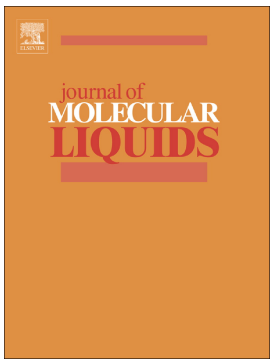

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Peptides in Aqueous Protic Ionic Liquid Solutions: Apparent and Transfer Volumes at 298.15 K and at 0.1 MPa

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ABSTRACT: The densities of glycine based peptides namely glycyl-glycine (digly), glycine-L-valine (gly-val), glycyl-L-leucine (gly-leu) and glycyl-glycyl-glycine (triglycine/trigly) in aqueous solutions containing ~0.20 mol·kg^{-1} of triethylammonium acetate [TEAA], triethylammonium glycolate [TEAG], triethylammonium pyruvate [TEAPy] and triethylammonium propionate [TEAP] protic ionic liquids (PILs) are reported at 298.15 K and at atmospheric pressure. Experimental density data obtained for these systems were used to estimate apparent molar volume (ϕ_V), the limiting apparent molar volume (ϕ_V^0), partial molar volume of solute ($\overline{V}_2$) and partial molar volume of mixed solvent (PILs+water) ($\overline{V}_1$). The change in limiting apparent molar volume due to transfer ($\Delta_{tr}\phi_V^0$) of peptides from water to aqueous ionic liquid solutions were also estimated. The outcomes obtained from all these thermodynamic parameters were discussed in terms of ion−peptide interactions, ion−ion interactions, hydrophobic solvation, peptide group contributions to limiting volumes, etc.

Keywords: peptides; protic ionic liquids; partial molar volume; transfer molar volume; hydrophobic and hydrophilic Interaction; group contributions

1. Introduction

Structure and function of biological macromolecules and assemblies is decided by the surrounding environment of these molecules. Stability and the native structure of such

molecules is controlled by collective efforts of various non−covalent forces such as hydrogen bonding, ionic and dipolar interactions, dispersion forces, hydrophobic interactions, etc. [1,2]. Denaturation of globular proteins in aqueous medium results due to alteration in hydrogen bonding (H–bond) interactions, α–helix, β−sheets in proteins as compared to its native form which leads to the uncoiling of protein into a disorderly shape [3−6]. Due to complex nature of such non-covalent interactions between proteins and co-solutes/solvents, it is preferable to investigate small building blocks such as amino acids or small peptides which help largely to interpolate or scale up the outcomes to larger polypeptides or proteins as some structural features and functional groups are common [7−10]. Thus, to enhance our understanding about various ionic and/or molecular interactions existing in aqueous biomolecular solutions, measurement and analysis of effect of additive concentrations and of temperature variation on the thermodynamic properties helps a lot. In this line, thermodynamic and physicochemical investigation of amino acids and peptides in aqueous and in aqueous cosolute solutions are well documented to get fruitful information about ion−ion and ion−solvent interactions which helps to increase our deep learning towards naturally occurring bioprocesses [7−23].

Ionic liquids (ILs) are showing notable presence in almost every scientific field of basic and applied sciences as well as in technological innovations and in medicinal sciences [24−26] due to large probabilities in their designs through combinations of structurally different types of cations and anions as per the need. Especially, bio-ionic liquids (ionic liquids from biological resources) are gaining much attention due to their potential role in protein chemistry as osmolytes or denaturants or stabilizer, in pharmaceutical chemistry as a part of drugs or as solubilizing and stabilizing agents for many unstable and insoluble drugs etc. due to less or nontoxic nature of bio-ionic liquids. To understand the potential role of traditional ILs and such bio-ILs in protein chemistry and medicinal fields, many researchers are engaged in investigations on molecular level understanding of interactions between

biomolecules and ionic liquids. In this regard, thermodynamic and physicochemical properties of amino acids and peptides in aqueous aprotic ionic liquids (APILs) are well-studied [27−48], however, a limited work is available in aqueous solutions of protic ionic liquids (PILs) [49]. Volumetric investigations can give novel data as far as the folding/defolding phenomenon, conformational stability and ligand−binding properties of a protein are at central place [50]. Volumetric studies of amino acids and peptides in imidazolium based bromide ionic liquids studied by Fang [27] and Wen [40] revealed that the increased concentration of imidazolium-based bromide ionic liquids causes dehydration effect on the amino acids/peptides which gets enhanced with temperature increase. Furthermore, this study revealed that the hydrophilic–hydrophilic interactions are more prominent amongst amino acids/dipeptide and imidazolium-based bromide ILs rather than with the head groups or hydrophobic side chains. Recently, we reported thermodynamic properties of bio-ionic liquids using volumetric, speed of sound and osmotic vapor pressure measurements for the ammonium based carboxylate/nitrate PILs and imidazolium based amino acids ([C$_n$Mim][AA] (n=3,4)) in aqueous solutions [51−58]. We observed that these bio-ionic liquids gets strongly hydrated and shows the high hydration number due to cooperative hydrogen bonding of carboxylate and amino acid anions with water structure making effect i.e. kosmotropic effect which gets further enhanced with increase in hydrophobicity of ions.

In this work, we are reporting volumetric studies for peptides (glycyl-glycine, glycine-L-valine, glycyl-L-leucine and glycyl-glycyl-glycine) in aqueous solutions containing ~0.2 mol·kg^{-1} protic ionic liquids (triethylammonium acetate [TEAA], triethylammonium glycolate [TEAG], triethylammonium pyruvate [TEAPy] and triethylammonium propionate [TEAP]) at 298.15 K. Volumetric properties such as apparent molar volume (ϕ_V), the limiting apparent molar volume (ϕ_V^0), limiting apparent transfer molar volume ($\Delta_{tr}\phi_V^0$), partial molar volume of solute ($\bar{V}_2$) and partial molar volume of mixed solvent (PILs+water) ($\bar{V}_1$) were

estimated using density data are reported. The group contributions method is presented and used to estimate group contribution to limiting apparent molar volume (ϕ_V^0) for peptides in aqueous and aqueous ~0.20 mol·kg^{-1} PIL solutions. The details of measurements, data analysis and discussion on results obtained are outlined below.

2. Experimental

2.1. Materials

The glycine based peptides namely glycyl-glycine (Sigma, mass fraction >0.99), glycine-L-valine (Hi-Media, mass fraction 0.98), glycyl-L-leucine (Hi-media, mass fraction 0.98) and glycyl-glycyl-glycine i.e. triglycine (Sigma, mass fraction 0.99), triethylamine (Merck, mass fraction ≥0.99) and glacial acetic acid (Merck, mass fraction ≥0.99) were used without further purification. Sodium chloride (NaCl) (BDH, mass fraction >0.999) and potassium chloride (KCl) (BDH, mass fraction >0.998), were used without further purification but dried under a vacuum at 393 K for 24 hours before use. The source, purity method, purity and CAS number for the chemicals used are reported in Table 1.

Table 1 Chemical name, CAS No., molecular mass, purity, source and analysis method of chemicals used.

Chemical name	CAS no.	Molecular mass ($g \cdot mol^{-1}$)	Mass fraction purity	Water content (mass fraction)	Source	Analysis method
Glycyl-glycine	556-50-3	132.12	>0.99[a]		Sigma	
Glycyl-L-valine	1963-21-9	174.20	0.98[a]		Hi-Media	
Glycyl-L-leucine	869-19-2	188.22	0.98[a]		Hi-Media	
Triglycine	556-33-2	189.17	0.99[a]		Sigma	
Triethylamine	121-44-8	101.19	>0.99[a]		Merck	
Acetic acid	64-19-7	60.05	>0.99[a]		Merck	
Phosphorous pentaoxide	1314-56-3	141.95	>0.98[a]		Merck	
Sodium chloride	7647-14-5	58.44	>0.999[a]		BDH	
Potassium chloride	7447-40-7	74.55	>0.998[a]		BDH	
TEAA[b]	5204-74-0	161.24	≥ 0.98[b]	0.0179[d]	Synthesized in lab	NMR
TEAP[c]	51009-80-4	175.27	≥ 0.98[c]	0.0021[d]	Synthesized in lab	NMR
TEAG[c]	178461-51-3	177.24	≥ 0.98[c]	0.0109 [d]	Synthesized in lab	NMR
TEAPy[c]	–	189.25	≥ 0.99[c]	0.0059 [d]	Synthesized in lab	NMR

[a]As stated by supplier.

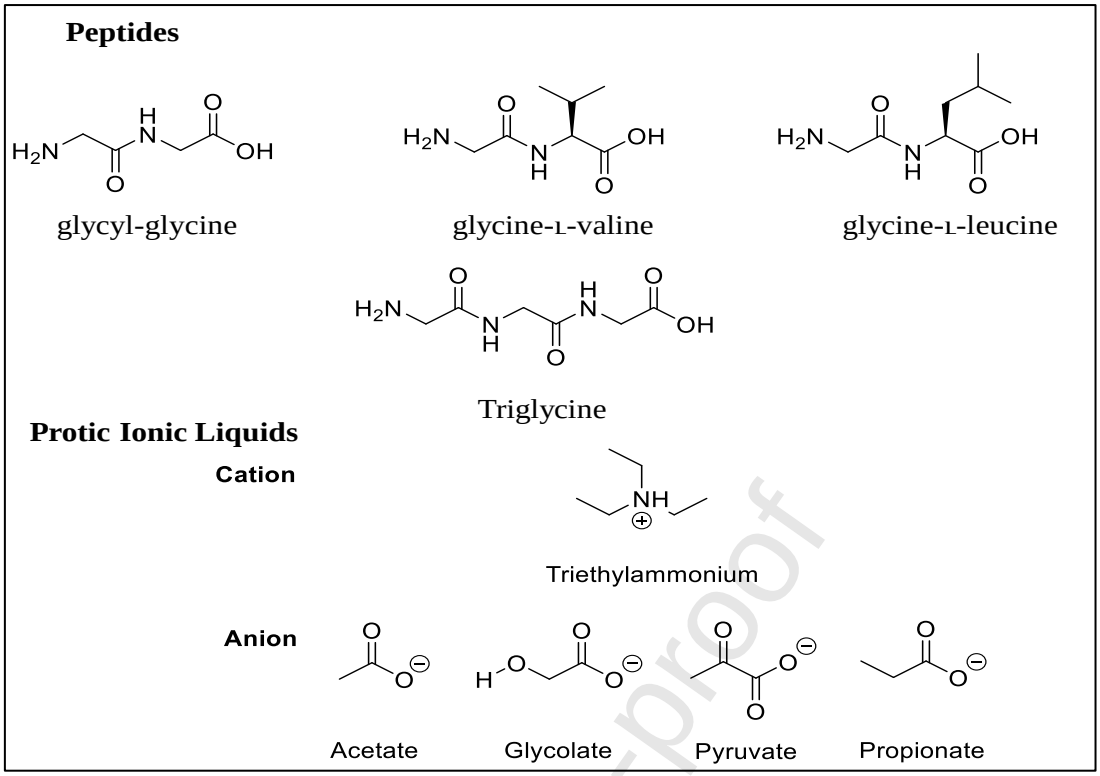
[b]The PIL, Triethylammonium acetate [TEAA] is synthesized in laboratory using procedure reported earlier [51]. The purity was checked by ^{1}H NMR spectroscopic techniques. No traces of impurities were detected in ^{1}H NMR spectra (See Supplementary Material).

[c]PILs namely Triethylammonium propionate [TEAP], Triethylammonium glycolate [TEAG] and Triethylammonium pyruvate [TEAPy] used here are synthesized and characterized earlier [51].

[d]Water content in the PILs estimated using TKF-55 Karl-Fischer titrator and values reported are average of at least three measurements for each PIL. This water content was treated as an impurity while reporting the PIL purity and considered as a part of solvent while preparing aqueous solutions of PILs to correct the concentrations accordingly.

2.2. Synthesis of Protic Ionic Liquids

All the studied protic ionic liquids (PILs) namely triethylammonium acetate [TEAA], triethylammonium glycolate [TEAG], triethylammonium pyruvate [TEAPy] and triethylammonium propionate [TEAP] were synthesized in our laboratory. The details on synthesis, purification and characterization of these PILs are reported already in our recent article [51] except triethylammonium acetate [TEAA] for which also a similar procedure of synthesis and purification were adopted. Accordingly, for synthesis of TEAA PIL, equimolar quantities of triethylamine and acetic acid were used. The triethylamine were taken into a round bottom flask equipped with a reflux condenser and a dropping funnel with the tip under stopcock. An equimolar quantity of glacial acetic acid was added dropwise with constant stirring into triethylamine with the help of dropping funnel. An equimolar mixture in the round bottom flask with constant stirring was kept in a circulating heated water-bath initially at 333.15 K for 1 hour and then at 343.15 K for 2 hour for completion of reaction to form TEAA. The traces of water molecules in the TEAA PIL were removed by drying at 343.15 K under vacuum for 48 hours. Finally, the resulting PIL was stored under dry conditions i.e. over phosphorous pentaoxide (P_2O_5) in a desiccator. The structure of the TEAA was confirmed by ^{1}H NMR spectra in $CDCl_3$ solvent obtained using Bruker 300 MHz NMR instrument (see Supplementary Material, Fig. S1). The details of purity estimation and water content analysis of studied PILs using the ^{1}H NMR spectral data were reported already in our recent work [51]. The chemical structures of studied peptides and PILs are schematically represented in scheme 1. Water contents in the synthesized PILs were estimated using TKF-55 Karl-Fischer titrator and found to be 0.0179 mass fraction in TEAA, 0.0109 mass fraction in TEAG, 0.0059 mass fraction in TEAPy and 0.0021 mass fraction in TEAP which were taken into consideration during the preparation of binary aqueous solutions of PILs. The water content given here is the average over at least three measurements for each PIL.



Scheme 1. Chemical structures of peptides and cations and anions of protic ionic liquids.

2.3. Preparation of Sample Solutions

Fresh doubly quartz distilled water was used for the entire work. All aqueous binary (PIL + water) and ternary (peptide + PIL + water) solutions were prepared on the molality basis at room temperature in the airtight glass vessels using a Mettler Toledo ML204/A01 weighing balance having readability of ± 0.0001 g. The standard uncertainties in the molality of solutions were found to be ± $1 \cdot 10^{-4}$ mol·kg^{-1}.

2.4. Density Measurements

The densities (ρ) of the ternary solutions were measured using vibrating–tube digital densitometer (Anton Paar, DMA 60/602 M) at 298.15 K and at atmospheric pressure. The temperature of the measuring cell was maintained constant at 298.15 K using Julabo F-34HE cryostat, having temperature stability of ± 0.01 K. The densitometer was calibrated using water and air, the densities of which are known at 298.15 K and at atmospheric pressure.

Density of air used for calibration was corrected for laboratory pressure and humidity. Oscillation period τ of the vibrating tube (either filled with water or with air) was measured at 298.15 K and used to determine instrumental calibration constants *A* and *B* of the equation $\rho = A + B\tau^2$. After knowing constants, *A* and *B*, the densities of peptides in aqueous PIL solutions at different concentrations were obtained from measured τ values of the corresponding sample solutions at 298.15 K. The combined uncertainty in the density measurements was found to be ± $1 \cdot 10^{-2}$ kg·m^{-3}. The authenticity of density measurements was ascertained by comparing the density data of known system i.e. of aqueous solutions of NaCl and KCl, with those available in literature [59] (see Supplementary Material, Fig. S2). The information regarding the calibration, testing and error analysis for density measurements are also reported in earlier work [56].

3. Result and discussion

3.1. Apparent and Partial Molar Volumes

The experimental density (ρ) data for peptides in the aqueous PIL solutions (Peptides + PIL + water ternary systems) at 298.15 K are reported in Tables 2 to 5 which were used to calculate apparent molar volume (ϕ_V), using the following standard Eq. (1) [60].

$$\phi_V = \left(\frac{(\rho_0 - \rho)}{m_2 \rho \rho_0} \right) + \left(\frac{M_2}{\rho} \right) \quad (1)$$

where M_2 is the molar mass of a peptide in kg·mol^{-1}, m_2 is the molality of peptide in aqueous PIL expressed in mol·kg^{-1} and, ρ_0 and ρ represents the density in kg·m^{-3} of solvent (~0.2 mol·kg^{-1} PIL + water) and of solution respectively. The data of apparent molar volume (ϕ_V) of peptides thus obtained are collected in Tables 2 to 5 along with estimated uncertainties at

each concentration. Fig. 1 represents comparison of concentration dependent ϕ_V data for studied glycine-based peptides in aqueous ~0.2 mol·kg^{-1} PIL solutions at 298.15 K. It has been observed that the apparent molar volume for different peptides in aqueous PIL solutions increases initially with increase in concentration of peptide and remains more or less constant at studied higher concentrations. Increase in ϕ_V at low concentration is due to peptide solvation through hydrogen bonding between PIL and peptide which gets dominated by peptide association at higher peptide concentration in aqueous PIL solutions. The apparent molar volume at infinite dilution (ϕ_V^0) was obtained from the extrapolation of the plot of apparent molar volume (ϕ_V) versus the molal concentration of the peptide (m_2) (see Fig. 1) to infinite dilution using the Eq. (2).

$$\phi_V = \phi_V^0 + B_V m_2 \tag{2}$$

Table 2 Experimental densities (ρ), apparent molar volume (ϕ_V), partial molal volume of solute ($\bar{V}_2$) and solvent ($\bar{V}_1$) at different molality (m_2) of peptides in aqueous solution containing 0.2000 mol·kg^{-1} of TEAA (m_1) at 298.15 K and 0.1 MPa.[a]

m_2 /mol·kg^{-1}	ρ /kg·m^{-3}	$10^6 \cdot \phi_V$ /m^3·mol^{-1}	$10^6 \cdot \bar{V}_2$ /m^3·mol^{-1}	$10^6 \cdot \bar{V}_1$ /m^3·mol^{-1}	m_2 /mol·kg^{-1}	ρ /kg·m^{-3}	$10^6 \cdot \phi_V$ /m^3·mol^{-1}	$10^6 \cdot \bar{V}_2$ /m^3·mol^{-1}	$10^6 \cdot \bar{V}_1$ /m^3·mol^{-1}
Glycyl-glycine + 0.2000 mol·kg^{-1} of TEAA + Water					**Glycyl-L-valine + 0.2000 mol·kg^{-1} of TEAA + Water**				
0.0000	999.02			18.547	0.0000	999.00			18.547
0.0214	1000.22	76.01 ± 0.47	76.07 ± 0.47	18.547	0.0203	1000.06	121.86 ± 0.49	121.98 ± 0.49	18.547
0.0415	1001.33	76.31 ± 0.24	76.41 ± 0.24	18.547	0.0399	1001.07	122.10 ± 0.25	122.34 ± 0.25	18.547
0.0603	1002.36	76.49 ± 0.17	76.64 ± 0.17	18.547	0.0605	1002.12	122.36 ± 0.17	122.72 ± 0.17	18.547
0.0798	1003.43	76.56 ± 0.13	76.76 ± 0.13	18.547	0.0794	1003.08	122.47 ± 0.13	122.94 ± 0.13	18.547
0.0972	1004.38	76.61 ± 0.10	76.85 ± 0.10	18.547	0.0987	1004.04	122.56 ± 0.10	123.15 ± 0.10	18.546
0.1468	1007.05	76.79 ± 0.07	77.14 ±0.07	18.546	0.1449	1006.33	122.73 ± 0.07	123.60 ± 0.07	18.545
0.1937	1009.57	76.85 ± 0.05	77.33 ± 0.05	18.545	0.1689	1007.50	122.89 ± 0.06	123.89 ± 0.06	18.544
0.2447	1012.27	76.97 ± 0.04	77.57 ± 0.04	18.544	0.2006	1009.03	123.03 ± 0.05	124.22 ± 0.05	18.543
0.2962	1014.98	77.05 ± 0.03	77.77 ± 0.03	18.543					
0.3438	1017.46	77.08 ± 0.03	77.92 ± 0.03	18.542					

0.3974	1020.25	77.08 ± 0.03	78.05 ± 0.03	18.540					
Glycyl-L-leucine + 0.2000 mol·kg^{-1} of TEAA + Water					**Triglycine + 0.2000 mol·kg^{-1} of TEAA + Water**				
0.0000	999.02			18.547	0.0000	999.02			18.547
0.0203	1000.01	139.31 ± 0.49	139.41 ± 0.49	18.547	0.0199	1000.55	111.93 ± 0.50	112.01 ± 0.50	18.547
0.0298	1000.47	139.32 ± 0.34	139.46 ± 0.34	18.547	0.0385	1001.98	112.12 ± 0.26	112.28 ± 0.26	18.547
0.0391	1000.92	139.34 ± 0.26	139.52 ± 0.26	18.547	0.0584	1003.48	112.23 ± 0.17	112.46 ± 0.17	18.547
0.0490	1001.40	139.39 ± 0.20	139.62 ± 0.20	18.547	0.0789	1005.03	112.34 ± 0.13	112.66 ± 0.13	18.547
0.0592	1001.89	139.41 ± 0.17	139.69 ± 0.17	18.547	0.1178	1007.94	112.51 ± 0.08	112.98 ± 0.08	18.546
0.0695	1002.38	139.46 ± 0.14	139.79 ± 0.14	18.547	0.1607	1011.11	112.58 ± 0.06	113.22 ± 0.06	18.545
0.0844	1003.09	139.52 ± 0.12	139.92 ± 0.12	18.546	0.2009	1014.05	112.70 ± 0.05	113.50 ± 0.05	18.544
0.1000	1003.82	139.69 ± 0.10	140.15 ± 0.10	18.546					

[a]Standard uncertainties u are $u(T)$ = 0.02 K, $u(m_2)$ = 1·10^{-4} mol·kg^{-1}. The combined standard uncertainties in $u_c(\rho)$ = 1·10^{-2} kg·m^{-3} and $u_c(\overline{V}_1)$ = 2·10^{-9} m^3·mol^{-1}.

Table 3 Experimental densities (ρ), apparent molar volume (ϕ_V), partial molal volume of solute ($\overline{V}_2$) and solvent ($\overline{V}_1$) at different molality (m_2) of peptides in aqueous solution containing 0.2009 mol·kg^{-1} of TEAG (m_1) at 298.15 K and 0.1 MPa.[a]

m_2 /mol·kg^{-1}	ρ kg·m^{-3}	$10^6 \cdot \phi_V$ /m^3·mol^{-1}	$10^6 \cdot \overline{V}_2$ /m^3·mol^{-1}	$10^6 \cdot \overline{V}_1$ /m^3·mol^{-1}	m_2 /mol·kg^{-1}	ρ /kg·m^{-3}	$10^6 \cdot \phi_V$ /m^3·mol^{-1}	$10^6 \cdot \overline{V}_2$ /m^3·mol^{-1}	$10^6 \cdot \overline{V}_1$ /m^3·mol^{-1}
Glycyl-glycine + 0.2009 mol·kg^{-1} of TEAG + Water					**Glycyl-L-valine + 0.2009 mol·kg^{-1} of TEAG + Water**				
	1003.00					1003.00			
0.0000	1002.75[b]			18.533	0.0000	1002.75[b]			18.533
0.0400	1005.23	76.06 ± 0.25	76.17 ± 0.25	18.533	0.0206	1004.07	121.75 ± 0.48	121.94 ± 0.48	18.533
0.0600	1006.34	76.13 ± 0.17	76.31 ± 0.17	18.533	0.0383	1004.98	122.03 ± 0.26	122.38 ± 0.26	18.533
0.0811	1007.48	76.34 ± 0.12	76.57 ± 0.12	18.533	0.0595	1006.05	122.31 ± 0.17	122.85 ± 0.17	18.533
0.0976	1008.39	76.40 ± 0.10	76.68 ± 0.10	18.533	0.0783	1006.96	122.83 ± 0.13	123.54 ± 0.13	18.532
0.1449	1010.94	76.60 ± 0.07	77.02 ± 0.07	18.532	0.0984	1007.98	122.78 ± 0.10	123.68 ± 0.10	18.531
0.1953	1013.65	76.71 ± 0.05	77.27 ± 0.05	18.531	0.1285	1009.44	123.07 ± 0.08	124.23 ± 0.08	18.530
0.2443	1016.24	76.84 ± 0.04	77.54 ± 0.04	18.530	0.1683	1011.35	123.31 ± 0.06	124.83 ± 0.06	18.528
0.2950	1018.90	76.91 ± 0.03	77.75 ± 0.03	18.528	0.2001	1012.87	123.40 ± 0.05	125.21 ± 0.05	18.526
0.3442	1021.45	77.01 ± 0.03	78.00 ± 0.03	18.527					

0.3995	1024.27	77.14 ± 0.03	78.29 ± 0.03	18.525					
Glycyl-L-leucine + 0.2009 mol·kg^{-1} of TEAG + Water					**Triglycine + 0.2009 mol·kg^{-1} of TEAG + Water**				
	1003.00					1003.00			
0.0000	1002.75[b]			18.533	0.0000	1002.75[b]			18.533
0.0195	1003.95	138.98 ± 0.51	139.20 ± 0.51	18.533	0.0206	1004.58	111.82 ± 0.48	111.86 ± 0.48	18.533
0.0298	1004.45	139.11 ± 0.33	139.45 ± 0.33	18.533	0.0390	1005.99	111.93 ± 0.25	112.01 ± 0.25	18.533
0.0398	1004.92	139.25 ± 0.25	139.71 ± 0.25	18.533	0.0589	1007.51	111.99 ± 0.17	112.11 ± 0.17	18.533
0.0497	1005.39	139.41 ± 0.20	139.99 ± 0.20	18.533	0.0798	1009.09	112.03 ± 0.12	112.19 ± 0.12	18.533
0.0591	1005.84	139.42 ± 0.17	140.11 ± 0.17	18.532	0.1174	1011.92	112.06 ± 0.08	112.30 ± 0.08	18.533
0.0689	1006.30	139.52 ± 0.14	140.31 ± 0.14	18.532	0.1600	1015.08	112.19 ± 0.06	112.52 ± 0.06	18.532
0.0844	1007.02	139.69 ± 0.12	140.66 ± 0.12	18.532	0.1999	1018.02	112.21 ± 0.05	112.61 ± 0.05	18.532
0.0998	1007.72	139.98 ± 0.10	141.13 ± 0.10	18.531					

[a] Standard uncertainties u are $u(T)$ = 0.02 K, $u(m_2)$ = 1·10^{-4} mol·kg^{-1}. The combined standard uncertainties in $u_c(\rho)$ = 1·10^{-2} kg·m^{-3} and u_c ($\overline{V}_1$) = 2·10^{-9} m^3·mol^{-1}.

[b] Density data for TEAG + water solutions taken from Reference [51].

Table 4 Experimental densities (ρ), apparent molar volume (ϕ_V), partial molal volume of solute ($\overline{V}_2$) and solvent ($\overline{V}_1$) at different molality (m_2) of peptides in aqueous solution containing 0.1994 mol·kg^{-1} of TEAPy (m_1) at 298.15 K and 0.1 MPa.[a]

m_2 /mol·kg^{-1}	ρ /kg·m^{-3}	$10^6 \cdot \phi_V$ /m^3·mol^{-1}	$10^6 \cdot \overline{V}_2$ /m^3·mol^{-1}	$10^6 \cdot \overline{V}_1$ /m^3·mol^{-1}	m_2 /mol·kg^{-1}	ρ /kg·m^{-3}	$10^6 \cdot \phi_V$ /m^3·mol^{-1}	$10^6 \cdot \overline{V}_2$ /m^3·mol^{-1}	$10^6 \cdot \overline{V}_1$ /m^3·mol^{-1}
Glycyl-glycine + 0.1994 mol·kg^{-1} of TEAPy + Water					**Glycyl-L-valine + 0.1994 mol·kg^{-1} of TEAPy + Water**				
	1000.91					1000.91			
0.0000	1001.13[b]			18.610	0.0000	1001.13[b]			18.610
0.0212	1002.10	75.86 ± 0.47	75.94 ± 0.47	18.610	0.0229	1002.12	121.10 ± 0.44	121.27 ± 0.44	18.610
0.0412	1003.22	75.95 ± 0.24	76.10 ± 0.24	18.610	0.0409	1003.05	121.66 ± 0.24	121.96 ± 0.24	18.610
0.0598	1004.26	75.96 ± 0.17	76.18 ± 0.17	18.610	0.0597	1004.00	122.01 ± 0.17	122.46 ± 0.17	18.610
0.0793	1005.33	76.06 ± 0.13	76.35 ± 0.13	18.610	0.0784	1004.94	122.28 ± 0.13	122.87 ± 0.13	18.610
0.0983	1006.37	76.14 ± 0.10	76.50 ± 0.10	18.610	0.0995	1006.00	122.41 ± 0.10	123.16 ± 0.10	18.609
0.1478	1009.07	76.31 ± 0.07	76.84 ± 0.07	18.609	0.1283	1007.45	122.38 ± 0.08	123.34 ± 0.08	18.608
0.1954	1011.64	76.41 ± 0.05	77.12 ± 0.05	18.608	0.1681	1009.40	122.61 ± 0.06	123.88 ± 0.06	18.606
0.2432	1014.17	76.58 ± 0.04	77.46 ± 0.04	18.606	0.2006	1010.98	122.69 ± 0.05	124.20 ± 0.05	18.605
0.2979	1017.01	76.82 ± 0.03	77.90 ± 0.03	18.604					

0.3469	1019.51	77.07 ± 0.03	78.33 ± 0.03	18.602					
0.3959	1021.99	77.24 ± 0.03	78.67 ± 0.03	18.600					
Glycyl-ʟ-leucine + 0.1994 mol·kg^{-1} of TEAPy + Water					**Triglycine + 0.1994 mol·kg^{-1} of TEAPy + Water**				
	1000.91					1000.91			
0.0000	1001.13[b]			18.610	0.0000	1001.13[b]			18.610
0.0198	1001.89	138.91 ± 0.50	139.04 ± 0.50	18.610	0.0204	1002.49	111.63 ± 0.49	111.74 ± 0.49	18.610
0.0298	1002.37	138.98 ± 0.33	139.18 ± 0.33	18.610	0.0393	1003.93	111.86 ± 0.25	112.08 ± 0.25	18.610
0.0399	1002.86	139.08 ± 0.25	139.35 ± 0.25	18.610	0.0591	1005.43	112.19 ± 0.17	112.52 ± 0.17	18.610
0.0492	1003.31	139.17 ± 0.20	139.50 ± 0.20	18.610	0.0787	1006.90	112.39 ± 0.13	112.84 ± 0.13	18.610
0.0594	1003.80	139.22 ± 0.17	139.62 ± 0.17	18.610	0.1181	1009.84	112.55 ± 0.08	113.22 ± 0.08	18.609
0.0695	1004.28	139.26 ± 0.14	139.72 ± 0.14	18.610	0.1581	1012.79	112.69 ± 0.06	113.59 ± 0.06	18.608
0.0846	1004.99	139.35 ± 0.12	139.91 ± 0.12	18.610	0.2029	1016.07	112.70 ± 0.05	113.85 ± 0.05	18.606
0.1001	1005.72	139.46 ± 0.10	140.13 ± 0.10	18.609					

[a] Standard uncertainties u are $u(T)$ = 0.02 K, $u(m_2) = 1\cdot10^{-4}$ mol·kg^{-1}. The combined standard uncertainties in $u_c(\rho) = 1\cdot10^{-2}$ kg·m^{-3} and $u_c(\overline{V}_1) = 2\cdot10^{-9}$ m^3·mol^{-1}.

[b] Density data for TEAPy + water solutions taken from Reference [51].

Table 5 Experimental densities (ρ), apparent molar volume (ϕ_V), partial molal volume of solute ($\overline{V}_2$) and solvent ($\overline{V}_1$) at different molality (m_2) of peptides in aqueous solution containing ~0.2116 mol·kg^{-1} of TEAP (m_1) at 298.15 K and 0.1 MPa.[a]

m_2 /mol·kg^{-1}	ρ /kg·m^{-3}	$10^6 \cdot \phi_V$ /m^3·mol^{-1}	$10^6 \cdot \overline{V}_2$ /m^3·mol^{-1}	$10^6 \cdot \overline{V}_1$ /m^3·mol^{-1}	m_2 /mol·kg^{-1}	ρ /kg·m^{-3}	$10^6 \cdot \phi_V$ /m^3·mol^{-1}	$10^6 \cdot \overline{V}_2$ /m^3·mol^{-1}	$10^6 \cdot \overline{V}_1$ /m^3·mol^{-1}
Glycyl-glycine + 0.2116 mol·kg^{-1} of TEAP + Water					**Glycyl-L-valine + 0.2116 mol·kg^{-1} of TEAP + Water**				
	998.57					998.57			
0.0000	998.43[b]			18.638	0.0000	998.43[b]			18.638
0.0398	1000.80	75.83 ± 0.25	75.99 ± 0.25	18.638	0.0191	999.59	120.96 ± 0.53	121.12 ± 0.53	18.638
0.0590	1001.87	75.96 ± 0.17	76.19 ± 0.17	18.638	0.0402	1000.67	121.64 ± 0.25	121.99 ± 0.25	18.638
0.0801	1003.04	76.03 ± 0.12	76.34 ± 0.12	18.638	0.0588	1001.62	122.15 ± 0.17	122.65 ± 0.17	18.638
0.0981	1004.04	75.99 ± 0.10	76.38 ± 0.10	18.638	0.0797	1002.67	122.40 ± 0.13	123.08 ± 0.13	18.637
0.1453	1006.57	76.45 ± 0.07	77.02 ± 0.07	18.637	0.0990	1003.63	122.54 ± 0.10	123.39 ± 0.10	18.637
0.1973	1009.32	76.82 ± 0.05	77.60 ± 0.05	18.636	0.1291	1005.15	122.56 ± 0.08	123.66 ± 0.08	18.636
0.2467	1011.95	76.91 ± 0.04	77.89 ± 0.04	18.634	0.1684	1007.09	122.70 ± 0.06	124.14 ± 0.06	18.634
0.3481	1017.26	77.02 ± 0.03	78.40 ± 0.03	18.629	0.1994	1008.59	122.80 ± 0.05	124.51 ± 0.05	18.632

0.4166	1020.71	77.30 ± 0.03	78.96 ± 0.03	18.626					
Glycyl-L-leucine + 0.2115 mol·kg^{-1} of TEAP + Water					**Triglycine + 0.2115 mol·kg^{-1} of TEAP + Water**				
	998.55					998.55			
0.0000	998.43[b]			18.639	0.0000	998.43[b]			18.639
0.0198	999.53	138.74 ± 0.51	138.90 ± 0.51	18.639	0.0211	1000.20	111.16 ± 0.47	111.31 ± 0.47	18.639
0.0349	1000.27	139.01 ± 0.29	139.30 ± 0.29	18.638	0.0394	1001.59	111.72 ± 0.25	112.00 ± 0.25	18.638
0.0392	1000.48	139.10 ± 0.26	139.43 ± 0.26	18.638	0.0593	1003.09	112.16 ± 0.17	112.57 ± 0.17	18.638
0.0494	1000.96	139.20 ± 0.20	139.62 ± 0.20	18.638	0.0783	1004.53	112.27 ± 0.13	112.82 ± 0.13	18.638
0.0595	1001.45	139.29 ± 0.17	139.80 ± 0.17	18.638	0.1184	1007.53	112.37 ± 0.08	113.21 ± 0.08	18.637
0.0695	1001.93	139.34 ± 0.14	139.93 ± 0.14	18.638	0.1588	1010.52	112.51 ± 0.06	113.63 ± 0.06	18.635
0.0894	1002.87	139.43 ± 0.11	140.19 ± 0.11	18.637	0.2003	1013.54	112.71 ± 0.05	114.12 ± 0.05	18.633
0.1003	1003.39	139.49 ± 0.10	140.34 ± 0.10	18.637					

[a]Standard uncertainties u are $u(T)$ = 0.02 K, $u(m_2)$ = 1·10^{-4} mol·kg^{-1}. The combined standard uncertainties in $u_c(\rho)$ = 1·10^{-2} kg·m^{-3} and u_c ($\overline{V}_1$) = 2·10^{-9} m^3·mol^{-1}.

[b]Density data for TEAP + water solutions taken from Reference [51].

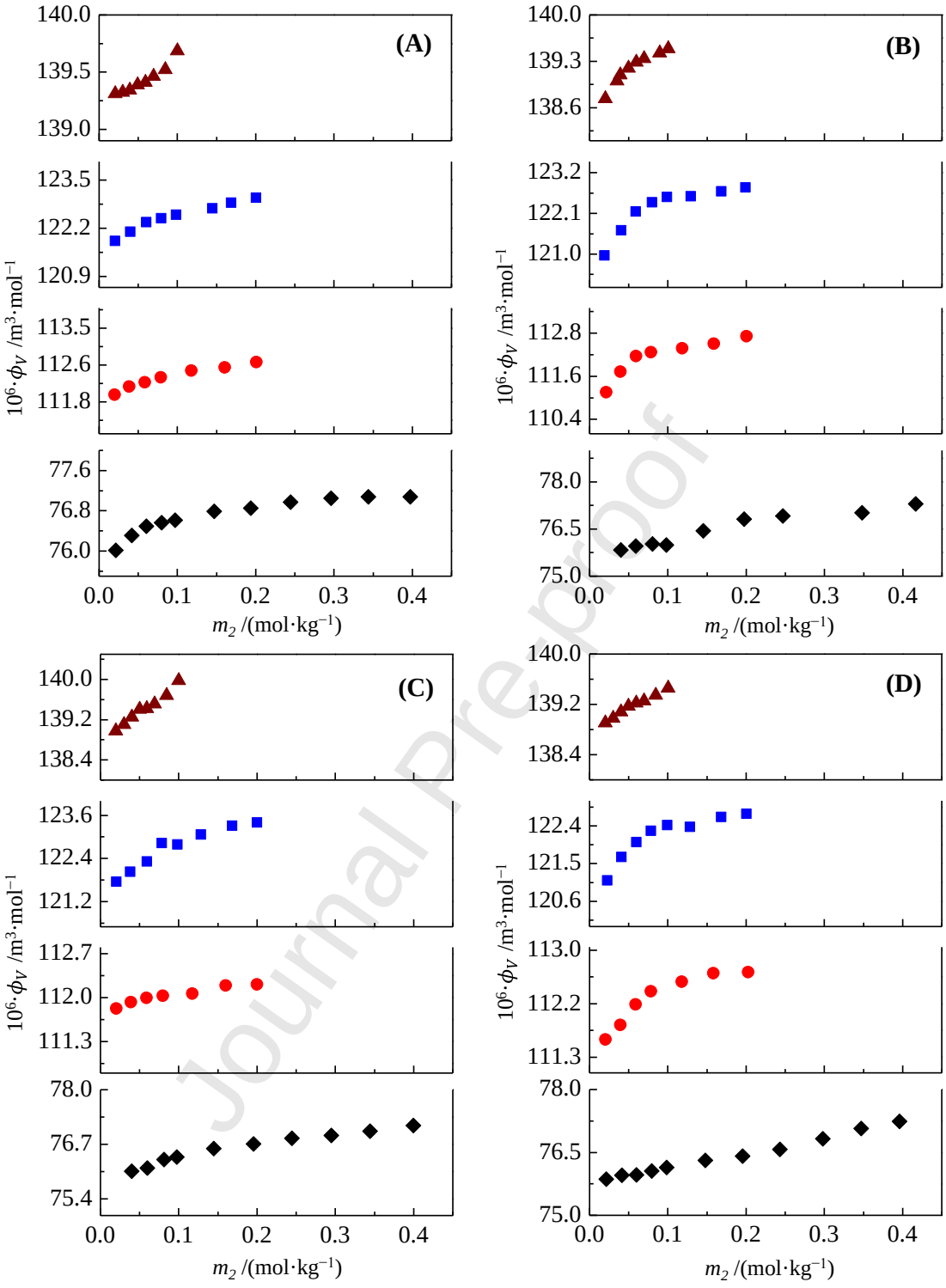


Fig. 1. Variation of apparent molar volume (ϕ_V) for different peptide: digly, ♦; trigly, ●; gly-val, ■; gly-leu, ▲ in ~0.2 mol·kg^{-1} aqueous solutions of different PILs: A) TEAA, B) TEAP, C) TEAG and D) TEAPy at 298.15 K.

wherein ϕ_V^0 is the limiting apparent molar volume of a peptide in aqueous PIL solution and B_V is the experimental slope. The data of ϕ_V^0 and B_V thus obtained for studied glycine based peptides in aqueous PIL solutions at 298.15 K are compiled in Table 6. The parameter ϕ_V^0 excludes ion–ion interactions and hence is helpful to understand precisely the ion–solvent interaction. It is noted that the limiting apparent molar volume of peptides in aqueous PIL solutions is lower than that in water which indicates the hydrophobic solvation of peptides in aqueous PIL solutions more important in presence PIL in water that its absence. Furthermore, the effect of hydrophobic solvation of peptides in aqueous PIL solutions becomes more apparent with increase in hydrophobicity of PILs as revealed from more decrease in ϕ_V^0 values with PIL hydrophobicity (see the Table 6). A similar trends were also observed for amino acids and peptides in the aqueous of tetra-*n*-alkylammonium bromide and 1-*n*-pentyl-3-methylimidazolium chloride ([C_5mim] Cl) solutions [18−20,29] resulting an increased electrostriction in the vicinity of charged centers of the peptide. The reorganization and strengthening of hydrogen bonded network of water molecules in the vicinity of peptide functionality arises due to electric field exerted by charged centers in solution and hence diminution of the void spaces leads to decrease in ϕ_V^0 values for peptides implying hydrophobic solvation. Since, the effect is more prominent if ternary mixture contains relatively more hydrophobic species i.e. either gly-leu or TEAP.

Table 6 The limiting apparent molar volume (ϕ_V^0) and experimental slope (B_V) for the different peptides in the ~0.20 mol·kg^{-1} of different PILs aqueous solutions at 298.15 K and 0.1MPa.[a]

Solvents	Glycyl-glycine		Triglycine		Glycine-ʟ-valine		Glycyl-ʟ-leucine	
	ϕ_V^0	B_V	ϕ_V^0	B_V	ϕ_V^0	B_V	ϕ_V^0	B_V
Water	76.30 ± 0.07[b]	2.55 ± 0.35[b]	112.11 ± 0.03[c]	5.4 ± 0.4[c]	121.99 ± 0.02[c]	1.7 ± 0.4[c]	139.70 ± 0.07[c]	0.9 ± 1[c]
TEAA	76.58 ± 0.07	1.41 ± 0.22	112.10 ± 0.05	3.01 ± 0.30	122.04 ± 0.05	5.00 ± 0.28	139.10 ± 0.05	5.60 ± 0.69
TEAG	76.23 ± 0.04	2.30 ± 0.14	111.89 ± 0.04	1.64 ± 0.24	122.16 ± 0.14	6.46 ± 0.87	138.72 ± 0.08	12.14 ± 0.97
TEAPy	75.67 ± 0.04	3.93 ± 0.13	112.11 ± 0.13	3.11 ± 0.82	121.83 ± 0.13	4.40 ± 0.83	138.83 ± 0.03	6.24 ± 0.31
TEAP	76.04 ± 0.13	3.03 ± 0.40	111.82 ± 0.11	4.44 ± 0.70	121.97 ± 0.15	4.27 ± 0.94	138.85 ± 0.06	6.51 ± 0.72

[a]Units: $10^6 \cdot \phi_V^0$ /(m^3.mol^{-1}); $10^6 \cdot B_V$ /(m^3·kg·mol^{-2}).

[b,c] ϕ_V^0 values for peptides + water solutions taken from Reference [10,12].

The partial molar volumes of non-electrolytes (peptide) ($\overline{V}_2$) and partial molar volume of solvent ($\overline{V}_1$) (i.e. water + ~0.2 mol·kg^{-1}of PIL) were obtained from the Eqs. (3) and (4), respectively [60]

$$\overline{V_2} = \phi_V + m_2\left(\frac{\partial \phi_V}{\partial m_2}\right) \tag{3}$$

$$\overline{V_1} = V_1^0 - \left(\frac{M_S}{\upsilon}\right) m_2^2 \left(\frac{\partial \phi_V}{\partial m_2}\right) \tag{4}$$

where, V_1^0 is the molar volume of solvent obtained from density of mixed solvent system, υ is the number of ions produced by peptides (υ =1). M_S is the molecular weight of mixed solvent (water + ~0.2 mol·kg^{-1} of PIL) which was estimated using Eq. (5) [60].

$$M_S = \frac{1}{\left(\frac{w_{water}}{M_{water}} + \frac{w_{PIL}}{M_{PIL}}\right)} \tag{5}$$

where, w_{water} and w_{PIL} are mass fraction of water and PIL respectively for the binary mixtures used here as a mixed solvent, whereas M_{water} and M_{PILs} are molar masses in kg·mol^{-1} of water and PILs respectively. The data estimated for partial molar volume of solute (peptides) and solvent (~0.2 mol·kg^{-1}of aqueous solutions of PILs) are given in Tables 2 to 5 and graphically shown in Figs. 2 to 5. From Figs. 2 to 5, it is observed that, the partial molar volumes of peptides ($\overline{V}_2$) increases with increasing concentration. Further in case of increasing hydrophobicity of PIL in aqueous solutions, the decrease in partial molar volume of peptides is observed. The partial molar volume of solute increases at lower concentration however at higher concentration the values becomes more or less constant for all studied peptides and the trends observed are in line with the ϕ_V data so Supplementary the above interpretation offered

to concentration dependent behavior of ϕ_v. The partial molar volumes of solvent (water + ~0.2 mol.kg^{-1} of PIL) ($\overline{V}_1$) decreases with increasing the concentration of peptides and increases slightly with increasing the hydrophobicity of PILs indicating hydrophobic solvation of peptides in aqueous PIL solutions with PIL mediated water structure making effect [58]. This kosmotropic effect in peptide solutions in presence of PILs is further supported by sign and magnitude of limiting transfer molar volumes obtained for transfer of peptides from water to aqueous PIL solutions which accounts the interactions between solute peptide and cosolute PIL, the details of which are given below.

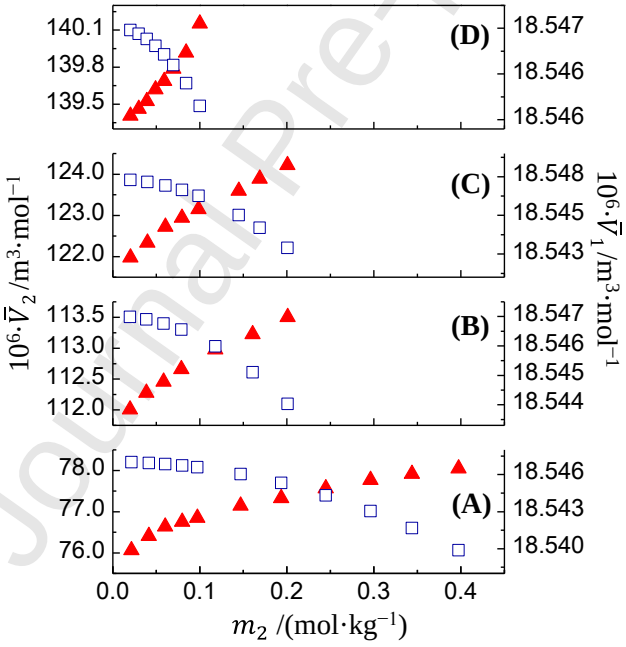


Fig. 2. The variation of the partial molar volume of peptide ($\overline{V}_2$) (rigid triangle ▲) and solvent (water + ~0.2 mol·kg^{-1} of TEAA ($\overline{V}_1$)) (open square □) as function of the molality of peptides (m_2) at 298.15 K: A) digly, B) trigly, C) gly-val and D) gly-leu.

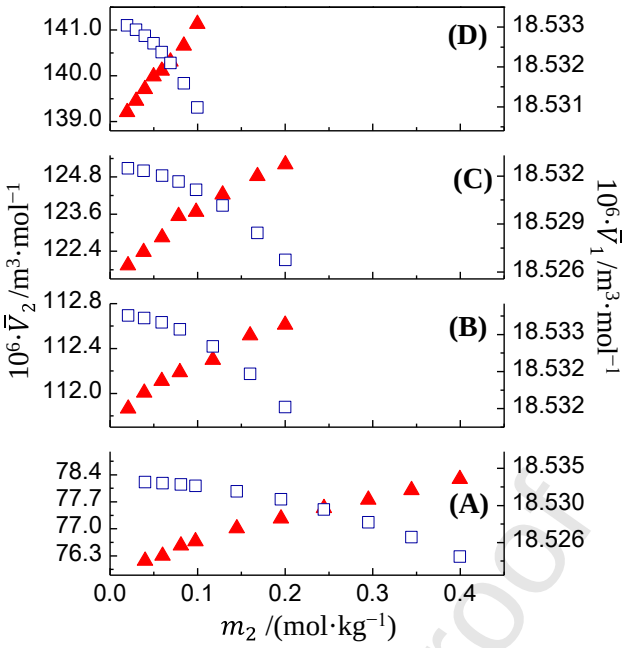


Fig. 3. The variation of the partial molar volume of peptide ($\bar{V}_2$) (rigid triangle ▲) and solvent (water + ~0.2 mol·kg^{-1} of TEAG ($\bar{V}_1$)) (open square □) as function of the molality of peptides (m_2) at 298.15 K: A) digly, B) trigly, C) gly-val and D) gly-leu.

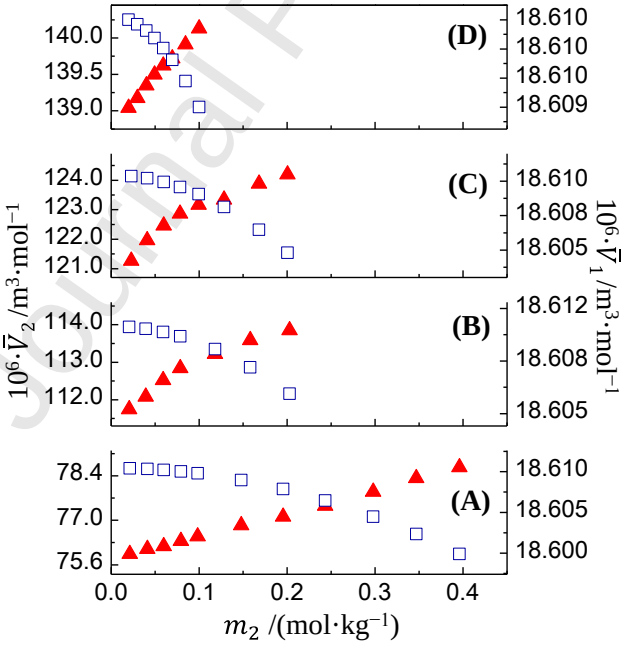


Fig. 4. The variation of the partial molar volume of peptide ($\bar{V}_2$) (rigid triangle ▲) and solvent (water + ~0.2 mol·kg^{-1} of TEAPy ($\bar{V}_1$)) (open square □) as function of the molality of peptides (m_2) at 298.15 K: A) digly, B) trigly, C) gly-val and D) gly-leu.

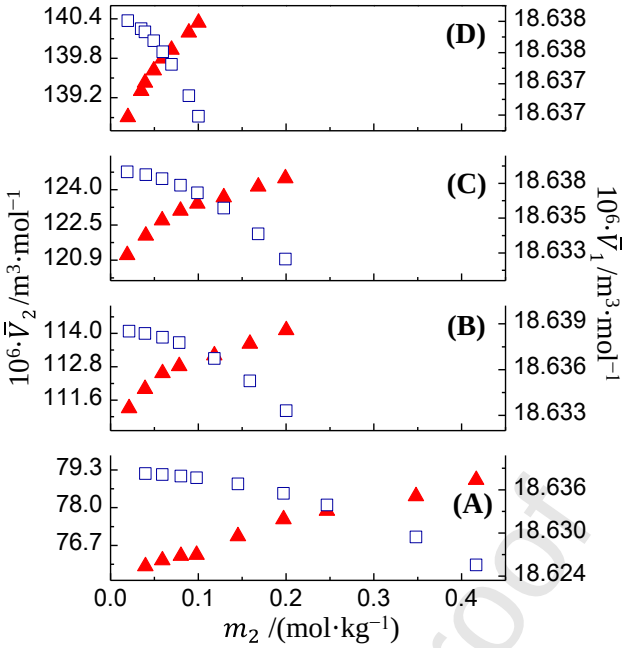


Fig. 5. The variation of the partial molar volume of peptide ($\bar{V}_2$) (rigid triangle ▲) and solvent (water + ~0.2 mol·kg^{-1} of TEAP ($\bar{V}_1$)) (open square □) as function of the molality of peptides (m_2) at 298.15 K: A) digly, B) trigly, C) gly-val and D) gly-leu.

3.2. Apparent Transfer Molar Volume

Limiting apparent transfer molar volumes ($\Delta_{tr}\phi_V^0$) for the peptides due to its transfer from water to aqueous PILs solutions were calculated using Eq. (6) and the data obtained for $\Delta_{tr}\phi_V^0$ of studied peptides are collected in Table 7.

$$\Delta_{tr}\phi_V^0 = \phi_V^0(\sim 0.2 mol \cdot kg^{-1} aqueous PIL\ Solution) - \phi_V^0(water) \quad (6)$$

where, ϕ_V^0 (water) is the limiting apparent molar volume of peptide in pure water at 298.15 K data of which were taken from the literature [10,12] and ϕ_V^0 (~0.2 mol·kg^{-1} aqueous PIL solution) is the limiting apparent molar volume of peptides in ~0.2 mol·kg^{-1} aqueous PIL solution. The obtained $\Delta_{tr}\phi_V^0$ values are negative (Table 7 and Fig. 6) for all studied peptides in aqueous PIL solutions which means that the limiting apparent molar volumes for studied

peptides in aqueous PILs solutions are smaller than those in pure water. From Table 7, it is observed that the $\Delta_{tr}\phi_V^0$ for digly in ~0.2 mol·kg^{-1} of aqueous TEAA solutions at 298.15 K are close to zero indicating that the zwitterionic $-NH_3^+$ and $-COO^-$ groups interacts water molecules with same strength as the ions of TEAA with water. However, $\Delta_{tr}\phi_V^0$ values become more negative by increasing the hydrophobicity of PILs (see Fig. 6) due to hydrophobic–hydrophobic interaction between non–polar group of PILs and non−polar group of peptides dominating over ion−ion interactions. Furthermore, the $\Delta_{tr}\phi_V^0$ value shift towards more negative with increasing the hydrophobicity of peptides and the effect is more pronounced for peptides in aqueous solutions of TEAPy and TEAP (shown in Scheme 2). A comparison in $\Delta_{tr}\phi_V^0$ values for studied peptides in different APILs, traditional salts, tetra-*n*-alkyl ammonium halide salt, carbohydrates and surfactants within studied concentration range of PILs are given in the Table 7. Banipal [15], Ali [20] and Yan [21] have also observed similar trends for amino acids and peptides in sodium salts of carboxylic acids, tetra-*n*-alkylammonium bromide and sodium butyrate in aqueous solutions, respectively. Fang et al. [27] reported the negative magnitudes of $\Delta_{tr}\phi_V^0$ for amino acids such as glycine, L-phenylalanine and L-alanine for their transfer from water to aqueous solution of APIL namely 1-ethyl-3-methylimidazolium bromide ([Emim][Br]). Similarly, few other researchers [37,38,49] also reported negative magnitude of $\Delta_{tr}\phi_V^0$ for amino acids on transfer them from water to aqueous 1-*n*-alkyl-3-methylimidazolium based ionic liquids. Furthermore, Singh et al. [49] demonstrated that that DL-α-alanine and glycine have negative $\Delta_{tr}\phi_V^0$ for their transfer from water to aqueous PIL namely 3-hydroxypropylammonium formate (3-HPAF). Recently, we observed that ion−hydrophobic interaction between the for amino acids ionic liquids (AAILs) and polyethylene glycols (PEGs) assisted with solute–cosolute H−bonding along with electrostriction effect exists in ternary aqueous solutions containing AAILs and

PEGs as revealed from observed negative magnitude of $\Delta_{tr}\phi_V^0$ [58]. A similar behavior has also been seen for the amino acids/peptides with aqueous tetraalkylammonium salts solutions [18−20]. The positive $\Delta_{tr}\phi_V^0$ are reported for peptides in aqueous NaCl [7], sodium butyrate [21], K_2SO_4 [23], KNO_3 [23], imidazolium based ionic liquids [29−46], glucose [8,61], sucrose [8], D-galactose [62], xylitol [63], CPC and CPB [64] and tartrazine [65] solution at 298.15 K (See the Table 7). The positive values of $\Delta_{tr}\phi_V^0$ represent stronger hydrophilic interactions between solute and co-solute in aqueous medium causing expansion of volume with release of some hydrated water molecules on transfer of solute from to aqueous co-solute solutions. Seen in this light, H−bond assisted hydrophobic solvation of peptides occurs in aqueous protic ionic liquid solutions. These results can be justified based on co-sphere overlap model [12]. Accordingly, when interactions between ionic (or dipolar) groups between two different molecules occurs through electrostatic forces then they are called as ion-ion interactions or dipolar-dipolar interactions or ion-dipolar interaction which results in increase in volume on transferring solute from binary to ternary aqueous solutions. On contrary if interactions between non-polar moieties of two molecules occurs in aqueous medium then they are called as hydrophobic-hydrophobic interactions which causes large decrease in volumes on transferring solute from aqueous binary to aqueous ternary solutions. Similar decrease is in volume of transfer is observed but with less negative magnitudes when the ionic (or dipolar) groups of one molecule interact with non-polar moieties of other molecules in aqueous solutions. Thus, according to co-sphere overlap model, the positive $\Delta_{tr}\phi_V^0$ values signify ion (or dipolar)−ion (or dipolar) interactions whereas negative $\Delta_{tr}\phi_V^0$ denotes ion (or dipolar)−hydrophobic and/or hydrophobic−hydrophobic interactions. The nature of solute species affects the properties of water molecules in the cosphere hydration shell. In the perspective of co-sphere overlap model, for ternary systems peptides + PILs +

water, three different types of interactions exist based on sign and magnitude of $\Delta_{tr}\phi_V^0$ values. These are: (i) ion−ion interactions, (ii) ion−hydrophobic interactions and (iii) hydrophobic−hydrophobic interactions. Accordingly, ionic–hydrophobic interaction between ionic group of PILs/peptides and non−polar group of peptides/PILs along with H-bond assisted hydrophobic association of peptides dominates over ion–ion interactions causing more electrostriction alongwith water structure making effect (Kosmotropic effect) (shown in Scheme 2). Thus, aqueous peptide solutions containing PIL TEAA favors ion-ion or ion-diploar interactions over hydrophobic-hydrophobic interactions when the peptides less hydrophobic e.g. gly-gly, trigly and gly-$_L$-val. However, as the hydrophobicity increases either for the PIL or for the peptide, the hydrophobic-hydrophobic interactions amongst the PIL and peptide molecules dominates over ion-ion or ion-diploar interactions as noted from the sign and magnitude of $\Delta_{tr}\phi_V^0$ reported in Table 7. This observation indicates that fine structural changes in PILs can help to tune the physicochemical behavior of aqueous PIL solutions for their appropriation use in different fields such as protein purification, stabilization, drug formulations etc.

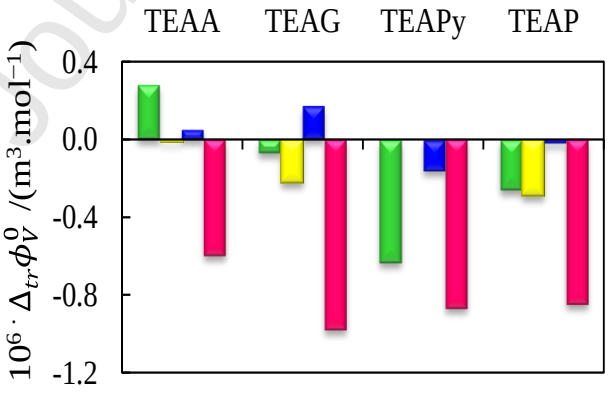
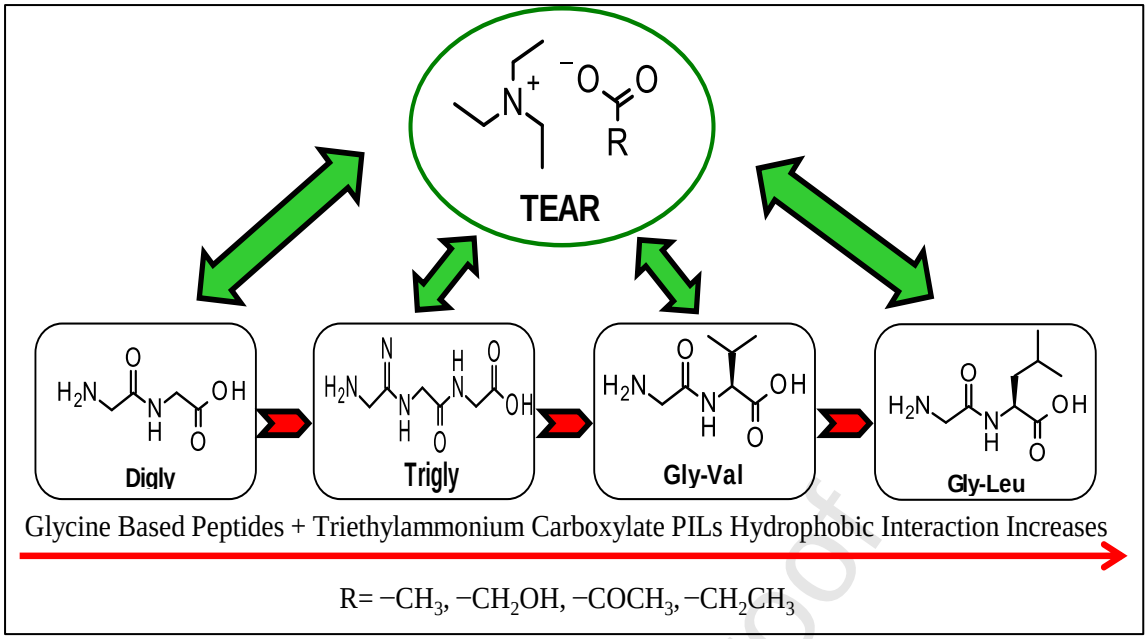


Fig. 6. Apparent transfer molar volume ($\Delta_{tr}\phi_V^0$) of peptides: digly,■; trigly,■; gly-val, ■; gly-leu, ■ in ~0.2 mol·kg^{-1} PILs aqueous solution at 298.15 K.



Scheme 2. Glycine based peptides and triethylammonium based carboxylate PILs interactions.

Table 7. The comparison of observed apparent transfer molar volume ($\Delta_{tr}\phi_V^0$) of studied peptides with the literature values at 298.15 K.

Peptides	This work		Literature value	
	Solvent (~0.2 mol·kg^{-1} PILs)[a]	$10^6\cdot\Delta_{tr}\phi_V^0$ /m^3.mol^{-1}	Solvent (Aqueous electrolyte/non-electrolyte solutions)	$10^6\cdot\Delta_{tr}\phi_V^0$ /m^3.mol^{-1}
Glycyl-glycine	TEAA	0.28 ± 0.10	0.2581[a] TMABr[b]	0.67
	TEAG	−0.07 ± 0.08	0.2623[a] TEABr[b]	0.82
	TEAPy	−0.63 ± 0.08	0.2709 [a] TBABr[b]	0.76
	TEAP	−0.26 ±0.15	0.5312[a] TMABr[b]	0.62
			0.5491[a] TEABr[b]	0.80
			0.5889[a] TBABr[b]	1.76
			0.5[a] Sodium butyrate (NaB_4)[d]	2.96
			0.5[a] , 1.0[a] SA[e]	2.93, 4.23
			0.5[a] , 1.0[a] MA[e]	3.47, 5.18
			0.512[a] K_2SO_4[f]	0.25
			0.512[a] KNO_3[f]	0.11

	0.01[a] [C_5mim]Cl[g]	−0.08
	0.03[a] [C_5mim]Cl[g]	0.70
	0.1[a] [C_4mim]Br[h]	8.86
	0.3[a] [C_4mim]Br[h]	11.75
	0.1[a], 0.3 [a] [mim]Cl[i]	1.79, 1.19
	0.1[a], 0.3 [a] [C_2mim]Cl[i]	0.80, 2.33
	0.1[a], 0.3 [a] [C_8mim]Cl[i]	4.54, 0.38
	0.03[a] [C_6mim]Cl[j]	0.92
	0.1[a] [C_{10}mim]Br[k]	1.11
	0.1[a] [C_{14}mim]Br[k]	1.61
	0.05[a] [C_{12}mim]Br[l]	0.30
	0.10[a] [C_{12}mim]Br[l]	1.10
	0.05[a] D-Galactose[m]	1.223
	2.06 and 3.96 mass % Glucose[n]	0.55, 0.67
	0.1[a], 0.15[a] Xylitol[o]	1.13, 1.96
	0.05[a] CPC[p]	0.5322

			0.05[a] CPB[p]	0.2271
			0.01[a] Tartrazine[q]	5.9546
			1.0[a] CH3COONa[r]	3.62
			1.0[a] NaSCN[r]	1.19
			1.0[a] NaSCN[r]	5.58
Triglycine	TEAA	−0.01 ± 0.06	0.5[a], 1.0[a] SA[e]	3.64, 5.66
	TEAG	−0.22 ± 0.05	0.5[a], 1.0[a] MA[e]	4.67, 6.87
	TEAPy	0.00 ± 0.13	2.06 and 3.96 mass % Glucose[n]	0.42, 0.43
	TEAP	−0.29 ± 0.11	0.1[a], 0.15[a] Xylitol[o]	1.3, 1.63
			0.01[a] Tartrazine[q]	8.2129
			1.0[a] CH_3COONa[r]	3.70
			1.0[a] NaSCN[r]	2.23
			1.0[a] NaSCN[r]	–
Glycine-ʟ-valine	TEAA	0.05 ± 0.05	0.5[a] Sodium butyrate[d]	2.74
	TEAG	0.17 ± 0.14	0.1[a] $[C_{10}mim]Br$[k]	1.61
	TEAPy	−0.16 ± 0.13	0.1[a] $[C_{14}mim]Br$[k]	2.37

	TEAP	−0.02 ± 0.15	0.05[a] $[C_{12}mim]Br$[l]	0.28
			0.10[a] $[C_{12}mim]Br$[l]	1.57
Glycyl-ʟ-leucine	TEAA	−0.60 ± 0.09	0.25[a] TEABr[c]	−0.83
	TEAG	−0.98 ± 0.11	0.5[a] Sodium butyrate[d]	2.43
	TEAPy	−0.87 ± 0.08	0.5[a], 1.0[a] SA[e]	1.79, 2.9
	TEAP	−0.85 ± 0.09	0.5[a], 1.0[a] MA[e]	2.25, 3.68
			0.1[a] $[C_{10}mim]Br$[k]	2.00
			0.1[a] $[C_{14}mim]Br$[k]	3.86
			0.05[a] $[C_{12}mim]Br$[l]	0.49
			0.10[a] $[C_{12}mim]Br$[l]	1.44

[a]Units refer to molality ($mol \cdot kg^{-1}$) of the solution in the presence of cosolute.

[b]Literature values of $\Delta_{tr}\phi_V^0$ for peptides in aqueous cosolute solution [16], [c]Reference [19], [d]Reference [21], [e]Reference [22], [f]Reference [23], [g]Reference [29], [h]Reference [30], [i]Reference [31], [j]Reference [32], [k]Reference [40], [l]Reference [46], [m]Reference [61], [n]Reference [62], [o]Reference [63], [p]Reference [64], [q]Reference [65] and [r]Reference [66].

TEAA (Triethylammonium acetate), **TEAG** (Triethylammonium glycolate), **TEAPy** (Triethylammonium pyruvate), **TEAP** (Triethylammonium propionate), **TMABr** (Tetramethyl ammonium bromide), **TEABr** (Tetraethyl ammonium bromide), **TBABr** (Tetrabutyl ammonium bromide), **SA** (Sodium acetate), **MA** (Magnesium acetate), **[mim]Cl** (1-methylimidazolium chloride), **$[C_2mim]Cl$** (1-ethyl-3-methylimidazolium chloride), **$[C_4mim]Br$** (1-Butyl-3-methylimidazolium bromide), **$[C_5mim]Cl$** (1-pentyl-3-methylimidazoliumchloride), **$[C_8mim]Cl$** (1-methyl-3-octylimidazolium chloride), **$[C_6mim]Cl$** (1-hexyl-3-methylimidazolium chloride), **$[C_{10}mim]Br$** (1-Decyl-3-methylimidazolium bromide), **$[C_{12}mim]Br$** (1-Dodecyl-3-methylimidazolium bromide) **$[C_{14}mim]Br$** (1-tetradecyl-3-methyl-imidazolium bromide), **CPC** (Cetylpyridinium chloride) and **CPB** (Cetylpyridinium bromide).

3.3. Group Contribution in Limiting Apparent Molar Volume (ϕ_V^0) of Peptides

A linear relationship has been observed between the ϕ_V^0 of the peptides and the number of carbon atoms n_C, in their alkyl side chains which is represented by Eq. (7) [18,21].

$$\phi_V^0 = \phi_V^0(-CH_2CONH-,-NH_3^+,-COO^-) + n_C\phi_V^0(CH_2) \quad (7)$$

where n_C is the number of carbon atoms in the side alkyl chain of peptides, $\phi_V^0(-CH_2CONH-,-NH_3^+,-COO^-)$ is the sum of zwitterionic end groups and peptide backbone unit i.e. $-CH_2CONH-$ group contribution whereas $\phi_V^0(CH_2)$ represents methylene group contribution to ϕ_V^0. The values of $\phi_V^0(-CH_2CONH-,-NH_3^+,-COO^-)$ and $\phi_V^0(CH_2)$, were estimated by least-square fit method and are summarized in Table 8 for all the studied peptide in aqueous PIL solutions. The ϕ_V^0 values of the peptide backbone unit (CH_2CONH), an amide group (–CONH), alkyl side chain of glycyl-valine and glycyl-leucine contributions to the ϕ_V^0 values have been calculated from the difference in ϕ_V^0 values of homologous glycyl peptides (Eqs. (8 to (12)) [18,21,22,36].

$$\phi_V^0(-CH_2CONH) = \phi_V^0(Trigly) - \phi_V^0(Digly) \quad (8)$$

$$\phi_V^0(-CH(CH_3)_2) = \phi_V^0(Gly-val) - \phi_V^0(Digly) \quad (9)$$

$$\phi_V^0(-CH_2CH(CH_3)_2) = \phi_V^0(Gly-leu) - \phi_V^0(Digly) \quad (10)$$

$$\phi_V^0(-NH_3^+,-COO^-) = \phi_V^0(-CH_2CONH-,-NH_3^+,-COO^-) - \phi_V^0(-CH_2CONH-) \quad (11)$$

$$\phi_V^0(-CONH-) = \phi_V^0(-CH_2CONH-) - \phi_V^0(-CH_2) \quad (12)$$

Table 8. Contribution of zwitter ionic group ($-NH_3^+$, $-COO^-$), $-CH_2$ group and the other alkyl chain to apparent molar volume (ϕ_V^0) for peptides in aqueous ~0.20 mol·kg^{-1} of different PILs solutions at 298.15 K and 0.1MPa.[a]

Group	$10^6 \cdot \phi_V^0$ /(m^3.mol^{-1})					Method
	Water	TEAA	TEAG	TEAPy	TEAP	
$-CH_2CONH-$, $-NH_3^+$, $-COO^-$	60.12 ± 2.80	60.58 ± 1.91	60.63 ± 0.33	59.85 ± 0.46	60.13 ± 1.18	
$-CH_2-$	15.52 ± 0.71 15.91[a]	15.57 ± 0.50	15.57 ± 0.13	15.78 ± 0.11	15.72 ± 0.26	Eq. 7
$-CH_2CONH-$	35.81 ± 0.08 33.04[b]	35.52 ± 0.09	35.66 ± 0.06	36.44 ± 0.26	35.78 ± 0.21	Eq. 8
$-NH_3^+$, $-COO^-$	24.31 ± 2.80 27.68[a]	25.06 ± 1.91	24.97 ± 0.34	23.41 ± 0.53	24.35 ± 1.20	Eq. 11
$-CONH-$	20.29 ± 0.71 15.81[b]	19.95 ± 0.51	20.09 ± 0.14	20.66 ± 0.28	20.66 ± 0.34	Eq. 12
$-CH(CH_3)_2$	45.69 ± 0.07 45.96[b]	45.46 ± 0.10	45.93 ± 0.14	46.16 ± 0.29	45.93 ± 0.23	Eq. 9
$-CH_2CH(CH_3)_2$	63.40 ± 0.10 63.40[b]	62.52 ± 0.08	62.49 ± 0.06	63.16 ± 0.22	62.81 ± 0.12	Eq. 10

[a] ϕ_V^0 Values for the respective group contribution in aqueous solution at 298.15 K are taken from the Reference [18] and [b]Reference [21].

Group contributions to ϕ_V^0 for these groups in aqueous and aqueous PILs solution are also listed in Table 8. The zwitterionic end groups $\phi_V^0(-NH_3^+,-COO^-)$ and methylene group $\phi_V^0(-CH_2)$ contributions, in water, agree well with literature values (See Table 8) [18,21]. From Table 8, it is observed for studied glycyl based peptides in aqueous PIL solutions that the $\phi_V^0(-CH_2)$ increases with increasing hydrophobicity of PIL while $\phi_V^0(-NH_3^+,-COO^-)$ decreases with increasing hydrophobicity of PIL. Furthermore, $\phi_V^0(-CH_2CONH)$ and $\phi_V^0(-CONH-)$ are greater in aqueous PIL solutions than those in water, while reverse is true for the alkyl side chain contributions. These results suggest that peptide groups have a different hydration effect in mixed solutions. The presence of PILs causes more organized water around these moieties due to hydrophobic effect. Combined effect of all these is the hydrophobic solvation of peptides in aqueous PIL solutions leading overall decrease in ϕ_V^0 values on transferring peptides from water to aqueous PIL solutions.

4. Conclusion

Experimental density (ρ) data for glycine-based peptides in aqueous protic ionic liquids (PILs) at 298.15 K and at atmospheric pressure were reported and used to study apparent, partial and transfer molar volumes and group contributions. Concentration dependent trends observed for apparent molar volume (ϕ_v) signifies hydrophobic solvation of peptides through H-bond formation between PIL and peptide at lower concentration. ϕ_V^0 values of peptides found to be smaller in aqueous PIL solution as compared to those in water also supports above conclusion on hydrophobic solvation. Concentration dependence of partial molar volumes of peptides in aqueous PIL solutions was offered similar explanations

as those provided to ϕ_V data analysis. Trends for partial molar volumes of solvent (i.e. water + ~0.2 mol.kg^{-1} of PIL) ($\overline{V}_1$) with concentration variation and hydrophobicity of peptides revealed hydrophobic solvation of peptides along-with kosmotropic effects in aqueous PIL solutions. The limiting transfer volumes ($\Delta_{tr}\phi_V^0$) of peptides on their transfer from water to aqueous PIL solutions at 298.15 K are found to be negative and becomes more negative when either the hydrophobicity of a peptide or a PIL increases. In terms of cosphere overlap model, we found that ionic–hydrophobic interaction between ionic group of PILs/peptides and non−polar group of peptides/PILs along with H−bond assisted hydrophobic association of peptides which dominates over ion–ion interactions causing more electrostriction alongwith water structure making effect (Kosmotropic effect) except for some peptide in aqueous TEAA solutions wherein electrostatic ion-ion interactions are much stronger. Finally, the group contribution to ϕ_V^0 of peptides in aqueous PIL solutions shows values of $\phi_V^0(-CH_2)$, $\phi_V^0(-CH_2CONH)$ and $\phi_V^0(-CONH-)$ are larger in aqueous PIL solutions as compared to those in water and effect gets enhanced with increase in hydrophobicity of PILs. However, reverse effect is observed for $\phi_V^0(-NH_3^+,-COO^-)$ i.e. for zwitterionic end group contributions. Thus, we observe that peptide groups have different hydration properties in mixed solvents than in water and are governed by the nature and strength of non−covalent interactions existing in mixed solvent i.e. aqueous PIL solutions in present case. All these signify that the hydrophobic solvation of peptides in aqueous PIL solutions causes decrease in ϕ_V^0 values peptides on transferring them from water to aqueous PIL solutions. Careful understanding of findings in this work signify that the fine structural changes in PILs can help to tune the physicochemical behavior of aqueous PIL solutions for their appropriation use in different fields such as protein purification, stabilization, drug formulations etc.

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Disclosure statement

The authors declare no conflict of interests.

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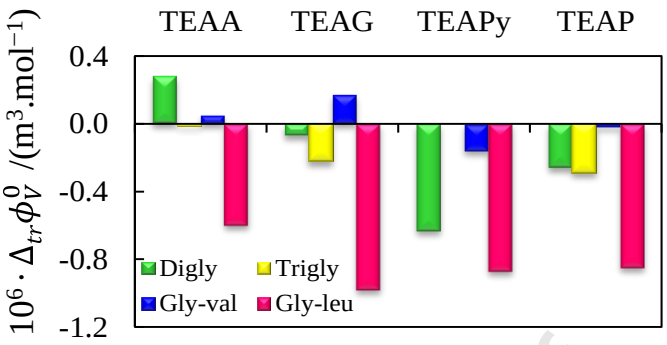
Highlights

- Densities for ternary solutions of (Glycine based peptides + Protic Ionic Liquid + H_2O) are measured.
- Volumetric properties of peptide in aqueous PILs are discussed.
- A hydrophobic interaction dominates over ion–ion interactions.
- Significant effect of hydrophobicity of PILs is observed on hydration of peptides.
- Peptides were structure maker in all aqueous PILs solutions.

Graphical abstract:



Author Statements:

Kumal R. Patil: Execution of Experimental Work and Data Processing

Shrikant P Musale: Data analysis and visualization

Dilip H Dagade: Supervisor (Research guide), work idea, data interpretation, model development and discussion.

Conflict of interests

The authors declare no conflict of interests.

Highlights

- Densities for ternary solutions of (Glycine based peptides + Protic Ionic Liquid + H_2O) are measured.
- Volumetric properties of peptide in aqueous PILs are discussed.
- A hydrophobic interaction dominates over ion–ion interactions.
- Significant effect of hydrophobicity of PILs is observed on hydration of peptides.
- Peptides were structure maker in all aqueous PILs solutions.