG. 6.** Plot of the difference between the apparent molar volumes of transfer,  $\Delta_t V_{\Phi}^0$ , from water to aqueous solution of NMA obtained at 308.15 K and 288.15 K against the concentration  $m_{\text{NMA}}$  of NMA (mol/kg of water) for: glycine ( $\circ$ ), betaine ( $\bullet$ ), TMAO ( $\square$ ) [1] and n-butylurea ( $\Delta$ ) [2].

**FIG. 7.** Plot of the difference between apparent molar isentropic compressions of transfer,  $\Delta_t K_{S,\Phi}^0$  from water to aqueous solution of NMA obtained at 308.15 K and 288.15 K against the concentration  $m_{\text{NMA}}$  of NMA (mol/kg of water) for: glycine ( $\circ$ ), betaine ( $\bullet$ ), TMAO ( $\square$ ) [1] and n-butylurea ( $\Delta$ ) [2].

**FIG. 8.** Plot of the apparent molar volume of transfer,  $\Delta_t V_{\Phi}^0$ , from water to aqueous solution of NMA against the concentration  $m_{\text{NMA}}$  of NMA (mol/kg of water) at 298.15 K for: glycine ( $\circ$ ), betaine ( $\bullet$ ), TMAO ( $\square$ ) [1] and n-butylurea ( $\Delta$ ) [2].

Fig.1  
a)



b)

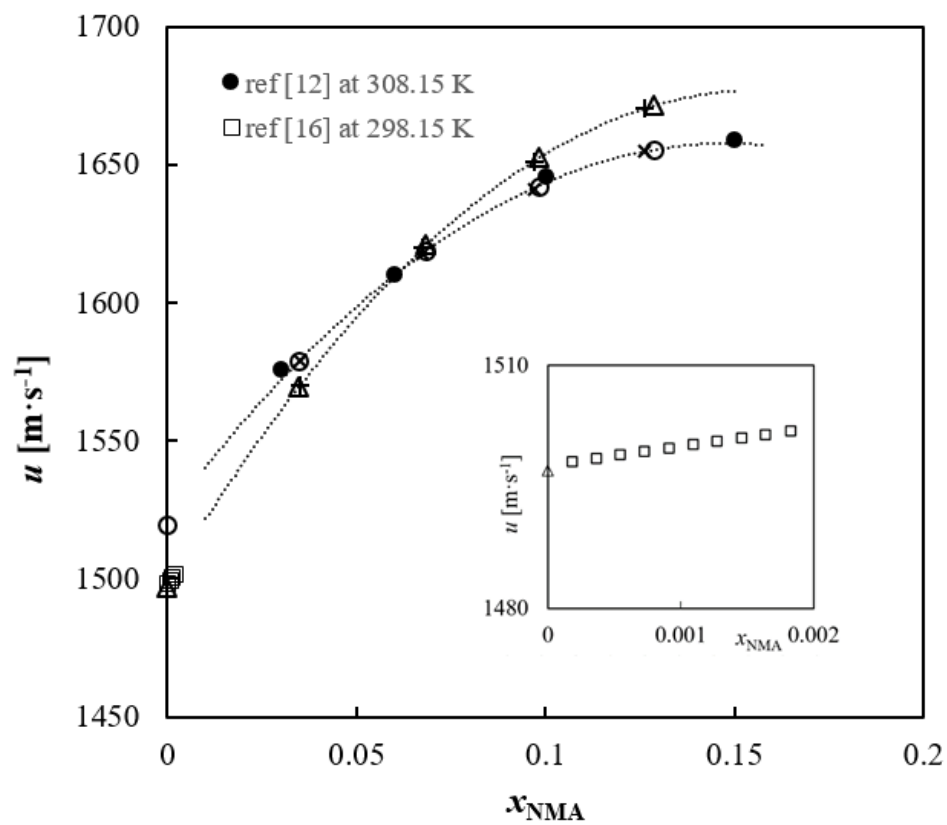
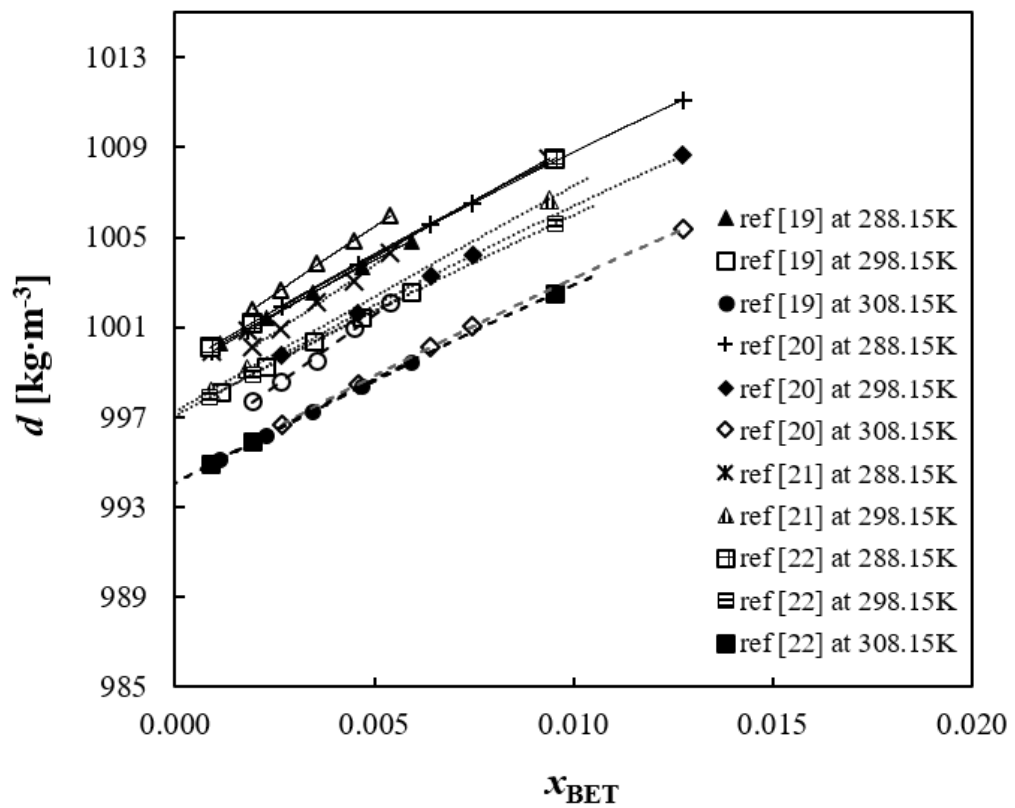


Fig. 2

a)



b)

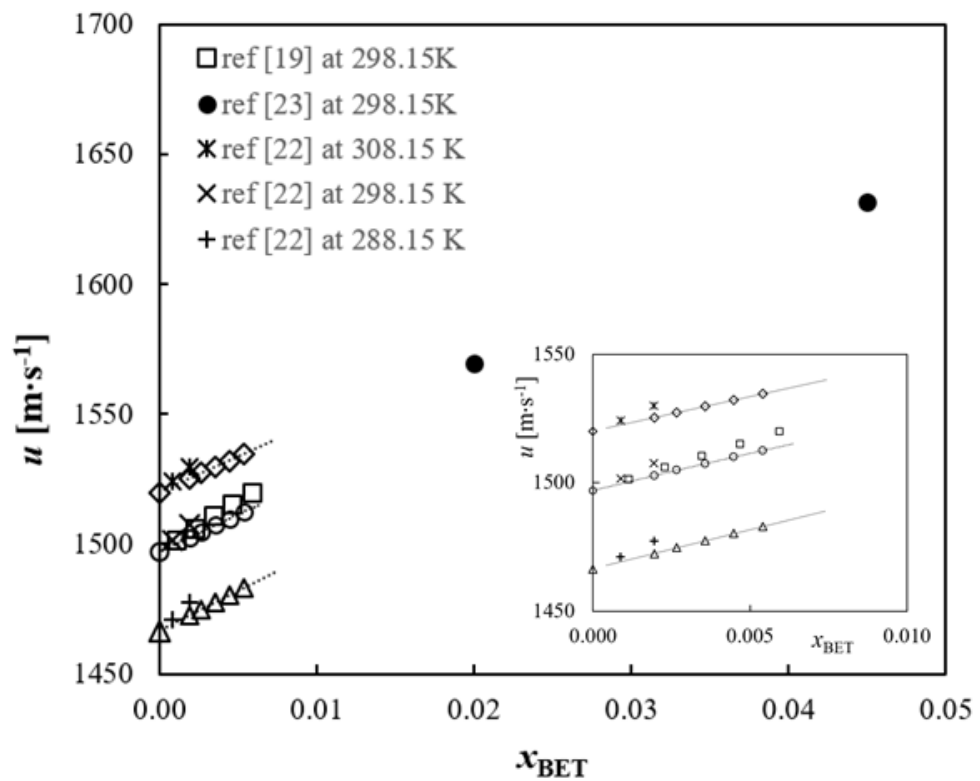


Fig. 3

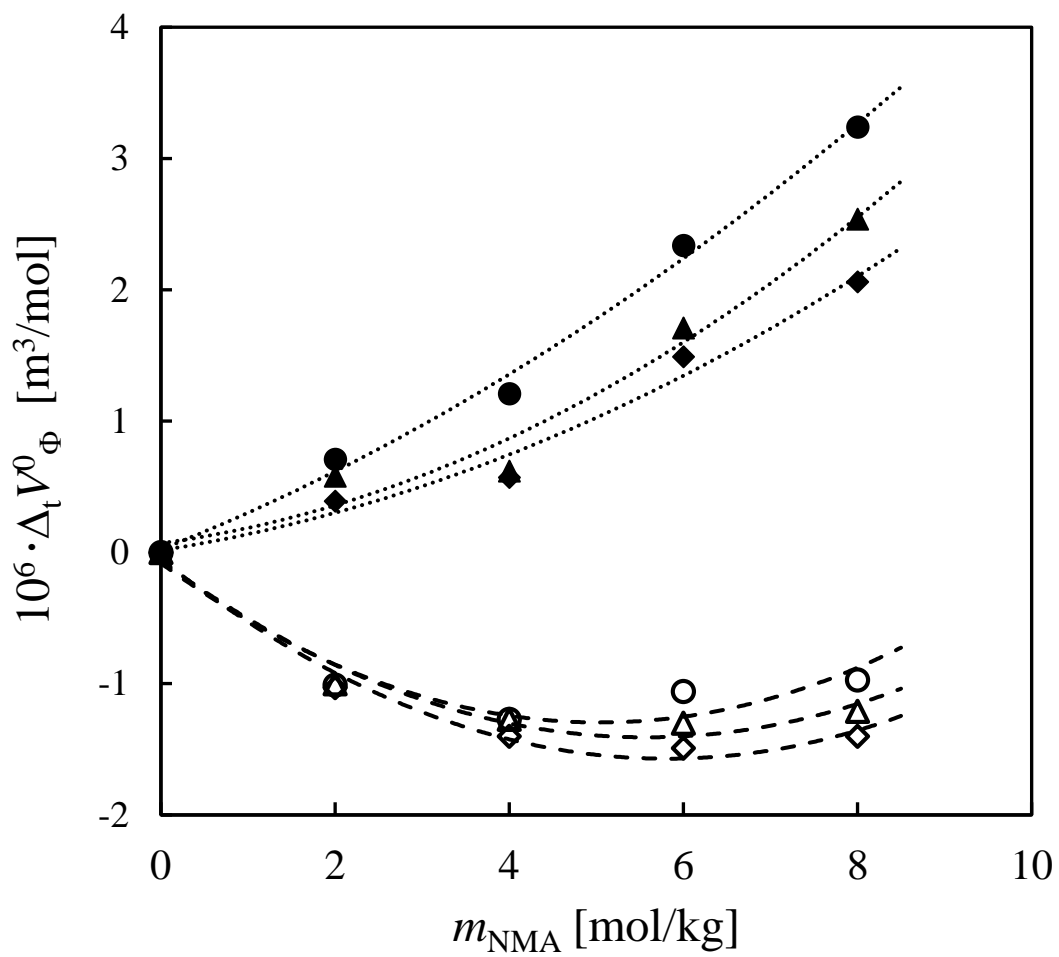


Fig. 4

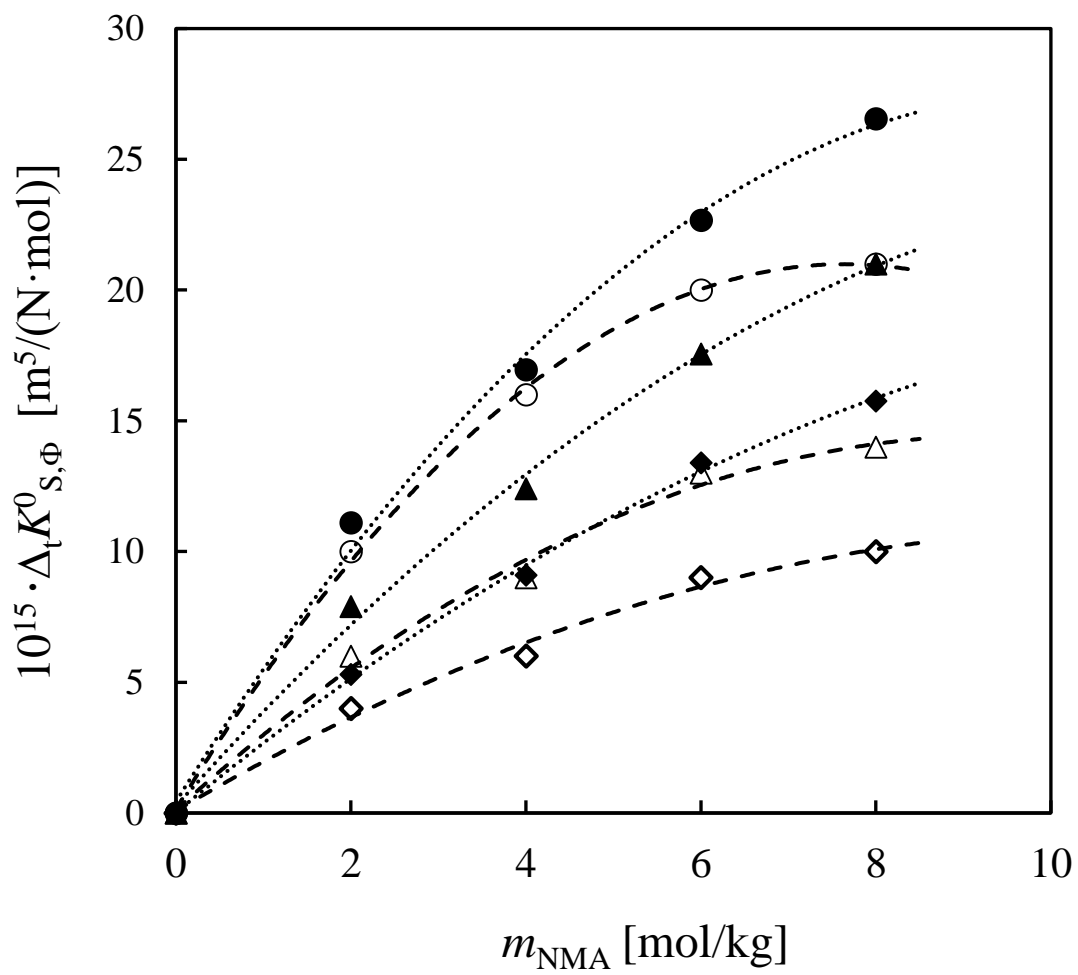


Fig. 5

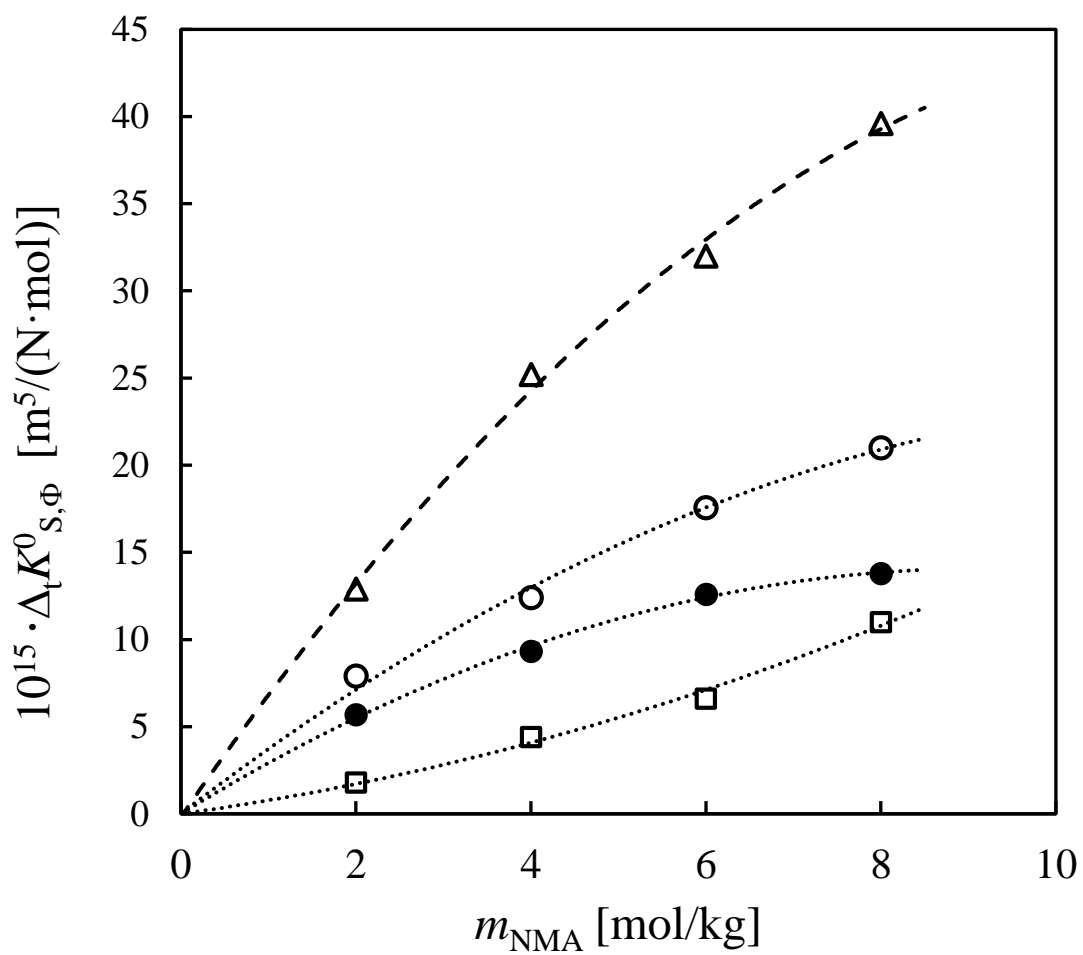


Fig. 6

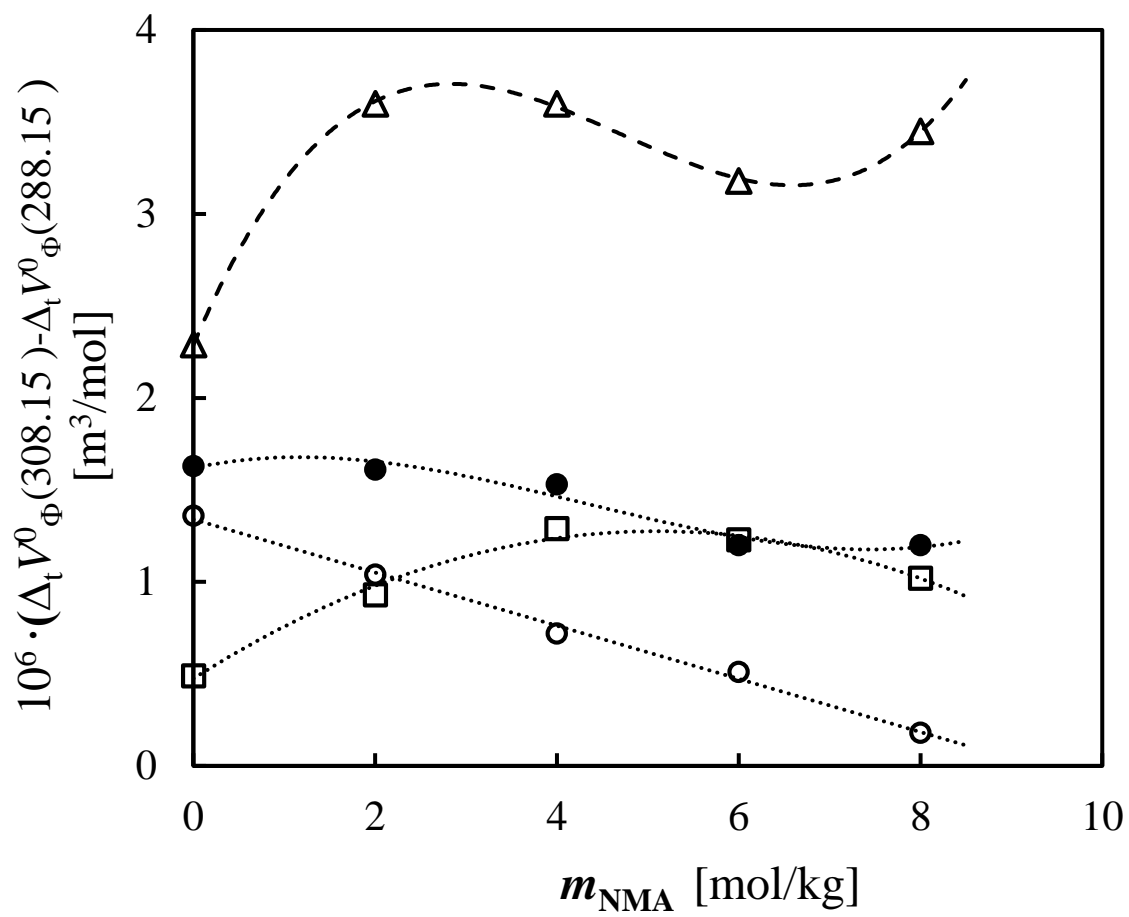


Fig. 7

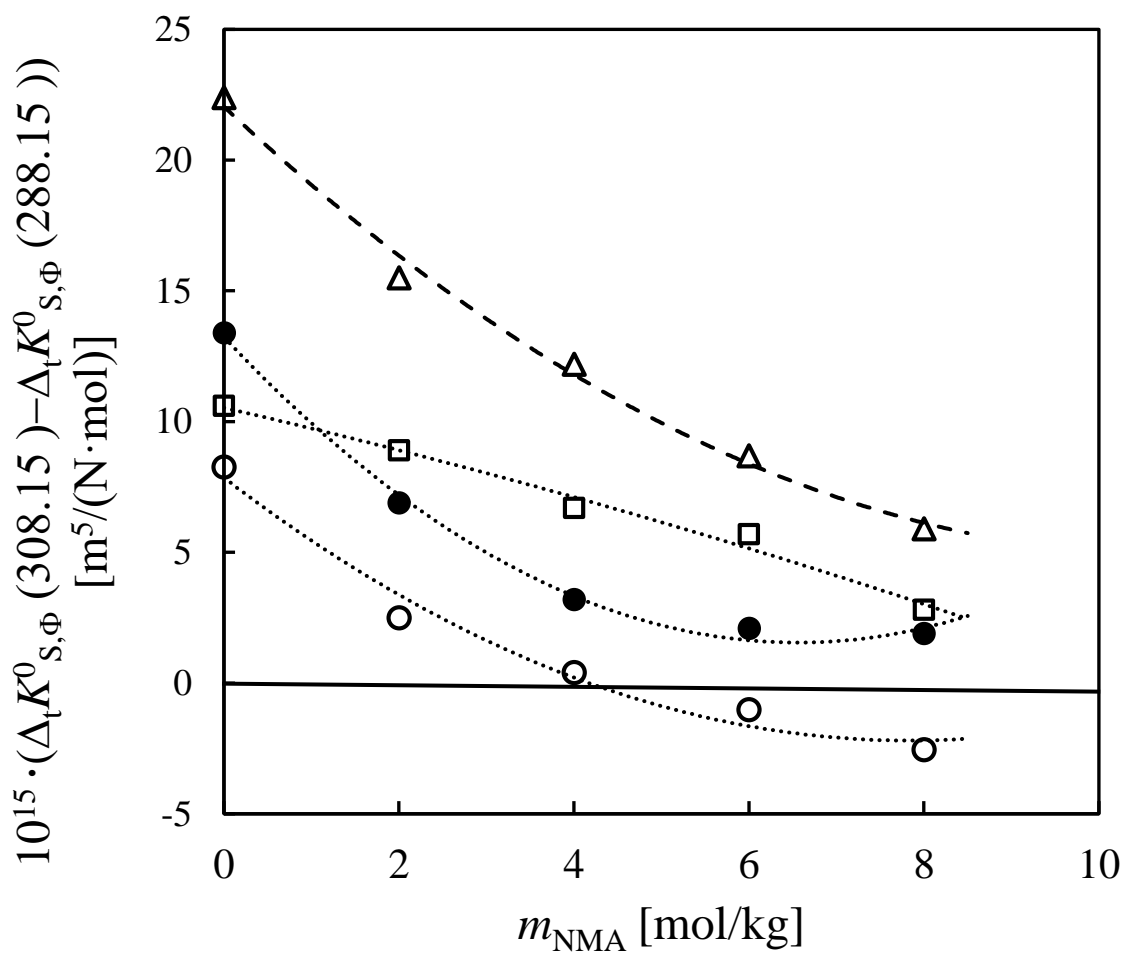




Fig. 8

