Parallel Developments in Aprotic and Protic Ionic Liquids: Physical Chemistry and Applications

C. AUSTEN ANGELL,* NOLENE BYRNE, AND JEAN-PHILIPPE BELIERES
Department of Chemistry and Biochemistry, Arizona State University, Tempe, Arizona 85287-1604

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ABSTRACT
This Account covers research dating from the early 1960s in the field of low-melting molten salts and hydrates, which has recently become popular under the rubric of "ionic liquids". It covers understanding gained in the principal author's laboratories (initially in Australia, but mostly in the U.S.A.) from spectroscopic, dynamic, and thermodynamic studies and includes recent applications of this understanding in the fields of energy conversion and bio-preservation. Both protic and aprotic varieties of ionic liquids are included, but recent studies have focused on the protic class because of the special applications made possible by the highly variable proton activities available in these liquids.

In this Account, we take a broad view of ionic liquids (using the term in its current sense of liquids comprised of ions and melting below 100 °C). We include, along with the general cases of organic cations, liquids in which common inorganic cations (like Mg²⁺ and Ca²⁺) strongly bind a hydration or solvation shell and then behave like large cation systems and melt below 100 °C.

Angell obtained his bachelor’s and Masters’ degrees from the University of Melbourne, Australia, and his Ph.D. from Imperial College of Science, University of London. He is currently Regents’ Professor of Chemistry at Arizona State University, Tempe, AZ, having moved to ASU in 1989 after 23 years at Purdue University. His first work in the sub-100 °C ionic liquid field was done at Melbourne University. He has since worked on a wide range of problems related to liquids and glasses, including electrolytes for lithium batteries and most recently ionic liquids for fuel cells and protein folding/preservation studies. He has been especially intrigued by the problem of supercooled water and related liquids in which the phenomenon of polymorphism is encountered. Awards for research have included the MRS Turnbull Lecture (2006), ACS Joel Henry Hildebrand award for the study of liquids (2004), the Neville Mott award of the Journal of Non-Crystalline Solids (1992), and the Morey award of the American Ceramic Society (1990). He has also been the recipient of an Alexander von Humboldt senior research award, and a Fulbright award. He has served on the editorial boards of a number of major journals including the Journal of Physical Chemistry, the Journal of Chemical Physics, and Annual Review of Physical Chemistry. In 1998, he was honored with a special issue of the Journal of Physical Chemistry.

Byrne received her B.E.(Math)(Hons) in 2003 then a Ph.D. in 2006 from Monash University, Australia, in the area of improved electrolytes for lithium metal batteries. She is currently in a postdoctoral role at Arizona State University, Tempe, AZ. Her research involves the use of ionic liquids as media for improved biopreservation and electrochemical devices.

Belieres did his undergraduate studies at the Université Joseph Fourier in Grenoble, France, and his Master degree in electrochemistry at the Institut National Polytechnique Grenoble, France. He received his Ph.D. in Chemistry from Arizona State University, and he is currently a Research Assistant Professor in the Chemistry and Biochemistry department at ASU. His research has been focused on fuel cells, lithium batteries, and ionic liquids.

We will try to organize a wide variety of measurements on low-melting ionic liquid systems into a coherent body of knowledge that is relevant to the ionic liquid field as it is currently developing.

Under the expanded concept of the field, ionic liquid studies commenced in the Angell laboratory in 1962 with a study of the transport properties of the hydrates of Mg(NO₃)₂ and Ca(NO₃)₂. These were described as melts of "large weak-field cations" and their properties correlated with those of normal anhydrous molten salts via their effective cation radii, the sum of the normal radius plus one water molecule diameter. This notion was followed in 1966 with a full study under the title “A new class of molten salt mixtures” in which the hydrated cations were treated as independent cation species. The large cations mixed ideally with ordinary inorganic cations such as Na⁺ and K⁺, to give solutions in which such cohesion indicators as the glass transition temperature, Tg, changed linearly with composition. The concept proved quite fruitful, and a field (“hydrate melts” or “solvate melts”) developed in its wake in which asymmetric anions like SCN⁻ and NO₂⁻ were used with solvated cations to create a very wide range of systems that were liquid at room temperature, while remaining ionic in their general properties.

The validity of the molten salt analogy was given additional conviction by the observation that transition metal ions such as Ni(II) and Co(II) could be found in the complex anion states NiCl₄²⁻ and CoCl₄²⁻ when added to the “hydrate melt” chlorides.²

Of special interest here was the observation³ of extreme downfield NMR proton chemical shifts of hydrate protons when the hydrated cation was Al³⁺. These lay much further downfield than did the protons in strong mineral acids at the same concentration, leading to the design of solutions of highly acidic character from salts that would usually be considered neutral. Indeed it was shown in ref 3 that platinum can be dissolved much more rapidly in a hot ionic liquid formed from AlCl₃(H₂O)₆ + Al(NO₃)₃·(H₂O)₆ than in boiling aqua regia. This scenario of variable proton activity in solvent-free, nominally neutral ionic liquids will be revisited in the context of protic ionic liquids at the end of this Account.

Most of the interest in the field lies with room temperature liquids in which the cations are indeed of organic origin, and the story takes up naturally with these because of the manner in which the organic cation ionic liquids can be put to use in providing excellent complex anions for spectroscopic study. A good example is that shown in Figure 1 from the work of Gruen and McBeth⁴ in whose laboratory one of us first encountered melts such as pyridinium chloride, PyHCl, and first used them in the (unpublished) study of Ni(II) complexes in low-melting systems such as PyHCl–ZnCl₂. We were impressed by the sharp changes in electronic spectra of Ni(II), reflecting...
distinct and different structures, which were produced by small changes in solution composition.

The knowledge that these liquids could be used for molten salt studies at low temperatures quickly became useful when the new science of high-pressure physical chemistry using the diamond anvil high-pressure cell (DAC) burst on the scene. Little was known at that time of the effect of pressure on ion coordination.

The DAC was then only available for room-temperature studies, and a room-temperature molten chloride for the application of this newly invented high-pressure technique was needed. Even these days, ambient-temperature ionic liquid chlorides are not common, but Angell and Abke- meier found that ethanolaminium chloride could serve as the ambient-temperature noncrystallizing component that induced ambient-temperature stability on mixtures with ethylammonium chloride. This chloride ion melt proved ideally suited for the study of transition metal ion spectra up to 3.5 GPa, pressure being calibrated in the range to 0.3 GPa by observing the glass transition spectroscopically and relating it to independently determined values from high-pressure differential thermal analysis (DTA) studies.

Ni(II) electronic spectra of good quality, shown in Figure 2, were obtained, and the conversion from NiCl₂⁻ at ambient pressure to NiCl₄⁴⁻ at higher pressures was observed. Impressively simple, the change with pressure of the equilibrium constant

\[
d\ln K_\text{eq}/dP = -\Delta V/RT
\]

for the process NiCl₂⁻ + 2Cl⁻ → NiCl₄⁴⁻, yielded a \(\Delta V\) within 10% of the volume of 2 mol of chloride ion.⁵

**Role of Complex Anions in the Development of Ambient Temperature Air- and Water-Stable Ionic Liquids**

While the latter study of transition metal spectra used organic cation ionic liquids as a tool for the study of complex ions, Hodge and co-workers⁶ used complex anions in organic cation salts for the study of ionic liquid properties, indeed properties that have found recent applications.

The dramatically different effects of complexation on the liquid viscosity are well illustrated by a 1976 study⁷ that used the lower-melting \(\alpha\)-methyl derivative of pyridinium chloride as the source of the Lewis base chloride.

Data seen in Figure 3 show that the cohesive energy of an ionic liquid is lowered most strongly when chloride ions are complexed by FeCl₃ to produce the large singly charged FeCl₄⁻ anion. This observation leads to the prediction that, among ionic liquids of a given cation, the tetrachloroferrate salt should be the most fluid and also most conductive, even more so than the well-studied ionic liquids containing AlCl₄⁻ anions.⁸ The veracity of this expectation is demonstrated in Figure 4 for salts of the butylmethylimidazolium cation taken from a very recent study by Xu et al.⁹ For the 25 °C conductivity, the tetrachloroferrate is half an order of magnitude above any others in the group. The increase of ionic mobility by complexation seen here is no different in principle from the use of BeF₂ additions to alkali fluoride melts utilized in the molten salt reactor technology that had provided much of the driving force, and also funding, for molten salt studies in the 1950–1970 period. There, the addition of 1 mol of BeF₂ replaced two high charge intensity fluoride anions with a doubly charged anion of much larger dimensions, for a net decrease in cohesion.

The fluorinated anion salts (containing BF₄⁻ and PF₆⁻) that are most frequently cited in connection to practical applications of the ambient-temperature ionic liquids, are to be understood in the same terms (unfortunately, the tetrachloroferrate anion is slowly hydrolyzed by water, and the applications of ambient temperature ionic liquids generally require the presence of water-stable anions). Fluorinated species in addition to being water-stable have the advantage of being unpolarizable and so minimize the effect of van der Waals contributions to the liquid cohesion. The importance of this characteristic of fluorinated anions to ionic liquid properties was first emphasized in a 1983 study of binary solutions of ionic liquid halides with lithium halides by Cooper.⁹ There it was predicted that the replacement of iodide anions by the equivalent number of BF₄⁻ anions would lead to an increase of ambient temperature conductivity, which was then proven true (unfortunately only in a “note added in proof” in ref 9) by some 2 orders of magnitude.⁹,¹⁰ Cooper’s further extensive syntheses and measurements on viscosity and conductivity of a family of tetraalkylammonium tetrafluoroborate salts remained unpublished (for over 2 decades) until 2003.⁷

In the meantime, Cooper and O’Sullivan¹¹ reported, in 1992, the first systematic study of ambient-temperature water-stable ionic liquids, using triflate and related anions combined with imidazolium type cations. This announce-ment, presented at one of the Electrochemical Society’s International Molten Salt Conferences and eventually published in its proceedings,¹¹ was immediately followed by a communication by Wilkes and co-workers¹² who had been working on similar salts, and the field of high-fluiddity, water-stable ionic liquids was launched.
Ionicity of Ionic Liquids: Relation to Vapor Pressure and Conductivity

The “ionicity” of aprotic ionic liquids is the property that is responsible for their characteristic low vapor pressures. Low vapor pressure is the single most important property of this class of liquid, the property that, more than any other, is responsible for high level of interest in the field. However there is very little written about what exactly is involved in achieving high ionicity.\(^\text{13}\) If the ions created in forming the ionic liquid were to remain locked together in pairs, the liquids would not be found to be of very low vapor pressure, and their conductivities would also be poor. They would just represent an unusual group of polar liquids. Indeed there are cases of aprotic ionic liquids in which for one reason or another the ions are largely paired up, and these are characterized by a high vapor pressure and a poor conductivity (see below). To obtain the low vapor pressures for which ionic liquids are generally reputed, the ions must distribute themselves in a uniform manner each ion surrounded by a symmetrical shell of the opposite charge. This is the distribution that minimizes the electrostatic potential energy, developing the liquid equivalent of the ionic crystal Madelung energy.\(^\text{14}\) It is the difficulty of overcoming this electrostatic free energy to produce individual ion pairs that is responsible for the low vapor pressures of “good” ionic liquids.

An example of an ion-paired aprotic salt (unsymmetrical charge shell) is shown in Figure 5, which is a plot based on the classical concept, due to Walden, that ionic mobility is determined by the viscous friction of the liquid dragging on the ion as it attempts to move under the electrochemical force exerted by the DC electric field. This

![Figure 2](image2.png)  
**FIGURE 2.** Electronic spectra (d–d transitions) of Ni(II) in a chloride environment both at 1 atm pressure and variable temperature down into the glass state (panel a) as a function of pressure up to 0.3 GPa at 24.5 °C and as a function of both up to 0.91 GPa, showing conversion from tetrahedral to octahedral coordination at high pressures and low temperatures. Reprinted with permission from ref 5. Copyright 1973 American Chemical Society.

![Figure 3](image3.png)  
**FIGURE 3.** Variation of \(T_g\) of the simple chloride glass former \(\alpha\)-methylpyridinium chloride on chloride complexation with various Lewis acids. Adapted from ref 6 by permission.

![Figure 4](image4.png)  
**FIGURE 4.** Fluidity data for a number of salts of the butylmethylimidazolium cation, showing that the tetrachloroferrate is more fluid than any of the fluorinated anion salts, in accord with its lowest \(T_g\) seen here (log \(\eta^{-1} = 10^{-13}\)) and in the Figure 6 legend. Reproduced with permission from ref 8. Copyright 2003 American Chemical Society.
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salt, methoxymethyl-dimethyl-ethyl-ammonium tetrafluoborate (MOMNM\(_2\)E-BF\(_4\) or \([N_1-O-1,211]\)[BF\(_4\)]

Figure 5 can be used to classify liquids between the groups “ionic” (whose members lie near the diagonal line), “subionic” (whose members lie below the line in the region labeled as “poor” ionic liquids), and superionic (whose members lie above the line by virtue of having some ionic species, usually small and low-charged, that can “slip through the cracks” in a nanoscopic sense and thus escape the full viscous drag of the surrounding medium).

**Cohesive Energy of Ionic Liquids**

The salts that develop the full Madelung potential are, by the above understanding, those that will have the largest cohesive energy (for given ionic sizes). Generally they will be the cases that have ions of comparable size and uniform shape so that they pack well and occupy a small volume. However salts that conform to this description will in general find it easy to crystallize into some high lattice energy structure and will have high melting points. They will therefore not be among the group known as “ionic liquids”. To qualify as ionic liquids there must be some factor that lowers the lattice energy and thereby lowers the melting point. The “good” ionic liquids then must be those with some optimum departure from high symmetry, achieved by judicious (or serendipitous) choice of ion shape and interaction factors.

Those that achieve a sufficiently low melting point will then fail to crystallize and, on further cooling, will terminate in the glassy state. The temperature at which the liquid vitrifies, \(T_g\), provides a second measure of the liquid cohesion. The plot is expected to have a minimum where decreasing Coulomb attractions balance increasing van der Waals attractions. Such a minimum is indeed found\(^8\) in the vicinity of a volume that corresponds to a charge concentration of ~4 M (Figure 6).

The case of the “poor” ionic liquid \([N_1-O-1,211][BF_4]\), in which the cations and anions are largely paired, is of interest in this connection. This aprotic ionic liquid was seen, in Figure 10 of ref 8 to have the lowest cohesion, by the \(T_g\) criterion, of any aprotic IL. This is fully consistent with its poorly developed Madelung potential. It is only underlain by the protic cases, I–IV, discussed below.

**Aprotic Ionic Liquid Applications: Lithium Battery Electrolytes**

Despite the interest widely expressed in ionic liquid-based lithium battery electrolytes, we omit reference to our recent studies in this area. This application is limited by a fundamental problem, discussed in ref 9 and illustrated in Figure 3, where it is seen that all small cations, including Li\(^+\), get trapped as anionic complexes (by outcompeting the large solvent cations) and hence must lose mobility. This problem is reduced by use of fluorinated anions\(^9\) but not overcome.
**Protic Ionic Liquids: Properties and Applications**

In contrast to the systems on which most of the work in the ionic liquid field has been performed (represented here by data in Figure 4), the protic ionic liquids are formed very simply by proton transfer from an inorganic acid to a Bronsted base. The base is usually organic in character, but there are also inorganic examples. The salt hydrazinium nitrate was reported in 1971 to have a melting point of only 70 °C,\(^{16}\) and hydrazinium formate and hydrazinium acetate are both molten below 100 °C and can easily be studied through room temperature down to their glass transition temperatures.\(^{16}\) The electrical conductance of hydrazinium formate at room temperature (measured in 1970 but only reported in the open literature in 2003\(^{17}\)) is higher than that of any aprotic ionic liquid and is among the highest known. Many eutectic mixtures of ammonium salts are liquid below 100 °C, and these have recently been shown to be very interesting in application as fuel cell electrolytes,\(^{18}\) as will be discussed further below. And of course there are the hydronium compounds such as hydronium triflate, which is congruently melting, \(T_m=35\) °C, and has a very high electrical conductivity. Triflic acid and other superacids will presumably protonate many inorganic molecules that would not normally be considered as having cationic forms, so there are likely to be many additional inorganic protic ionic liquids recognized in the future. However the organic bases are far more versatile for protic ionic liquid formation.

There are organic bases not only from the primary, secondary or tertiary amines, but also with nitrogen atoms in heterocyclic rings (which may or may not be resonance stabilized).\(^{19}\) There are also protonatable phosphorus analogs available, and sulfur-, selenium-, and iodine-based examples also exist.

An important aspect of protic ionic liquids is that the properties of the ionic liquid formed on proton transfer depend very strongly on the relative strengths of the acid and base between which the proton is transferred.\(^{20}\) While the concentration of protonated species is always very high, of order 5–10 M in most cases, the properties may vary from strongly acidic to strongly basic in character, as is readily seen by simple tests with proton sensitive probes, such as the common indicator ions. For instance, a wide pH range litmus paper registers bright pink in contact with triethylammonium triflate but dark blue in contact with triethylammonium acetate. The systematic and quantitative measurement of basicity in these solvent-free liquids is a recent achievement.

In order to assist the prediction of protic ionic liquid properties, the authors\(^ {20,21}\) have generalized the Gurney proton energy level diagram\(^ {22}\) not just to deal with proton transfers between acid and base species in an aqueous environment but, rather, to describe the energy changes when a proton moves between any proton donor and proton acceptor pair in the absence of any solvent at all. So far, for lack of alternatives, levels in the diagram relative to that of the \(\text{H}_2\text{O}^+\)/\(\text{H}_2\text{O}\) level have been based on the known aqueous \(\text{pK}_a\) values, but this is of course only approximate because of the different dehydration energies of acid and base species in passing from aqueous to anhydrous systems. The approximate values will in time be replaced by energy levels based on NMR chemical shift studies\(^ {23}\) and other direct protic ionic liquids measurements, for instance, vapor pressure changes,\(^ {20}\) and particularly on voltages obtained from appropriate electrochemical measurements. The latter are just beginning.\(^ {24,25}\)

An example of the energy level diagram is provided in Figure 7.\(^ {21}\) It is constructed, following Gurney, by grouping acid and conjugate base forms of each molecule or ion together at a free energy level (in electronvolts) based on the arbitrary assignment of 0 eV to the couple \(\text{H}_2\text{O}^+\)/\(\text{H}_2\text{O}\). The right-hand member of the couple is described as possessing an unoccupied quantized proton energy level, while the left-hand member has this level "occupied". The proton in the occupied level of any couple can "fall into" the vacant level of any couple lower on the diagram. When the occupied level of a couple lies on a neutral molecule, the fall of its proton to the unoccupied level of a couple at a lower level leaves behind a negatively charged species and creates a new positive species from the neutral molecule into whose unoccupied proton level it fell, and an ionic liquid is created if the melting point of the

![FIGURE 7](image-url)
product is below 100 °C, as is frequently the case. The
relation between the level values is based on \( E \left( \Delta G / F \right) \).

We can now group the products of such proton transfers together into three protic electrolyte categories, acid, neutral, and basic. Acid protic electrolytes result when the proton falls from an occupied level on a superacid to a vacant level on a weak base, for example
from triflic acid to fluoroaniline or to water, as in common aqueous acids. Neutral electrolytes arise when the proton falls from a moderately strong acid like nitric or methanesulfonic acid to a strong base like ethylamine or cyclopentylamine. Provided the proton falls across a gap of 0.7 eV or more, the electrolyte is a “good” ionic liquid, meaning the proton resides on the base for at least 99% of the time. Finally, basic electrolytes, for example, the product of proton transfer from a weak acid such as acetic acid to a base such as ethylamine, will give a poor ionic liquid. To obtain a truly basic ionic liquid, the cation of an initial proton transfer process yielding a product like EAN must be combined with the product of second proton transfer process that yields a basic anion like OH\(^-\) or NH\(_2\)^- by some metathetical reaction, for example, \([\text{CH}_3\text{CH}_2\text{NH}_3]^+|\text{Cl}^-| + \text{NaOH} \rightarrow [\text{CH}_3\text{CH}_2\text{NH}_3]^+|\text{OH}^-| + \text{NaCl}\). Such compounds tend to be high melting and have been little explored to date. The properties of low melting liquids produced by reducing the charge density of the anion by complexation, for example, with a weak Lewis acid, such as Al(OH)\(_3\), have yet to be explored.

The ionic conductances of some protic ionic liquids are shown in Figure 8 along with those of aqueous LiCl to show that for some salts like dimethylammonium nitrate\(^{17}\) the conductivities at ambient temperature can be as high as those found in aqueous solutions. The

![Figure 8](image-url) Conductivities of various protic ionic liquids (and one aprotic case, \([\text{N}_1\cdot\text{O}-2.211]|\text{BF}_4\]) compared with those of aqueous LiCl solutions to show how the aqueous solutions are no longer unique with respect to high ionic conductivity. II is dimethylammonium nitrate; V is methoxypropylammonium nitrate. Reprinted from ref 17 with permission of AAAS.

In Figure 9, the conductivities, while indeed very high, do not reach the values of the best organic cation ILs except in the case of the hydrazinium formate. The case of ammonium bifluoride is exceptional, and it is possible that this unusual liquid, extensively used as a cleaning and a fluorinating agent in inorganic fluoride chemistry\(^{26}\) may be a “dry” proton conductor.
provide electrolytes of a type that are simply not available in systems in which water acts as acid or base in the proton transfer process.

It is even more astonishing that until 2006 it had not been realized that mixtures of inorganic salts (namely, the ammonium salts) can be used as protic electrolytes for fuel cells running above 80 °C (hydrazinium salts could be used to much lower temperatures). Applied as fuel cell electrolytes, the ammonium salts give more stable performance than electrolytes containing organic cations. The IR-corrected polarization curves for some inorganic electrolyte fuel cells are shown in Figure 10, using the logarithmic current ("Tafel plot") form. The plateau current at the theoretical voltage can now be extended out to 50 mA cm⁻² in some inorganic systems to be described in future articles, beyond the previously recognized limits. The increase in efficiency relative to the phosphoric acid cell is about 20%, and the implied reduction in generated heat may be very important in some applications, which do not require high power.

While the ionic liquids offer the possibility of higher efficiency cells, the phosphoric acid (PA) cell has a significant advantage in kinetics. The kinetic advantage lies in the higher conductivity of the phosphoric acid, which is a molecular liquid that exhibits not only considerable autoionization but also "dry" proton mobility. This superfractional component, which is illustrated in Figure 11, is unfortunately not present in the protic ionic liquids, which mostly lie on or below the "ideal" Walden line. It may be necessary to compromise on power output for the sake of energy efficiency, though the NH₄HF₂ data in Figure 9 suggest that breakthroughs may eventuate.

In an initial report on the relation of fuel cell voltage output to other properties of the PILs, we noted a relation between the output voltage and the difference in pKₐ values for the acid and base components of the electrolyte. The voltage increased strongly with increasing ΔpKₐ and reached the theoretical value when the ΔpKₐ value for the electrolyte reached about 14. There is now an urgent need to develop direct measures of the free energy of proton transfer for the anhydrous case.

**In Situ Measures of Proton Transfer Energetics and Relative Proton Activities in Ionic Liquids**

Two steps have now been taken toward direct proton transfer energy characterization. The first has been to study the NMR proton chemical shift for the proton that has been transferred to the base nitrogen but is still being counteracted by the conjugate base of the acid from which it came. The stronger the "pull" from the conjugate base (which means the smaller the free energy of transfer), the more deshielded will be the N-H proton. The more deshielded the proton, the further downfield its resonance frequency will lie relative to a standard reference, for example, C(CH₃)₄. Thus the N-H chemical shift (δN-H) can serve as an in situ probe of the proton transfer energy. We find immediately that it does not enjoy a linear relation with the value of ΔpKₐ but rather gives a relationship similar to that between the fuel cell open circuit voltage (OCV) and the ΔpKₐ value. The second step has been to develop a method of measuring directly the voltage necessary to displace a proton from its normal site on a nitrogen onto the anion from which it originally came. This will be described elsewhere. These direct measures have already found application in another recently developing application of ionic liquids, namely, the stabilization of biomolecules discussed in the next section.

**Proton Activity and Protein Stabilization in PILs**

An exciting new role for ionic liquids may lie in the storage and manipulation of sensitive biomolecules. Fujita et al. have recently reported that the protein cytochrome c is remarkably stabilized in solution in a nontoxic dihydrogen phosphate IL, and we have found that lysozyme in...
200 mg/mL concentration is still folded after 3 years in ambient solutions with large ethylammonium nitrate concentrations. The source and full extent of this stability is not yet properly understood.

It is well-known from standard biological concentration solution studies that each protein has pH range in which its native state is most preferred and that deviations from that pH range can affect its folding energy and its stability against such undesirable behavior as aggregation, and recently, fibrillation. It might be expected that the proton activity of the solvent will exert a strong effect on the stability of proteins in the ionic liquid protective solution, and this is verified by our findings,32 which are shown in Figure 12. In Figure 12, the proton activity is represented by the δ(N–H) while, for stability on the vertical axis, we use a refoldability index pending the necessary long-term assessments.

The refoldability index is the percentage of the initial enthalpy of unfolding obtained after lowering the temperature to allow thermally denatured molecules to refold and then remeasuring the unfolding enthalpy. The difference between the two is a measure of the fraction of the protein lost to the aggregation process and is found to be 3%. The stability index is then given by the ratio (second unfolding enthalpy)/(first unfolding enthalpy) and is 0.97, or 97%, under favorable circumstances. The "% refoldability" for hen egg lysozyme is shown as a function of the N–H chemical shift (δ(N–H)) determined for the solution in which the lysozyme was dissolved, in Figure 12. The presence of a δ(N–H) range equivalent to about 2 pH units in which the same high refoldability index is obtained is quite striking. The refoldability index varies as a function of water content at constant ionic liquid content. But when the δ(N–H) of the solution is measured, it is found that the δ(N–H) has also changed with water content, and it is evidently only the proton activity that is important. The solution in which the 3 years stability was recorded31 happened to have been formulated near the center of the maximum stability zone of Figure 12 for lysozyme.

Different proteins have different favorable proton activity stability ranges. We hope to establish these optimum conditions for many normally fragile biomolecules so that they can be preserved indefinitely at ambient temperatures, thereby opening the door to unrefrigerated shipping and storage of drugs and other biologically important materials.

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