

Density and viscosity of α -amino acids in aqueous solutions of cetyltrimethylammonium bromide

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The densities and viscosities of aqueous solution of cetyltrimethylammonium bromide (0.01 mol kg^{-1}) (CTAB) and solutions of CTAB containing amino acids, viz., glycine, L-serine, and L-valine (0.01 – 0.05 mol kg^{-1}), were determined in the temperature range 298.15 – 313.15 K . Apparent molar volumes of the amino acids were calculated from the density and viscosity values. The calculated apparent molar volumes were used to calculate standard partial molar volumes (\bar{V}_2^0) and standard partial molar volumes of transfer of amino acids from water to an aqueous solution of CTAB. The viscosity values were used for the calculation of the viscosity coefficients A and B in the Jones–Dole equation. The linear dependences of \bar{V}_2^0 and B on the number of carbon atoms in the alkyl chains of the amino acids were found. The results obtained were used in analysis of hydrophilic-hydrophilic, hydrophilic-hydrophobic, and hydrophobic-hydrophobic interactions that occur during dissolution of amino acids in an aqueous solution of CTAB.

Key words: amino acids, cetyltrimethylammonium bromide, standard partial molar volume, viscosity coefficients, hydrophobic-hydrophobic interactions, hydrophilic-hydrophobic interactions, hydrophilic-hydrophilic interactions.

The stable globular native conformation of proteins results from the delicate balance established due to interactions between various groups in protein molecules. The character of these interactions depends on the pH of medium, temperature, and the presence of low-molecular-weight substances, such as substrates, coenzymes, inhibitors, and activators that specifically bind to the native structure. The interaction of surfactants with globular proteins attracts much attention of researchers.^{1–6} Studies of these interactions favor understanding the denaturation and solubilization action of surfactants on membrane proteins and lipids. Since conformations and configurations of proteins are complicated, it is more convenient to study these processes in systems modeling proteins, for instance, in aqueous solutions of amino acids (AA) and aqueous solutions of CTAB.

Surfactants at low concentrations in aqueous solutions behave much as normal electrolytes, but in more concentrated solutions they exhibit different behavior. Such behavior is due to the formation of aggregates consisting of many surfactant molecules. At specific concentration known as the critical micelle concentration (CMC), surfactant molecules undergo aggregation to form micelles. For CTAB, the CMC is achieved at $8.2 \cdot 10^{-4} \text{ mol L}^{-1}$ (see Ref. 7). The physicochemical properties of surfactant solutions differ noticeably in solutions with concentrations

higher and lower than the CMC.^{1,2} At concentrations below the CMC, the physicochemical properties of ionic surfactants resemble those of strong electrolytes. At concentrations exceeding the CMC, the properties of surfactants change considerably, indicating the formation of highly cooperative molecular associates.

Volumetric measurements have been reported to be a sensitive tool for analysis of interactions in solutions.⁸ In the present work we evaluated various volumetric parameters, such as apparent molar volumes, partial molar volumes, and volumes of transfer of amino acids from water to an aqueous solution of CTAB. The determined viscosity values were used to estimate the viscosity coefficients of amino acids (A and B in the Jones–Dole equations) in an aqueous solution of CTAB. These data can throw light on the interactions of biomolecules in aqueous CTAB solutions.

Experimental

The following amino acids were used: glycine (Merck, Germany, fraction 99 wt.%), L-serine, and L-valine (Thomas Baker, USA, fractions 98.5 and 99.0 wt.%, respectively). The amino acids were recrystallized from aqueous ethanol, dried *in vacuo* for 72 h, and stored at 20°C in a desiccator over P_2O_5 .

Cetyltrimethylammonium bromide (analytical reagent grade) was additionally purified by recrystallization and then dried

in vacuo. Solutions of Gly, Ser, and Val were prepared in concentrations of 0.01, 0.02, 0.03, 0.04, and 0.05 mol kg⁻¹ in an aqueous solution of CTAB (0.01 mol kg⁻¹). Doubly distilled and deionized water was used for the preparation of fresh solutions. Samples were weighed on a Precisa XB-220A balance (Switzerland) with a precision of ± 0.0001 g.

A single-stem pycnometer (bulb capacity $8 \cdot 10^{-6}$ m³) of Borosil glass equipped with a calibrated capillary and a well-fitted cap was used to measure the densities of solutions at 298.15, 303.15, 308.15, and 318.15 K. Viscosities were measured with a Ubbelohde suspended level viscosimeter. Densities and viscosities were measured with an accuracy to ± 0.01 kg m⁻³ and $\pm 3 \cdot 10^{-6}$ N s m⁻², respectively. Experimental procedures of measuring density and viscosity were described in detail in our earlier papers.⁹

Results and Discussion

Viscosimetric analysis. The densities (ρ) of amino acid solutions of various molality (m) measured at 298.15, 303.15, 308.15, and 313.15 K are given in Table 1. Apparent molar volumes (\bar{V}_2) were calculated from the values of ρ

$$\bar{V}_2 = \frac{M}{\rho} - \frac{1000(\rho - \rho_0)}{m\rho\rho_0}, \quad (1)$$

where M is the molar weight of AA and ρ_0 is the density of an aqueous solution of CTAB.

The calculated apparent molar volumes of the AA are presented in Table 2. It can be seen from Table 2 that \bar{V}_2 is a linear function of molality. As the AA concentration increases, the apparent molar volumes decrease both for Gly and Ser, while Val values follow the opposite trend.

The results obtained are well described by the equation

$$\bar{V}_2 = \bar{V}_2^0 + S_v^* m^{1/2}, \quad (2)$$

where \bar{V}_2^0 is the standard partial molar volume of AA corresponding to the apparent molar volume at infinite dilution; and S_v^* is the experimental slope.

The respective data are presented in Table 3. At infinite dilution there are no solute–solute interactions and only the solute–solvent interactions are observed. Thus, the value of \bar{V}_2^0 characterizes the solute–solvent interactions, whereas parameter S_v^* can serve as a quantitative estimate of interactions between solutes. The high positive values of \bar{V}_2^0 are observed for all the three AA at all temperatures studied, indicating the presence of strong solute–solvent interactions in this system. The values of \bar{V}_2^0 increase in the following order: Gly < Ser < Val.

An increase in \bar{V}_2^0 on going from Gly to Val is attributed to the enhancement of hydrophobicity of the side chain as the H atom of glycine is replaced by a hydrophobic

Table 1. Density (ρ) and viscosity (η) of glycine (Gly), serine (Ser), and valine (Val) in aqueous solutions of CTAB at different temperatures

m^* /mol kg ⁻¹	ρ /kg m ⁻³				η /N s m ⁻²			
	298.15 K	303.15 K	308.15 K	313.15 K	298.15 K	303.15 K	308.15 K	313.15 K
Gly + Solution of CTAB (0.01 mol kg ⁻¹)								
0	0.9367	0.9353	0.9338	0.9323	0.8836	0.7923	0.7077	0.6468
0.01	0.9369	0.9354	0.9339	0.9324	0.8855	0.7981	0.7159	0.6571
0.02	0.9371	0.9356	0.9340	0.9325	0.9030	0.8149	0.7329	0.6731
0.03	0.9373	0.9358	0.9342	0.9327	0.9237	0.8345	0.7511	0.6915
0.04	0.9376	0.9360	0.9344	0.9328	0.9465	0.8558	0.7713	0.7111
0.05	0.9378	0.9362	0.9346	0.9330	0.9694	0.8804	0.7936	0.7312
Ser + Solution of CTAB (0.01 mol kg ⁻¹)								
0	0.9367	0.9353	0.9338	0.9323	0.8836	0.7923	0.7077	0.6468
0.01	0.9370	0.9355	0.9340	0.9326	0.8865	0.7958	0.7128	0.6522
0.02	0.9374	0.9359	0.9343	0.9329	0.8913	0.8005	0.7180	0.6573
0.03	0.9377	0.9362	0.9347	0.9332	0.8966	0.8057	0.7234	0.6624
0.04	0.9382	0.9366	0.9351	0.9336	0.9019	0.8111	0.7285	0.6671
0.05	0.9386	0.9371	0.9355	0.9340	0.9071	0.8162	0.7341	0.6725
Val + Solution of CTAB (0.01 mol kg ⁻¹)								
0	0.9367	0.9353	0.9338	0.9323	0.8836	0.7923	0.7077	0.6468
0.01	0.9369	0.9354	0.9340	0.9325	0.8841	0.7949	0.7108	0.6511
0.02	0.9371	0.9356	0.9341	0.9326	0.8881	0.7997	0.7157	0.6564
0.03	0.9372	0.9357	0.9342	0.9327	0.8928	0.8049	0.7210	0.6616
0.04	0.9373	0.9358	0.9343	0.9328	0.8978	0.8102	0.7267	0.6673
0.05	0.9374	0.9359	0.9344	0.9329	0.9026	0.8152	0.7312	0.6733

* Here and in Table 2, m is molality of an amino acid solution in 0.01 m CTAB.

Table 2. Apparent molar volumes (\bar{V}_2) of glycine, serine, and valine in aqueous solutions of CTAB at different temperatures

m /mol kg ⁻¹	$\bar{V}_2 \cdot 10^5$ /m ³ mol ⁻¹			
	298.15 K	303.15 K	308.15 K	313.15 K
Gly + Solution of CTAB (0.01 mol kg ⁻¹)				
0.01	5.9616	6.3108	6.7770	7.0964
0.02	5.8464	6.1382	6.6040	6.9000
0.03	5.7311	6.0036	6.4691	6.7456
0.04	5.6443	5.8784	6.3437	6.6103
0.05	5.5460	5.7799	6.1760	6.4826
Ser + Solution of CTAB (0.01 mol kg ⁻¹)				
0.01	8.0254	8.2618	8.4995	8.6237
0.02	7.6238	7.8018	8.0783	8.2185
0.03	7.3361	7.5322	7.7678	7.8898
0.04	7.1052	7.2816	7.4877	7.6664
0.05	6.9199	7.0614	7.3186	7.3930
Val + Solution of CTAB (0.01 mol kg ⁻¹)				
0.01	10.4530	10.5806	10.6970	10.8150
0.02	10.6222	10.7500	10.8679	10.9803
0.03	10.7612	10.8705	10.9927	11.1037
0.04	10.8758	10.9763	11.0918	11.2080
0.05	10.9485	11.0551	11.1715	11.2701

group CH₂OH in serine. Serine, in turn, is less hydrophobic than valine in which the H atom is replaced by still more hydrophobic group Me₂CHCH. It was also found that the values of \bar{V}_2^0 increase with temperature due to reduced electrostriction, *i.e.*, weakening of deformation of dissolved molecules in the electric field.

The data in Table 3 show that the values of S_v^* are lower than \bar{V}_2^0 . This implies weak interactions between solutes. The decrease in S_v^* with temperature for all the three AA under study indicates that the three amino acids behave as the net-structure breakers in the presence of surfactant (CTAB).

The partial molar volumes of transfer of the amino acids from water to an aqueous solution of CTAB ($\Delta_{tr}\bar{V}_2^0$) are given in Table 3. They were determined from the values of \bar{V}_2^0 by the equation

$$\Delta_{tr}\bar{V}_2^0 = \bar{V}_{2(aq,CTAB)}^0 - \bar{V}_{2(aq)}^0 \quad (3)$$

The values of $\bar{V}_{2(aq)}^0$ are taken from Ref. 10.

Thus, interactions of the following types are observed in an amino acid—aqueous CTAB solution system: (1) hydrophilic-hydrophilic interactions between the zwitterions of AA and the polar group of CTAB; (2) hydrophilic-hydrophobic interactions between the zwitterions of AA and the hydrophobic group of CTAB, as well as between the side alkyl chains of AA and the polar group of CTAB; and (3) hydrophobic-hydrophobic

Table 3. Values of \bar{V}_2^0 , S_v^* , $\bar{V}_{2(aq)}^0$, $\Delta_{tr}\bar{V}_2^0$, and n_H for glycine, serine, and valine in aqueous solutions of CTAB at different temperatures

Parameter	298.15 K	303.15 K	308.15 K	313.15 K
Gly + Solution of CTAB (0.01 mol kg ⁻¹)				
$\bar{V}_2^0 \cdot 10^5$ /m ³ mol ⁻¹	6.3083	6.7457	7.2671	7.5966
$S_v^* \cdot 10^{-5}$ /m ³ mol ^{-3/2} L ^{-1/2}	-3.3546	-4.3157	-4.7313	-4.9484
$\bar{V}_{2(aq)}^0 \cdot 10^5$ /m ³ mol ⁻¹	4.3249*	—	4.3787*	4.4009*
$\Delta_{tr}\bar{V}_2^0 \cdot 10^5$ /m ³ mol ⁻¹	1.9834	—	2.8884	3.1957
n_H	-3.9783	—	—	—
Ser + Solution of CTAB (0.01 mol kg ⁻¹)				
$\bar{V}_2^0 \cdot 10^5$ /m ³ mol ⁻¹	8.9053	9.1961	9.4558	9.6093
$S_v^* \cdot 10^{-5}$ /m ³ mol ^{-3/2} L ^{-1/2}	-8.9667	-9.5937	-9.6954	-9.8483
$\bar{V}_{2(aq)}^0 \cdot 10^5$ /m ³ mol ⁻¹	6.060*	—	6.115*	6.163*
$\Delta_{tr}\bar{V}_2^0 \cdot 10^5$ /m ³ mol ⁻¹	2.8453	—	3.3408	3.4463
n_H	-5.3864	—	—	—
Val + Solution of CTAB (0.01 mol kg ⁻¹)				
$\bar{V}_2^0 \cdot 10^5$ /m ³ mol ⁻¹	10.0476	10.2004	10.3190	10.4479
$S_v^* \cdot 10^{-5}$ /m ³ mol ^{-3/2} L ^{-1/2}	4.0834	3.8548	3.8488	3.7428
$\bar{V}_{2(aq)}^0 \cdot 10^5$ /m ³ mol ⁻¹	9.098*	—	9.1556*	9.1676*
$\Delta_{tr}\bar{V}_2^0 \cdot 10^5$ /m ³ mol ⁻¹	0.9496	—	1.1634	1.2803
n_H	0.5374	—	—	—

* The data are taken from Ref. 10.

interactions between the hydrophobic groups of AA and CTAB.

The results can be interpreted in the light of the cosphere overlap model,¹¹ according to which the effect of overlap of hydration cospheres results is destructive. It was found¹² that the overlap of cospheres of two ionic species increases the volume and the overlap of hydrophobic-hydrophobic and ion-hydrophobic groups results in net decrease in volume. Since the positive values of $\Delta_{tr}\bar{V}_2^0$ were obtained for all the three AA studied, we conclude that interactions of the first type predominate over interactions of the second and third types in this system.

Some researchers¹³ explained the partial molar volume of a non-electrolyte (\bar{V}_2^0) as a combination of the intrinsic volume of the solute and changes in volume due to its interaction with solvent molecules. It was shown¹⁴ that the intrinsic volume (V_{int}) is the sum of the van der

Waals volume of the solute¹⁵ (V_{vw}) and the volume associated with the voids and empty spaces present therein (V_{void})¹⁶

$$V_{int} = V_{vw} + V_{void} \quad (4)$$

The modified variant of Eq. (4) can be used¹⁷ to evaluate the contribution of a solute molecule to $\Delta_{tr}\bar{V}_2^0$

$$\bar{V}_2^0 = V_{vw} + V_{void} - n\sigma_s, \quad (5)$$

where σ_s is the shrinkage in volume due to the interaction of hydrogen bonding groups present in a solute with water molecules and n is the number of sites in the molecule capable of hydrogen bonding.

Therefore, the value of \bar{V}_2^0 of an AA molecule can be viewed as:

$$\bar{V}_2^0 = V_{vw} + V_{void} - V_s, \quad (6)$$

where $V_s = n\sigma_s$.

It is assumed that V_{vw} and V_{void} remain nearly unchanged upon dissolution of amino acids in water or in an aqueous solution of surfactant. Then, the positive values of the volumes of AA transfer can be rationalized in terms of decrease in σ_s in the presence of CTAB in aqueous solutions.

Surfactant molecules self-aggregate to form micelles at concentrations higher than the CMC. Since the CMC⁷ for CTAB is $8.2 \cdot 10^{-4}$ mol L⁻¹, the surfactant exists in the micellar form under the conditions chosen for the present study.

A simple model can be used to express standard partial molar volumes of AA. According to this model, \bar{V}_2^0 for AA is the combination of the contribution of the intrinsic partial molar volume ($\bar{V}_{2(int)}^0$) and the change in the partial molar volume due to the effect of electrostriction ($\bar{V}_{2(elect)}^0$) due to the hydration of AA

$$\bar{V}_2^0 = \bar{V}_{2(int)}^0 + \bar{V}_{2(elect)}^0 \quad (7)$$

Further, as $\bar{V}_{2(int)}^0$ comprises of the van der Waals volume and the volume due to molecular packing, $\bar{V}_{2(int)}^0$ can be calculated¹⁸ by the equation including the molar volume of crystals ($\bar{V}_{2(cryst)}^0$)

$$\bar{V}_{2(int)}^0 = (0.7/0.634)\bar{V}_{2(cryst)}^0, \quad (8)$$

where 0.7 is the packing density for molecules in organic crystals and 0.634 is the packing density of randomly packed spheres. The crystal molar volumes can be determined from the crystal densities of AA¹⁹ by the equation

$$\bar{V}_{2(cryst)}^0 = M/\rho_{cryst} \quad (9)$$

The value of $\bar{V}_{2(elect)}^0$ can be estimated from the experimental values of \bar{V}_2^0 by Eq. (7). Thus, the number of

water molecules (n_H) in the hydrate shell of AA can be calculated by the formula

$$n_H = \frac{\bar{V}_{2(elect)}^0}{(\bar{V}_E^0 - \bar{V}_B^0)}, \quad (10)$$

where \bar{V}_E^0 and \bar{V}_B^0 are molar volumes of electrostricted water and bulk water at 298.15 K, respectively. The model assumes that a decrease in volume accompanies the transfer of any water molecule from solution to the hydrate shell of AA. The number n_H can be calculated inserting $\bar{V}_E^0 - \bar{V}_B^0 \approx -3.0$ cm³ mol⁻¹ (determined for electrolyte solutions²⁰) into Eq. (10). The obtained values of n_H are given in Table 3. On going from the neat solvent (water) to an aqueous solution of CTAB, the values of n_H vary substantially. This suggests that amino acids undergo strong dehydration in the presence of an aqueous solution of CTAB.

The densities of solutions and the viscosities of the solvent and solution determined are presented in Table 1 as a function of AA concentration and temperature. The following equation²¹ can be used to analyze the data:

$$\eta_r = \eta/\eta_0 + Am^{1/2} + Bm, \quad (11)$$

where η_r , η , and η_0 are the relative viscosity, viscosity of the solution, and viscosity of the solvent, respectively; A and B are coefficients. The values of A and B are presented in Table 4. Coefficients A signify the solute–solute interactions, while coefficients B are concerned with solute–solvent interactions. The data in Table 4 show that coefficients B are positive and their values exceed those of coefficient A . This suggests the dominance of strong solute–solvent interactions over solute–solute interactions.

Table 4. Coefficients A and B (see Eq. (11)) for glycine, serine, and valine in aqueous solutions of CTAB at different temperatures

Coefficient	298.15 K	303.15 K	308.15 K	313.15 K
Gly + Solution of CTAB (0.01 mol kg ⁻¹)				
$A \cdot 10^2$ /dm ^{3/2} mol ^{-1/2}	-0.3152	-0.2747	-0.2305	-0.1945
$B \cdot 10$ /dm ³ mol ⁻¹	0.3347	0.3404	0.4218	0.3455
Ser + Solution of CTAB (0.01 mol kg ⁻¹)				
$A \cdot 10^2$ /dm ^{3/2} mol ^{-1/2}	-3.7710	-3.0900	-3.6303	6.4035
$B \cdot 10$ /dm ³ mol ⁻¹	7.0442	7.4227	7.5839	7.6115
Val + Solution of CTAB (0.01 mol kg ⁻¹)				
$A \cdot 10^2$ /dm ^{3/2} mol ^{-1/2}	-6.7751	-4.5682	-4.2683	-2.8234
$B \cdot 10$ /dm ³ mol ⁻¹	7.3727	7.8711	8.6818	9.3664

Coefficients B increase in the series Gly < Ser < Val, coinciding with the sequence obtained from the standard partial molar volumes.

The value of $(dB/dT)_p$ rather than size of coefficient B plays a significant role in determining the structure-making/breaking tendency of a solute.²² The negative values of $(dB/dT)_p$ signify structure making, while positive values represent structure-breaking ability.²³ The temperature plots of coefficient B are presented in Fig. 1. It is seen that all the three AA studied act as structure-breakers under the experimental conditions. This is confirmed by the results obtained by analysis of the values of S_v^* .

Contribution of various groups. The values of \bar{V}_2^0 for amino acids vary linearly with an increase in the number of carbon atoms (n_C) in side alkyl chains. Similar linear correlations for α -AA in aqueous solutions were reported.^{5,24–26} We attempted to use linear regression analysis for processing the following equation:

$$\bar{V}_2^0(\text{CH}_3) = 1.5\bar{V}_2^0(\text{CH}_2), \quad (12)$$

where $\bar{V}_2^0(\text{NH}_3^+, \text{COO}^-)$ and $\bar{V}_2^0(\text{CH}_2)$ are the contributions to \bar{V}_2^0 of the terminal zwitterion and methylene group, respectively. Amino acids with the following alkyl chains were studied in the present work: CH_2 (Gly), CH_2CH (Ser), and Me_2CHCH (Val). The contributions to \bar{V}_2^0 from other alkyl chains can be estimated by the following equations^{27,28}:

$$\bar{V}_2^0(\text{CH}_3) = 1.5\bar{V}_2^0(\text{CH}_2), \quad (13)$$

$$\bar{V}_2^0(\text{CH}) = 0.5\bar{V}_2^0(\text{CH}_2). \quad (14)$$

The contribution of the OH groups of serine can be estimated using the equation

$$\bar{V}_2^0(\text{OH}) = \bar{V}_2^0(\text{Ser}) - \bar{V}_2^0(\text{NH}_3^+, \text{COO}^-) - \bar{V}_2^0(\text{CH}_2) - \bar{V}_2^0(\text{CH}). \quad (15)$$

Table 5. Contribution of moieties (NH_3^+ , COO^-), CH_2 and OH groups, and other alkyl chains to the values of \bar{V}_2^0 and coefficients B for glycine, serine, and valine in aqueous solutions of CTAB at different temperatures

Moiety, group	298.15 K	303.15 K	308.15 K	313.15 K
$\bar{V}_2^0 \cdot 10^5 / \text{m}^3 \text{mol}^{-1}$				
$\text{NH}_3^+, \text{COO}^-$	5.7371	6.2435	6.8748	7.4245
CH_2	1.1499	1.0588	0.9280	0.8392
OH	1.4432	1.3644	1.2675	1.4205
CH_2CH	1.7249	1.5882	1.3920	1.2589
Me_2CHCH	4.5998	4.2352	3.7120	3.3570
$B \cdot 10 / \text{dm}^3 \text{mol}^{-1}$				
$\text{NH}_3^+, \text{COO}^-$	0.3311	0.33821	0.3366	0.33673
CH_2	-7.4344	-7.4463	-7.2177	-7.0700

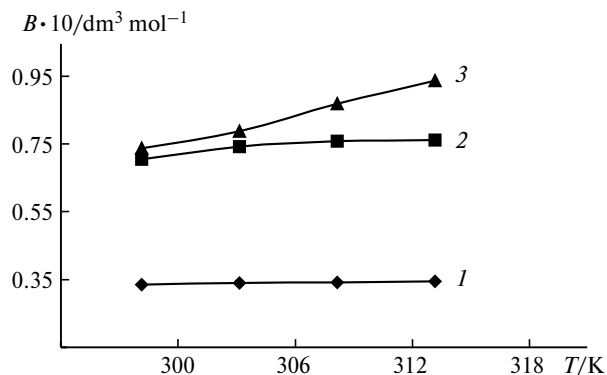


Fig. 1. Temperature plots of coefficient B for Gly (1), Ser (2), and Val (3) in aqueous solutions of CTAB at 298.15, 303.15, 308.15, and 313.15 K (points).

The obtained results are summarized in Table 5. We may conclude from the data in Table 5 that $\bar{V}_2^0(\text{NH}_3^+, \text{COO}^-)$ are higher than $\bar{V}_2^0(\text{CH}_2)$. This confirms the suggestion that the hydrophilic-hydrophilic interactions in this system are more pronounced than the hydrophilic-hydrophobic and hydrophobic-hydrophobic interactions.

Coefficients B reflect the net structural effects of charged groups and hydrophobic CH_2 groups of amino acids. The influence of these groups can be estimated separately by noting that the B coefficients like \bar{V}_2^0 also vary linearly with n_C

$$B = B(\text{NH}_3^+, \text{COO}^-) - n_C B(\text{CH}_2), \quad (16)$$

where $B(\text{NH}_3^+, \text{COO}^-)$ and $B(\text{CH}_2)$ are the contributions to coefficient B from the zwitterion and methylene group, respectively. The data in Table 5 show that $B(\text{NH}_3^+, \text{COO}^-)$ increase and $B(\text{CH}_2)$ decrease with temperature. This also confirms that the hydrophilic-hydrophilic interactions predominate over the hydrophilic-hydrophobic and hydrophobic-hydrophobic interactions in the studied system. Therefore, both the analysis of the contribution of alkyl chains to \bar{V}_2^0 and the coefficients B obtained confirm the above regularities of variation of $\Delta_{tr}\bar{V}_2^0$.

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