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Interactions of Some Amino Acids with Aqueous Tetraethylammonium Bromide at 298.15 K: A Volumetric Approach

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The apparent molar volumes, $V_{\phi,2}$, of glycine, L-alanine, DL-α-amino-*n*-butyric acid, L-valine, and L-leucine have been determined in aqueous 0.25, 0.75, 1.0, and 1.5 mol-dm^{-3} tetraethylammonium bromide (TEAB) solutions by density measurements at 298.15 K. These data have been used to calculate the infinite dilution apparent molar volumes, $V^{\circ}_{2,m}$, for the amino acids in aqueous tetraethylammonium bromide and the standard partial molar volumes of transfer ($\Delta_{tr}V^{\circ}_{2,m}$) of the amino acids from water to the aqueous salt solutions. The linear correlation of $V^{\circ}_{2,m}$ for a homologous series of amino acids has been utilized to calculate the contribution of the charged end groups (NH_3^+, COO^-), CH_2 group, and other alkyl chains of the amino acids to $V^{\circ}_{2,m}$. The results of the standard partial molar volumes of transfer from water to aqueous tetraethylammonium bromide have been interpreted in terms of ion–ion, ion–polar, and hydrophobic–hydrophobic group interactions. The volume of transfer data suggest that ion–ion or ion–hydrophilic interactions are predominant in the case of glycine and alanine, and hydrophobic–hydrophobic group interactions are predominant in the case of DL-α-amino butyric acid, L-valine, and L-leucine.

KEY WORDS: Standard partial molar volume; amino acids; hydration number; tetraethylammonium bromide.

1. INTRODUCTION

It is well documented that various cosolutes/cosolvents such as guanidine hydrochloride, sodium thiocyanate, magnesium chloride, urea, and alcohols affect proteins in different ways, acting as effective probes of their conformations in solutions.[1–6] Investigations of these conformational changes provide valuable information on the role of the solvent in maintaining the native, intermediate, and denatured states of the proteins. The stabilizing or destabilizing effect of the

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additives on proteins can be due to either direct binding or interactions indirectly through solvent-mediated effects, and needs to be experimentally investigated in each case. Thermodynamic properties of amino acids in aqueous electrolyte solutions provide important information about solute–solvent and solute–solute interactions that can be of great help in understanding the effect of electrolytes on biologically important systems.[7–10]

Tetraalkylammonium salts can give better insight into the effect of electrostatic and hydrophobic interactions on the stability of proteins as these salts are expected to influence macromolecular conformations by weakening attractive or repulsive inter- and intrachain charge–charge interactions and by affecting hydrophobic interactions through the side chains of the alkyl groups. Tetraalkylammonim salts are bulky in nature and are known to orient water molecules around them depending on their alkyl chain. These salts undergo hydrophobic hydration in water that is usually understood as the formation of more ordered and rigid structures of water surrounding the solute molecules. The effect of tetraalkylammonium salts on the stability of lysozyme and phycocyanin has been reported in the literature.[11–13]

Among the various physical parameters, the standard partial molar volume has been recognized as a quantity that is sensitive to structural changes occurring in solutions. In order to understand the finer details of the interaction of the functional groups of the proteins with one of these salts we have studied the standard partial molar volumes of transfer of some amino acids at five different concentrations of tetraethylammonium bromide.

2. EXPERIMENTAL

The amino acids glycine, L-alanine, DL-α-amino-n-butyric acid, L-valine, and L-leucine were procured from Sigma-Aldrich Co., USA. Tetraethylammonium bromide (TEAB) was of extra-pure analytical reagent grade purchased from Sisco Research Laboratories, India. The purity of all the chemicals as reported by the vendors is listed in Table I. All the amino acids were dried over P_2O_5 in a vacuum desiccator. The moisture content in the amino acids was determined using a Karl Fisher Titrator (Systronics, India). Appropriate corrections were applied wherever necessary. The water used for making the solutions of amino acids was double distilled and de-ionized by passing it through a Cole-Parmer Barnstead mixed-bed, ion-exchange resin column followed by degassing. Solution densities were measured with a vibrating tube digital densimeter (model DA-210 from Kyoto Electronics, Japan), details of which have been described elsewhere.[14] The temperature of the densimeter cell was maintained by circulating water from a Julabo constant temperature circulation bath. This arrangement gave a temperature stability of ± 0.01 K. The vibrational period of the densimeter tube containing the solution of interest was measured three times in each case. The reproducibility of

Table I. Compounds Used in This Study with Their Empirical Formula, Molecular Weight (M_r), Source (S = Sigma-Aldrich Company, USA; SRL = Sisco Research Laboratories, India), and Their Mole Fraction Purity (x) as Reported by the Vendors

Compoud	Formula	M_r	Source	x
Glycine	$C_2H_5NO_2$	75.07	S	>0.99
L-Alanine	$C_3H_7NO_2$	89.09	S	>0.99
DL-α-Amino-n-butyric acid	$C_4H_9NO_2$	103.1	S	>0.99
L-Valine	$C_5H_{11}NO_2$	117.1	S	>0.98
L-Leucine	$C_6H_{13}NO_2$	131.2	S	>0.98
TEAB	$C_8H_{20}BrN$	210.16	SRL	>0.98

the density measurements on the average was $\pm(3 \times 10^{-6})$ g-cm^{-3}. The calibration of the densimeter was performed every day and the accuracy was checked by measuring the densities of aqueous sodium chloride solutions in the concentration range of 0.08860 to 0.82435 mol-kg^{-1}. The results of measurements of sodium chloride solutions were found to be in excellent agreement with the literature values[15] with a maximum difference of 0.03 cm^3-mol^{-1} in the values of $V_{\phi,2}$.

3. RESULTS AND DISCUSSION

The values of apparent molar volume, $V_{\phi,2}$, were calculated from the measured densities using the following equation,

$$V_{\phi,2} = \frac{M}{\rho} - \frac{\rho - \rho_o}{m\rho\rho_o} \tag{1}$$

where M is the molar mass of the solute, m is the molality of the amino acid in TEAB–water mixtures, ρ and ρ_o are the densities of the amino acid–salt–water ternary system and reference solvent (desired molality of aqueous TEAB), respectively. The results of the density measurements at 298.15 K are given in Table II. In the cases where the molality dependence of $V_{\phi,2}$ was found to be either negligible or having no definite trend, the value of the standard partial molar volume at infinite dilution, $V^\circ_{2,m}$, was evaluated by taking an average of all the data points. In all other cases, the value of the standard partial molar volume was obtained by least-squares fitting to the following equation:

$$V_{\phi,2} = V^\circ_{2,m} + S_v m \tag{2}$$

The parameter, S_v, is the volumetric virial coefficient that characterizes the pairwise interaction of solvated solute species in solution.[16–18] The values of the standard partial molar volume along with those of S_v are given in Table III. The sign of S_v is determined by the nature of the interaction between the solute species. For zwitterionic amino acids, the positive values of S_v suggest that the pairwise

Table II. Molalities, Densities and Apparent Molar Volumes of the Amino Acids in Aqueous TEAB Solutions at 298.15 K

m (mol-kg^{-1})	ρ (g-cm^{-3})	$V_{\phi,2}$ (cm^3-mol^{-1})	m (mol-kg^{-1})	ρ (g-cm^{-3})	$V_{\phi,2}$ (cm^3-mol^{-1})
		0.25 mol-dm^{-3} TEAB			
Glycine			L-Alanine		
0.000000	1.006280		0.000000	1.006280	
0.069538	1.008497	43.02	0.050259	1.007711	60.33
0.101665	1.009510	43.09	0.104556	1.009237	60.43
0.148979	1.010990	43.18	0.200569	1.011893	60.56
0.298740	1.015550	43.57	0.307936	1.014827	60.61
0.410123	1.018865	43.76	0.389276	1.017005	60.68
0.502861	1.021634	43.79	0.519781	1.020432	60.79
0.608303	1.024727	43.86	0.607024	1.022693	60.84
DL-α-Amino-n-butyric acid			L-Valine		
0.000000	1.006285		0.000000	1.006285	
0.054146	1.007772	75.23	0.044861	1.007473	90.10
0.197499	1.011640	75.28	0.104527	1.009032	90.17
0.302002	1.014386	75.36	0.163949	1.010551	90.29
0.399819	1.016908	75.42	0.207828	1.011665	90.32
0.399966	1.016892	75.47	0.254778	1.012845	90.35
0.491826	1.019221	75.51	0.300150	1.013998	90.30
0.623628	1.022489	75.58	0.403966	1.016579	90.28
L-Leucine					
0.000000	1.006285				
0.028195	1.006956	106.80			
0.033526	1.007080	106.88			
0.041540	1.007282	106.57			
0.046097	1.007386	106.62			
0.046284	1.007367	107.18			
0.054144	1.007572	106.77			
0.068250	1.007875	107.20			
0.069135	1.007901	107.12			

Table II. Continued

m (mol-kg^{-1})	ρ (g-cm^{-3})	$V_{\phi,2}$ (cm^3-mol^{-1})	m (mol-kg^{-1})	ρ (g-cm^{-3})	$V_{\phi,2}$ (cm^3-mol^{-1})
		0.50 mol-dm^{-3} TEAB			
Glycine			L-Alanine		
0.000000	1.015857		0.000000	1.015857	
0.100879	1.018995	43.62	0.198816	1.021370	60.50
0.200471	1.022021	43.84	0.297211	1.024025	60.58
0.303852	1.025120	43.96	0.394685	1.026609	60.66
0.404325	1.028105	44.01	0.505133	1.029496	60.72
0.499660	1.030884	44.10	0.599950	1.031922	60.79
0.601464	1.033824	44.17			
DL-α-Amino-n-butyric acid			L-Valine		
0.000000	1.015850		0.000000	1.015850	
0.049894	1.017210	74.98	0.052283	1.017232	89.54
0.100691	1.018572	75.09	0.101714	1.018481	89.97
0.206616	1.021375	75.17	0.157057	1.019899	89.93
0.292645	1.023598	75.26	0.201577	1.021010	90.01
0.402614	1.026403	75.31	0.296825	1.023369	90.06
0.502397	1.028901	75.35	0.360357	1.024941	90.02
0.591153	1.031071	75.41			
L-Leucine					
0.000000	1.015850				
0.026075	1.016467	106.16			
0.040096	1.016789	106.36			
0.051192	1.017032	106.65			
0.052329	1.017072	106.40			
0.065312	1.017383	106.25			
0.069277	1.017481	106.17			
0.075052	1.017623	106.07			

Table II. Continued

m (mol-kg^{-1})	ρ (g-cm^{-3})	$V_{\phi,2}$ (cm^3-mol^{-1})	m (mol-kg^{-1})	ρ (g-cm^{-3})	$V_{\phi,2}$ (cm^3-mol^{-1})
		0.75 mol-dm^{-3} TEAB			
Glycine			L-Alanine		
0.000000	1.025578		0.000000	1.025542	
0.098699	1.028571	44.24	0.107129	1.028494	60.50
0.200844	1.031626	44.31	0.194542	1.030866	60.54
0.288233	1.034205	44.37	0.298598	1.033641	60.60
0.398514	1.037419	44.44	0.393275	1.036111	60.69
0.590717	1.042959	44.47	0.490599	1.038622	60.75
			0.590067	1.041124	60.84
DL-α-Amino-n-butyric acid			L-Valine		
0.000000	1.025547		0.000000	1.025547	
0.045238	1.026772	74.70	0.051368	1.026877	89.44
0.097499	1.028172	74.74	0.101928	1.028178	89.41
0.202906	1.030946	74.84	0.153068	1.029467	89.49
0.297157	1.033361	74.96	0.200259	1.030625	89.63
0.396555	1.035823	75.14	0.248361	1.031807	89.67
0.516973	1.038794	75.20	0.294088	1.032939	89.64
0.613705	1.041085	75.31	0.343604	1.034124	89.70
L-Leucine					
0.000000	1.025547				
0.025926	1.026145	105.93			
0.033701	1.026338	105.53			
0.044627	1.026568	106.07			
0.051977	1.026746	105.87			
0.063161	1.027018	105.63			
0.077410	1.027325	105.91			
0.080086	1.027357	106.25			

Table II. Continued

m (mol-kg^{-1})	ρ (g-cm^{-3})	$V_{\phi,2}$ (cm^3-mol^{-1})	m (mol-kg^{-1})	ρ (g-cm^{-3})	$V_{\phi,2}$ (cm^3-mol^{-1})
		1.0 mol-dm^{-3} TEAB			
L-Glycine			L-Alanine		
0.000000	1.035732		0.000000	1.035739	
0.095988	1.038611	44.40	0.097107	1.038379	60.52
0.291835	1.044374	44.50	0.195898	1.041017	60.59
0.294548	1.044449	44.52	0.295593	1.043622	60.69
0.392803	1.047279	44.58	0.386899.	1.045969	60.77
0.488761	1.050011	44.63	0.494627	1.048706	60.82
0.574294	1.052420	44.67	0.592375	1.051116	60.91
DL-α-Amino-n-butyric acid			L-Valine		
0.000000	1.035739		0.000000	1.035739	
0.101349	1.038433	74.57	0.048770	1.037008	88.70
0.198098	1.040913	74.82	0.051520	1.037063	88.99
0.298287	1.043411	75.01	0.088183	1.038024	88.71
0.392212	1.045747	75.03	0.097531	1.038255	88.80
0.482091	1.047908	75.13	0.098389	1.038251	89.04
0.592078	1.050457	75.30	0.104377	1.038445	88.66
			0.113725	1.038676	88.73
			0.144276	1.039403	89.07
			0.145457	1.039428	89.10
			0.158788	1.039790	88.93
L-Leucine					
0.000000	1.035739				
0.048709	1.036852	105.25			
0.050915	1.036896	105.38			
0.058957	1.037070	105.49			
0.060892	1.037104	105.64			
0.071855	1.037346	105.66			
0.072806	1.037392	105.34			
0.079667	1.037517	105.69			

Table II. Continued

m (mol-kg^{-1})	ρ (g-cm^{-3})	$V_{\phi,2}$ (cm^3-mol^{-1})	m (mol-kg^{-1})	ρ (g-cm^{-3})	$V_{\phi,2}$ (cm^3-mol^{-1})
		1.5 mol-dm^{-3} TEAB			
Glycine			L-Alanine		
0.000000	1.056975		0.000000	1.056975	
0.098951	1.059846	44.93	0.094742	1.059468	60.59
0.195699	1.062614	44.99	0.192247	1.061964	60.77
0.292514	1.065359	45.01	0.291536	1.064446	60.92
0.388194	1.068025	45.07	0.295282	1.064550	60.89
0.491164	1.070839	45.17	0.386791	1.066802	60.98
0.571862	1.072969	45.30	0.478567	1.069011	61.08
			0.587174	1.071585	61.17
DL-α-Amino-n-butyric acid			L-Valine		
0.000000	1.056980		0.000000	1.056980	
0.050913	1.058284	74.52	0.045421	1.058155	87.53
0.097310	1.059459	74.57	0.054869	1.058383	87.78
0.197181	1.061883	74.94	0.074613	1.058866	88.01
0.250515	1.063163	75.01	0.090056	1.059279	87.75
0.375942	1.066088	75.21	0.123316	1.060085	87.99
0.466021	1.068111	75.37	0.124889	1.060137	87.90
			0.137655	1.060414	88.17
L-Leucine					
0.000000	1.056980				
0.031286	1.057674	104.19			
0.032391	1.057678	104.76			
0.037630	1.057808	104.34			
0.038243	1.057826	104.24			
0.039531	1.057851	104.33			
0.044620	1.057959	104.40			
0.045899	1.057996	104.21			
0.047415	1.058037	104.08			

Table III. Volumetric Parameters of Amino Acids in Aqueous TEAB Solutions at 298.15 K[a,b,c]

Amino acids		Water	Tetraethylammonium bromide (mol-dm^{-3})				
			0.25	0.50	0.75	1.0	1.50
Glycine	$V^{\circ}_{2,m}$	43.14 (0.06)[d]	42.96 (0.07)	43.59 (0.05)	44.21 (0.03)	44.33 (0.01)	44.83 (0.04)
	S_v	0.86 (0.09)	1.68 (0.18)	1.02 (0.13)	0.5 (0.08)	0.58 (0.02)	0.73 (0.10)
	R	0.97	0.97	0.97	0.96	1.0	0.96
L-Alanine	$V^{\circ}_{2,m}$	60.43 (0.04)[d]	60.33 (0.02)	60.37 (0.01)	60.41 (0.01)	60.45 (0.01)	60.55 (0.03)
	S_v	0.73 (0.06)	0.87 (0.07)	0.71 (0.03)	0.71 (0.03)	0.78 (0.03)	1.12 (1.00)
	R	0.99	0.99	1.0	1.0	1.0	0.98
DL-α-Amino-n-butyric acid	$V^{\circ}_{2,m}$	75.51 (0.02)[e]	75.18 (0.02)	75.00 (0.03)	74.64 (0.02)	74.51 (0.06)	74.44 (0.05)
	S_v		0.66 (0.05)	0.732 (0.07)	1.11 (0.06)	1.37 (0.17)	2.09 (0.19)
	R		0.99	0.91	0.99	0.97	0.98
L-Valine	$V^{\circ}_{2,m}$	90.39 (0.14)[d]	90.26 (0.09)	89.92 (0.19)	89.55 (0.11)	88.87 (0.17)	87.88 (0.21)
L-Leucine	$V^{\circ}_{2,m}$	107.72 (0.24)[d]	106.89 (0.25)	106.29 (0.19)	105.88 (0.24)	105.49 (0.17)	104.32 (0.21)

[a]Units: $V^{\circ}_{2,m}$ (cm^3-mol^{-1}); S_v (cm^3-mol^{-2}-kg).
[b]R: Regression coefficient of the linear fit.
[c]Entries in the parentheses are the uncertainties.
[d]Ref. [19].
[e]Ref. [20].

interaction is dominated by the interaction of the charged functional groups. The variation in the values of S_v with side-chain position indicates that the methyl group modulates the interaction of the charged end-groups in the pairwise interaction.

The values of $V^{\circ}_{2,m}$ are positive for all the amino acids in aqueous tetraethyammonium bromide solutions at all the molalities studied. These values show an increase in the case of glycine and L-alanine, but a decrease in the case of DL-α-amino-n-butyric acid, L-valine, and L-leucine with an increase in the molality of the salt. For glycine, the value of $V^{\circ}_{2,m}$ increases by (1.87 ± 0.08) cm^3-mol^{-1} with an increase in the concentration of aqueous TEAB from 0.25 to 1.50 mol-dm^{-3}. However, for L-alanine, the increase in the $V^{\circ}_{2,m}$ value is only (0.22 ± 0.04) cm^3-mol^{-1}. In the case of DL-α-amino-n-butyric acid, L-valine and L-leucine, the value of $V^{\circ}_{2,m}$ decreases by $-(0.74 \pm 0.05)$, $-(2.38 \pm 0.23)$ and $-(2.57 \pm 0.33)$ cm^3-mol^{-1}, respectively, for the same increase in the molality of TEAB in each case. At each molality, the value of $V^{\circ}_{2,m}$ (Table III) showed a linear variation with the number of carbon atoms in the alkyl chain of the amino acids with an average correlation coefficient of 0.999. Similar linear correlations have been observed earlier for homologous series of ω-amino acids in aqueous potassium thiocyanate solution and α-amino acids.[21] This linear variation is represented by

$$V^{\circ}_{2,m} = V^{\circ}_{2,m}(NH_3^+, COO^-) + n_c V^{\circ}_{2,m}(CH_2) \tag{3}$$

where n_c is the number of carbon atoms in the alkyl chain of the α-amino acids; $V^{\circ}_{2,m}(NH_3^+, COO^-)$ and $V^{\circ}_{2,m}(CH_2)$ are the zwitterionic end groups and the methylene group contribution to $V^{\circ}_{2,m}$, respectively. The values of $V^{\circ}_{2,m}(NH_3^+, COO^-)$ and $V^{\circ}_{2,m}(CH_2)$, calculated by a least-squares regression analysis are listed in Table IV. Since the alkyl chains of the homologous series of the α-amino acids studied in this work are CH_2-(glycine), CH_3CH-(alanine), CH_3CH_2CH-(α-amino-n-butyric acid), CH_3CH_3CHCH-(valine), and $CH_3CH_3CHCH_2CH$-(leucine), the value of $V^{\circ}_2(CH_2)$ obtained by this procedure characterizes the mean contribution of the CH– and CH_3– groups to $V^{\circ}_{2,m}$ of the α-amino acids. As suggested by Hakin and coworkers,[22,23] the contributions of the other alkyl chains of the α-amino acids reported in Table IV were calculated as follows:

$$V^{\circ}_{2,m}(CH_3) = 1.5\, V^{\circ}_{2,m}(CH_2) \tag{4}$$

$$V^{\circ}_{2,m}(CH) = 0.5\, V^{\circ}_{2,m}(CH_2) \tag{5}$$

Larger values of $V^{\circ}_{2,m}(NH_3^+, COO^-)$ than $V^{\circ}_{2,m}(CH_2)$ indicate that the interactions of the ions of TEAB with the zwitterionic groups of the amino acids dominate compared to those of the hydrophobic group–TEAB interactions. In addition, the difference in the molar masses of the ($NH_3{}^+$, COO^-) and CH_2 groups also accounts for the larger $V^{\circ}_{2,m}$ values of the former. The number of water molecules hydrated to the amino acids, N_w, were calculated from the values of the measured standard partial molar volume of the latter by using the method described below.

Table IV. Contributions of Zwitterionic Group (NH_3^+, COO^-), CH_2 Group, and the Other Alkyl Chains to the Infinite Dilution Apparent Molar Volume in Aqueous TEAB at 298.15 K

	$V_{2,m}^{\circ}$ (cm^3-mol^{-1})					
		Tetraethylammonium bromide (mol-dm^{-3})				
Group	Water	0.25	0.50	0.75	1.0	1.5
NH_3^+,COO^-	27.68 (1.12)	27.79 (0.84)	28.54 (0.70)	29.21 (0.66)	29.50 (0.81)	30.4 (0.56)
CH_2–	15.91 (0.33)	15.78 (0.25)	15.50 (0.21)	15.25 (0.18)	15.07 (0.24)	14.73 (0.17)
CH_3CH–	31.82 (0.40)	31.56 (0.40)	31.00 (0.40)	30.50 (0.50)	30.14 (0.40)	29.46 (0.40)
CH_3CH_2CH–	47.73 (0.40)	47.34 (0.40)	46.50 (0.40)	45.75 (0.40)	45.21 (0.40)	44.19 (0.50)
CH_3CH_3CHCH–	63.64 (0.40)	63.12 (0.40)	62.00 (0.40)	61.00 (0.40)	60.28 (0.40)	58.92 (0.40)
$CH_3CH_3CHCH_2CH$–	79.55 (0.50)	78.90 (0.50)	77.50 (0.50)	76.25 (0.50)	75.35 (0.55)	73.65 (0.40)

Note. Values in the parentheses are the uncertainties. Average correlation coefficient: 0.99953.

Table V. Hydration Number (N_W) of Amino Acids in Aqueous TEAB at 298.15 K

	Concentration (mol-dm^{-3})				
Amino acids	0.25	0.5	0.75	1.0	1.5
Glycine	3.0	2.8	2.6	2.5	2.3
L-Alanine	3.8	3.8	3.8	3.8	3.7
L-Valine	3.9	4.0	4.2	4.4	4.7
L-Leucine	5.8	5.9	6.1	6.2	6.6

The values of $V^{\circ}_{2,m}$ for the amino acids can be expressed by[24,25]

$$V^{\circ}_{2,m} = V^{\circ}_{2}(\text{int}) + V^{\circ}_{2}(\text{elect}) \tag{6}$$

where V°_{2}(int) is the intrinsic partial molar volume of the amino acid and V°_{2}(elect) is the electrostriction partial molar volume due to the hydration of the amino acids. The V°_{2}(int) term can be further divided into two terms, one for the van der Waals volume and the other for the volume due to packing effects. Millero *et al.*[25] have obtained values of V°_{2}(int) for amino acids from their molar crystal volumes by using the relationship:

$$V^{\circ}_{2}(\text{int}) = \frac{0.7}{0.634}\, V^{\circ}_{2}(\text{cryst}) \tag{7}$$

where 0.7 is the packing density for the molecule in an organic crystal and 0.634 is the packing density for the randomly packed spheres. The molar volume of the crystal was calculated from the crystal densities of the amino acids reported by Berlin and Pallansh[26] at 298.15 K. The values of V^{o}_{2}(elect) were obtained from the experimentally measured $V^{\circ}_{2,m}$ values using Eq. (6).

Further, the decrease in the volume due to electrostriction can be related to the hydration number, N_w, of the amino acids as[25]

$$N_{\text{w}} = \frac{V^{\circ}_{2}(\text{elect})}{V^{\circ}_{\text{E}} - V^{\circ}_{\text{B}}} \tag{8}$$

where V°_{E} is the molar volume of the electrostricted water and V°_{B} is the molar volume of the bulk water at 298.15 K. This model assumes that for every water molecule taken from the bulk phase to the region near the amino acid, the volume is decreased by $(V^{\circ}_{\text{E}} - V^{\circ}_{\text{B}})$. Using $(V^{\circ}_{\text{E}} - V^{\circ}_{\text{B}}) \approx -3.0\,\text{cm}^3\text{–mol}^{-1}$[25] for electrolytes at 298.15 K, the hydration numbers have been calculated (Table V).

The calculated values of N_w for the amino acids in aqueous TEAB are observed to vary in the following order:

$$N_{\text{w}}(\text{leucine}) > N_{\text{w}}(\text{valine}) \approx N_{\text{w}}(\text{alanine}) > N_{\text{w}}(\text{glycine})$$

This trend is maintained at all the concentrations of TEAB studied. In general, with an increase in the concentration of TEAB, the N_w value exhibits a decreasing trend in the case of glycine leading to reduction in the electrostriction. In the case of L-alanine, the value of the hydration number is unaffected by the increase in the concentration of TEAB. This indicates that the CH_2 group in alanine reduces the ion–ion interaction between the amino acid and the salt. However, a slight increase in the hydration number is observed in the case of valine and leucine. This indicates that the increase in the interaction of the hydrophobic groups of these two amino acids with those of the salt does not lead to a reduction in the electrostriction of water, thereby leading to a slight increase in the hydration number N_{w}.

3.1. Standard Partial Molar Volumes of Transfer of Amino Acids From Water to Aqueous TEAB

Limiting thermodynamic properties of transfer yield qualitative and quantitative information regarding the interactions of a cosolvent and a solute without having to take into account the effects of solute–solute interactions. The standard partial molar volumes of transfer ($\Delta_{\mathrm{tr}} V^{\circ}_{2,m}$) for the amino acids from water to TEAB solutions presented in Table VI were calculated as follows:

$$\Delta_{\mathrm{tr}}\, V^{\circ}_{2,m}\,[\text{water to TEAB(aq)}] = V^{\circ}_{2,m}\,[\text{TEAB (aq)}] - V^{\circ}_{2,m}\,[\text{water}] \qquad (9)$$

The uncertainties in the values of volume of transfer are calculated as the root mean squares of the uncertainties associated with the apparent molar properties at infinite dilution. Both positive and negative volume of transfer values were observed for the amino acids. The values of $\Delta_{\mathrm{tr}} V^{\circ}_{2,m}$ for glycine increase positively with increasing concentration of TEAB. This trend also seems to be followed by L-alanine, although the value of $\Delta_{\mathrm{tr}} V^{\circ}_{2,m}$ is very small. However, for α-amino-*n*-butyric acid, valine and leucine, the values of $\Delta_{\mathrm{tr}} V^{\circ}_{2,m}$ become more negative with increasing concentration of TEAB in solution.

Table VI. Transfer Volumes of Amino Acids [$\Delta_{\mathrm{tr}} V^{\circ}_{2,m}$ ($\mathrm{cm^3\text{-}mol^{-1}}$)] from Water to Aqueous Tetraethylammonium Bromide at 298.15 K

	Tetraethylammonium bromide ($\mathrm{mol\text{-}dm^{-3}}$)				
Amino acids	0.25	0.50	0.75	1.00	1.50
Glycine	−0.18 (0.09)	0.45 (0.08)	1.07 (0.07)	1.20 (0.00)	1.69 (0.07)
L-Alanine	−0.10 (0.05)	−0.06 (0.04)	−0.02 (0.04)	0.02 (0.04)	0.12 (0.05)
DL-α-amino-*n*-butyric acid	−0.36 (0.02)	−0.52 (0.03)	−0.87 (0.02)	−1.01 (0.07)	−1.07 (0.05)
L-Valine	−0.13 (0.17)	−0.47 (0.24)	−0.84 (0.19)	−1.52 (0.22)	−2.51 (0.25)
L-Leucine	−0.83 (0.34)	−1.43 (0.31)	−1.91 (0.4)	−2.24 (0.30)	−3.40 (0.32)

Franks *et al.*[24] explained the standard partial molar volume of a nonelectrolyte as being a combination of the intrinsic volume of the solute and the volume changes due to its interactions with the solvent. The intrinsic volume is considered to consist of two contributions:[27]

$$V_{2,m}^{\circ}(\text{int}) = V_{\text{vW}} + V_{\text{void}} \tag{10}$$

where V_{vW} is the volume occupied by the solute due to its van der Waals volume[28] and V_{void} is the volume associated with the voids and empty spaces present therein.[29] The above equation was modified by Shahidi *et al.*[30] in order to evaluate the contribution of a solute molecule to its standard partial molar volume as

$$V_{2,m}^{\circ}(\text{int}) = V_{\text{vW}} + V_{\text{void}} - n\sigma_{\text{s}} \tag{11}$$

where σ_{s} is the shrinkage in the volume produced by the interaction of hydrogen bonding groups present in the solute with water molecules and n is the number of potential hydrogen bonding sites in the molecule. Thus, $V_{2,m}^{\circ}$ of an amino acid can be viewed as

$$V_{2,m}^{\circ} = V_{\text{vW}} + V_{\text{void}} - V_{\text{shrinkage}} \tag{12}$$

If it is assumed that V_{vW} and V_{void} remain approximately the same in water and in the aqueous TEAB solutions, the positive volume of transfer for the amino acids, glycine and alanine can be rationalized in terms of a decrease in the volume of shrinkage in the presence of the TEAB molecules in aqueous solutions.

The following types of interactions can occur in the ternary system of amino acid, TEAB and water: (a) ion–ion interactions between the Br^- of TEAB and the $-NH_3^+$ group of the amino acid; (b) ion–ion interactions between the $(C_2H_5)_4N^+$ of TEAB and the COO^- group of the amino acid; (c) hydrophobic–hydrophobic interactions between the ethyl groups of TEAB and the hydrophobic group of the amino acid.

Taking the cosphere overlap model[31] as the guideline, (a) and (b) type interactions would lead to a positive $\Delta_{\text{tr}}V_{2,m}^{\circ}$ since there is a reduction in the electrostriction effect and the overall water structure is enhanced. Interactions of type (c) would lead to a negative $\Delta_{\text{tr}}V_{2,m}^{\circ}$, because the introduction of the alkyl group provides an additional tendency of hydrophilic–hydrophobic and hydrophobic–hydrophobic groups to interact and as a result there will be a reduction in the structure of water formed as a result of their cospheres overlapping.

The value of $\Delta_{\text{tr}}V_{2,m}^{\circ}$ for glycine from water to 0.25 mol-dm^{-3} TEAB is negative, but is very small. This indicates a balance of type (a) to (c) interactions. An increase in the concentration of cosolute TEAB, results in a positive $\Delta_{\text{tr}}V_{2,m}^{\circ}$ indicating an enhancement in the ion–ion interaction between the zwitterionic centers of the amino acids and the ion of the salt. A similar trend is observed

in the case of alanine, although an additional $-CH_2-$ group compared to glycine increases the hydrophobic interactions, leading to a reduction in the positive value of $\Delta_{tr}V^{\circ}_{2,m}$. The number of hydrophobic groups increases in the order, α-amino-*n*-butyric acid, valine, leucine. The increased number of hydrophobic groups in these amino acids interacts strongly with the hydrophobic groups of TEAB, thereby leading to negative volumes of transfer, which increases with increasing the concentration of TEAB.

Jain and Ahluwalia[11] have observed that the transition temperature of lysozyme decreases in the presence of tetraethylammonium bromide with increasing concentration of the salt. The change in the transition temperature relative to that in the buffer varied from -6.8 K in the presence of 0.5 mol-dm^{-3} to -14.4 K in the presence of 2.0 mol-dm^{-3} tetraethylammonium bromide. Similarly the change in calorimetric enthalpy observed by them varied from $-(47 \pm 16)$ kJ-mol^{-1} to $-(91 \pm 12)$ kJ-mol^{-1} for the same change in the concentration of the salt. The authors explained their results on the basis that the tetraalkylammonium halides destabilize lysozyme by interacting with the exposed hydrophobic groups of the denatured state, simultaneously weakening the hydrophobic interactions between the nonpolar groups of the proteins as well as perturbing the characteristic water structure around the protein molecule. Our discussion supports this explanation.

The denaturing action of the tetraalkylammonium halides on proteins has been attributed by Timasheff and coworkers[32–34] to the binding of the denaturant molecules to the denatured state of the protein that is stronger than the exclusion of cosolvent from the protein surface. Hydrophobic interactions between the protein and bulky alkyl groups on tetraalkylammonium ions are believed to play an essential role in inhibiting the aggregation properties of phycocyanin.[12,13] On the basis of enthalpy and entropy data obtained for the protein-quaternary bromides, Chen and Berns[12,13] have postulated that the direct contact region between the protein and hydrophobic solute is mainly on the hydrophobic area of the protein and the bulky alkyl groups of the hydrophobic solutes that interact principally by means of hydrophobic forces instead of a direct contact between the charged ions and polar groups of the protein favored by electrostatic interactions. The results obtained in this work are consistent with the above-mentioned observations because the increase in the hydrophobic content of the amino acids consistently leads to a more negative volume of transfer due to hydrophobic interactions.

4. CONCLUSIONS

The values of the standard partial molar volume of the homologous series of amino acids with the number of carbon atoms from one to five in the chain are positive in aqueous tetraethylammonium bromide solutions. The contribution of the zwitterionic (NH_3^+, COO^-) group to the value of the standard

partial molar volume increases with increasing concentration of tetraethylammonium bromide. However, in general, the contribution of $–CH_2–$ and other alkyl groups has a decreasing trend. The standard partial molar volumes of transfer for the amino acids with the number of carbon atoms from three to five in the chain show a decrease with increasing concentration of the salt indicating the predominance of hydrophobic interactions between the hydrophobic groups of the amino acids and those of the cosolute over the ionic or hydrophobic interactions. The increase in the hydrophobic content of the amino acids increases the number of water molecules hydrated to the charged centers of the salt, indicating the predominance of the hydrophobic interaction between the amino acid and tetraethylammonium bromide with increasing number of carbon atoms in the former.

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