Biophysical Reviews (2018) 10:841–846
https://doi.org/10.1007/s12551-018-0411-x

REVIEW

Innovative aspects of protein stability in ionic liquid mixtures

Awanish Kumar[1] · Pannuru Venkatesu[1]

Received: 1 February 2018 / Accepted: 5 March 2018 / Published online: 16 March 2018

Abstract
Mixtures of ionic liquids (ILs) have attracted our attention because of their extraordinary performances in extraction technologies and in absorbing large amount of CO_2 gas. It has been observed that when two or more ILs are mixed in different proportions, a new solvent is obtained which is much better than that of each component of ILs from which the mixture is obtained. Within a mixture of ILs, several unidentified interactions occur among several ions which give rise to unique solvent properties to the mixture. Herein, in this review, we have highlighted the utilization of the advantageous properties of the IL mixtures in protein stability studies. This approach is exceptional and opens new directions to the use of ILs in biotechnology.

Keywords Ionic liquids · IL mixtures · Cations and anions

Introduction

Over the past years, ionic liquids (ILs) have been considered as novel biocompatible solvents for biotechnology. The ILs are composed of organic ions that have certain characteristics that hinder crystallization, and therefore, these salts are liquid near room temperature (Podgoršek et al. 2016). Due to this reason, they are also termed as room temperature ILs too. ILs are environmentally benign and chemically stable. Their physical properties can be easily tuned based on the experimental requirements. Interestingly, ILs have very low vapor pressure and hence are highly stable (Greaves and Drummond 2015; Smith et al. 2014). Very few of the literature reports that the ILs have very low freezing points in their purest state (Matsumoto et al. 2006; Kareem et al. 2010).

Most of the ILs represented themselves as promising candidates for the proteins. An interesting aspect of the ILs is their self-buffering characteristics which have been recently explored in the literature. ILs offer high self-buffering capacity for the protein solutions in the physiological pH range (Taha et al. 2014; Gupta et al. 2016). Overall, ILs can adjust themselves in any required experimental conditions starting from protein stability, its extraction, crystallization, aggregation control, and enzyme catalysis under non-native environment (Wu et al. 2014; Zhao 2016). Additionally, it has been reported recently that some of the ILs can significantly reduce the viscosities of concentrated mAbs solutions (Weight et al. 2015). Hence, the use of ILs is beneficial not only for controlling protein denaturation but also for reducing the viscosities of highly concentrated protein solutions in pharmacy.

To date, direct experiments using ILs have been performed in our laboratory (some of them are listed as references) on the stability in proteins against chemical- and temperature-induced denaturation (Jha and Venkatesu 2016; Bisht et al. 2016; Bisht et al. 2017; Attri and Venkatesu 2012; Attri et al. 2014; Bisht et al. 2015; Jha et al. 2016; Kumar et al. 2015; Bisht and Venkatesu 2017; Attri et al. 2012; Jha et al. 2014; Kumar et al. 2014a, b). Additionally, ILs also counteracted the pH-induced stability of proteins (Jha et al. 2017). For the first time, we were successful in controlling the self-aggregation in insulin at pH 7 using biocompatible ILs (Kumar and Venkatesu 2013). We would like to emphasize that we observed that a controlled amount of ILs can not only control the insulin self-aggregation but also stabilize its monomeric structure against thermal stresses (Kumar and Venkatesu 2013).

As stated earlier, in the present situation, ILs have proven to be the novel solvents in protein-based studies. Using the unique tunable physical properties of the ILs, we can now fulfill the requirements of the enzymatic reactions in the

This article is part of a Special Issue on "Ionic Liquids and Biomolecules" edited by Antonio Benedetto and Hans-Joachim Galla.

✉ Pannuru Venkatesu
venkatesup@hotmail.com; pvenkatesu@chemistry.du.ac.in

[1] Department of Chemistry, University of Delhi, Delhi 110007, India

non-aqueous medium (Bi et al. 2015; Park and Kazlauskas 2003). ILs are now used for successful and selective recovery of proteins using aqueous biphasic mixtures (Desai et al. 2016; Lee et al. 2017). Reports are available where clear crystals of proteins have been obtained in the presence of various ILs (Schröder 2017). The most interesting aspect is that the crystals of proteins obtained using ILs were similar to those of the native forms.

There are several reviews available in the literature summarizing the unique effects of ILs on protein stability (Sivapragasam et al. 2016). Accordingly, we have also reviewed the sustainable and biophysical properties of proteins in various families of the ILs on several occasions (Kumar et al. 2017; Kumar et al. 2016; Kumar et al. 2014a, b; Kumar and Venkatesu 2014; Kumar and Venkatesu 2012). Therefore, in this highlight, we will not be focusing on the effect of an IL over proteins. We will try to expose the untouched area in the field of protein stability using ILs. However, in the upcoming section, we wish to briefly introduce the basic definition of ILs and their application and their properties of their mixtures in the field of protein stability.

A brief introduction to ionic liquids

Ionic liquids (ILs) are organic salts with positive and negative ions. ILs consist of organic nitrogen-containing heterocyclic cations and inorganic anions. Most of the ILs (but not all) have the melting temperature below ~ 100 °C. Excellent story of ILs has been explicitly elucidated in several elegant reviews (Wang et al. 2017; Lei 2017; Zhang et al. 2017). Commonly available IL contains cations such as ammonium, phosphonium, sulfonium, guanidinium, pyridinium, imidazolium, and pyrrolidinium with counter anions such as chloride, bromide, tetrafluoroborate, hexafluorophosphate, trifluoromethanesulfonyl, *bis*(trifluoromethanesulfonyl) imide, dicyanamide, and alkylsulfate. ILs attracted synthetic chemists because of its large number of multi-functional properties. Due to the combination of various organic and inorganic ions in ILs, their bulk and interfacial behavior is complex that is governed by Coulombic, van der Waals, dipole-dipole, hydrogen-bonding, and solvophobic forces (Zahn et al. 2008; Gebbie et al. 2017; Daschakraborty and Biswas 2014; Hayes et al. 2013). On the other hand, they have negligible vapor pressure, high thermal stability, tunable viscosity, high conductivity, and heat capacity, and they are miscible with water and organic solvents. Several reviews and research articles are available that explain the advantageous properties of ILs that have been proven very useful in industries and biotechnology, also promoting the environmental safety protocols such as absorption of CO_2 (Ramdin et al. 2012; Lei et al. 2014; Zeng et al. 2017). These ILs can be recovered easily and can recycle again and again retaining their physical and chemical integrity (Mai et al. 2014). Moreover, because of the size and complexity of ILs, their interaction with the biomolecules such as proteins is dependent on their dispersion interactions, steric effects, and hydrogen bonding (Benedetto and Ballone 2016).

Novel IL mixtures

As stated in the above sections, ILs have proven to be an important candidate to absorb harmful and toxic gases from the environment. In this context, CO_2 absorption is the most important as this gas is responsible to a major extent in disturbing our natural environment. It has been observed in the literature that the efficiency of absorbing CO_2 is increased dramatically in the presence of the ILs (Chen et al. 2016; Vijayraghavan et al. 2013; Bates et al. 2002). The most interesting aspect is that when various ILs are mixed in defined proportions, they cancel their negative effects and only their advantageous characters are increased. For example, Wang et al. (Wang et al. 2018) examined a series of binary liquid mixtures of $[NH_2emim][BF_4]$ and $[Bmim][BF_4]$ for CO_2 capture. This approach provided opportunity exploiting their advantages and reducing the disadvantages of each of the components. The authors pointed that the estimated physicochemical properties in a mixture with a mole fraction of $[NH_2emim][BF_4]$ of 0.4 had variation characteristics significantly different from those in mixtures with other mole fractions, which might be attributed to the large interaction between the two kinds of IL components and showed a positive effect on CO_2 absorption (Wang et al. 2018).

Thus, mixtures of ILs hold the advantage of using existing synthetic procedures and having well-defined parameters. Further, mixture of ILs finds application as effective antibacterial agents by effectively disrupting the biomimetic membranes (Losada-Pérez et al. 2016). Mixtures of ILs are used successfully in dissolving large amount of cellulose (~ 40%), and the dissolved cellulose could be easily reconstituted from its solution in ionic liquid mixtures by addition of water (Stolarska et al. 2017).

It has been predicted that in the mixture of ILs, various interactions interplay giving rise to complex solvent structures (Nakajima et al. 2017). The analysis of optical Kerr effect (OKE) spectra for mixtures of ILs by Quitevis and group (Xiao et al. 2006, 2008) showed that in a mixture of ILs, as presented in Fig. 1, the interactions between ILs in the mixture are governed by the intermolecular vibrational modes which added to the solvent structure and its intrinsic properties.

On the other hand, it has been illustrated that the additivity in the observed spectra is because of the reason the ions of the ILs experience the same environment as what they experience in the IL. For example, mixtures of $[C_4C_1im][NTf_2]$ with $[C_4C_1im][I]$ were found to show perfect additivity in both

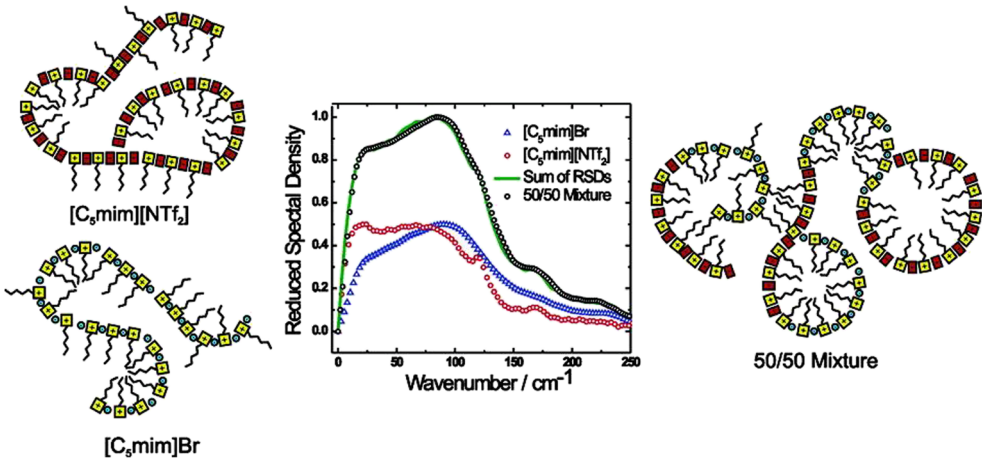

Fig. 1 Simplified structure between cations and anions in ionic liquid mixture. Adopted from ref. Xiao et al. 2006. With permission from American Chemical Society

their UV absorption and their IR spectra, indicating that the I^- ions in the mixtures were in the same environment as those in $[C_4C_1im][I]$ itself (Katoh et al. 2008). It is evident that the mixtures of ILs are complex and the interactions occurring in the mixture is still unknown. However, we emphasize the readers to refer to Niedermeyer et al. (2012) that have summarized the available literature and several important information very elegantly in their review on the mixture of ILs.

A new approach to stabilize proteins in the mixture of ILs

Based on the above description on the mixture of ILs, it seems that mixing of ILs together could potentially have a huge economical and scientific impact on the application of ILs in protein stability and refolding studies. However, considering the large amount of publications concerned with ILs, the number of publications specifically on the effect of mixtures of ILs on protein stability is rare or missing. Thus, the major aim of this review is to highlight the use of IL mixtures in the protein world. Recently, Xu et al. (2016) observed an increased biocatalysis in the mixture of ChCl/EG and $C_4MIM{\cdot}PF_6$ (choline chloride/ethylene glycol and 1-butyl-3-methylimidazolium hexafluorophosphate), and the mixture showed good biocompatibility with the cells.

However, the first step in this field of research has been presented by our group. We reported the direct interaction of the IL mixture on the stability of protein (Reddy et al. 2015). Herein, we reported that the deleterious action of [Bmim][I] was counteracted by the presence of [Bmim][Br]. However, it is interesting to note that Br^- and I^- containing ILs act as denaturants when used "alone" as a cosolvent in protein solutions (Sasmal et al. 2011; Mojumdar et al. 2012; Ghosh et al. 2015; Kumar and Venkatesu 2012). In the contrary, for the first time, we have shown the counteracting effect of [Bmim][Br] on the denaturation action of [Bmim][I] on α-chymotrypsin (CT). As presented in Fig. 2, the fluorescence and thermal denaturation results demonstrated that [Bmim][Br] acts as a stabilizer at low concentrations, whereas it is denaturant at high concentrations. On the other hand, [Bmim][I] is reported to be a denaturing agent at all concentrations. However, the denaturing ability of [Bmim][I] was compensated by [Bmim][Br]. The counteraction action of [Bmim][Br] on [Bmim][I] was observed to be more pronounced at lower concentrations of [Bmim][Br] (0.025 M).

As presented in Fig. 2a, the fluorescence maximum (I_{max}) for CT in buffer was 73.6 a.u. In the presence of 0.025 M [Bmim][Br] and 0.2 M [Bmim][I], it was 71.5 and 39.7 a.u., respectively. Interestingly, after the addition of 0.025 M [Bmim][Br] to 0.2 M [Bmim][I], it is noticeable that an increase in the tryptophan (Trp) fluorescence spectra of CT was observed. It should be pointed that the absolute value of I_{max} was reduced to half with respect to that of the native CT because of the enhanced exposure of Trp residues to the bulk solvent. It is necessary to take into account that under denaturing conditions, the absolute intensity value was reduced to nearly half in the presence of [Bmim][I]. Interestingly, after the addition of 0.025 M [Bmim][Br] to 0.2 M [Bmim][I], it is noticeable that the fluorescence spectra were clearly modified with enhancement of the I_{max} values. This is an evidence that the protein again experiences the native-like environment in the mixture of ILs, which is reflected from the denaturation temperature (T_m) data presented in Fig. 2b.

The main mechanism due to which [Bmim][Br] counteracts the denaturation action of [Bmim][I] is still unexplored, and we are actively investigating it in detail in our laboratory. It is very interesting to find out the cause due to which a tenfold lower concentration of the [Bmim][Br] counteracts 0.2 M of [Bmim][I] action. We hypothesize based on the information available in the literature that this might be due to the internal interactions between ions of the [Bmim][Br] and [Bmim][I] in the aqueous mixture. It is possible that in a mixture, the negative ions get accumulated near the cation (since the cation is similar); thereby, the charges are decreased. In

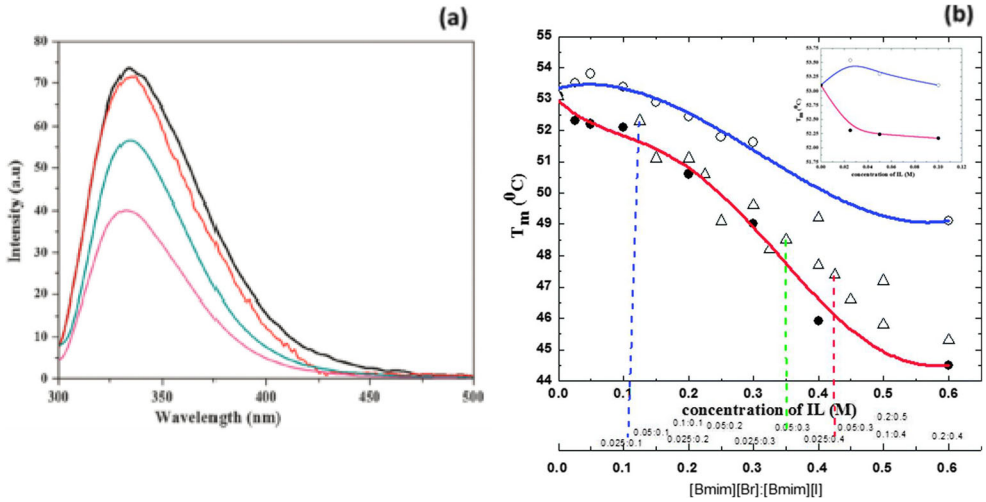

Fig. 2 **a** Fluorescence intensity changes for α-chymotrypsin (CT) in Tris-HCl buffer (black color line), 0.025 M [Bmim][Br] (red color line), 0.2 M [Bmim][I] (magenta color line), and 0.025 M [Bmim][Br] + 0.2 M [Bmim][I] mixture (dark cyan color). **b** Transition temperatures (T_m) for CT in Tris-HCl buffer in the presence of [Bmim][Br] (white circle), [Bmim][I] (black circle), and a [Bmim][I] + [Bmim][Br] (triangle) mixture at various concentrations, obtained from the temperature-dependent fluorescence intensities. Inset shows the change in T_m of CT in the presence of [Bmim][Br] (white circle) and [Bmim][I] (black circle) at lower concentrations (≤ 1.0 M). Adopted from ref. Reddy et al. (2015). With permission from The Royal Society of Chemistry

[Bmim] ILs, the charge is expected to delocalize over multiple atoms of a molecule and furthermore shows spatial anisotropy and asymmetry and allows directional interactions. Reports on the analysis of the [Bmim][Cl] ion pair showed that the butyl chain can give rise to a number of different conformers with several different stable positions of the Cl around the cation (Fig. 3) (Hunt et al. 2006; Hunt and Gould 2006; Kossmann et al. 2006).

Moreover, as mentioned by Reddy et al. (2015), both the anions (Br^- and I^-) are of similar size; therefore, in the mixture of ILs with same cation and different but similar sized anions, because of charge dispersion throughout the imidazolium cation, the cation can be in contact with a number of anions. Hence, as indicated in Fig. 3, the intermolecular modes of a cation are thus governed not by one anion, but by several of anions, not necessarily all equal. Thus, direct cation–anion contacts are formed that can be expected to exhibit localization of the anions around the cation, with in the mixture, possibly restricting the mobility of the anions to interact with the protein surface. Hence, due to this reason, we observed an increase in the Trp fluorescence in the mixture of [Bmim][Br] + [Bmim][I] (Reddy et al. 2015). Although the fluorescence intensity was lower in the presence of [Bmim][Br] + [Bmim][I] than that of the native protein, it was certainly larger than that of [Bmim][I]. The main reason behind the enhancement of intensity with the addition of [Bmim][Br] into the protein solution with [Bmim][I] is the movement of Trp towards the more hydrophobic environment, and therefore a high fluorescence intensity is observed.

The exact mechanism for the protective action of the mixture of two denaturant ILs for protein in a solution is still unclear. However, as it is undoubtedly seen from the literature, it is the rearrangement game between the ILs and water molecules around the protein surface that leads to protein stability. The support to our hypothesis is due to the use of computational methods by Xia et al. (2012), where it was observed that when a mixture of urea and GdmHCl (guanidine hydrochloride) was used in lysozyme solution, the protein undergoes to a collapse state instead of unfolding. The authors stated that the collapse of the protein conformational ensembles was accompanied with decreased solubility and increase in non-native self-interactions of hydrophobic residues in the urea/GdmCl mixture. According to the authors, this increase of non-native interactions resulted in protein's collapse transition from the fully denatured states. The authors also observed that during the protein collapse, GdmCl was absorbed onto the protein surface and at the same time, urea molecules accumulated near the protein surface, resulting in an enhanced "local crowding" for the protein near its first solvation shell (Xia et al. 2012).

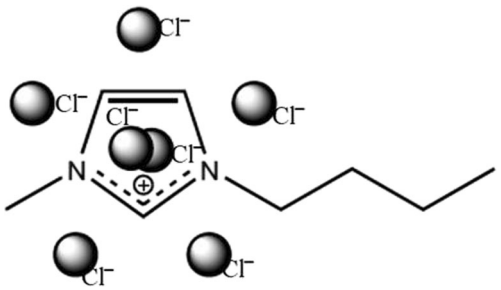

Fig. 3 Expected schematic representation of the chloride positions in the [Bmim][Cl] IL. Adopted from Heiko Jannes Niedermeyer Thesis. (https://spiral.imperial.ac.uk/bitstream/10044/1/11645/1/Niedermeyer-HJ-2012-PhD-Thesis.pdf)

These results improve our knowledge of the excellent properties of IL mixtures as stabilizers for the native conformation of protein, since IL mixtures are able to stabilize enzymes and are suitable as reaction media for enzymatic biotransformations of industrial interest.

Conclusion and future advantages of the mixture of ILs with respect to protein stability

There are lots of possibilities, we see, on using mixture of ILs for protein stability and refolding studies. IL mixtures can be used in controlling protein aggregation. The mixtures can be used to create unique protein formulations which will be useful for pharmaceutical industry as well as academic purpose. Moreover, ILs have tunable physical properties and low freezing point; hence, their mixtures can be prepared wisely, in order to prevent the unfolding and aggregation of proteins in cold. Some of the properties that are unique and will overcome the drawbacks of aqueous medium containing proteins include as follows: (1) if suitably selected IL mixtures can possess self-buffering properties, hence, as mentioned above, drastic decrease in the pH of the protein system can be significantly controlled. (2) As having very low freezing point, hence, ice nucleation in the solutions of IL mixtures can be significantly reduced, thereby facilitating the spectroscopic investigations of the proteins. (3) IL mixtures containing ammonium and cholinium cations are highly biocompatible and non-toxic, and hence, their formulations with antibodies or other proteins will not pose any health hazard when subjected in vivo. (4) Catalytic activities of various proteins can be efficiently examined in the IL mixtures. A lot of experimental approach is required to explore and identify the hidden physical properties of the IL mixtures. This identification will help in making the mixture of ILs more suitable for protein stabilities. We strongly believe that this approach will facilitate us to find out a suitable universal solvent IL-based solvent system which is biocompatible and can be efficiently used to stabilize antibodies and proteins against denaturation and aggregation due to temperature changes (low/high), chemical effect, pH changes, etc.

Acknowledgements We gratefully acknowledge the Council of Scientific and Industrial Research, New Delhi, India, through the Grant No. 01(2871)/17/EMR-II) for financial support.

Compliance with ethical standards

Conflict of interest Awanish Kumar declares that he has no conflicts of interest. Pannuru Venkatesu declares that he has no conflicts of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

References

Attri P, Venkatesu P, Kumar A (2012) Water and a protic ionic liquid acted as refolding additives for chemically denatured enzymes. Org Biomol Chem 10:7475–7478

Attri P, Venkatesu P (2012) Influence of protic ionic liquids on the structure and stability of succinylated Con A. Int J Biol Macromol 51: 119–128

Attri P, Jha I, Choi EH, Venkatesu P (2014) Variation in the structural changes of myoglobin in the presence of several protic ionic liquid. Int J Biol Macromol 69:114–123

Bisht M, Venkatesu P (2017) Influence of cholinium-based ionic liquids on the structural stability and activity of α-chymotrypsin. New J Chem 41:13902–13911

Bisht M, Kumar A, Venkatesu P (2015) Analysis of the driving force that rule the stability of lysozyme in alkylammonium-based ionic liquids. Int J Biol Macromol 81:1074–1081

Bisht M, Jha I, Venkatesu P (2016) Comprehensive evaluation of biomolecular interactions between protein and amino acid based-ionic liquids: a comparable study between [Bmim][Br] and [Bmim][Gly] ionic liquids. Chemistry Select 1:3510–3519

Bisht M, Mondal D, Pereira MM et al (2017) Long-term protein packaging in cholinium-based ionic liquids: improved catalytic activity and enhanced stability of cytochrome c against multiple stresses. Green Chem 19:4900–4911

Bi YH, Duan ZQ, Li XQ et al (2015) Introducing biobased ionic liquids as the nonaqueous media for enzymatic synthesis of phosphatidylserine. Agric Food Chem 63:1558–1561

Bates ED, Mayton RD, Ntai I et al (2002) CO_2 capture by a task-specific ionic liquid. J Am Chem Soc 124:926–927

Benedetto A, Ballone P (2016) Room temperature ionic liquids meet biomolecules: a microscopic view of structure and dynamics. ACS Sus Chem Eng 4:392–412

Chen FF, Huang K, Zhou Y et al (2016) Multi-molar absorption of CO_2 by the activation of carboxylate groups in amino acid ionic liquids. Angew Chem 128:7282–7286

Desai R K, Streefland M, Wijffels R H, et al (2016) Extraction of proteins with ABS. In: Freire M. (eds) Ionic-liquid-based aqueous biphasic systems. Green Chemistry and Sustainable Technology. Springer, Berlin, Heidelberg

Daschakraborty S, Biswas R (2014) Dielectric relaxation in ionic liquids: role of ion-ion and ion-dipole interactions, and effects of heterogeneity. J Chem Phys 140:014504

Greaves TL, Drummond CJ (2015) Protic ionic liquids: evolving structure–property relationships and expanding applications. Chem Rev 115:11379–11448

Gupta BS, Taha M, Lee MJ (2016) Extraction of an active enzyme by self-buffering ionic liquids: a green medium for enzymatic research. RSC Adv 6:18567–18576

Gebbie MA, Smith AM, Dobbs HA et al (2017) Long range electrostatic forces in ionic liquids. Chem Commun 53:1214–1224

Ghosh S, Parui S, Jana B et al (2015) Ionic liquid induced dehydration and domain closure in lysozyme: FCS and MD simulation. J Chem Phys 143:125103

Hayes R, Imberti S Dr., Warr GG et al (2013) The nature of hydrogen bonding in protic ionic liquids. Angew Chem 52:4623–4627

Hunt PA, Kirchner B, Welton T (2006) Characterising the electronic structure of ionic liquids: an examination of the 1-butyl-3-methylimidazolium chloride ion pair. Chem Eur J 12:6762–6775

Hunt PA, Gould IR (2006) Structural characterization of the 1-butyl-3-methylimidazolium chloride ion pair using ab initio methods. J Phys Chem A 110:2269–2282

Jha I, Venkatesu P (2016) Unprecedented improvement in the stability of Haemoglobin in the presence of promising green solvent 1-allyl-3-methylimidazolium chloride. ACS Sustain Chem Eng 4:413−421

Jha I, Attri P, Venkatesu P (2014) Unexpected effects of the alteration of structure and stability of myoglobin and hemoglobin in ammonium-based ionic liquids. Phys Chem Chem Phys 16:5514–5526

Jha I, Rani A, Venkatesu P (2017) Sustained stability and activity of lysozyme in choline chloride against pH induced denaturation. ACS Sus Chem Eng 5:8344–8355

Jha I, Bisht M, Venkatesu P (2016) Does 1-allyl-3-methylimidazolium chloride acts as a biocompatible solvent for stem bromelain? J Phys Chem B 120:5625–5633

Kossmann S, Thar J, Kirchner B, Hunt PA (2006) Cooperativity in ionic liquids. J Chem Phys 124:174506–174512

Kumar A, Rani A, Venkatesu P (2015) A comparative study of the Hofmeister series of anions of the ionic salts and ionic liquids on the stability of α-chymotrypsin. New J Chem 39:938–952

Kumar A, Rani A, Venkatesu P et al (2014a) Quantitative evaluation of the ability of ionic liquids to offset the cold-induced unfolding of proteins. Phys Chem Chem Phys 16:15806–15810

Kumar A, Venkatesu P (2013) Prevention of insulin self-aggregation by protic ionic liquid. RSC Adv 3:362–367

Kumar A, Bisht M, Venkatesu P (2016) Exploring the structure and stability of amino acids and glycine peptides in biocompatible ionic liquids. RSC Adv 6:18763–18777

Kumar A, Venkatesu P, Taha M, Lee MJ (2014b) Thermodynamic contribution of amino acids in ionic liquids towards protein stability. Curr Biochem Eng 1:125–140

Kumar A, Venkatesu P (2014) Does the stability of proteins in ionic liquids obeys the Hofmeister series? Int J Biol Macromol 63:244–253

Kumar A, Venkatesu P (2012) Overview of the stability of α-chymotrypsin in different solvent media. Chem Rev 112:4283–4307

Kumar A, Bisht M, Venkatesu P (2017) Biocompatibility of ionic liquids towards protein stability: a comprehensive overview on the current understanding and their implications. Int J Biol Macromol 96:611–651

Katoh R, Hara M, Tsuzuki S (2008) Ion pair formation in [bmim]I ionic liquids. J Phys Chem B 112:15426–15430

Kareem MA, Mjalli FS, Hashim Mohd A, AlNashef IM (2010) Phosphonium-based ionic liquids analogues and their physical properties. J Chem Eng Data 55:4632–4637

Losada-Pérez P, Khorshid M, Renner FU (2016) Interactions of aqueous imidazolium-based ionic liquid mixtures with solid-supported phospholipid vesicles. PLoS One 11:e0163518

Lee SY, Khoiroh I, Ooi CW et al (2017) Recent advances in protein extraction using ionic liquid-based aqueous two-phase systems. Sep Purif Rev 46:291–304

Lei Z (2017) Introduction: Ionic Liquids. Chem Rev 117:6633–6635

Lei Z, Dai C, Chen B (2014) Gas solubility in ionic liquids. Chem Rev 114:1289–1326

Matsumoto K, Hagiwara R, Mazej Z, Benkič P, Žemva B (2006) Crystal structures of frozen room temperature ionic liquids, 1-ethyl-3-methylimidazolium tetrafluoroborate ($EMImBF_4$), hexafluoroniobate ($EMImNbF_6$) and hexafluorotantalate ($EMImTaF_6$), determined by low-temperature X-ray diffraction. Solid State Sci 8:1250–1257

Mai NL, Ahn K, Koo YM (2014) Methods for recovery of ionic liquids—a review. Process Biochem 49:872–881

Mojumdar SS, Chowdhury R, Chattoraj S et al (2012) Role of ionic liquid on the conformational dynamics in the native, molten globule, and unfolded states of cytochrome C: a fluorescence correlation spectroscopy study. J Phys Chem B 116:12189–12198

Niedermeyer H, Hallett JP, Villar-Garcia IJ et al (2012) Mixtures of ionic liquids. Chem Soc Rev 41:7780–7802

Nakajima K, Nakanishi S, Lísal M, Kimura K (2017) Surface structures of binary mixture of ionic liquids. J Mol Liq 230:542–549

Podgoršek A, Jacquemin J, Pádua AAH et al (2016) Mixing enthalpy for binary mixtures containing ionic liquids. Chem Rev 116:6075−6106

Park S, Kazlauskas RJ (2003) Biocatalysis in ionic liquids—advantages beyond green technology. Cur Opin Biotechnol 14:432–437

Reddy PM, Umapathi R, Venkatesu P (2015) A green approach to offset the perturbation action of 1-butyl-3-methylimidazolium iodide on α-chymotrypsin. Phys Chem Chem Phys 17:184–190

Ramdin M, de Loos TW, Vlugt TJH (2012) State-of-the-art of CO_2 capture with ionic liquids. Ind Eng Chem Res 51:8149–8177

Sasmal DK, Mondal T, Mojumdar SS et al (2011) An FCS study of unfolding and refolding of CPM-labeled human serum albumin: role of ionic liquid. J Phys Chem B 115:13075–13083

Schröder C (2017) Proteins in ionic liquids: current status of experiments and simulations. Top Curr Chem 375–425

Stolarska O, Pawlowska-Zygarowicz A, Soto A, Rodríguez H et al (2017) Mixtures of ionic liquids as more efficient media for cellulose dissolution. Carbohydr Polym 178:2017

Sivapragasam M, Moniruzzaman M, Goto M (2016) Recent advances in exploiting ionic liquids for biomolecules: solubility, stability and applications. Biotechnol J 11:1000–1013

Smith EL, Abbott AP, Ryder KS (2014) Deep eutectic solvents (DESs) and their applications. Chem Rev 114:11060–11082

Taha ME, Silva FA, Quental MV, Ventura Sónia PM, Freirea MG, Coutinho JAP (2014) Good's buffers as a basis for developing self-buffering and biocompatible ionic liquids for biological research. Green Chem 16:3149–3159

Vijayraghavan R, Pas SJ, Izgorodina EI et al (2013) Diamino protic ionic liquids for CO_2 capture. Phys Chem Chem Phys 15:19994–19999

Weight A K, Larson A M, Langer R S, et al (2015) U S Patent No. WO 2015038811 A2, Liquid protein formulations containing ionic liquids

Wu BP, Wen Q, Xu H, Yang Z (2014) Insights into the impact of deep eutectic solvents on horseradish peroxidase: activity, stability and structure. J Mol Catal B Enzym 101:101–107

Wang B, Qin L, Mu T et al (2017) Are ionic liquids chemically stable? Chem Rev 117:7113–7131

Wang M, Rao N, Wang M et al (2018) Properties of ionic liquid mixtures of [NH2e-mim][BF4] and [bmim][BF4] as absorbents for CO_2 capture. Greenhouse Gas Sci Technol 0:1–10

Xia Z, Das P, Shakhnovich E et al (2012) Collapse of unfolded proteins in a mixture of denaturants. J Am Chem Soc 134:18266–18274

Xiao D, Rajian JR, Li S et al (2006) Additivity in the optical Kerr effect spectra of binary ionic liquid mixtures: implications for nanostructural organization. J Phys Chem B 110:16174–16178

Xiao D, Rajian JR, Hines LG, Li S et al (2008) Nanostructural organization and anion effects in the optical Kerr effect spectra of binary ionic liquid mixtures. J Phys Chem B 112:13316–13325

Xu P, Du P, Zong MH et al (2016) Combination of deep eutectic solvent and ionic liquid to improve biocatalytic reduction of 2-octanone with Acetobacter pasteurianus GIM1.158 cell. Sci Rep 6:1–10

Zhao H (2016) Protein stabilization and enzyme activation in ionic liquids: specific ion effects. J Chem Technol Biotechnol 91:25–50

Zhang S, Zhang J, Zhang Y et al (2017) Nanoconfined ionic liquids. Chem Rev 117:6755–6833

Zahn S, Uhlig F, Thar J, Spickermann C et al (2008) Intermolecular forces in an ionic liquid ([Mmim][Cl]) versus those in a typical salt (NaCl). Angew Chem 47:3639–3641

Zeng S, Zhang X, Bai L et al (2017) Ionic-liquid-based CO_2 capture systems: structure, interaction and process. Chem Rev 117:9625–9673