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The hydration properties of protein stabilizer, trimethylamine-*N*-oxide in aqueous solutions of *N*-methylacetamide – The volumetric and compressibility studies between 288.15 and 308.15 K

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Apparent molar volumes, V_Φ, and apparent molar isentropic compressions, $K_{S,\Phi}$, of the protein stabilizer, trimethylamine-*N*-oxide (TMAO) were determined from the densities and speed of sound measured at T = (288.15, 298.15 and 308.15) K in aqueous solutions of *N*-methylacetamide (NMA) at four different concentrations (2, 4, 6 and 8 mol/kg). The concentration dependencies of the calculated quantities extrapolated to the infinite dilution lead to the standard partial molar properties. The latter values were combined with the previously published data for TMAO in pure water, to obtain the partial molar properties of transfer from water to aqueous NMA solutions. From the transfer data the interaction parameters were determined according to the McMillan-Mayer theory formalism. The calculated parameters and their temperature characteristics are discussed in terms of solute-solvent, solute-solute and solute-cosolute interactions and compared with analogous data for protein denaturant, n-butylurea.

KEYWORDS: trimethylamine-*N*-oxide; apparent molar volume; apparent molar isentropic compression; *N*-methylacetamide; protein model

1. Introduction

This article is a continuation of our systematic study on the acoustic and volumetric properties of low molecular weight molecules influencing the behaviour of protein. *N*-methylacetamide has been chosen to mimic specific aspects of protein features [1,2]. This compound is a model of peptide bond from backbone chain of the polypeptide. Such approach has been applied previously by us [3] and by other research teams [2,4]. Our latest study examined the properties of solutions of n-butylurea, which is an effective protein denaturant [5]. In the present work, the ternary system with TMAO was investigated. This osmolyte stabilizes proteins against the thermal and pressure induced denaturation and counteracts the denaturing effect of urea [6–8]. TMAO is a small compact molecule with hydrophilic and hydrophobic groups at its opposite ends and strongly interacts with water [9–15]. It is a great interest to establish the mechanism of the influence of the osmolyte on protein stability. The studies for binary systems (osmolyte in water) do not allow to point out the specific parameter which can qualify the solute as stabilizer or denaturant. However, our previous and presented volumetric and acoustic studies for ternary systems seem to indicate the possibility to examine the impact of the osmolyte on protein stabilizing at moderate conditions.

In current work, the apparent molar volume, V_{Φ}, isentropic compressibility, κ_S, and apparent molar isentropic compression, $K_{S,\Phi}$, were calculated for TMAO in aqueous solutions of NMA (at concentration of 2, 4, 6 and 8 mol/kg) using measured densities and speed of sound at T = (288.15, 298.15 and 308.15) K. The standard partial molar volumes and compressions were estimated by extrapolation of the apparent molar quantities to infinite dilution. The standard partial molar volume and compression, $\Delta_t V_{\Phi}^0$ and $\Delta_t K_{S,\Phi}^0$, of transfer of TMAO from water to aqueous solutions of NMA were determined and used to estimate the interaction parameters according to the McMillan-Mayer theory formalism [16].

The obtained parameters and their temperature characteristics are discussed in terms of alteration of hydration phenomena, solute-solvent, solute-solute and solute-cosolute interactions and compared with the analogous parameters for n-butylurea (i.e. strong destabilizer of protein [3,5]).

2. Experimental

2.1. Materials

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Source and grade of the chemicals employed in this work are presented in Table 1. *N*-methylacetamide was used as received, without further purification. Trimethylamine-*N*-oxide was dried at the temperature of 323 K under reduced pressure to obtain the anhydrous compound. The effectiveness of drying was determined by measurement of the melting temperature and was continued until the substance melted at the temperature of 503.5 K [17]. Water for solution preparation was distilled and then degassed by boiling for about 20 minutes at reduced pressure.

The solutions were prepared by weight using an analytical balance (RADWAG XA 60/220, Poland) with the precision 0.1 mg. The combined accuracy of the molality determination was around 0.0030 $mol \cdot kg^{-1}$.

2.2. Density and speed of sound

The densities of the solutions and the speeds of sound were measured using Anton Paar DSA 5000 M densimeter at the atmospheric pressure (0.100±0.0025 MPa). The combined accuracy for a single measurement of the density and speed of sound are $3 \cdot 10^{-2}$ $kg \cdot m^{-3}$ and 0.5 $m \cdot s^{-1}$, respectively. The values of speed of sound in studied systems were measured at the frequency of 3 MHz. The instrument was equipped with the Peltier-type thermostating unit and temperature was kept constant at (288.15, 298.15 and 308.15) K with accuracy of 0.01 K.

In the densimeter and sound speed cells, an adjustment procedure was performed before the measurements with ultra-pure Type 1 degassed water and air at 293.15 K and at 0.1002 MPa pressure. The reference data of water densities [18] and speed of sound [19] were taken from literature.

3. Results

3.1. Density and speed of sound

The density (*d*) and the speed of sound (*u*) data obtained for solutions of trimethylamine-*N*-oxide in aqueous solutions of *N*-methylacetamide (NMA) at the temperature ranging from (288.15 to 308.15) K are shown in Table 2. The exact concentrations of NMA in water in studied systems were: 1.997, 4.004, 6.048 and 8.054 mol per one kilogram of pure water. In the descriptions in the tables and in the figures these values have been rounded to 2, 4, 6 and 8 for the display purposes only. For the calculations (see below) the exact numbers have been taken.

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The increase in the density of the solution and the speed of sound with the increase in the amount of dissolved solute (TMAO) is observed in all studied systems. However, as the concentration of NMA increases this effect is less pronounced for the speed of sound and becomes more evident for the density.

3.2. Isentropic compressibility

Addition of a non-electrolyte to water results in the decrease in compressibility. The Laplace equation:

$$\kappa_S = 1/(u^2 d) \tag{1}$$

has been used to obtain the isentropic compressibility of solvent, $\kappa_{S,0}$, and solution, κ_S, from the measured density, d, and the speed of sound, u, data. In the studied range of concentration, the compressibility of solution decreases linearly in accordance with the relation:

$$\kappa_S = \kappa_{S,0} + A_\kappa m \tag{2}$$

where m (mol·kg^{-1}) is the molality of a solution and A_κ is the empirical coefficient. The parameters of the Eq. (2) are presented in Table 3.

3.3. Apparent molar volume and apparent molar isentropic compression

The apparent molar volume, V_Φ (m^3·mol^{-1}), describes the change of the volume of the system related to introduction of 1 mol of a solute to the solution. The V_Φ values were calculated from experimental density data using the equation:

$$V_\Phi = M_2 / d - (d - d_0)/(m d d_0) \tag{3}$$

where d_0 (kg·m^{-3}) is the density of a solvent (i.e. the aqueous solution of NMA); and M_2 (kg·mol^{-1}) is the molar mass of the solute (TMAO).

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The apparent molar volumes change with concentration of TMAO and the linear function of molality, *m*:

$$V_{\Phi} = V_{\Phi}^{0} + S_V m \tag{4}$$

describes the relation in the studied systems at all measured temperatures. The least squares fitting permits to obtain the experimental coefficient, S_v, and the limiting apparent molar volume of the solute, V_{Φ}^{0}, which is equal to the partial molar volume, $\bar{V}^{0}$. The calculated V_{Φ} data for the solutions with 4 moles of NMA/kg are shown in Fig. 1 as an example. The obtained parameters of the Eq. (4) are presented in Table 4.

The presence of the solute changes noticeably the compression of the liquid. Density and ultrasound speed measurements were combined to calculate the apparent molar isentropic compression, $K_{S,\Phi}$($m^5 \cdot mol^{-1} \cdot N^{-1}$):

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

For all studied systems, the obtained apparent molar isentropic compression changes with the concentration of TMAO (an example in Fig. 2) and the linear function of the concentration at all measured temperatures was used to describe this relation:

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

$K_{S,\Phi}^{0}$ parameter in the Eq.(6) is the limiting apparent isentropic compression (equal to the standard partial molar compression) and the $S_{K,S}$ factor is the experimental slope. The parameters of the Eq. (6) are collected in Table 3.

3.4. Standard partial molar quantities of transfer

In the solution at infinite dilution the solute-solute interactions are not present and the difference between the apparent molar volumes in NMA aqueous solutions and in pure water reflects the influence of cosolute on TMAO hydration. The standard transfer partial molar

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volumes, $\Delta_t V_\Phi^0$, of TMAO from water to aqueous solutions of NMA have been determined from the data of V_Φ^0 provided in Table 4 and the literature data [13], according to the relation:

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

The obtained $\Delta_t V_\Phi^0$ values are shown in Table 5. As it can be seen, all the data of $\Delta_t V_\Phi^0$ are negative and their variation with the concentration of NMA and temperature is presented in Fig. 3 .

The values of the standard transfer molar compressions, $\Delta_t K_{S,\Phi}^0$, from water to aqueous solution of NMA have been calculated using the analogous relation:

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

The $\Delta_t K_{S,\Phi}^0$ data are presented in Table 5. All obtained $\Delta_t K_{S,\Phi}^0$values are positive and the influence of the concentration of NMA and temperature on $\Delta_t K_{S,\Phi}^0$ is shown in Fig. 4.

3.5. Volumetric and compression interaction parameters

The McMillan-Mayer theory formalism of solutions formulates the methodology of identification of the contributions, to the total measured solvation effect, originating from interactions in clusters (pair, triplet etc.) of solute-cosolute molecules [16,20,21]. The interaction parameters are calculated from transfer properties by the following equation:

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

where A and B refer to trimethylaminne-*N*-oxide and NMA, respectively and y_{AB}, y_{ABB}, y_{ABBB} are the pair, triplet and quartet interaction parameters, respectively. For the partial molar volume the thermodynamic transfer function can be expressed as:

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

For the partial molar isentropic compression the equation is analogous:

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

The parameters of the Eqs 10 and 11 have been calculated using the least squares method and are presented in Table 6. As it can be seen, the pair volumetric interaction parameter is negative at all measured temperatures, whereas the triplet volumetric parameter is positive with the absolute value smaller than the v_{AB} value. In the case of the compression interaction parameters, both quantities (i.e. the pair interaction and triplet interaction parameter) are positive. These effects are presented in Figs 5 and 6.

The standard deviations, σ, were calculated for all relations as follows: $\sigma = \left(\sum\left(Y_{cal} - Y_{exp}\right)^2 / f\right)^{1/2}$ where: Y_{exp} is the value obtained experimentally; Y_{cal} is the value calculated from fitted equation and f is the number of degrees of freedom. The parameters of the discussed equations collected in the tables 3-6 are shown with the uncertainties determined for 0.95 level of confidence accordance to the least-squares linear regression or multiple regression.

4. Discussion

The hydration of TMAO was analysed with variety of methods [9–12,14] which have shown that oxygen atom in carbonyl moiety strongly interacts with water molecules. This osmolyte in pure water has relatively big apparent molar volume, moderate negative compression and both quantities, the apparent molar volume and the apparent molar compression, decrease slightly with concentration of TMAO [13].

The volumetric and acoustic parameters of TMAO in aqueous NMA solutions are to some extent different than those present in pure water.

The limiting apparent molar volume of TMAO in aqueous NMA decreases as the concentration of NMA increases and the value is smaller than this obtained for TMAO in pure water. As a result, the standard partial molar volume, $\Delta_t V_\Phi^0$, of transfer of TMAO from water [13] to aqueous solutions of NMA is lower than zero and becomes even more negative as the concentration of NMA increases. This effect is caused by the influence of NMA on the hydration sphere of TMAO (as compared to solution in pure water). It seems that the presence of the cosolute does not allow formation of voluminous hydrophobic hydration sphere in the vicinity of the group of three methyl moieties from TMAO molecule. The same effect is observed for BU in NMA aqueous solutions [3] but it reveals different characteristic as a

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function of amount of NMA, i.e. the biggest volumetric effect is observed when BU is transferred from water to the aqueous solution of NMA at the lowest measured concentration and systematically decreases to zero as the concentration of NMA increases.

The contribution of the pair and triplet interactions to the total volumetric effect is shown in Fig. 5. As it can be seen, the solute-cosolute pair interaction has dominant negative impact on $\Delta_t V_\Phi^0$ and the solute-cosolute triplet interactions lead to positive volume effect for the transfer of TMAO from water to aqueous solution of NMA.

The influence of temperature on the limiting apparent molar volume of TMAO is consistent with the expectation, i.e. V_Φ^0 increases as temperature rises in all studied systems, but the change of V_Φ^0 with temperature in measured interval of 20 deg seems to be independent of the NMA concentration. Opposite effect was observed for BU in NMA aqueous solutions [3]. The biggest changes of the limiting apparent molar volume with temperature are observed for the solution with the lowest dissolved amount of NMA and they clearly decrease~~s~~ as the concentration of NMA increases. However, the influence of temperature on V_Φ^0 is always bigger in aqueous solution of NMA than in pure water (i.e. for TMAO and BU).

The solute-solute interactions influence on the solute solvation and determine the character of changes of V_ϕ with concentration. The V_ϕ value for TMAO changes with its concentration only slightly and in general decreases as the amount of the solute increases. In essence, this phenomenon is not sensitive to temperature alteration and does not change with the NMA concentration. The S_v parameters for TMAO in pure water are small and negative [13], similarly to the S_v values in NMA solutions and they do not change with temperature.

The acoustic characteristic of the investigated systems is much more sensitive to the changes of concentration of both solute and cosolute as well as temperature. Addition of a non-electrolyte to water results in the decrease in compressibility. The presence of the cosolute in aqueous solution causes smaller reduction of the compressibility with the concentration of the solute. The higher concentration of NMA is, the weaker influence of TMAO on compressibility is noticed, the A_κ parameter from the Eq. 2 is closer to zero. However, these effects (especially the impact of the NMA concentration) are much stronger for BU in NMA aqueous solutions [3] than for the studied systems with TMAO.

As it was mentioned above, the apparent molar compression of TMAO in water has moderate negative value and it has been determined that this quantity stays negative for most of the studied systems except for the series in 8M NMA solution at 298.15 and 308.15 K. The calculated values of apparent molar compression change slightly with the concentration of

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TMAO and, in general, increase with the concentration of the solute. The small impact of the TMAO concentration on $K_{S,\Phi}$ was also reported for a binary system (pure water-TMAO) but the trend was opposite ($S_{K,S}$ has small negative value in pure water [13]). The obtained $K_{S,\Phi}$ data in NMA solutions are always higher than those in pure water and increase markedly as the concentration of NMA increases. However, this effect is a few times smaller than for the analogous system for BU [3]. In our opinion, the most important difference between the systems with TMAO and BU lies in the fact that $K^0_{S,\Phi}$ for trimethylamine-*N*-oxide does not change its sign in the presence of NMA, as compared to $K^0_{S,\Phi}$(TMAO) in pure water, and stays negative (for most of the systems from present work).

The rise of temperature brings the increase in the apparent molar compression of TMAO, but this trend decreases as the concentration of NMA increases. The same effect is noticed for BU in NMA solutions but again the change of $K^0_{S,\Phi}$ associated with the increase in NMA concentration is clearly bigger for BU [3] than for TMAO.

The determined compression interaction parameters show that both solute-cosolute interaction parameters k_{AB}, k_{ABB} are positive and the pair interactions are dominant and much stronger than the triplet interactions. Moreover, as it can be seen in Fig. 6, the triplet interactions are practically negligibly small in 2, 4 and 6 M solutions of NMA.

5. Conclusions

The calculated volumetric and acoustic quantities clearly reveal the impact of the presence of NMA on TMAO parameters and show prominent differences as compared to the data obtained for BU in aqueous solution of NMA. TMAO is one of the best known protein structure stabilizers, whereas BU is a strong denaturing agent [5].

The distinctive feature of TMAO uncovered in the present study is the scarce influence of the concentration of this solute on its apparent molar quantities in all studied systems (i.e. at all used concentrations of NMA and at all measured temperatures). Deeper investigation shows relatively low impact of temperature on investigated parameters as compared to the solutions of BU. The partial molar volume of TMAO in infinitely diluted solutions with NMA as a cosolute is always notably smaller than in pure water and it does not vary much with the concentration of amide. In our opinion, the most important difference between the systems with TMAO and BU lies in the fact that $K^0_{S,\Phi}$ for trimethylamine-*N*-oxide does not change its sign in the presence of NMA, as compared to $K^0_{S,\Phi}$ (TMAO) in pure water, and stays negative (for most

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of the systems from present work). The negative value of the molar compression means that the compression of the solvent is bigger than the compression of the solution. This effect can impede the change of the conformation of the protein molecule as compared to the system containing pure solvent or to the protein solution in presence of the solute with positive $K_{S,\Phi}^{0}$ (e.g. BU) [3]. Our supposition is that the negative value of the apparent molar compression decreases the possibility of the conformation change of the protein molecule and thus increases stability of its native state.

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

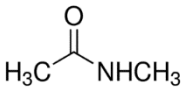
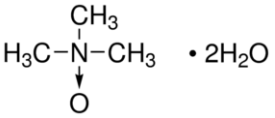
Chemical	Chemical formula	Chemical structure	Source	Purity	CAS number
N-methylacetamide (NMA)	C_3H_7NO		Aldrich Chemicals	≥99%	79-16-3
N,N,N-Trimethylamine *N*-oxide dihydrate (TMAO)	$(CH_3)_3NO{\cdot}2H_2O$		Fluka	≥99%	62637-93-8

Table 2. Densities, *d,* and speed of sound, *u,* of solutions of TMAO in aqueous solution of *N*-methylacetamide (2, 4, 6 and 8 mol/kg) at different temperatures and at pressure p = 0.10 MPa[a].

T/K	288.15	298.15	308.15	288.15	298.15	308.15
m /(mol·kg^{-1})		d/(kg·m^{-3})			u/(m·s^{-1})	
in 2 mol/kg of NMA						
0	1000.62	997.66	993.91	1555.4	1569.4	1578.4
0.1466	1001.20	998.20	994.42	1566.7	1579.5	1587.5
0.2358	1001.56	998.53	994.72	1573.4	1585.5	1592.9
0.2765	1001.73	998.69	994.87	1576.7	1588.4	1595.5
0.3158	1001.89	998.84	995.01	1579.8	1591.1	1598.0
0.3468	1002.00	998.94	995.11	1581.8	1593.0	1599.7
in 4 mol/kg of NMA						
0	1003.65	999.66	995.01	1618.4	1619.6	1617.7
0.1435	1004.34	1000.28	995.60	1628.9	1629.1	1626.3
0.1833	1004.53	1000.46	995.77	1632.0	1631.8	1628.8
0.2318	1004.76	1000.66	995.96	1635.5	1635.0	1631.6
0.2728	1004.94	1000.84	996.13	1638.4	1637.6	1634.0
0.3118	1005.13	1001.01	996.29	1641.3	1640.2	1636.4
0.3426	1005.27	1001.14	996.41	1643.4	1642.1	1638.1
in 6 mol/kg of NMA						
0	1 006.41	1 001.47	996.03	1660.1	1652.0	1642.0
0.1447	1 007.12	1 002.13	996.66	1670.0	1660.9	1650.1
0.2326	1 007.55	1 002.53	997.04	1675.7	1666.1	1654.8
0.2730	1 007.74	1 002.70	997.20	1678.3	1668.5	1657.1
0.3125	1 007.93	1 002.88	997.37	1680.9	1670.9	1659.2

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0.3426	1 008.06	1 003.00	997.49	1682.8	1672.6	1660.8
in 8 mol/kg of NMA						
0	1008.43	1002.74	996.66	1685.5	1670.8	1654.9
0.1441	1009.08	1003.36	997.27	1693.8	1678.5	1662.1
0.1833	1009.27	1003.54	997.44	1696.1	1680.6	1664.1
0.2317	1009.49	1003.75	997.64	1698.9	1683.2	1666.5
0.2716	1009.67	1003.92	997.81	1701.2	1685.3	1668.5
0.3111	1009.85	1004.09	997.97	1703.5	1687.4	1670.4
0.3414	1009.99	1004.22	998.10	1705.3	1689.1	1671.9

[a] Combined extended uncertainties calculated at 0.95 lever of confidence U are U(d)= $3\cdot10^{-2}$ kg·m^{-3}, U(u)= 0.5 m·s^{-1}, U(T) = 0.01K, U(m) = 0.0030 mol·kg^{-1} and U(p) = 0.0025 MPa

Table 3. Parameter (A_κ) of Eq. (2), and parameters of Eq. (6) for TMAO in aqueous solutions of NMA at different temperatures and at pressure p = 0.1MPa. Standard deviation (σ) is presented for discussed relations.

T/K	m_{NMA}/ (mol·kg^{-1})	$10^{15}\cdot K^0_{\Phi}$/ (m^5·(N·mol)$^{-1}$)	$10^{15}\cdot S_K$ / (kg·m^5·N^{-1}·mol^{-2})	$10^{16}\cdot\sigma$/ (m^5·(N·mol)$^{-1}$)	$10^{11}\cdot A_k$/ (kg·m^2·(N·mol)$^{-1}$)	$10^{14}\sigma$ m^2·N^{-1}
288.15	2	-13.3±0.054	1±2.0	3.1	-4.15±0.018	11
298.15	2	-8.3±0.34	1±1.3	1.3	-3.63±0.013	8
308.15	2	-4.4±0.33	0.4±1.2	1.9	-3.24±0.011	7
288.15	4	-9.4±0.26	0.5±1.1	1.7	-3.53±0.011	7
298.15	4	-5.7±0.13	0.4±0.50	0.9	-3.200±0.009	6
308.15	4	-2.7±0.18	0.2±0.68	1.2	-2.932±0.008	5
288.15	6	-6.7±0.16	4.5±0.58	0.9	-3.02±0.016	10
298.15	6	-3.5±0.14	2.6±0.51	0.8	-2.80±0.012	7
308.15	6	-1.0±0.17	2.0±0.63	1.0	-2.62±0.010	6
288.15	8	-0.71±0.071	-1.2±0.28	0.5	-2.517±0.003	1.4
298.15	8	0.90±0.070	-0.8±0.28	0.5	-2.408±0.004	2.0
308.15	8	2.1±0.10	0.0±0.39	0.7	-2.336±0.006	3.5

Table 4. Parameters (S_v and V^0_{Φ}) of Eq. (4) for TMAO in aqueous solutions of NMA at different temperatures. Standard deviation (σ) is presented for discussed relation.

T/K	m_{NMA}/ (mol·kg^{-1})	$10^6\cdot V^0_{\Phi}$/ (m^3·mol^{-1})	$10^6\cdot S_v$/ (kg·m^3·mol^{-2})	$10^8\cdot\sigma$ / (m^3·mol^{-1})
288.15	2	71.13±0.044	-0.5±0.16	2.5
298.15	2	71.64±0.053	-0.5±0.19	3.0
308.15	2	72.06±0.042	-0.2±0.16	2.4

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288.15	4	70.06±0.016	0.00±0.06	1.1
298.15	4	70.84±0.025	-0.3±0.10	1.7
308.15	4	71.35±0.023	-0.21±0.09	1.5
288.15	6	69.71±0.035	0.1±0.13	2.0
298.15	6	70.37±0.038	0.1±0.14	2.2
308.15	6	70.94±0.031	0.2±0.12	1.7
288.15	8	70.04±0.038	-0.5±0.15	2.5
298.15	8	70.60±0.037	-0.4±0.14	2.4
308.15	8	71.06±0.019	-0.15±0.072	1.8

Table 5. Standard partial molar volume of transfer, $\Delta_t V_{\Phi}^{0}$, and standard molar compression of transfer, $\Delta_t K_{S,\Phi}^{0}$, of TMAO 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^{-1})		$10^6 \cdot \Delta_t V^0_{\Phi}$/ (m^3·mol^{-1})			$10^{15} \cdot \Delta_t K^0_{\Phi}$/ (m^5·(N·mol)$^{-1}$)	
2	-1.94±0.06	-1.68±0.07	-1.50±0.06	3.6±0.2	1.8±0.6	1.9±0.6
4	-3.01±0.04	-2.48±0.05	-2.21±0.05	7.5±0.4	4.3±0.4	3.6±0.4
6	-3.36±0.06	-2.95±0.06	-2.62±0.06	10.1±0.4	6.6±0.4	5.35±0.4
8	-3.03±0.06	-2.72±0.06	-2.50±0.04	16.2±0.2	11.0±0.3	8.4±0.3

Table 6. Interaction parameters for Eq. (10) and (11) at different temperatures.

T/K	$10^6 \cdot v_{AB}$ / (kg·m^3·mol^{-2})	$10^6 \cdot v_{ABB}$ / (kg^2·m^3·mol^{-3})	$10^{15} \cdot k_{AB}$ / (kg·m^2·(N·mol)$^{-1}$)	$10^{15} \cdot k_{ABB}$ / (kg^2·m^2·(N·mol^2)$^{-1}$)
288.15	-0.578±0.009	0.033±0.001	0.87±0.09	0.01±0.01
298.15	-0.49±0.02	0.027±0.003	0.38±0.05	0.023±0.006
308.15	-0.43±0.02	0.024±0.003	0.44±0.05	0.004±0.006

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LEGEND TO THE FIGURES

FIG. 1. Apparent molar volume, V_{Φ}, against the concentration m for TMAO in aqueous NMA solution (4 mol/kg) at 288.15 K (●), 298.15 K (▲) and 308.15 K (×).

FIG. 2. Apparent molar isentropic compression, $K_{S,\Phi}$ against the concentration m for TMAO in aqueous NMA solution (4 mol/kg) at 288.15 K (●), 298.15 K (▲) and 308.15 K (×).

FIG. 3. Apparent molar volume of transfer for TMAO, $\Delta_t V_{\Phi}^0$, from water to aqueous solution of NMA against the concentration m_{NMA} of NMA (mol/kg of water) at 288.15 K (●), 298.15 K (▲) and 308.15 K (×).

FIG. 4. Apparent molar isentropic compression of transfer for TMAO, $\Delta_t K_{S,\Phi}^0$ from water to aqueous solution of NMA against the concentration m_{NMA} of NMA (mol/kg of water) at 288.15 K (●), 298.15 K (▲) and 308.15 K (×).

FIG. 5. Apparent molar volume of transfer for TMAO, $\Delta_t V_{\Phi}^0$, from water to aqueous solution of NMA against the concentration m_{NMA} of NMA (mol/kg of water) at 288.15 K (●) and the contribution of pair (×) and triplet (□) interactions parameters.

FIG. 6. Apparent molar isentropic compression of transfer for TMAO, $\Delta_t K_{S,\Phi}^0$ from water to aqueous solution of NMA against the concentration m_{NMA} of NMA (mol/kg of water) at 288.15 K (●) and the contribution of pair (×) and triplet (□) interaction parameters.

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Fig. 1

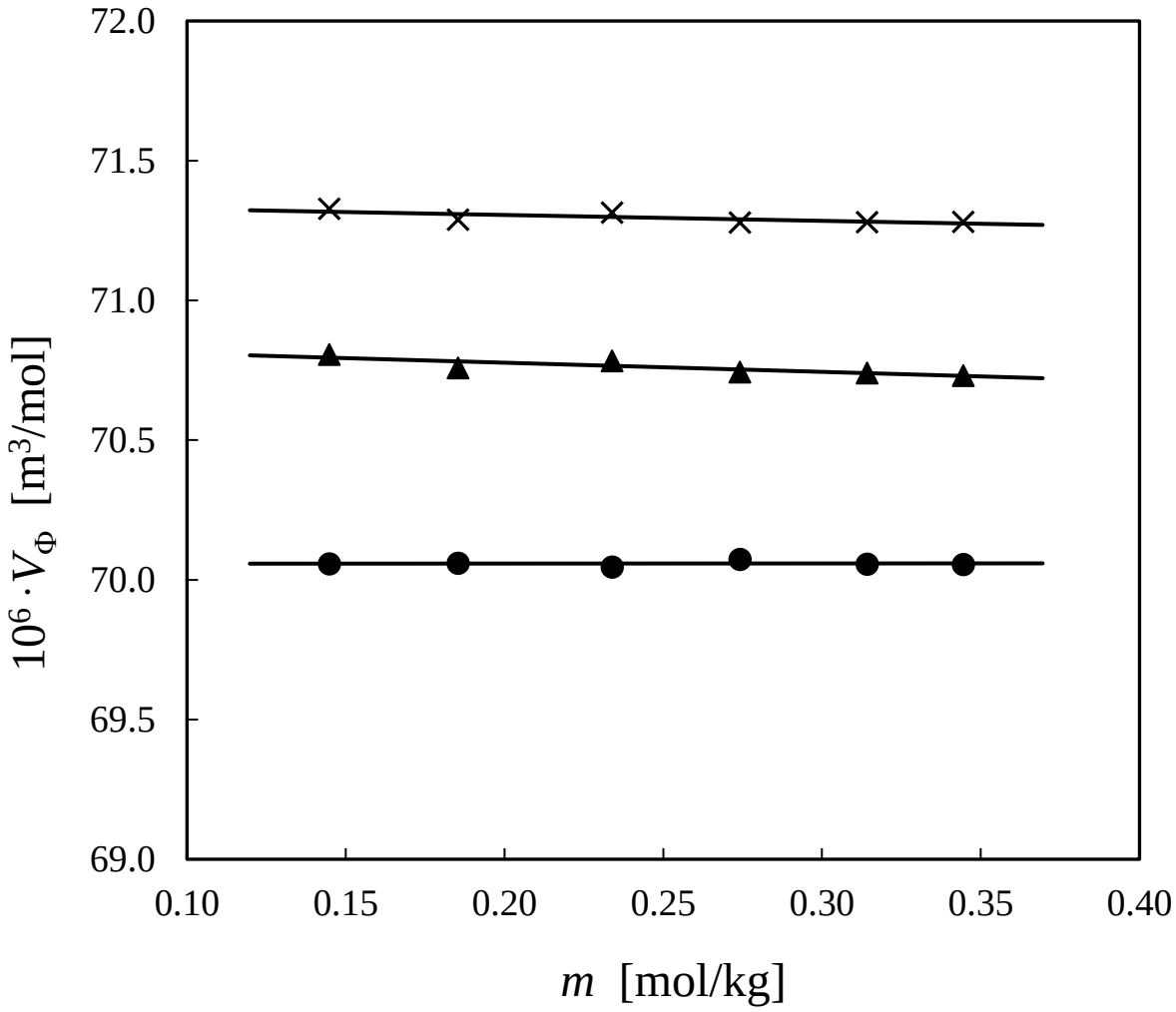

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Fig. 2

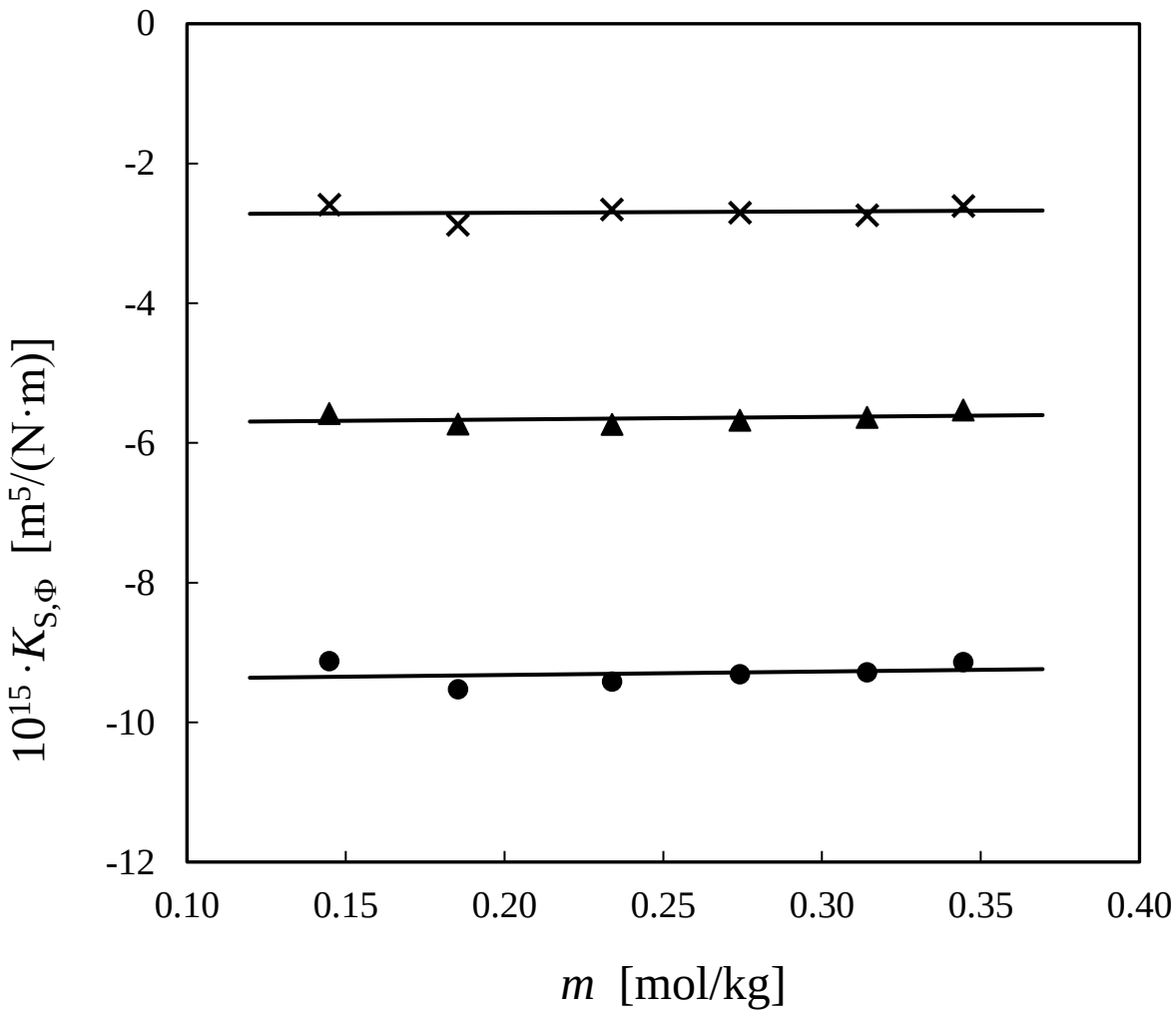

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Fig. 3

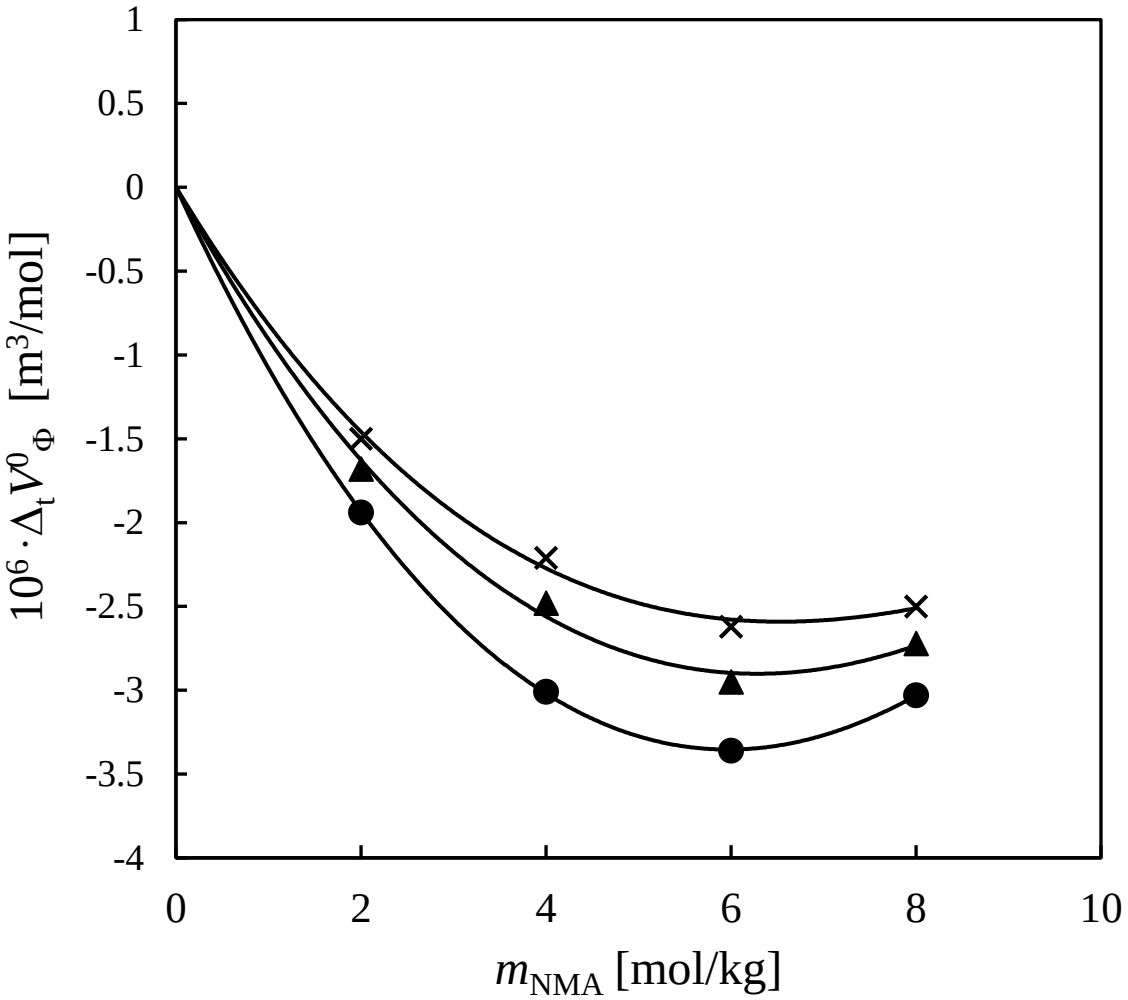

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Fig. 4

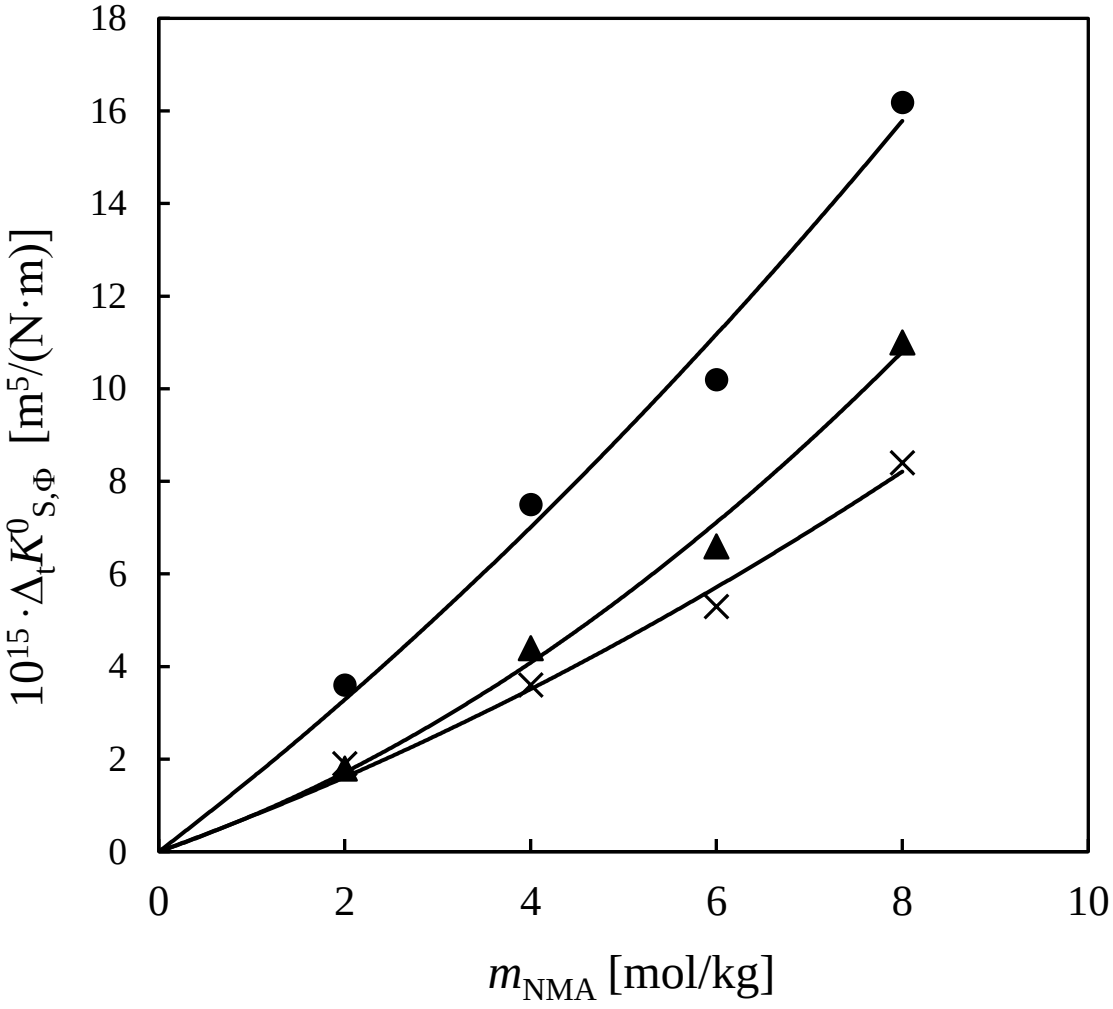

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Fig. 5

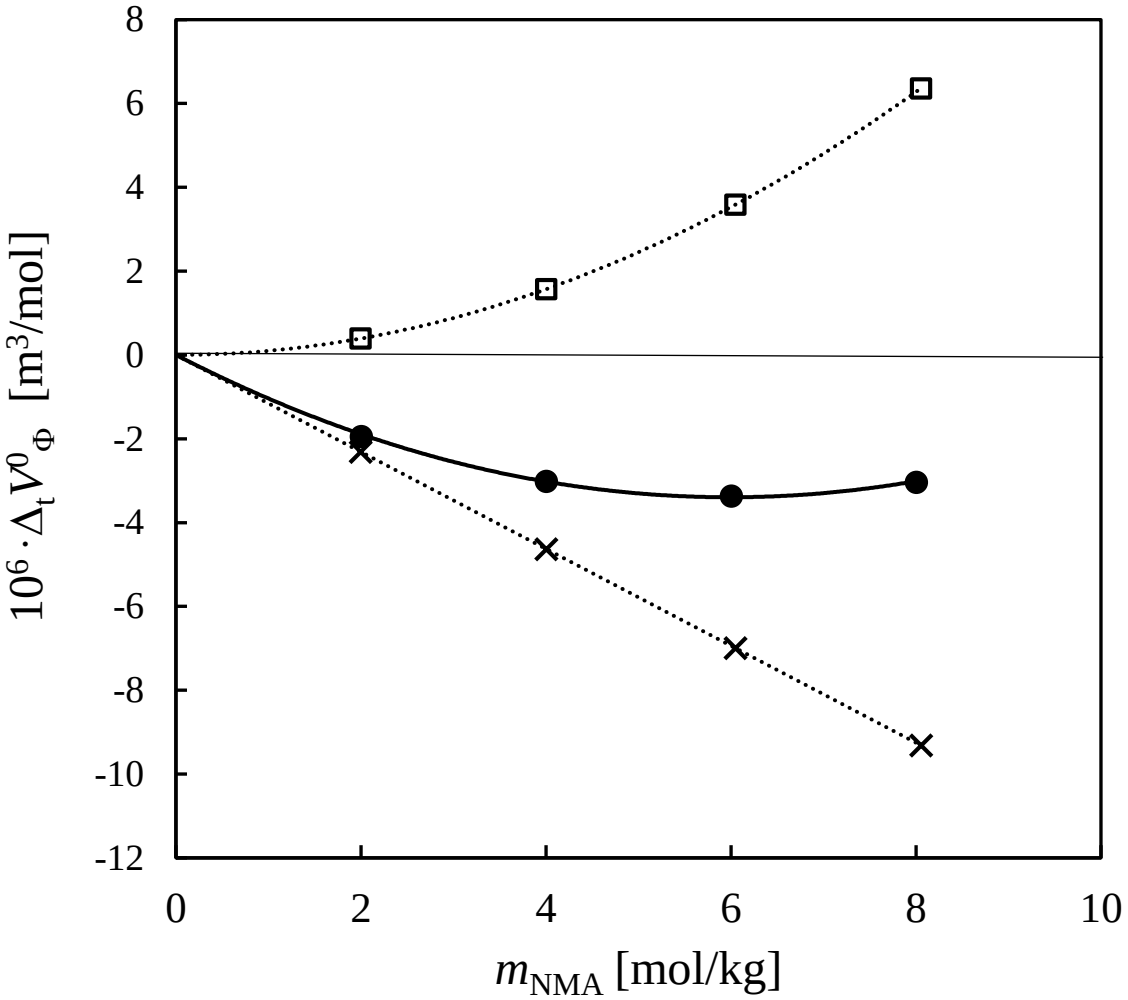

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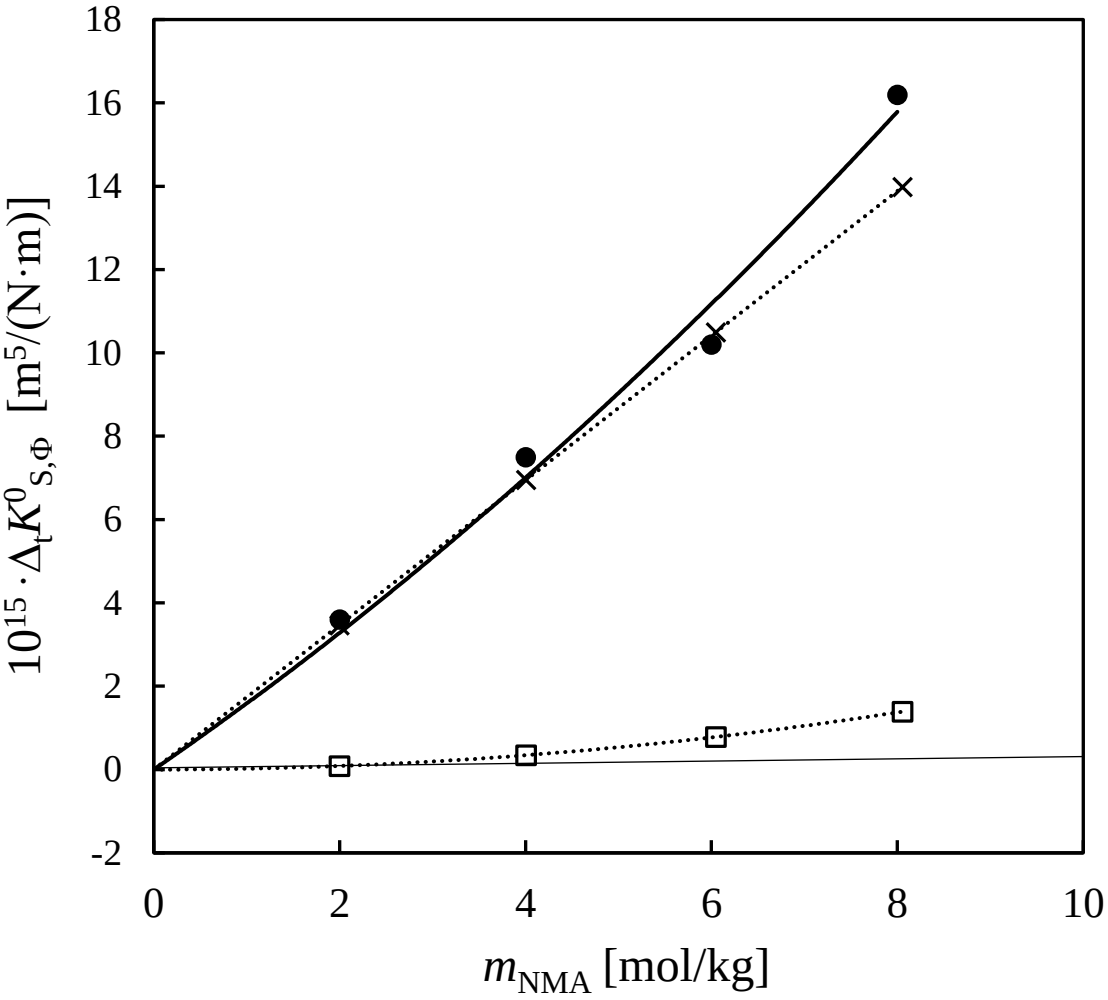
Fig. 6



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