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Influence of biocompatible ammonium ionic liquids on the solubility of L-alanine and L-valine in water

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ABSTRACT

Understanding the folding process of proteins or polypeptides in co-solvents is a fascinating and critical issue in biophysical chemistry. In recent years, ionic liquids (ILs) represent a versatility of the new class of co-solvents. To quantify the bimolecular interactions of amino acids (AA), such as L-alanine (Ala) and L-valine (Val) with biocompatible ILs, we report the apparent transfer free energies ($\Delta G'_{tr}$) for AA from water to aqueous solutions of six ammonium based ILs (diethylammonium acetate (DEAA), diethylammonium sulfate (DEAS), triethyl ammonium acetate (TEAA), triethylammonium sulfate (TEAS), triethylammonium dihydrogen phosphate (TEAP), and trimethylammonium acetate (TMAA)) through solubility measurements, as a function of IL concentration at 298.15 K under atmospheric pressure. Salting-out effect was found for AA in aqueous IL solutions with increasing IL concentrations. In addition, we observed positive values of $\Delta G'_{tr}$ for Ala and Val from water to ILs, indicating that the interactions between ILs and AA are unfavorable. From the solubility results, we envisage that the selected ammonium based-ILs provide stability to the structure of AA. Further, the stability of AA has been studied by means of the UV–vis spectroscopy.

1. Introduction

In recent years, ionic liquids (ILs) are emerging as greener solvents that can be contentedly adopted in numerous applications of thermodynamics and bio-thermodynamics [1–5]. ILs are salts that are entirely composed of ions having designable properties and applications [1,6,7]. Due to the ionic nature of the materials, ILs have essentially negligible vapor pressure and so can be envisioned as being useful in a variety of applications. Thus, the properties of ILs such as solubility, polarity, viscosity and density are not only changed on the nature of the solute but also dependent on the type of environmental effects. ILs those are less viscous and easy to handle, now a days are very attractive solvent media for the assay of protein stability [6]. In this context, the stabilization of protein, activity and structure in aqueous IL solutions has been systematically reported by our research group [5,8–10]. ILs are fascinating for chemists and chemical engineers in the view of their close association with AA, polypeptides and other biomolecules. Understanding of the protein folding in the co-solvents is critical, central and crucial roles in biophysical chemistry and molecular biology. Biomolecules are practically linear polymers of many amino acids (AA) and play a key role in several biological processes. The proteins need a distinct 3D structure within the surroundings to perform specific functions. Although AA are simplest and highly interesting molecules that are of fundamental importance in science, technology, in our daily life, are far from being completely understood and are currently under active research.

AA performs a variety of functional activities and their stability in the presence of co-solvents mainly depends on a delicate balance between ion–ion and biomolecule–ion interactions. From the thermodynamic point of view, the stability of protein is inferred through the difference in free energies of the unfolded state (U) and the native state (N). Moreover, the proteins properties are basically dependent on the nature and the arrangement of the AA side chain along the protein backbone. These AA are only responsible for the individual characteristics of the protein, and it has been recognized that all of the information pertaining to the protein is implicit in the AA sequence [11–13]. Moreover, the individual study on the thermodynamic stabilities of the AA in various solvents can be very much helpful in understanding the mechanism of protein stabilization in stressful environments.

Nowadays, a class of ILs has received attention for providing better natural environment for the biomolecules to overcome the physiological stresses [14–16]. In spite of the widespread application and popularity, there are, however, critical unsolved issues concerning the use of ILs in the biotechnological processes. In fact, to optimize and control the extraction of biomolecules by ILs from

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aqueous media, an effective manipulation of the thermodynamic parameters must be considered that influence the solvation of the biomolecules on their stability in ILs. In these context experimental studies on the AA and ILs is very limited [17,18]. Moreover, the stability of simple AA, which is building blocks of globular proteins, is entirely different from globular proteins and the relationship of AA structures and their stability in ILs is not fully understood and remains unclear.

In order to understand the influence of ILs on the stability of AA, various combinations of cations (small alkyl series) and anions (acetate, phosphate and sulfate) were synthesized in our laboratory. Herein, we present the effect of six ammonium based ILs; diethylammonium acetate ($[Et_2NH][CH_3COO]$, DEAA), diethylammonium sulfate ($[Et_2NH][HSO_4]$, DEAS), triethyl ammonium acetate ($[Et_3NH][CH_3COO]$, TEAA), triethylammonium sulfate ($[Et_3NH][HSO_4]$, TEAS), triethylammonium dihydrogen phosphate ($[Et_3NH][H_2PO_4]$, TEAP), and trimethylammonium acetate ($[Me_3NH][CH_3COO]$, TMAA) on AA such as, L-alanine (Ala) and L-valine (Val) by the apparent transfer free energies ($\Delta G'_{tr}$) from water to these six ammonium based ILs through solubility measurements. The schematic chemical structures of the ILs are shown in Fig. 1. By choosing AA, we intend to give a contribution toward the understanding of the solvation mechanisms which involves in protein folding studies by ILs. To ascertain whether the ILs has any consequences on the structure of both the AA, we further performed UV–vis spectroscopic measurements as a function of AA concentration in the ILs.

We observed positive values of $\Delta G'_{tr}$ for both the AA from water to ILs, indicating that the interactions between ILs and AA surface are unfavorable. The results demonstrate that the effect of IL concentration on AA may be due to their influence on the water structure around the AA thereby bringing about a change in the thermodynamic properties. Finally, the effects of anions and alkyl chain length are discussed to identify the factors that determine the stabilizing performance of ammonium ILs.

2. Materials and methods

2.1. Materials

Alanine (Ala) and Valine (Val) both having 99% purity were purchased from Across Organics (USA). All were used as received. The NANO water was used for preparing the aqueous ILs. The purified water was distilled and de-ionized with a resistivity of 18.3 Ω cm and all the solutions were prepared using a Mettler Toledo balance with a precision of ±0.0001 g.

2.2. Synthesis of ILs

All ammonium family of ILs used in this work, namely, DEAA, DEAS, TEAA, TEAP, TEAS and TMAA of general type [amine][anion] were synthesized in the following manner [8–10].

2.2.1. Synthesis of diethyl ammonium acetate (DEAA)

The synthesis of DEAA was carried out in a 250 ml round-bottomed flask, which was immersed in a water-bath and fitted with a reflux condenser. Acetic acid (1 mol) was added dropwise into the diethylamine (1 mol) at 343.15 K for 1 h under circulating water bath. The reaction mixture was heated to 343.15 K with stirring for 2 h to ensure completion of reaction. The reaction mixture was then dried at 353.15 K until the weight of the residue became constant. The sample analyzed by Karl Fisher titration revealed very low levels of water (below 70 ppm). The yield of DEAA was 97%. ^{1}H NMR ($CDCl_3$): δ (ppm) 1.3 (t, 6H), 1.97 (s, 3H), 2.95 (m, 3H), 9.20 (s, 2H).

2.2.2. Synthesis of diethylammonium hydrogen sulfate (DEAS)

The similar procedure as that delineated above for DEAA was followed with the exception of the use of sulfuric acid [anion] instead of acetic acid. The yield of DEAS was 95%. ^{1}H NMR (DMSO-d_6): 1.14 (t, 6H), 2.95 (m, 3H), 8.6 (s, 1H).

2.2.3. Synthesis of triethylammonium acetate (TEAA)

The synthesis of TEAA was carried out in a 250 ml round-bottomed flask, which was immersed in a water-bath and fitted with a reflux condenser. Acetic acid (1 mol) was added drop wise into the triethylamine (1 mol) at 343.15 K for 1 h under the circulating water bath. The reaction mixture was heated at 343.15 K with stirring for 2 h to ensure completion of reaction. The reaction mixture was then dried at 353.15 K until the weight of the residue remained constant. The sample was analyzed by Karl Fisher titration revealed very low levels of water (below 70 ppm). The yield of TEAA was 94%. ^{1}H NMR ($CDCl_3$): δ (ppm) 0.778 (t, 9H), 1.466 (s, 3H), 2.58 (m, 6H), 11.0 (s, 1H).

2.2.4. Synthesis of triethylammonium hydrogen sulfate (TEAS)

The similar procedure as that delineated above for TEAA was followed with the exception of the use of sulfuric acid [anion] instead of acetic acid. The yield of TEAS was 98%. ^{1}H NMR ($CDCl_3$): 1.3 (t, 9H), 3.16 (m, 6H), 5.04 (s, 1H).

2.2.5. Synthesis of triethylammonium dihydrogen phosphate (TEAP)

The similar procedure as that delineated above for TEAA was followed with the exception of the use of phosphoric acid [anion] instead of acetic acid. The yield of TEAP was 96%. ^{1}H NMR (DMSO-d_6): δ (ppm) 1.18 (t, 9H), 3.06 (m, 6H), 6.37 (s, 1H).

2.2.6. Synthesis of trimethylammonium acetate (TMAA)

The synthesis of TMAA was carried out in a 250 ml round-bottomed flask, which was immersed in a water-bath and fitted with a reflux condenser. Acetic acid (1 mol) was added drop wise into trimethylamine (1 mol) at 343.15 K for 1 h under circulating water bath. The reaction mixture was heated at 343.15 K with stirring for 2 h to ensure completion of reaction. The reaction mixture was then dried at 353.15 K until the weight of the residue remained constant. The sample analyzed by Karl Fisher titration revealed very low levels of water (below 70 ppm). The yield of TMAA was 98%. ^{1}H NMR (DMSO-d_6): δ (ppm) 2.51 (s, 1H), 2.10 (s, 3H).

The purity of the ILs was confirmed through ^{1}H NMR spectroscopy and they are found to be more than 99%.

2.3. Solubility measurements

The solubility of a species is a macroscopic property which reflects the strength of the interactions established with solvent molecules. The solubility of AA was measured using density (ρ) measurements, which is similar to the method of Nozaki and Tanford [19–21]. The detailed procedure and apparatus used in this work have been delineated in our previous articles [9,10]. The related procedure includes each of the sample vials containing a fixed amount of solvent (aqueous or aqueous IL solutions) that was added to a weighed amount of Ala and Val in order to make a series of mixtures with increasing composition of AA. Each of the vials was then sealed with a Teflon coated screw cap, and the cap was sealed with parafilm to ensure an airtight and watertight seal. The vials were completely immersed in a low temperature shaker equipped with a water bath (Metrex, New Delhi, India). The bath temperature was controlled to (298.15 ± 0.01) K and the bath was housed in a constant room temperature, also maintained at nearly 298.15 K. At least ten samples were prepared for each system. Among these sample vials, five to six were prepared with unsaturated solutions

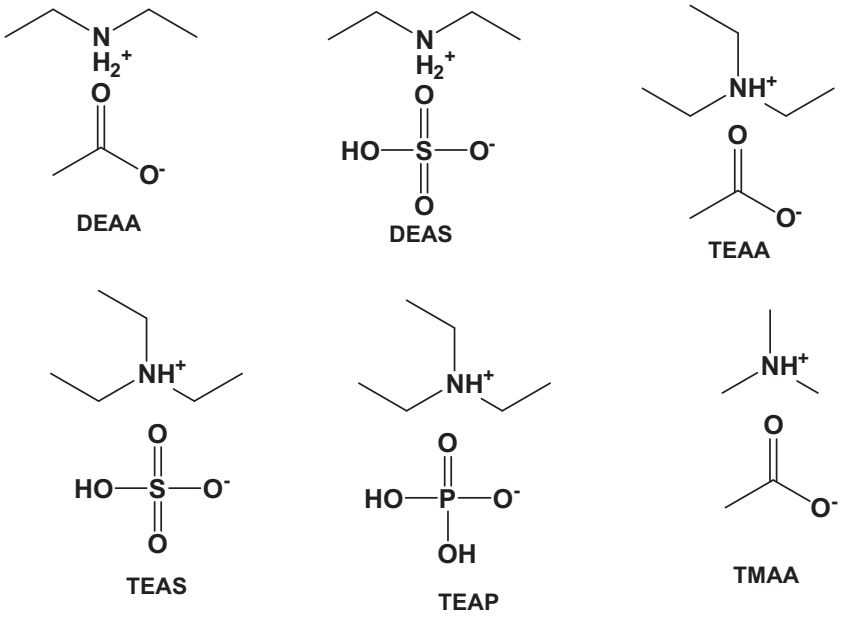

Fig. 1. Schematic chemical structures of ILs.

and the remaining vials with saturated solutions. In the present study, water and different concentrated IL aqueous solutions were chosen as co-solvents. After shaking to (36–48 h), the solution was left overnight so that the undissolved solid particles settle at the bottom of the sample vial. The supernatant of each solution was removed with the help of a syringe and filtered through a 0.47 μm disposal filter (Millipore, Millex-GS) before performing the density measurements. The density measurements were performed with an Anton-Paar DMA 4500 M (Austria) vibrating-tube densimeter, equipped with a built-in solid-state thermostat and a resident program with accuracy of temperature of ±0.03 K. The ρ values of the samples were measured at 298.15 K under ambient pressure with a precision of ±0.00005 g/cm^3. Proper calibration at each temperature was achieved with doubly distilled, de-ionized water and with air as standards. The uncertainty of the solubility limit is lower than ±1.2%.

2.4. UV–vis spectrophotometer

The UV–vis spectra for the samples were recorded using a double beam spectrophotometer (UV-1800, Shimadzu Co., Japan) within the range of 190–650 nm. Experiments were performed in 1 cm path length quartz cuvette. Spectrophotometer has matched quartz cells, with spectral 1 nm bandwidth and wavelength accuracy of ±0.3 nm with automatic wavelength corrections with a pair of 10 mm quartz cells. Each sample spectrum was obtained by subtracting appropriate blank media without AA from the experimental AA spectrum.

3. Results and discussion

Table S1 lists the concentration as well as experimental densities (ρ) of the samples of the AA in water and in aqueous ammonium IL solution. The solubilities of AA; Ala and Val were measured in the absence and presence of ILs at 298.15 K under atmospheric pressure. The solubility limits of each of the AA in the absence and in the presence of all six ILs aqueous solutions, expressed as grams of AA per 100 g of co-solvent, and the densities (ρ^*) of the saturated solutions at the solubility limit are summarized in Table 1 and graphically displayed in Fig. 2. Our results clearly reveal that different ILs have different influences on the solubilities of AA. As evident from the results in Table 1, the solubility of AA in water is higher for Ala as compared to Val. In Table 1, the obtained solubility data of AA in aqueous solutions are compared with available literature data [22]. The solubility for the Ala and Val was observed to be 16.62 and 5.75 g/100 g of water which correlates well with the observed solubility values by Liu and Bolen [22]. Ala shows the highest solubility in water and substantially lower solubility in water is prevailed for Val. Since, the observed partial volume for Ala is 86.4 Å, which is relatively smaller than those for the more hydrophobic residues Val having 136.8 Å partial volume [23]; the formation of a cage like structure or "clathrate" restricts the motion and number of possible arrangements of water molecules, thereby lowering the solubility of Val [11]. As seen from Fig. 2 the solubilities of AA decrease with increasing the IL concentration, indicating that salting-out effect is dominant. The interpretation of the solubility behavior of AA based on the specific individual effect of cation and anion of IL is difficult to predict since a balance between competitive interaction of the cation, anion and water exist with the AA. The combinations of which determines the preferential interaction as well as solvation of the IL with AA, helpful in predicting the differences in the solubility limits of the AA in the presence of varying concentrations of the ILs in aqueous solution. Moreover, Tome et al. proposed through experimental [24] as well as computational techniques [25] that salting-in and salting-out phenomena possess a common basis which is the competition between water–AA side chain, IL–AA side chain, and water–IL interactions. They concluded that the delicate balance between these interactions is dependent on the relative affinities of the biomolecules to water or to IL cation and anion and the magnitude of the solubilities. As presented the solubility values in Table 1 the water affinity of the AA decreases in the order Ala > Val, reflecting an increasing hydrophobicity as the side chain increases. Actually, the polarity of the Ala provokes an increase interaction with the more polar ILs. The more polar the AA, the stronger the interaction with the IL, consequently the magnitude of the interaction of IL with Val is expected to be low which is reflected through the lower solubility of Val in presence of ILs as compared to the solubilities of the Ala in ILs aqueous solution.

The apparent transfer free energy measurements ($\Delta G'_{tr}$) of the AA from water to aqueous ILs at 298.15 K under atmospheric

Table 1
Solubility of amino acids (S_{AA}) in water (or) in aqueous ILs and densities (ρ^*) at solubility limits at 298.15 K.[a]

Co-solvent	S_{AA} (g/100 g of solvent)				ρ^*_{AA} (g/cm^3)			
	0% (v/v)	30% (v/v)	50% (v/v)	70% (v/v)	0% (v/v)	30% (v/v)	50% (v/v)	70% (v/v)
Ala								
Water	16.62[b]				1.04289			
DEAA		9.61	7.43	4.88		1.02730	1.02746	1.02912
DEAS		6.36	4.15	2.16		1.10249	1.10693	1.10760
TEAA		8.51	6.35	4.02		1.02521	1.02574	1.02589
TEAS		5.45	3.33	1.30		1.07737	1.09212	1.10288
TEAP		7.43	5.16	2.96		1.09008	1.14368	1.15009
TMAA		10.55	8.18	5.71		1.05099	1.05228	1.05301
Val								
Water	5.75[b]				1.00945			
DEAA		3.52	2.74	1.92		1.01379	1.01845	1.01927
DEAS		2.38	1.59	0.89		1.05290	1.07525	1.10253
TEAA		3.13	2.26	1.52		1.01187	1.01772	1.01816
TEAS		2.02	1.25	0.59		1.06349	1.08676	1.10643
TEAP		2.73	1.92	1.16		1.07488	1.10279	1.14854
TMAA		3.81	3.02	2.25		1.02681	1.03583	1.04338

[a] The uncertainty in solubility limit is lower than ±1.2%.
[b] S_{AA} for L-Ala in water 16.60 and L-Val in water 5.73 [22].

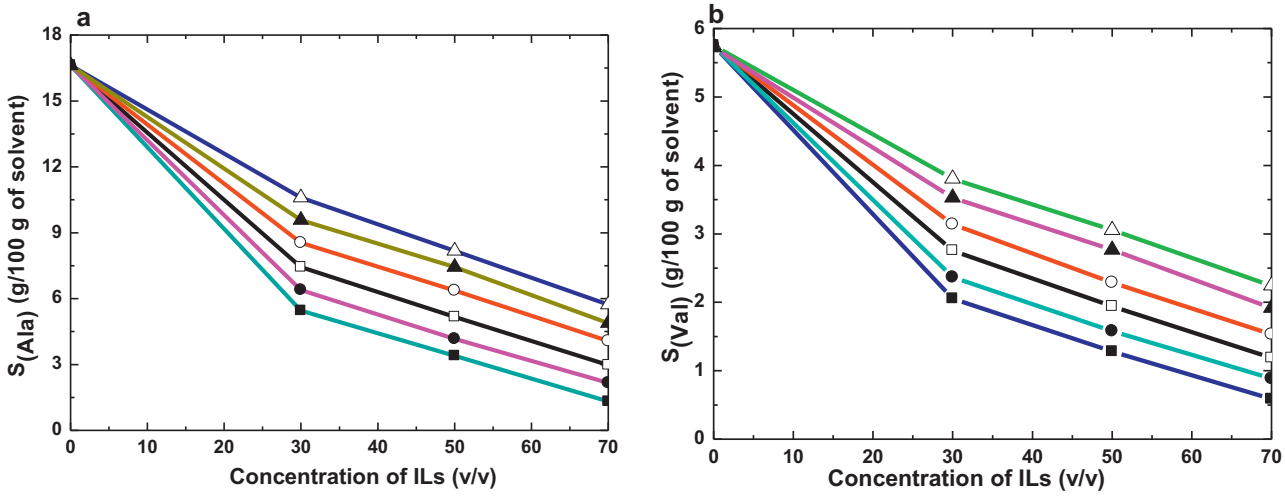

Fig. 2. Plot of solubility against concentration to illustrate the solubility limits (S_{AA}) for (a) Ala and (b) Val in an aqueous solution of ILs: TEAS (■), DEAS (●), TEAP (□), TEAA (○), DEAA (▲) and TMAA (△) at 298.15 K. Solid lines show the smoothness of the solubility points.

pressure was obtained from the measured solubility data. The $\Delta G'_{tr}$ have played a key role in understanding the effect of stabilization or destabilization of biomolecules in co-solvents. The solubilities were used to obtain the apparent transfer free energy ($\Delta G'_{tr}$) of GPs from water to ammonium ILs at 298.15 K. Nozaki and Tanford [19–21] developed a transfer free energy model that has utilized to assess the transfer free energies of proteins from water to different co-solvents. The detailed procedure for obtaining the $\Delta G'_{tr}$ of

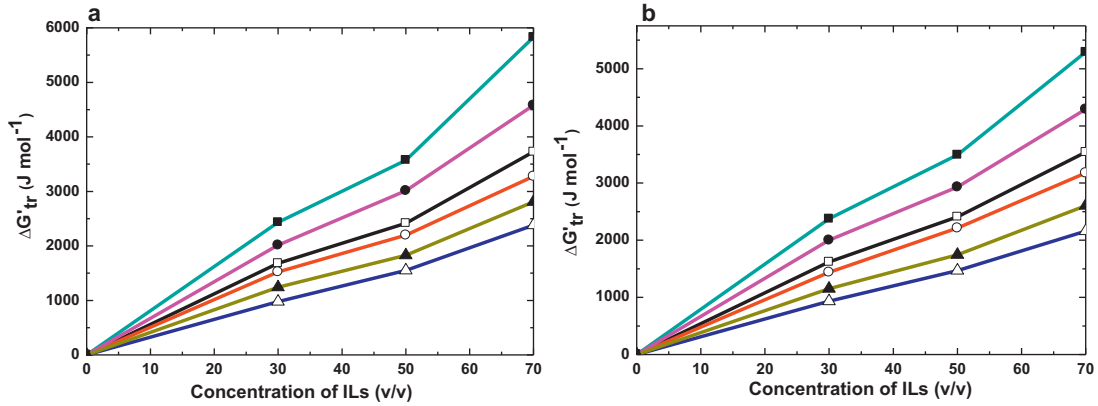

Fig. 3. Plot of the Gibbs free energy against concentration to show the apparent transfer Gibbs free energy ($\Delta G'_{tr}$) for (a) Ala and (b) Val in an aqueous solution of ILs: TEAS (■), DEAS (●), TEAP (□), TEAA (○), DEAA (▲) and TMAA (△) at 298.15 K. Solid lines show the smoothness of the solubility points.

Table 2
Apparent transfer free energies ($\Delta G'_{tr}$) of amino acids from water to aqueous ILs solutions at 298.15 K.

Amino acids	ILs	$\Delta G'_{tr}$ (J mol^{-1})		
		30% (v/v)	50% (v/v)	70% (v/v)
Ala	DEAA	1241.63	1829.23	2807.78
	DEAS	2015.16	3011.46	4580.90
	TEAA	1523.03	2197.71	3275.74
	TEAS	2433.78	3570.97	5829.20
	TEAP	1681.06	2414.44	3725.84
	TMAA	975.89	1548.91	2381.05
Val	DEAA	1153.04	1743.91	2603.65
	DEAS	2001.92	2930.71	4289.78
	TEAA	1439.49	2211.51	3175.72
	TEAS	2374.94	3492.28	5292.74
	TEAP	1619.05	2408.42	3538.64
	TMAA	932.09	1467.51	2160.53

this work has been depicted in our earlier paper [9,10,26,27]. The uncertainty of $\Delta G'_{tr}$ in the present experiment is lower than ±1.6%. Evidently, the unfavorable interactions of solvents with proteins account for the protein stability, indicating the positive contribution of the transfer free energy and that the solubility decreases with increasing the concentration of solvents (salting-out effect) as well. In contrast, $\Delta G'_{tr}$ that has a negative sign as well as the fact that the solubility increases with increasing concentration of co-solvents (salting-in effect) lead to the favorable interactions results in protein denaturation. We examined the ILs effect on the AA with the aid of the $\Delta G'_{tr}$ from water to IL solutions. Since, AA are ideal compounds to be studied as model biomolecules, therefore, by choosing AA, we intend to explore and predict the thermodynamic properties of the solvation mechanism which controls the stability of the proteins in ILs. This would help in improving the recovery efficiency as well as stability of proteins by ILs. The $\Delta G'_{tr}$ values are listed in Table 2 and graphically displayed in Fig. 3 as a function of IL concentrations at 298.15 K.

The experimental results compiled in Table 2 explicitly elucidate that there is an unfavorable interaction between ILs and AA that leads to stabilize the structure of AA. The salting-out effects observed can be understood in terms of a delicate balance between direct or indirect (water-mediated) interactions established by the AA and IL ions, dependent, on the polarity, size, and charge of the later. The delicate balance between these interactions is dependent on the relative affinities of the AA to water molecules or to ions of ILs and determines the observed trend and magnitude of the solubility effect. Moreover, the solubility limits decrease with increasing the concentration of ILs, indicating that its interaction with water becomes more important and starts to compete with its direct binding to the IL [24,25]. Further, from Fig. 3 we noticed that the positive $\Delta G'_{tr}$ values increase with increasing ILs concentration in AA aqueous solution. The positive contribution shows that these ILs are biocompatible co-solvents for AA and stabilize the structure of AA. Upon comparison in the set of various ILs, TEAS shows the highest $\Delta G'_{tr}$ values, whereas the TMAA IL has the lowest values of $\Delta G'_{tr}$ for the entire range of concentrations (v/v) viz. 30%, 50% and 70% of ILs. Moreover, the results noticeably forecast that the sulfate anions are strong stabilizers; acetates are moderate stabilizers for the AA which is obviously in correlation with our earlier results for the cyclic dipeptides (CDs) [9]. The $\Delta G'_{tr}$ values of TEAS with each of AA are significantly larger, and there is a large increase with increasing TEAS concentrations as compared to the other ILs. The $\Delta G'_{tr}$ values of the folding formed by the ILs shows that TEAS is the strongest stabilizer, and TMAA is weak stabilizer while the rest of the ILs are under the class of moderate stabilizers for the investigated model compounds. All the ILs exhibited comparable biocompatibility on both the AA. These AA tend to stabilize in the ILs in the following order: TEAS > DEAS > TEAP > TEAA > DEAA > TMAA. This order agrees well with the Hofmeister series of IL anions; where HSO_4^- plays the dominant role in the unfavorable interaction between the ILs and proteins. The molecular structures of IL may affect their ability of stabilizing the structure of AA. These results explicitly elucidate the delicate balance on the relative affinities of ILs on the biomolecules and determine the trend and magnitude of the stability of proteins in ILs. In fact, these results help us to optimize and to improve the effective manipulation of the proteins in ILs and aqueous IL solutions.

The difference in the stabilization behavior of the ILs can be attributed to the variation of the alkyl chain at the substituted ammonium cations led to variation in the efficacy of the ILs as stabilizers for the AA. Therefore, it is noteworthy to compare the stabilizing ability between alkyl chains at the substituted ammonium cations of the ILs. If for example and for the sake of comparison, we consider the case of triethylammonium cation (TEA^+) and the diethylammonium cation (DEA^+); TEA^+ acted as strong stabilizer, while DEA^+ behaved as weak stabilizer for AA. This difference in the behavior of water-soluble ILs may be attributed to an increase in the alkyl chain length of their cations that may lead to increase in the viscosity of the ILs. Apparently, we observed that TEAA has higher viscosity (24.12 mPa s) than DEAA (16.36 mPa s) thereby resulting in higher stabilizing ability in TEAA than DEAA for the structure of AA. The possible explanation is that the high viscosity of ILs slows down the conformational changes of biomolecules, allowing a more compact structure of AA. Moreover, because of the strong interaction of the HSO_4^- with water competes effectively for the water molecules associated with the AA surface, thus the strongly solvated IL is excluded from the AA surface. This encourages the AA surface to be primarily and preferentially solvated by the water molecules. Accordingly, we observe a more unfavorable interaction for the TEAS IL as compared to TEAA on the surface of the AA.

Our results specifically display that ILs bearing long alkyl chain in their cation show strong stabilizer for AA. The variations in $\Delta G'_{tr}$ provide evidence for IL induced stabilization of AA that basically depend upon different structural characteristic behavior of ILs on the solvent structure. Because of the difference in the cations and anion species, different ILs are solvated to different extent. Thereby, strongly solvated ILs stabilize AA and promote salting out. The solvated ILs interact poorly with weakly solvated AA and are excluded from the surface of AA. The explanation of IL induced protein model compounds stability is still not clearly understood however, this discrepancy may be primarily due to the weak hydration as well as an increase in hydrophobicity of the anion which has an indirect stabilizing effect on the structure of the proteins. Overall, the results of the anion and cation, demonstrated that the combination of sulfate and higher alkyl chain at the substituted ammonium cation, IL show essentially effective and strong stabilizer for the structure of AA. From Table 2, the stabilizing ability of diethyl ammonium ILs on both the AA (i.e. Ala and Val) follows the order $HSO_4^- > CH_3COO^-$. Similar results were observed in the case of triethyl ammonium ILs, with an existing order as $HSO_4^- > H_2PO_4^- > CH_3COO^-$. These comparisons in between the ILs conclude that the ability of ILs to stabilize AA structure follows the Hofmeister series order.

In support to these observations, the UV–vis spectra of changing concentration of Ala and Val near solubility limits in various ILs reveal that there is no variation in the absorption spectra at sufficiently high concentration of the AA (i.e. above solubility limit) (Fig. 4). On close investigation of the absorption spectra it seems that there is no shifting in the bands with increase in the concentrations of the ILs. However, the flattening of the absorbance bands is observed with increase in concentrations, indicative of the fact that most of the part of the radiations is being absorbed by the sample itself, indirectly reflecting the saturation limit of the

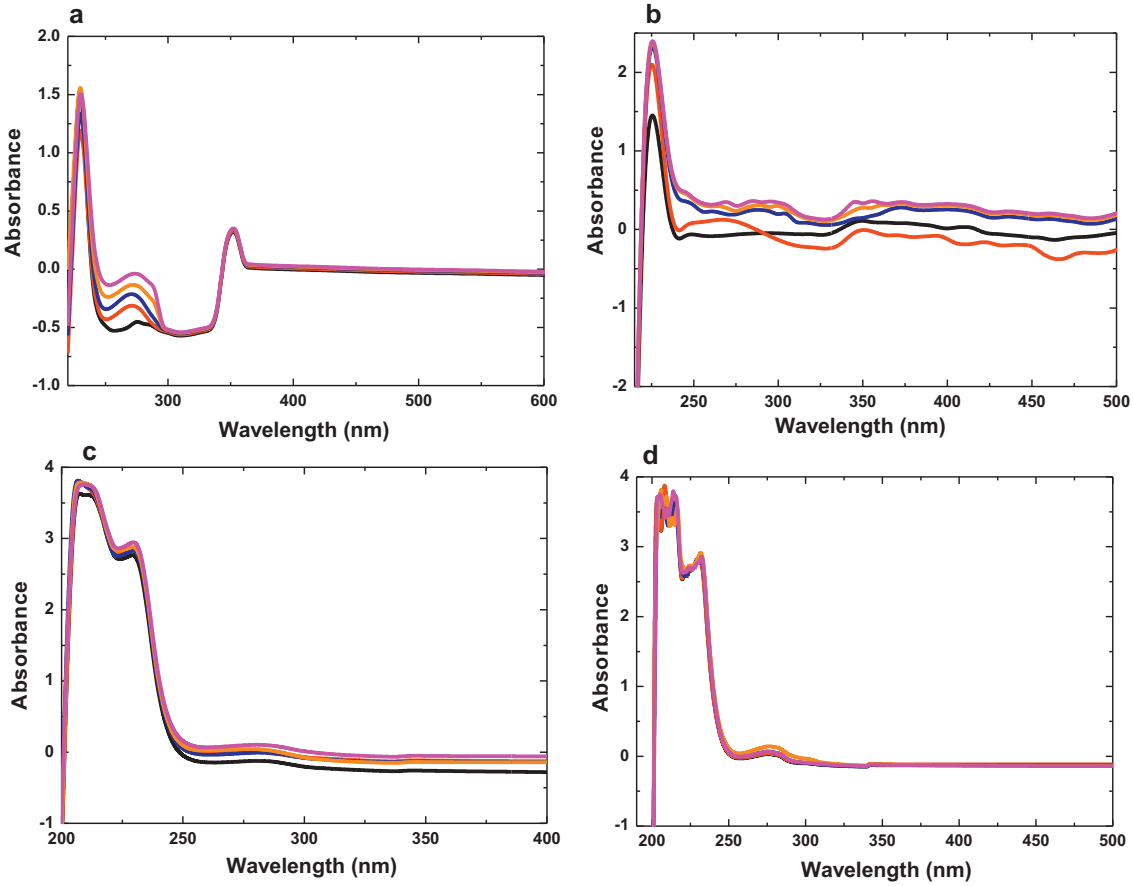

Fig. 4. UV–vis spectra of (a) Ala in water as function of Ala concentration: black (15.5), red (16.0), blue (16.62), orange (17.0), magenta (17.5); (b) Val in water as function of Val concentration: black (5.0), red (5.5), blue (5.75), orange (6.0), magenta (6.5); (c) Ala in 30% TEAS as function of Ala concentration: black (4.5), red (5.0), blue (5.45), orange (6.0), magenta (6.5); (d) Val in 30% TEAS as function of Val concentration: black (1.5), red (1.8), blue (2.05), orange (2.2), magenta (2.4). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

sample. For the sake of clarity and representation, we present here the absorption spectra of Ala (Fig. 4a) and Val (Fig. 4b) in water as well as in TEAS (Fig. 4c and d). Thereby, we conclude that the UV–vis spectrophotometer study provides useful information about the solubility limit of Ala and Val. The results indicate that there is slightly change in the wavelength of absorption spectra with addition of ILs to the solution as illustrated in Figs. 4, S1, S2 and Table S2. Absorbance for TEAP, TEAS and TMAA shows the more blue shift as compare to water, DEAA and DEAS for Ala and Val at various concentrations. Further, UV–vis spectrophotometer experiments were performed with other ILs at different concentrations with varying the concentrations of AA, these results are displayed in Figs. S1 and S2. These demonstrations are helpful to support and understand the vital forces that can drive the ensemble of microstates of the protein or polypeptide into species bearing little relationship to those that predominate in water in presence of ILs. In other words, strongly solvated ILs stabilize folded protein states and promote salting out. The solvated ILs interact poorly with weakly solvated protein groups and are excluded from the surface of proteins, promoting folded protein states.

4. Conclusions

In order to quantify the molecular interactions induced stability of the AA by ILs we have obtained the $\Delta G'_{tr}$ from water to aqueous IL solutions under atmospheric pressure through solubility measurements at 298.15 K. The results are informative which brings forward the influences of solvation behavior of ILs on the AA. Moreover, the results also provide significant information on the nature of interactions of the AA residues, water, and ILs. This detailed information, concludes that all biocompatible ILs can stabilize the structure of AA through unfavorable interactions between the protein's surface and ILs. Based on our experimental results, we therefore conclude that within the series of the ILs chosen, TEAS is the strongest stabilizer for AA and DEAA is moderate stabilizer while TMAA the weakest stabilizer for the AA.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.fluid.2012.08.021.

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