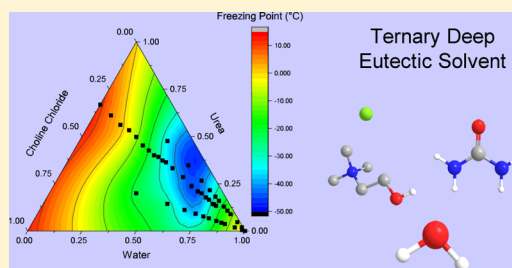


Ternary Deep Eutectic Solvent Behavior of Water and Urea–Choline Chloride Mixtures

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Supporting Information

ABSTRACT: Deep eutectic solvents (DESs) have potential as designer media whose physical, chemical, electrochemical, and spectroscopic properties are easily and broadly tuned. Due to the ubiquity of water and the hygroscopic nature of many DES components, it is important to understand how water affects DESs both on the nanoscale and in the bulk. Here, we present a study of the physical properties of DES/water mixtures. We found that water appears to form a ternary DES when mixed with 2:1 urea/ChCl.



INTRODUCTION

Deep eutectic solvents (DESs) have gained popularity as highly tunable environments for extractions, synthesis, and energy storage, among other uses.^{1–6} Even within the breadth of reported applications, many varieties of solvents remain unexplored; it has been proposed that there are more than 10⁶ unique combinations of molecules and salts that could produce a DES.^{2,7} This estimate prompts great promise for developing new DESs, as the material properties of these multicomponent ionic melts are easily tuned using molecular principles, and functionality can be built into the solvent itself. In other words, designing a DES provides the opportunity to design a solvent with viscosity, conductivity, and electrochemical and optical properties that are most favorable for a particular purpose.^{6,8–10}

Hydrogen bonding is likely a defining characteristic of metal-free DESs, at least for those designated type III (hydrogen bond donor mixed with an organic salt).^{2,11} While there is still debate about the boundaries of what should and should not be classified as a DES and about the role of hydrogen bonding in defining the resulting properties, most examples of metal-free DESs appear to exhibit hydrogen bonds.^{2,12} Molecular dynamics and other computational studies have elucidated the nanostructure of various DESs and DES/water mixtures and suggested mechanisms for the contributions of hydrogen bonding to the depressed freezing points.^{11,13,14} Stefanovic et al. showed that the freezing point depression differences between amide-based DESs such as 2:1 urea/choline chloride (urea/ChCl) and hydroxyl-based DESs like 2:1 ethylene glycol/choline chloride (EG/ChCl) are due to stronger interactions between the amide-based hydrogen bond donor (HBD) and the salt,¹¹ whereas Abbott has shown strong correlation between the molecular weight of the HBD and the freezing point depression, seemingly irrespective of (or indeed

perhaps dictating) the effective donor–acceptor interaction strength within a given pair of donor–acceptor moieties.¹⁵

In addition to moving toward a unifying definition for DESs, the community is also working to understand how DESs interact with other solvents. Abbott has shown that nonpolar solvents are typically immiscible with choline chloride-based DESs, and Maugeri has shown that while acetone is immiscible with choline chloride-based DESs, it can be used to recover the components by causing the choline chloride to precipitate.^{16,17} Of particular interest is the interaction between DESs and water. Many DESs comprise hygroscopic compounds like choline chloride that are practically challenging to dry completely. Though many authors show the water content to be low by NMR methods, it is likely that most “pure” DESs will, in fact, be mixed with some water.^{17–20} It is thus important for the community to develop a basic understanding of how DESs interact with varying quantities of water, especially for the most commonly used DES compositions. Several groups have studied the nanostructure of DES/water mixtures to develop our understanding of how water interacts with the hydrogen bond networks within DESs. They have shown that low quantities of water strengthen the hydrogen bonding within the DES, but that as the concentration of water increases, the water then weakens the interactions between each DES unit, and the DES components begin exhibiting behavior more typical of solutes in aqueous solution.^{14,19,21} In this work, we studied the physical properties of DES/water mixtures to provide context for the developing nanoscale understanding of the structure of DESs.

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METHODS

Choline chloride was dried to a constant mass by treatment in a vacuum oven at 50 °C for at least 8 h prior to use. It was weighed out into scintillation vials (for freezing point measurement) or water activity sample cups and then dried in a vacuum oven at 50 °C for 2 h. Urea was added, and the samples were returned to the vacuum oven, where they were heated to 50 °C for a minimum of 6 h.

Water Activity. Water activity was measured with an AquaLab 4TEV water activity meter in capacitance mode. All water activity measurements were made at 25 °C. “Dry” samples were removed from the drying oven and immediately stirred in a desiccator containing Dri-Rite until they cooled down to room temperature to minimize the uptake of atmospheric water before being placed directly into the water activity meter. After each water addition, samples were vigorously stirred for 1–2 min until a visually homogeneous solution was obtained.

Freezing Point. Freezing points were measured by immersing samples in a dry ice/acetone bath and monitoring the sample temperature with an infrared (IR) thermometer (HoldPeak, HP-981A, ± 2.0 °C as per manufacturer’s documentation). The technique was verified by measuring the freezing points of water (0 °C), dry 2:1 urea/ChCl (12 °C), and ethylene glycol (−13 °C).²² Equivalent results were obtained by inserting an alcohol thermometer and continuously monitoring the temperature during freezing. The IR temperature sensor was chosen because it measures temperature more quickly than the alcohol thermometer, thus reducing the exposure of the samples to outside contamination, particularly the absorption of water from the air. On freezing, the samples exhibited a rapid (~ 1 – 2 s) transition from clear to opaque. The freezing point was taken to be the temperature measured at this transition.

Freezing points of concentrated DES samples were measured for three independently prepared series of samples. After mixing and drying the DES samples in scintillation vials as described above, water was added to each sample to bring the mole fraction of water to the desired concentration. The vials were closed except when actively measuring the temperature with the IR thermometer. Freezing points for dilute (<3 molal, the maximum concentration for the instrument) aqueous samples of 2:1 urea/ChCl, urea, and choline chloride were measured using a Gonotec Osmomat 3000 osmometer. The dilute samples were prepared by weighing the needed mass of urea and choline chloride into a scintillation vial and adding the volume of water needed to reach the desired concentration. One set of dilute samples was prepared, and three aliquots of each concentration were measured in the osmometer.

RESULTS AND DISCUSSION

Understanding water activity in DESs is critical for their use in any biological context; below a water activity of ~ 0.6 , most microbial activity ceases.²³ More broadly, it has been shown that even dry DES samples contain a nonzero quantity of water.^{20,24} To complement recently published computational and neutron diffraction studies, we measured water activity in samples of 2:1 by mol urea/choline chloride because this parameter held the potential to show the point where the DES components transitioned from coordinating together as a solvent to solutes dissolved in water. Water activity has seen

extensive use in the food industry because it can be used to control the microbial activity and many of the textural properties of food.²⁵ The shape of water sorption isotherms has been linked to the structure of the substrate, and water activity has been used to determine how tightly bound the water is to the substrate.^{25,26} Urea has been shown to disrupt the structure of water strongly by reorienting water molecules so they do not form their standard solvation shells.²⁷ Additionally, water activity has been shown to affect the structure of DNA. Based on this, we aimed to use water activity measurements to gain insight into the structure of the 2:1 urea/ChCl/water mixtures.²⁸ The 2:1 urea/ChCl DES was selected due to its notoriety as an archetypical DES and the large volume of literature describing the solvent.

Overall, the water activity increases nonlinearly as water is added (Figure 1A); however, the water activity response is

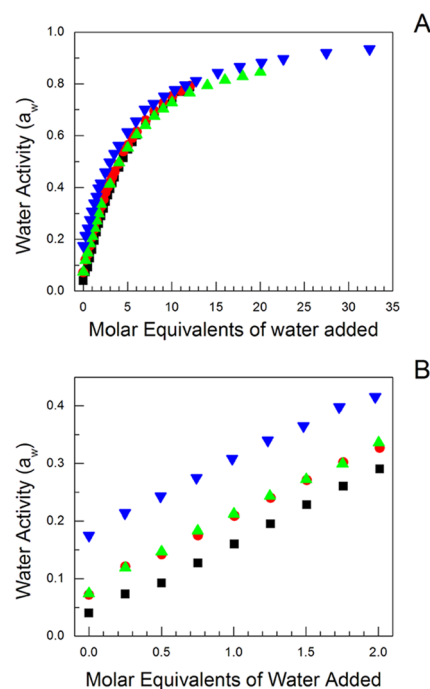


Figure 1. Water activity of 2:1 urea/ChCl vs equivalents of water per mole of choline chloride: (A) all additions and (B) linear region. Different symbols represent replicate measurements made with independently prepared samples.

quite linear as the first 2 equiv of water are added (Figure 1B). Four replicate measurements of the linear region had an average slope of 0.125 ± 0.002 for a linear regression model ($R^2 > 0.996$ for all trials) with varying initial values. Notably, the intercept of the linear equation for each replicate is likely the initial water activity with no water added. This is noteworthy for two reasons: the intercept is not expected to be the same, as different samples prepared have different water contents, and it leads to a straightforward methodology for the measurement of initial water content and changes in water activity over the course of a given experiment.

The initial water activity of each trial (obtained from the intercept) was converted into moles of water using the average slope of the linear region, and the apparent mole fraction of water in each sample at each water addition was calculated. The resulting water activity vs apparent mole fraction of water curve (Figure 2) was fit to a sigmoidal equation (eq 1) that

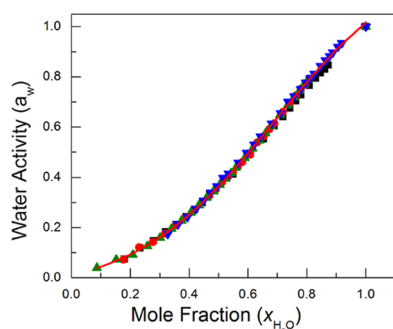


Figure 2. Water activity of 2:1 urea/ChCl vs corrected mole fraction of water. Cyan circles, black squares, green triangles, and blue stars are experimental measurements; the red line is the sigmoidal fit of the experimental data.

allows correlation of the water activity with the apparent mole fraction of water. Conversely, the apparent mole fraction of water in a sample of 2:1 urea/ChCl can be measured by water activity.⁴ Statistical analysis and fitting parameters for the nonlinear regression are provided in the [Supporting Information](#)

$$a_w = \frac{A_1 - A_2}{1 + e^{x-x_0/dx}} + A_1 \quad (1)$$

It has been reported that the shape of water sorption isotherms can be related to the structure of the substrate. For example, sigmoidal curves like those seen in [Figure 2](#) are indicative of a heterogeneous complex food.^{25,26} It is likely that the sigmoidal relationship we see between water activity and apparent water content is analogously showing that the 2:1 urea/ChCl DES is not composed of discrete homogeneous DES units of two ureas and one choline chloride but rather a variety of DES structures formed that have a net ratio of two ureas to one choline chloride. This is supported by the computational and neutron diffraction experiments that show the highly complex nature of DES structures.¹⁴

Understanding how water activity changes as water is added to 2:1 urea/ChCl is key for researchers looking to encourage or retard biological growth in addition to studies of proton-coupled electron transfer, solvation, or any other water-coupled or water-dependent process. Here, we demonstrate a method for determining how much added water will lead to a desired water activity for the 2:1 urea/ChCl DES. It should be noted that [eq 1](#) was empirically derived, and in the absence of further theoretical work, all constants must be empirically determined for each solvent environment. Additionally, we have shown that water activity measurement is a viable, nondestructive method for determining the apparent water content by mass or mole fraction in a DES. Water activity measurement merely requires the measurement of the vapor pressure of water above a sample, and with further analytical validation, this method could easily be integrated into manufacturing processes.

We now address the physicochemical properties of this DES/water mixture. Samples of 2:1 urea/ChCl were mixed with water to produce ternary solvents with ratios varying from 2:1:0.25 urea/ChCl/H₂O to 2:1:35 urea/ChCl/H₂O. This yielded mixtures where the mole fraction of water varied from 0.08 to 0.98. For the reader's convenience, we have included in the [Supporting Information](#) a table showing conversions between mole fraction, wt %, equivalents of water, and

molality. The freezing points of these mixtures decrease as water is added until a minimum is reached when 6 equiv of water (0.67 mol fraction) has been added ([Figure 3](#)). At that

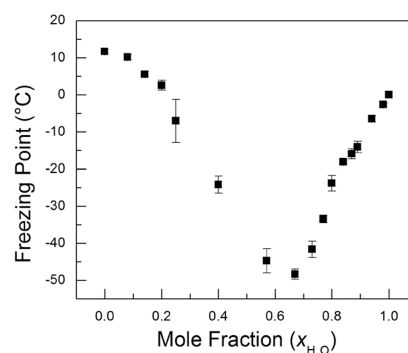


Figure 3. Freezing point of 2:1 urea/ChCl DES as a function of the mole fraction of water added.

ratio, 0.22:0.11:0.67 urea/ChCl/H₂O, the freezing point of the mixture is -48 ± 2 °C. With fractions of water greater than 0.67, the freezing point begins to increase. The mixture then approaches the limit of infinitely dilute urea and choline chloride in water. For reference, the freezing point of 2:1 urea/ChCl is reported to be 12 °C.¹⁷

Meng et al. examined the melting point of 2:1 urea/ChCl mixed with up to 10 wt % water and found a melting point depression trend similar to the trend we report for freezing points across a broader range of compositions ([Figure 4](#)).²⁰

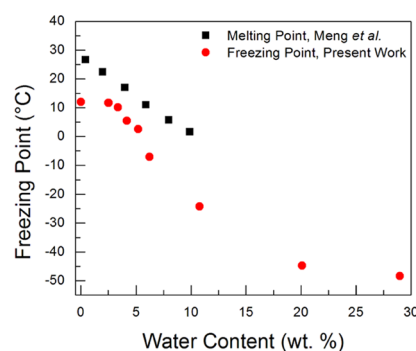


Figure 4. Melting point of 2:1 urea/ChCl measured by differential scanning calorimetry from Meng et al. and freezing points measured in the present work.²⁰

These authors point out that melting points have the advantage of avoiding any potential effects of supercooling.^{20,29} While this is an important point worthy of further study specific to the thermal properties of DESs, here we elect to move forward with an analysis of freezing points to facilitate discussion within the framework used by the majority of researchers working with these solvents.

Focusing in on the right side of [Figure 3](#), we see that from the freezing point minimum at $x_{H_2O} = 0.67$ – 0.98 , the freezing point increases linearly. This linear trend is similar to the freezing point depression of an ideal nondissociating solute in water ([Figure 5](#)). While this similarity could simply be a matter of scaling, all of the points to the right of $x_{H_2O} = 0.8$ deviate from the ideal line by a maximum of 2.6 °C, very close to the ± 2 °C accuracy of the IR thermometer used to collect these data. While we cannot provide conclusions without further

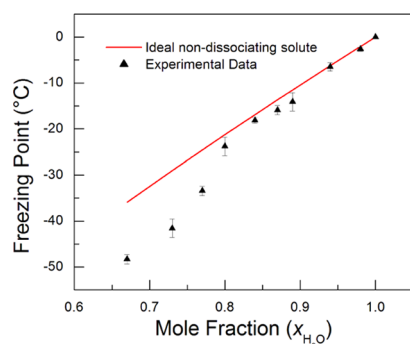


Figure 5. Freezing point of 2:1 urea/ChCl compared with the freezing point depression of an ideal nondissociating solute in water.

examination of the effects of urea and choline chloride individually, we believe this could indicate continued coordination between the urea and choline chloride even at relatively dilute conditions. In other words, at these concentrations, the DES components may behave as a weighted average single unit when solvated by water.

In addition to measuring the freezing point of 2:1 urea/ChCl/water mixtures, we tested two other ratios of urea/ChCl (Figure 6). Notably, the noneutectic ratios of urea/ChCl did

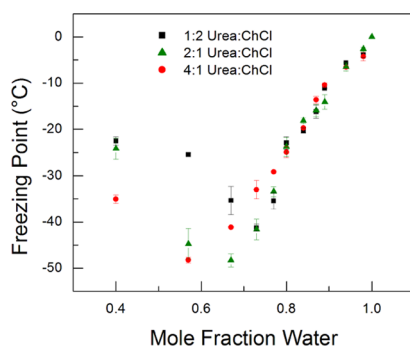


Figure 6. Freezing point of three ratios of urea/ChCl mixed with water.

not freeze cleanly with low concentrations of water. Instead, there was crystallization of one or more of the DES components when the samples were less than $0.4x_{\text{H}_2\text{O}}$. While the 4:1 urea/ChCl mixture has the same freezing minimum as the 2:1 eutectic ratio, we hesitate to call the mixture of 4:1 urea/ChCl/water the ternary eutectic ratio because of the anomalous precipitation at lower hydration levels.

CONCLUSIONS

Expanding our understanding of how DESs interact with other solvents is critical if DESs are to be used as substrates and solvents for studying other systems. Our research shows that this mixture of 6:1 water/[2:1 urea/ChCl] (i.e., $0.67 \text{ H}_2\text{O}/0.22 \text{ urea}/0.11 \text{ ChCl}$) is itself a deep eutectic solvent. From the perspective of water mixing with the DES as a unit, at the eutectic point, this mixture could be considered to be a binary mixture of 6:1 water/[2:1 urea/ChCl]. However, considering published reports of neutron diffraction studies and computational evidence for water's strong interactions with all components of the solvent, we propose that this DES/water mixture is better characterized as a ternary DES with a eutectic ratio of 6:2:1 water/urea/ChCl.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpcb.8b12322.

Water activity measurements during standard addition of water to 2:1 urea/ChCl (Figure S1); conversion of units commonly used in this publication and in the literature; molality of DES components assuming water is the solvent (Table S1); average freezing point measurements (Table S2) (PDF)

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

ChCl, choline chloride; DES(s), deep eutectic solvent(s); EG, ethylene glycol; HBD, hydrogen bond donor

ADDITIONAL NOTE

^aWhile our data show that water activity can be used to measure the water content of a DES, we note that a standard curve for the addition of water is required for each sample to account for the initial water content of the sample and the relative humidity. Otherwise, the time scale of the water activity measurement must be extended to 5–8 h.

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