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Unexpected Preferential Dehydration of Artemisinin in Ionic Liquids

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Thermodynamic measurements (at 298 K) reveal that a crucial step in the extraction process of the key antimalarial drug artemisinin by ionic liquids (ILs), namely, precipitation through the addition of water, is driven by artemisinin dehydration due to the differences in the water's interaction with the bulk ILs, rather than with the artemisinin itself.

Ionic liquids are molten salts that are liquid at room temperature. Due to the enormous number of potential ILs, it is possible, with the right insight, to design solvents to perform very specific tasks.[1] One outcome of this is that ILs have been very successful in the extraction of high-value natural products from various biomass sources, often showing significantly higher yields and selectivities than conventional molecular solvents.[1] These are compounds that can truly claim to be "rationally designed" molecular materials. Nevertheless, one aspect of the extraction procedures remains a major scientific and commercial hurdle to the widespread implementation of IL extraction technology; this is the reclamation of dissolved material from the IL. The key technique currently used is the addition of an antisolvent to precipitate the target material. However, this is a complex intermolecular process that is not at all well understood from both thermodynamic and molecular-level perspectives.[2]

What is the mechanism of precipitation through antisolvent addition? In this communication, we focus on the extraction of artemisinin, a potent naturally occurring antimalarial compound (Figure 1). This has been achieved by the authors using protic ionic liquids (PILs) with water as the antisolvent.[3] This approach not only overcomes shortcomings of the current commercial method (based upon *n*-hexane/ethyl acetate),[4] namely, impurities, toxicity, and environmental impact,[5] but also possesses increased artemisinin dissolution capacity.

The use of water as an antisolvent exploits the empirical fact that artemisinin solubility in the PILs is strongly dependent on the bulk water content. Obtaining optimum recovery through the addition of water is problematic and is underpinned by a complex intermolecular solvation process involving composition-dependent dynamic interactions between the PIL, water, and artemisinin.

This system is one of many well-documented cases in which water content and ionic liquid structure play crucial roles in solvation, yet the mechanisms are not at all well-understood.[2,6] To address this, we use two model ILs which provide contrasting solvent characteristics in an initial study that tackles these issues from both thermodynamic and molecular perspectives.

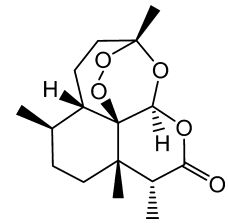

Figure 1. The structure of artemisinin.

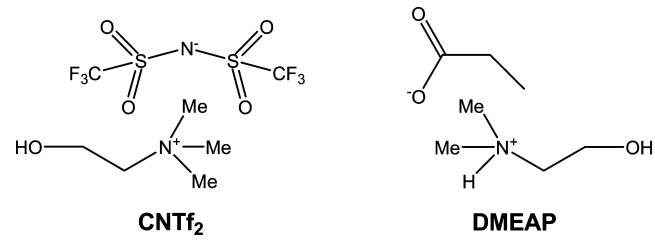

Figure 2. Structures of $CNTf_2$ and DMEAP.

The model ILs employed in this communication are *N*,*N*,-dimethylethanolammonium propanoate and choline bis(trifluoromethylsulfonyl)imide, abbreviated, respectively, as DMEAP and $CNTf_2$ (Figure 2). $CNTf_2$ is a hydrophobic aprotic ionic liquid in which all of the species exist as ions; DMEAP, on the other hand, is a hydrophilic protic ionic liquid, in which there is equilibrium between the ionic species and the neutral acid and base, where the ionic liquid is heavily favored. The differing characteristics of these ionic liquids serve as a probe for rationalizing the solvation processes.

The key question that we ask in this communication is, why does artemisinin solvation in ILs strongly depend on water content? To address this, the dependence of solvation free energy ΔG on water content in the ILs should be measured. To this end, artemisinin has been dissolved into IL/water mixtures of

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TABLE 1: Solubility of Artemisinin (mg) Per 1 mL of Ionic Liquid with a Specified Water Content at 298 K

ionic liquid	mol fraction of water in the solvent	solubility mg mL^{-1} solvent
DMEAP	0.020	47 ± 2
	0.17	27 ± 2
	0.29	21 ± 2
	0.39	7.9 ± 0.2
	0.47	5.2 ± 0.1
$CNTf_2$	0.053	86 ± 3
	0.17	10 ± 5
	0.26	9.3 ± 2
	0.34	8.5 ± 1
	0.40	6.2 ± 0.4

various compositions (Karl Fischer titration was employed to determine the precise water content of each sample).

Artemisinin was dissolved into 1 mL samples of the IL/water mixtures, which were sonicated first for 20 min, followed by standing for 1 h and subsequent filtration, before the dissolved artemisinin content was determined by HPLC.[7] Each measurement was repeated five times for error assessment. The resultant solubility data are summarized in Table 1. The water activity (a_w) measurements, carried out using a Rotronics relative humidity meter,[8] show the hydrophobic nature of $CNTf_2$ due to its higher-than-ideal water activity (see Figure 3), whereas DMEAP exhibits its hydrophilic characteristics through its lower-than-ideal water activity. This can be broadly interpreted as the DMEAP−water interactions being stronger, therefore reducing the humidity measurements above the DMEAP−water solution, in comparison to weaker $CNTf_2$−water interactions resulting in higher humidity measurements above the $CNTf_2$−water solutions. The two ILs employed in this communication thus exhibit opposite solvation environments in terms of their hydrophobicity. This can be anticipated due to the differences in anion structure, that is, the hydrogen bonding ability and short alkyl chain of DMEAP's propanoate and the highly fluoronated, charge-delocalized "soft" bis(trifluoromethylsulfonyl)imide of $CNTf_2$.

From the artemisinin solubilities (converted to mol fraction x), the solvation free energies of artemisinin in the ILs are calculated according to the well-established procedure[9]

$$\Delta G = -RT \ln x \tag{1}$$

where R is the gas constant ($R = 8.314$ J K^{-1} mol^{-1}) and T is the temperature. The free energies thus calculated are shown in Figure 4, which have been plotted against $-RT \ln a_w$ because

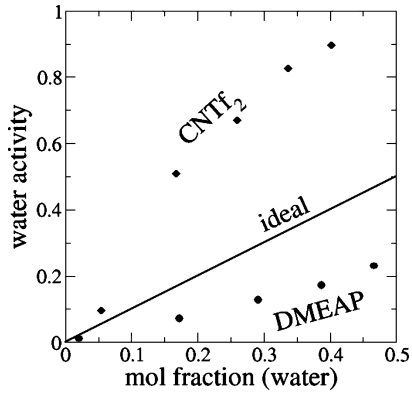

Figure 3. Water activity measurements of DMEAP and $CNTf_2$ with varying water contents.

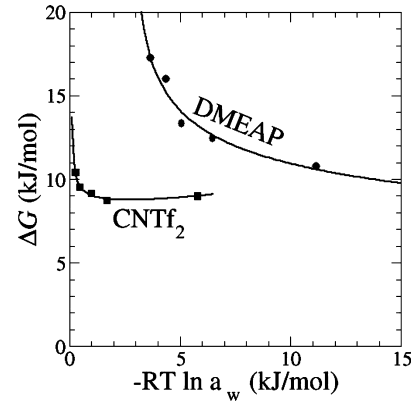

Figure 4. Solvation free energies of artemisinin in DMEAP and $CNTf_2$ with varying water contents.

TABLE 2: Equations Used to Fit the Water Activity Dependence ($\mu = RT \ln a_w$) of the Solvation Gibbs Free Energy (ΔG)[a]

ionic liquid	fitting equation
DMEAP	$\Delta G = a(-\mu - b)^c$
	$(\Gamma = ac(-\mu - b)^{c-1})$
	$a = 0.8196$, $b = 0.2370$, $c = -0.2152$
	$R^2 = 0.958$
$CNTf_2$	$\Delta G = a - b/\mu + c\mu^2$
	$(\Gamma = b\mu^{-2} - 2c\mu)$
	$a = 0.7116$, $b = 0.003651$, $c = 0.1503$
	$R^2 = 0.982$

[a] The preferential hydration parameter (Γ) is calculated by differentiating the fitting equation via eq 2.

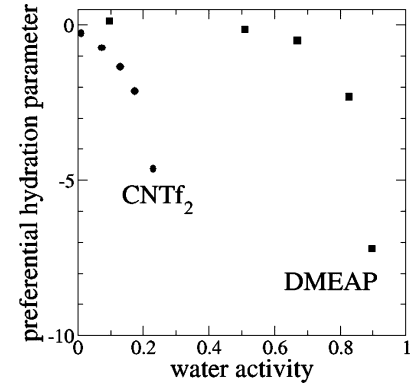

Figure 5. The preferential hydration parameters of artemisinin in DMEAP and $CNTf_2$ as a function of water activity.

the preferential hydration parameter Γ (the number of excess water molecules hydrating artemisinin relative to those of the solvating ionic liquid) can be calculated directly from this plot

$$\Gamma = -\mathrm{d}\Delta G/\mathrm{d}[RT \ln a_w] \tag{2}$$

It is then necessary to calculate the slope for each set of experimental solvation free energies in Figure 4.[10,11] To this end, nonlinear regression has been performed for the $-\mu$ ($= -RT \ln a_w$) dependence of ΔG, so that Γ could be calculated via differentiation. The fitting equations used and the resultant parameters are summarized in Table 2.

The preferential hydration parameters thus obtained show a strong dependence on water activity, as shown in Figure 5. The most striking feature common in both ionic liquids is that the more water there is in the solvent, the more negative the

preferential hydration parameter becomes. This means, at a molecular level, water molecules become preferentially excluded from the solvation shell of the artemisinin molecule as the water content in the ionic liquid is increased. The unfavorable interaction between water and artemisinin is itself hardly surprising because of its hydrophobic nature (its solubility in water is extremely low, 0.5 mg mL^{-1}).[3] However, the difference in the preferential dehydration between the two ionic liquids is striking; artemisinin is more preferentially dehydrated in the hydrophilic DMEAP than the hydrophobic $CNTf_2$.

The above observation seems counterintuitive if one attempts, in the following manner, to understand the preferential dehydration phenomena from a solute−solvent interaction perspective. It could well be expected that both the favorable interaction between the ILs and artemisinin and the unfavorable water−artemisinin interaction would contribute to preferential dehydration (as Γ signifies the relative abundance of excess water hydration over excess IL solvation of artemisinin).[10,11] Thus, comparing $CNTf_2$ and DMEAP, the hydrophobic artemisinin would be expected to interact more favorably with the hydrophobic $CNTf_2$ in the presence of water, in comparison to the hydrophilic DMEAP, thus leading to stronger preferential dehydration of the hydrophobic $CNTf_2$. However, the experimental data shown in Figure 5 exhibit the contrary trend, with DMEAP showing greater preferential dehydration. How, then, can we resolve the apparently paradoxical result posed above? The key lies in the molecular-level examination of the preferential hydration parameter; this is interpreted through the exchange of solvent molecules between the bulk phase and the solvation shell,[12,13] which indicates that the interaction between water and the bulk solvent phase must be considered to resolve the paradox. The water activity measurements in Figure 3 have shown that water molecules interact more strongly with bulk DMEAP than with bulk $CNTf_2$. The stronger water−bulk interaction for DMEAP (i.e., the hydrophilicity of DMEAP) makes it less favorable for water to leave the bulk environment to hydrate the artemisinin. This preference for water to reside in the bulk DMEAP in comparison to $CNTf_2$ is the scenario that rationalizes the stronger preferential dehydration in DMEAP compared to that in $CNTf_2$.

To summarize, the combination of preferential dehydration data and the solubility experiments has shown that it is the water−bulk solvent interactions which determine the preferential dehydration rather than direct IL or water interactions with artemisinin.

Caution must be taken, however, when one wishes to interpret the preferential hydration parameter when considering an actual extraction process; this caution arises due to the routine use of the volume/volume (v/v) scale (cf. water activity). Figure 6 shows the dependence of the preferential hydration parameter on water concentration (v/v %), in contrast with the water-activity-based plot in Figure 5. Using the v/v scale, $CNTf_2$ apparently exhibits more rapid dehydration than DMEAP, which implies an apparent reversal of the results obtained from Figure 5 and discussed above. With the v/v concentration scale (Figure 6), the onset of preferential dehydration is apparently shifted to higher water concentrations simply due to the low water activity of DMEAP itself. Thus the v/v concentration scale implicitly incorporates an additional (hidden) factor, that is, the water activity per unit quantity of water, which complicates analysis and leads to erroneous interpretation of the molecular-level rational for the process. It is for this reason that the water activity scale should be used when interpreting thermodynamic data at a molecular scale instead of the v/v scale, despite its widespread use in chemical engineering.

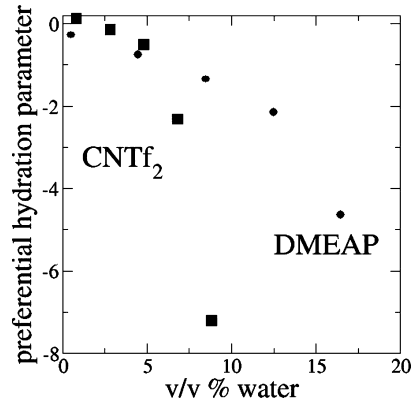

Figure 6. Preferential hydration parameters of artemisinin in DMEAP and $CNTf_2$ as a function of the v/v water/IL ratio.

In conclusion, we report evidence for increasing preferential dehydration of artemisinin in a hydrophobic IL and a hydrophilic IL ($CNTf_2$ and DMEAP, respectively) with increasing water content. The hydrophilicities of the ILs, and not the interactions with artemisinin itself, are shown to drive the preferential dehydration (and precipitation) of artemisinin via more-or-less favorable interactions between water and the bulk ILs. We believe that such quantitative characterization of solvation processes not only significantly contributes to our understanding of solvation thermodynamics and its mechanisms in the ILs but will additionally contribute to the rational design of the extraction process engineering of such important natural products with this class of solvents.

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References and Notes

(1) See: *Acc. Chem. Res.* **2007**, *40* (11) and all papers therein.
(2) (a) Seth, D; Sarkar, S.; Pramanik, R.; Ghatak, C.; Setua, P.; Sarkar, N. *J. Phys. Chem. B* **2009**, *113*, 6826. (b) Wakeham, D.; Hayes, R.; Warr, G. G.; Atkin, R. *J. Phys. Chem. B* **2009**, *113*, 5961. (c) Kobrak, M. N. *J. Chem. Phys.* **2007**, *127*, 099903.
(3) Bioniqs Ltd. *Further Devlopment of Artemisinin Extraction Methods Using Ionic Liquids*; Medicines for Malaria Venture: Geneva, Switzerland, 2008.
(4) Lapkin, A. A.; Plucinski, P. K.; Cutler, M. *J. Nat. Prod.* **2006**, *69*, 1653.
(5) *Toxicological Profile for n-Hexane*; U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry: Atlanta, GA, 1999.
(6) Greaves, T. L.; Drummond, C. J. *Chem. Rev.* **2008**, *108*, 206.
(7) Lapkin, A. A.; Walker, A.; Sullivan, N.; Khambay, B.; Mlambo, B.; Chemat, S. *J. Pharm. Biomed. Anal.* **2009**, *49*, 908.
(8) Hallworth, J. E.; Nomura, Y. *Biotechnol. Bioeng.* **1999**, *62*, 242.
(9) Nozaki, Y.; Tanford, C. *J. Biol. Chem.* **1971**, *246*, 2211.
(10) Timasheff, S. N. *Biochemistry* **2002**, *41*, 13473.
(11) (a) Shimizu, S. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *97*, 1195. (b) Shimizu, S.; McLaren, W. M.; Matubayasi, N. *J. Chem. Phys.* **2006**, *124*, 234905.
(12) (a) Schellman, A. *Biophys. J.* **2003**, *85*, 108. (b) Schurr, J. M.; Rangel, D. P.; Aragon, S. R. *Biophys. J.* **2005**, *89*, 2258.
(13) Record, M. T.; Anderson, C. T. *Biophys. J.* **1995**, *68*, 786.

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