

# In Competition for Water: Hydrated Choline Chloride:Urea vs Choline Acetate:Urea Deep Eutectic Solvents

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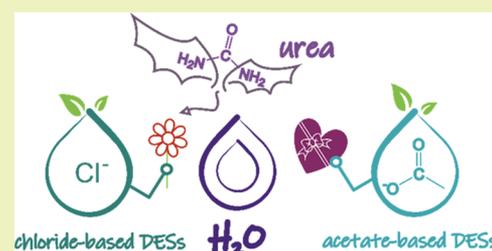
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Supporting Information

**ABSTRACT:** Unlike the archetypal deep eutectic solvent (DES) choline chloride:urea (ChCl:U), fundamental knowledge of the intermolecular network in choline acetate (ChOAc) DESs and how they change upon dilution is still missing. Here we jointly use UV resonance Raman (UVR) and NMR spectroscopy to comparatively explore how the strength and distribution of hydrogen bonding and the solvation of the components are modified in ChOAc:U and ChCl:U with increasing hydration. Overall, Raman and NMR data indicate that ChOAc:U is continuously affected by hydration and, even at low water concentrations, undergoes a breakage of DES–DES interactions, with rapid solvation of the urea portion and full exchange of mobile protons. On the contrary, ChCl:U seems to maintain its structure as small interplays gradually occur between urea in the DES and the surrounding water molecules. The combined approach provides a multifaceted consistent description of the systems, outlining the crucial role of the anion in driving the structure and dynamics of the materials and then yielding valuable data toward the exploitation of DESs as tunable systems.

**KEYWORDS:** deep eutectic systems, NADES, UV resonance Raman, nuclear magnetic resonance, hydrogen bond, solvation, structure, dynamics



## INTRODUCTION

Since the inaugural work by Abbott et al.,<sup>1</sup> the mixture of choline chloride and urea at a 1:2 mol ratio (ChCl:U, also known as reline) has gained a lot of attention, as witnessed by the 50-fold increase in the number of publications from 2003 to 2020.<sup>2</sup> ChCl:U is considered as the prototype of type III deep eutectic systems (DESs), a novel class of liquid materials obtained by mixing an ammonium, phosphonium, or sulfonium salt with a small organic molecule (i.e., amide, acid, amine, or alcohol) acting as the hydrogen bond donor (HBD).<sup>3</sup> Strong hydrogen bonding interactions between the species are believed to cause charge delocalization, which is responsible for the “deep” melting point depression of the mixture with respect to both the individual components and the ideal mixture.<sup>4,5</sup> The match between desirable physicochemical properties (good thermal and chemical stability, low flammability, and exceptional dissolution capabilities, among others) and environmental and economic advantages (ease of preparation from mostly largely available and environmentally friendly and safe raw materials, leading to low cost and high sustainability), makes these systems extremely appealing for a wide range of applications.<sup>3,4,6–8</sup>

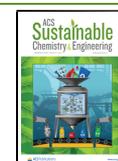
A strong—yet underexploited—feature of DESs is their potential as tunable solvents, due to the large number of possible combinations of constituents, leading to an estimated library of  $10^6$ – $10^8$  binary systems.<sup>4</sup> Moreover, multi-

component mixtures are particularly attractive, as the addition of a third (or even more) component may result in a positive impact on DESs' properties. Water, for instance, can reduce viscosity, while increasing polarity and conductivity,<sup>9–11</sup> thus being beneficial in processes such as electrodeposition<sup>12,13</sup> or solubilization and extraction.<sup>14–16</sup> However, a prerequisite to the practical exploitation of DESs' tunability is the development of a proper fundamental understanding of their structure, interactions, and dynamics at the molecular level. As DESs are hardly water-free,<sup>17</sup> a primary goal is also to obtain an understanding of the effect of progressive addition of water on DES's network. Indeed, although the presence of controlled amounts of water does not appear to disrupt the peculiar intermolecular connections,<sup>18,19</sup> the residual or intentionally added water has to be considered, as it will markedly affect the DES structure. Acting as an additional H-bond donor and acceptor, water participates in the intermolecular network and progressively weakens the intermolecular interactions between

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all ionic and neutral species until all DES components are fully solvated at high dilutions.<sup>11</sup>

An increasing body of work indicates that in ChCl-based DESs the chloride anion plays a major role in defining the structure and H-bond network of both neat and hydrated systems.<sup>19–31</sup> Moreover, the chloride significantly contributes to determining many important physicochemical properties of DESs, including Urbach and band gap energies,<sup>32,33</sup> and is the active ingredient when DESs are used as solubilizing agents, for instance in biomass delignification<sup>34</sup> or  $\beta$ -CD solvation.<sup>35</sup> In the framework of DESs as tunable systems, given the crucial role of the anion, investigating whether and how its replacement affects the DES nanostructure is of high relevance. Besides chloride, acetate (OAc) has emerged as an alternative anion in choline-based DESs, mainly based on the higher safety of its cholinium salt (choline acetate, ChOAc, an ionic liquid (IL) with a melting point of 51 °C<sup>36,37</sup>) with respect to ChCl.<sup>38,39</sup> Indeed, despite the claimed eco-friendliness and general safety of DESs, their toxicity profiles have to be scrutinized before any practical application. Toxicity studies concluded that both ChCl and ChOAc are “practically harmless”,<sup>38</sup> and all ChCl- and ChOAc-based DESs turned out to be even less toxic than their individual components.<sup>39</sup> However, comparing the two series, ChCl-based DESs were found to be more deleterious than ChOAc-based ones,<sup>39</sup> giving hence a boost to the design and application of OAc-based DESs. Unlike the parent ChCl-based DESs, a few mixtures of ChOAc and a HBD have been characterized,<sup>32,40</sup> and suggested as media for organic synthesis,<sup>41</sup> enzymatic reactions,<sup>42–47</sup> or biomass treatment.<sup>48</sup>

Although ChCl-based DESs, and in particular ChCl:U, are relatively well characterized in terms of structure and H-bond interactions, the network existing between ionic and neutral species in ChOAc-based DESs has scarcely been investigated in detail at the molecular level yet.<sup>49</sup> Moreover, unlike reline, no systematic study has been reported on the effect of progressive dilution on ChOAc-based DESs, to the best of our knowledge. As already seen for other DESs, the addition of a controlled amount of water may be beneficial in specific applications of ChOAc-based eutectics. It has been demonstrated for instance that variable amounts of water (up to 15 wt %) enhance the solubilization of hemicellulose from Kraft cellulose in DESs composed of ChOAc and levulinic acid, glycolic acid, or imidazole.<sup>48</sup> To fill these knowledge gaps we report here an in-depth study of ChOAc:U at a 1:2 mol ratio (also known as acetaline) under progressive dilution (up to 95 wt % of water) using a combination of UV resonance Raman (UVRR) and NMR spectroscopy. Structural and dynamic results are compared with those obtained under the same experimental conditions for the model system reline (ChCl:U 1:2), to single out the effect of the anion in the intermolecular network. The rationale behind this work is a comparative exploration of how the strength and distribution of intermolecular hydrogen bonding and the solvation of the components are modified in ChOAc- and ChCl-based DESs with increasing hydration. The combined UVRR and NMR analyses of the mixtures of DES and water provide a picture of the system from the atomic to the intermolecular scale and in a wide range of concentrations of water in DES.

## EXPERIMENTAL SECTION

The DESs ChCl:U and ChOAc:U (Figure 1) at a 1:2 molar ratio were prepared as previously described.<sup>29</sup> Seventeen samples were then

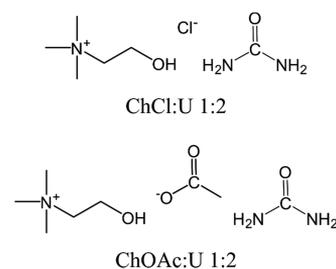


Figure 1. Structures of the two DESs studied in this work.

obtained diluting each DES with up to 95.2 wt % water (Table S1). More details on the sample preparation are given in the Supporting Information.

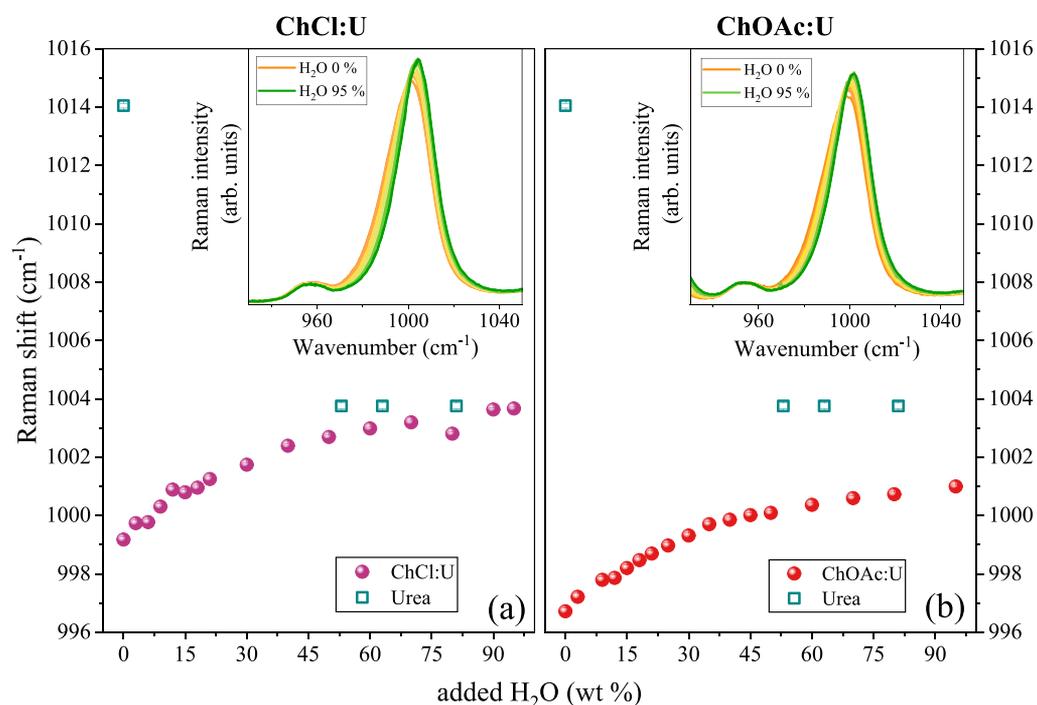
The samples for NMR analysis were placed in standard 5 mm tubes and multiple NMR experiments were performed at 305 K with a Bruker NEO 500 console (11.74 T) equipped with a direct observe BBFO (broadband including fluorine) iProbe and a variable-temperature unit (details are given in the Supporting Information).

UVRR experiments were carried out at the BL10.2-IUVS beamline of Elettra Sincrotrone Trieste (Italy) by exploiting the synchrotron-based UVRR setup.<sup>50,51</sup> The Raman spectra were collected using excitation wavelengths in the deep UV range at 226 and 266 nm. These excitation conditions allowed for the acquisition of suitable Raman signals of the DESs even at the higher dilution considered. Details on the employed Raman equipment and measurement conditions are reported in the Supporting Information. The analysis of the spectra obtained at the two excitation wavelengths for any given sample led to consistent results. The reported quantitative information specifically refers to the spectra with better features in terms of the spectral resolution and signal-to-noise ratio.

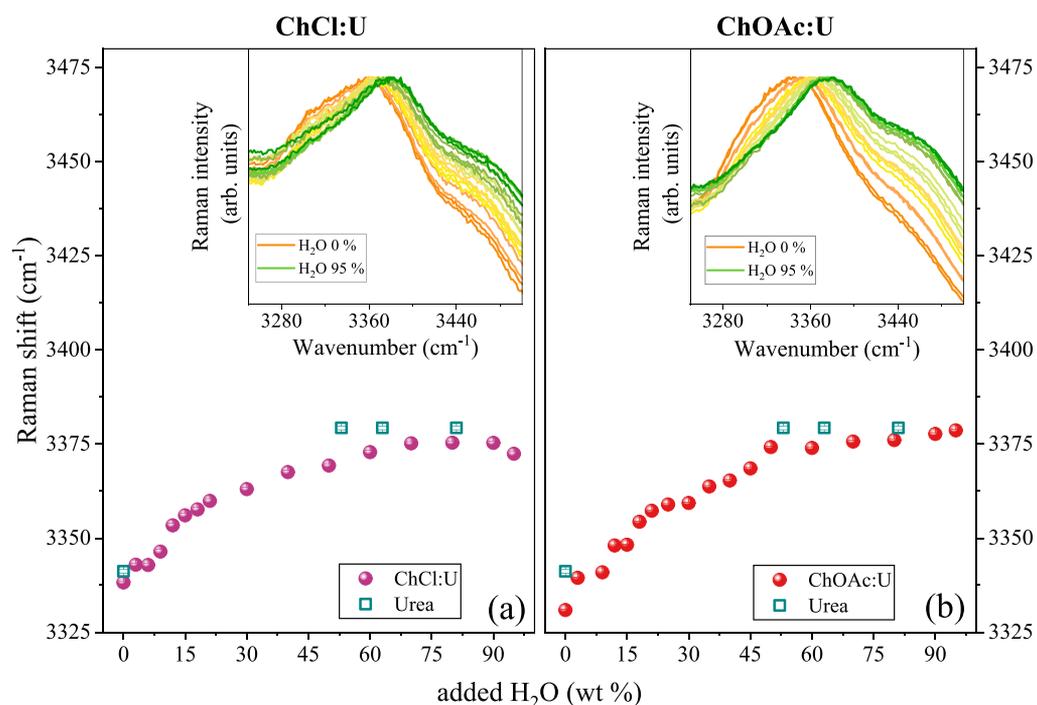
## RESULTS AND DISCUSSION

The systems, ChCl:U and ChOAc:U, which only differ in the anion, have been selected to evaluate to what extent the replacement of chloride with acetate affects the short- and long-range structure and mobility of the neat DES and its mixtures with water. The discussion will be structured as follows: (i) change in hydrogen bonding in the presence of increasing amounts of water as seen from the standpoint of UVRR and NMR, and (ii) dynamic behavior of DESs' components in the presence of increasing amounts of water.

**Change in the H-Bonding Network upon Hydration: The Urea Viewpoint via Raman Spectroscopy.** It is commonly accepted that H-bonds play a major role in defining DESs' organization. UVRR spectroscopy is a valid tool for tracking changes in H-bonding upon hydration in the DES/water mixtures, thanks to the marked increment of the detection limit owing to the resonance or pre-resonant enhancement and/or to the suppression of unwanted fluorescence contributions.<sup>52</sup> This allows the analysis of several characteristic Raman bands of DESs in the vibrational spectra of the mixtures with a relatively high water content, without significant reduction of the signal-to-noise ratio or interference from water signals. Figure S1a,b displays the UVRR spectra collected for ChCl:U/water and ChOAc:U/water mixtures in a wide spectral range, 800–3800  $\text{cm}^{-1}$ . Comparing the Raman profiles of DESs with those of the aqueous solutions of ChCl, ChOAc, and urea, enables recognizing the signals related to specific chemical moieties of the DES (Figure S1a,b). The prominent Raman band at  $\sim 998 \text{ cm}^{-1}$ , well visible in both ChCl:U and ChOAc:U UVRR spectra, can be assigned to the symmetric C–N stretching mode of urea.<sup>53–59</sup> This signal, labeled as  $\nu_s(\text{CN})$ , is located at about  $1010 \text{ cm}^{-1}$  for solid



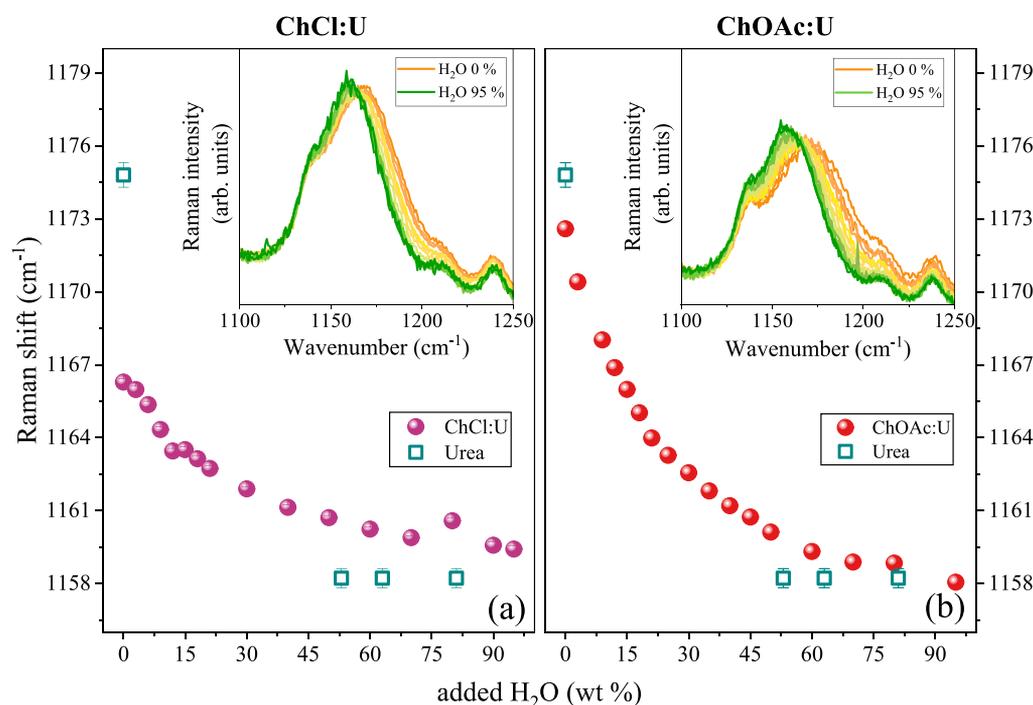
**Figure 2.** Wavenumber position of the Raman band  $\nu_s(\text{CN})$  at  $\sim 998 \text{ cm}^{-1}$  assigned to the CN stretching mode of urea for ChCl:U (a) and ChOAc:U (b) as a function of added water. The position of the band detected for solid urea and selected aqueous solutions is also reported. Insets: UVR spectra of ChCl:U and ChOAc:U at different water concentrations in the range of 940–1040  $\text{cm}^{-1}$  (the spectra have been arbitrarily normalized for better comparison).



**Figure 3.** Wavenumber position of the Raman band  $\nu(\text{NH}_2)$  at  $\sim 3340 \text{ cm}^{-1}$  assigned to the  $\text{NH}_2$  stretching mode of urea for ChCl:U (a) and ChOAc:U (b) DESs as a function of added water. The position of the band detected for solid urea and selected aqueous solutions is also reported. Insets: UVR spectra of ChCl:U and ChOAc:U as a function of water concentration in the spectral range of 3250–3450  $\text{cm}^{-1}$  (the spectra have been arbitrarily normalized for a better comparison).

urea<sup>56</sup> but it red-shifts down to  $\sim 1004 \text{ cm}^{-1}$  in water solutions (Figure 2), as also reported in previous studies.<sup>55,57</sup> The band further red-shifts to  $\sim 934 \text{ cm}^{-1}$  in the gas phase.<sup>56,58</sup> This situation can be explained considering the variations in the electronic density within the molecule in response to its

environment. In particular, urea is nonplanar in the gas phase, the C=O group exhibits a dominant double-bond character and the  $\text{NH}_2$  group a pyramidal conformation.<sup>58</sup> The formation of the  $\text{C}=\text{O}\cdots\text{H}$  hydrogen bond decreases the C=O bond order, increasing the double bond character of the



**Figure 4.** Wavenumber position of the Raman band  $\rho(\text{NH}_2)$  at  $\sim 1155 \text{ cm}^{-1}$  assigned to the  $\text{NH}_2$  rocking mode of urea for ChCl:U (a) and ChOAc:U (b) DESs as a function of added water. The position of the band observed for solid urea and selected aqueous solutions is also reported. Insets: Evolution of the UVR spectra of ChCl:U and ChOAc:U upon the increment of water in the spectral range of  $1100\text{--}1250 \text{ cm}^{-1}$  (the spectra have been arbitrarily normalized for a better comparison).

C–N groups and the planarity of the  $\text{NH}_2$  moiety. The intermolecular interactions are particularly effective in stabilizing the planar conformation of urea in the crystal, as reflected by the large frequency value of the  $\nu_s(\text{CN})$  signal ( $1010 \text{ cm}^{-1}$ ).<sup>59</sup> The planar conformation is expected to prevail also in the aqueous environment, a  $\nu_s(\text{CN})$  signal at  $\sim 1004 \text{ cm}^{-1}$ , owing to the formation of urea–water hydrogen bonds ( $\text{C}=\text{O}\cdots\text{HOH}$ ).<sup>59</sup> In ChCl:U, a further reduction of the strength of  $\text{C}=\text{O}\cdots\text{H}$  interactions, mostly related to urea–urea H-bonding in the neat DES, is reflected by the additional red-shift of the  $\nu_s(\text{CN})$  signal ( $999 \text{ cm}^{-1}$ ), yet urea remains mostly planar also in the DES system.<sup>58</sup> This is also the case of ChOAc:U, even though the observed small red-shift to  $997 \text{ cm}^{-1}$  (Figure 2) suggests that substituting the chloride with the acetate might cause an additional weakening of the H-bonds formed by the urea C=O group as H-acceptor.

Figure 2 shows the evolution of the wavenumber position of the  $\nu_s(\text{CN})$  band observed for ChCl:U and ChOAc:U as a result of the addition of water. A clear blue-shift with dilution is detected for both DESs (see insets in Figure 2) up to about 40–50 wt % water. However, upon further addition of water, the wavenumber position of the  $\nu_s(\text{CN})$  signal becomes almost concentration-independent. The plots indicate that in hydrated DESs, water molecules tend to form stronger H-bonds with the urea C=O group than those formed in the pure DESs. Moreover, the position of the  $\nu_s(\text{CN})$  signal becomes similar to that of diluted urea/water mixtures for water concentrations larger than 40–50 wt % (Figure 2), suggesting that the full hydration of the urea C=O groups might be attained under these conditions. Both ChCl:U and ChOAc:U behave similarly (Figure 2a,b), even though the urea C=O groups of ChOAc:U seem to be involved in weaker interactions compared to ChCl:U, in the whole concentration range. In particular, the high hydration range (added water wt % > 50)

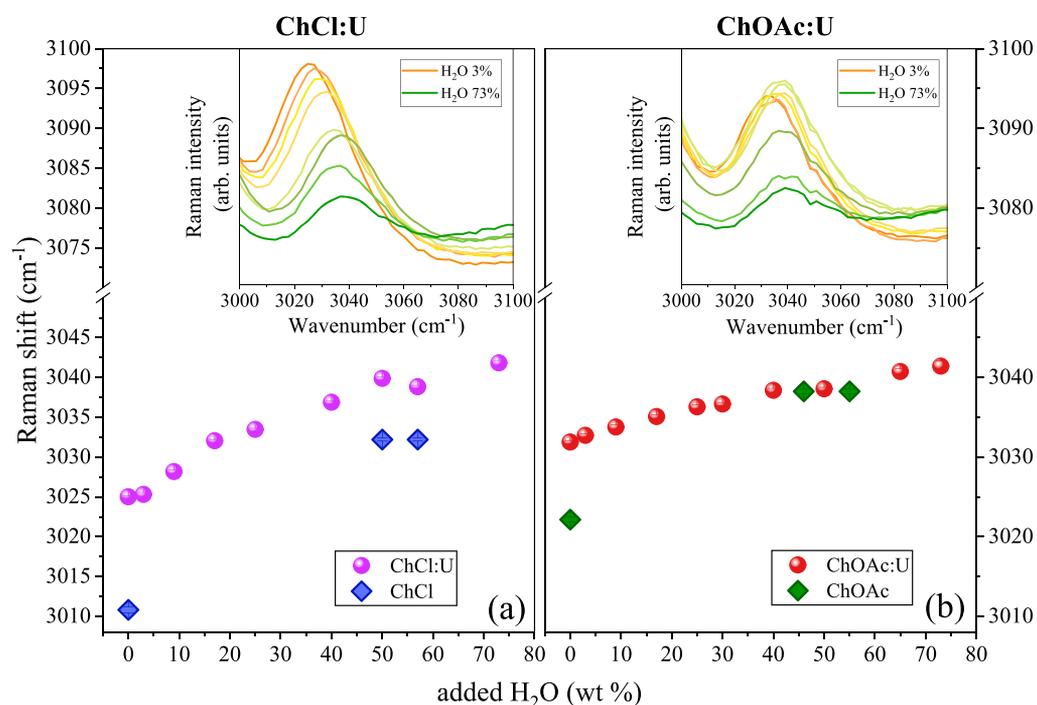
clearly shows the same Raman response of urea in ChCl:U and in the urea/water solutions, while a residual difference is present in the case of ChOAc:U vs U aqueous solutions after the addition of large amounts of water (compare bullets and squares in Figure 2a,b at ca. 50, 60, and 80 added H<sub>2</sub>O wt %). The presence of acetate, as a strong H-acceptor competitor of urea might play a relevant role in explaining the differences.

Further insights into the rearrangement of the DESs' H-bonding network upon water addition can be obtained by the analysis of the Raman band at  $\sim 3340 \text{ cm}^{-1}$ ,  $\nu(\text{NH}_2)$ , assigned to the NH stretching of urea (Figure 3a,b).<sup>58,59</sup> A red-shift of this signal is commonly related to the strengthening of H-bonding interactions involving the NH group, as a H-donor.<sup>59,60</sup> In this respect Figure 3 indicates that urea acts as a relatively stronger H-bond donor in neat ChOAc:U ( $\nu(\text{NH}_2)$  signal at  $3332 \text{ cm}^{-1}$ ) in comparison with neat ChCl:U ( $\nu(\text{NH}_2)$  signal at  $3338 \text{ cm}^{-1}$ ). Moreover, for both systems the band blue-shifts with the addition of water, reaching, at around 50 wt %, values comparable to those observed in diluted urea/water mixtures. The trend evidences a general weakening of the H-bond interactions around the  $\text{NH}_2$  groups, acting as H-donors.

Figure 4 illustrates the behavior of the Raman signal at  $\sim 1155 \text{ cm}^{-1}$  assigned to the  $\text{NH}_2$  rocking of urea ( $\rho(\text{NH}_2)$ ), which is also influenced by the overall H-bonding involvement of urea.<sup>61</sup>

In this case, the red-shift of this band with increasing water contents, likely accounts for the weakening of H-bond interactions around the NH groups, owing to their substitution with new  $\text{NH}\cdots\text{O}$  interactions with water, in line with the trends observed for the  $\nu(\text{NH}_2)$  signal (Figure 3).

Remarkably, the location of the  $\rho(\text{NH}_2)$  band differs significantly in the two neat DESs, thus indicating that urea acts as a stronger H-bond donor in ChOAc:U than ChCl:U,



**Figure 5.** Wavenumber position of the Raman band  $\nu(\text{CH})$  at  $\sim 3025 \text{ cm}^{-1}$  assigned to the stretching of the CH of choline for ChCl:U (a) and ChOAc:U (b) DESs as a function of added water. The positions of the bands observed for solid ChCl and selected ChCl/water mixtures (a) and solid ChOAc and selected ChOAc/water mixtures solutions are also reported. Insets: UVR spectra recorded at an excitation wavelength of 226 nm for ChCl:U and ChOAc:U at different water concentrations in the spectral range of  $3000\text{--}3100 \text{ cm}^{-1}$  (the spectra have been arbitrarily normalized for a better comparison).

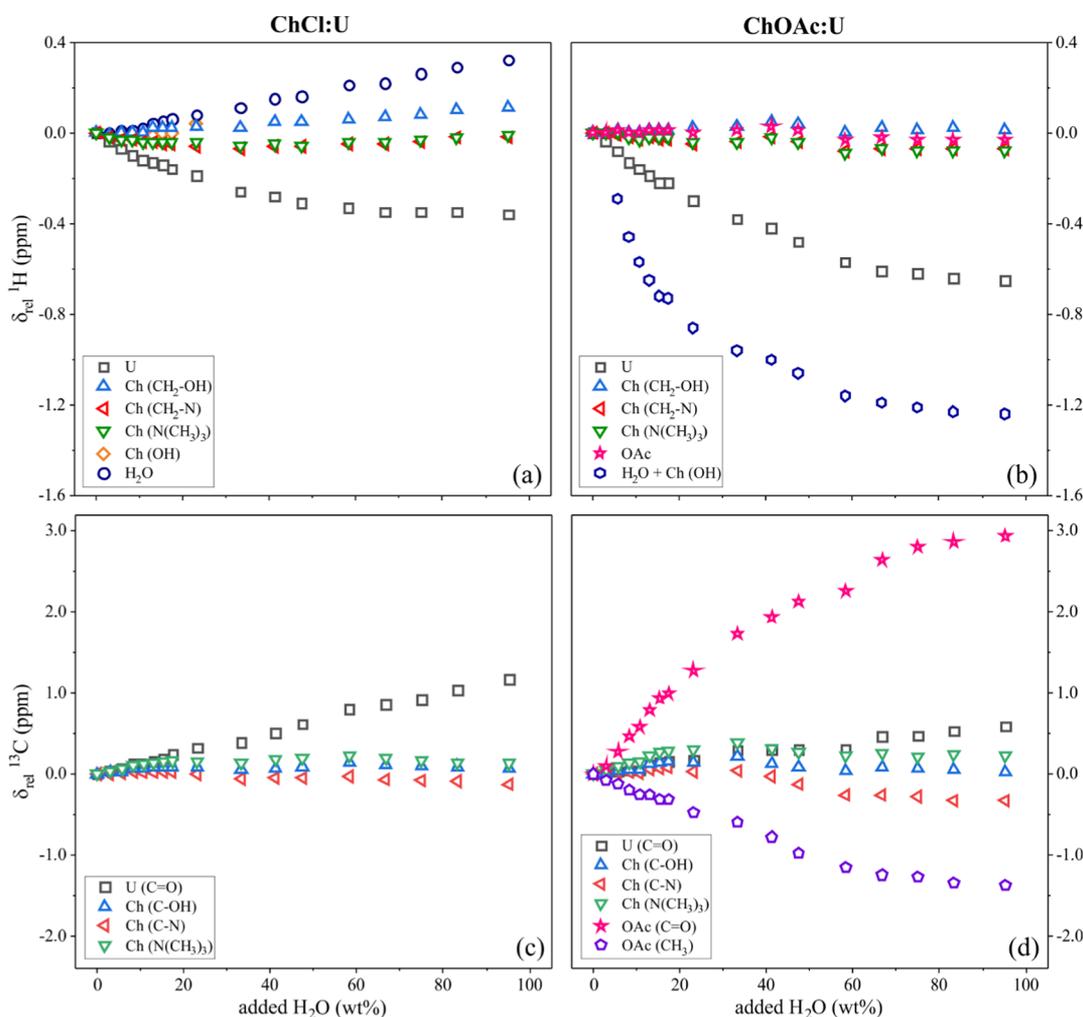
suggesting stronger urea–acetate interactions than urea–chloride ones. Note that using the N–H signals as a probe, we are specifically referring to H-bonding interactions around these groups, which are expected to be significant in determining the intermolecular organization of the systems.<sup>60</sup> Clearly, other contributions such as ion–ion, ion–dipole, dispersive, and steric interactions should also be considered.

Overall, Raman data evidence that the hydration process of both DESs occurs through the progressive insertion of water around urea molecules, whose vibrational properties tend to those of the fully hydrated species in their binary urea/water mixtures. This solvation process would clearly depend on a complex interplay of interactions among the different DES components and water.<sup>9,27</sup> Nevertheless the relevant role of urea–water interactions clearly emerges by the analysis of the Raman bands of the urea component. Clearly, the dual character of urea, which can act as both hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD) with the other species, appears crucial in determining the features of the extended H-bonding network that characterize these mixtures.

Raman signals arising from the choline's methylene and methyl groups in DES might provide further information on water–DESs interactions. Figure 5 reports the wavenumber position as a function of water concentration of the band at  $\sim 3025 \text{ cm}^{-1}$  that is mainly ascribable to the CH stretching mode ( $\nu(\text{CH})$ ) of choline in the DESs considered (Figure S1).<sup>62</sup>

In this case, the analysis has been limited to 73 wt % added water, since at higher water contents the interference arising from the broad OH stretching band of water ( $3100\text{--}3700 \text{ cm}^{-1}$ ) does not allow for a safe estimate of the  $\nu(\text{CH})$  position. For both systems, a blue-shift of the  $\nu(\text{CH})$  signal is observed upon water addition which might generally be ascribed to the

progressive hydration of the alkyl moiety of the choline cation.<sup>62,63</sup> Different from the bands of urea discussed above, Figure 5 evidences an almost linear behavior, suggesting that water insertion around choline is somewhat more gradual and that the hydration of its hydrophobic portion is not fully attained in the explored concentration range. At any rate, the data support the occurrence of a progressive substitution of cation–anion interactions with cation–water ones at high hydration degrees,<sup>27,64</sup> which is probably driven by the formation of new anion–water interactions (as will be discussed below). Note that in this case also, the position of the  $\nu(\text{CH})$  band is different in the two DESs with low water contents, which reflects the change in the interactions felt by the cation upon anion substitution. A similar situation is also observed by comparing the two ChCl to ChOAc solid samples, suggesting that anion-induced alterations around CH groups occurs also in the crystal phase. For both compounds, the  $\nu(\text{CH})$  band upshifts after dissolution in water, in line with the idea that the hydration of the cation promotes a blue-shift of the  $\nu(\text{CH})$  signals. In this respect, it seems that the hydration properties of choline in the binary ChCl/water solution containing ca. 50 wt % water are similar to those of choline in the ChCl:U/water mixture with about 20 wt % water. This result, albeit counterintuitive to some extent, could be tentatively explained considering that the presence of urea facilitates the cation hydration, possibly due to competitive urea–chloride interactions, which would contribute to increasing the exposition of choline to water. On the other hand, choline shows analogous hydration features in the ChOAc and ChOAc:U systems with a water content of about 50 wt %, suggesting that the cation exposition is not altered under these conditions. Overall, these results indicate that for the DES considered, the hydration properties of choline are



**Figure 6.**  $^1\text{H}$  (a, b) and  $^{13}\text{C}$  (c, d) relative shifts observed for ChCl:U (a–c) and ChOAc:U (b–d) upon dilution.

modulated by the nature of the anion, as will be further discussed below based on the analysis of NMR data.

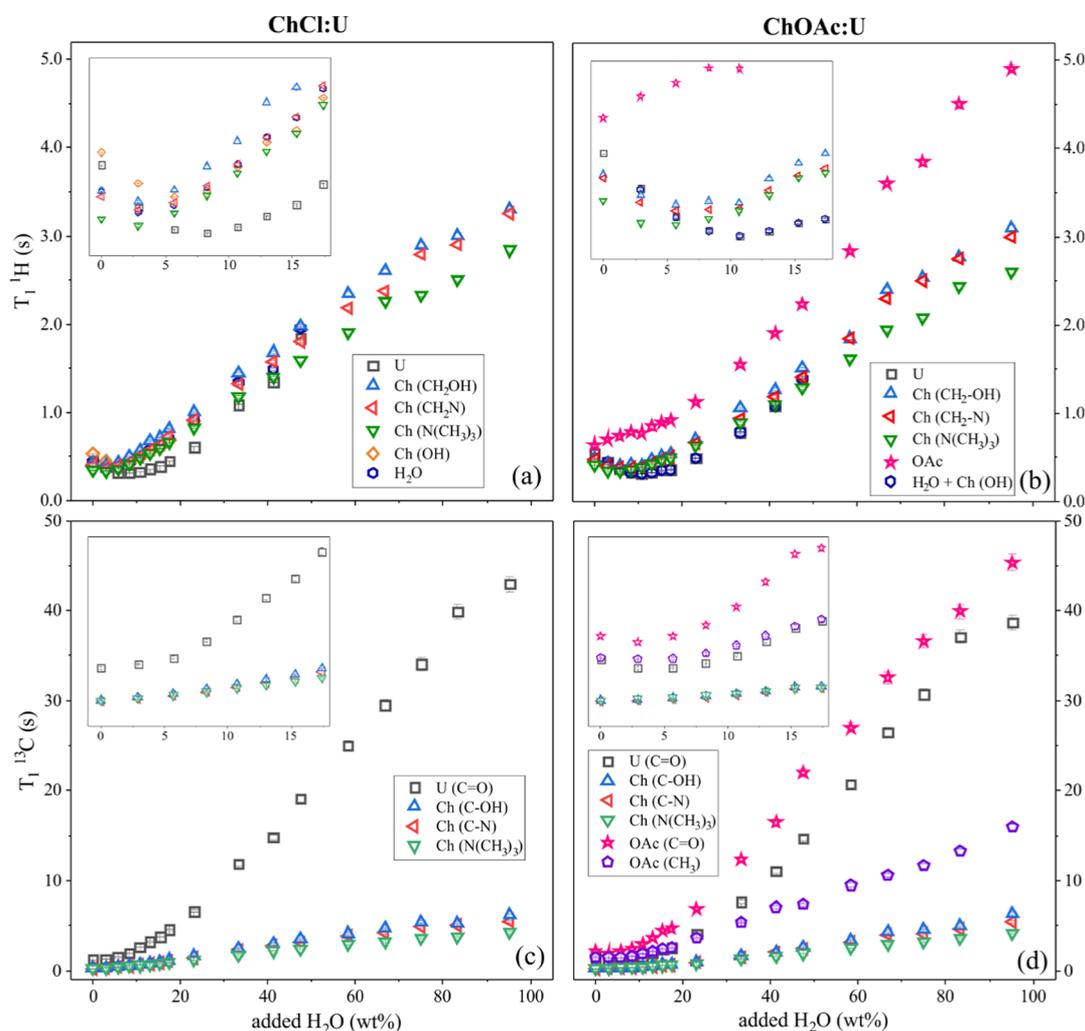
**Changes in the H-Bonding Network upon Hydration: The Role of the Anions Determined via NMR Spectroscopy.** The H-bond formation/weakening in DES/water mixtures can also be monitored following the upfield or downfield movement of the NMR chemical shifts.<sup>65</sup> To provide an easier visualization of the data, the relative shift can be used, defined as  $\delta_{\text{rel}} = \delta - \delta_0$ , where  $\delta_0$  is the chemical shift of a given nucleus in pure DES (or in the lowest dilution for H<sub>2</sub>O protons) and  $\delta$  is the chemical shift of the same nucleus for increased H<sub>2</sub>O contents.<sup>65,66</sup>  $^1\text{H}$  relative chemical shifts (Figure 6a,b) can be easily interpreted. In general, the high frequency (i.e., downfield) shift of  $^1\text{H}$  resonances is a very strong indicator of H-bond formation to electronegative atoms,<sup>67</sup> and would then suggest the formation of H-bonds in hydrated samples that are stronger than the existing ones.<sup>68</sup> Similarly, a low-frequency shift (i.e., upfield) would indicate the weakening of strong H-bonds of the DES network and their replacement with relatively less strong interactions with water.

In both ChCl:U and ChOAc:U series, a marked upfield shift of urea protons is observed (relative shift of 0.4 and 0.6 ppm, respectively). This is consistent with a weakening of the H-bond formed by the urea NH<sub>2</sub> functional groups in the presence of water and confirms the Raman findings that point

toward a replacement of H-bond interactions formed by the NH group—with the anions and with other H-acceptor components of the DESs—with weaker NH...O connections. This is also corroborated by the prominent upfield shift of  $^{35}\text{Cl}$  NMR signal already reported by some of us (Figure S2).<sup>29</sup>

Another similarity between the two systems is that there is almost no change in the signals of choline (the only exception is the CH<sub>2</sub>OH group, as discussed in the following), meaning they are not particularly affected by the addition of water. This is not surprising given the minor role of choline cations in the intermolecular network of nonhydrated ChCl:U and ChOAc:U,<sup>49</sup> and the fact that water is not expected to form specific interactions with its (hydrophobic) alkyl portion.

Interestingly, the Cl- and OAc- series shows a relevant difference in the behavior of the OH and H<sub>2</sub>O signals. In ChCl:U a distinct signal corresponding to the hydroxyl proton of Ch is detectable until sample 9 (23.1 wt % H<sub>2</sub>O), and is no longer visible from the sample with a water amount of 33.3 wt % onward (Figures S3 and 6a). This is in agreement with Posada et al.,<sup>69</sup> who found a complete depletion of the peak intensity assigned to hydroxyl protons of cholinium (i.e., indicating a complete exchange) only for ChCl:U contents of 75 wt %. Upon progressive dilution of ChCl:U, a downfield shift is observed for H<sub>2</sub>O and—less markedly—the methylene group vicinal to the hydroxyl group (Figure 6a). This is indicative of the progressive rupture of the H-bond of DES



**Figure 7.**  $^1\text{H}$  (a, b) and  $^{13}\text{C}$  (c, d)  $T_1$  relaxation times measured for ChCl:U (a–c) and ChOAc:U (b–d) upon dilution. Insets: enlargements of the low dilution region to better show the  $T_1$  minima.

during dilution with increased hydration of choline and the formation of relatively strong H-bonds between water and other species in the mixtures.<sup>65,70,71</sup> Contrarily, in ChOAc:U, a single peak is always observed for OH and H<sub>2</sub>O, even at low dilutions (Figures S4 and 6b), indicating that exchange is occurring in the OAc-based DES over the full range of concentration. The relatively easy proton exchange between the  $^1\text{H}$  resonances of –OH and H<sub>2</sub>O was also observed at room temperature in the ionic liquid ChOAc added with H<sub>2</sub>O at 30 wt %.<sup>68</sup> The more frequent and faster exchange occurring in ChOAc:U compared to ChCl:U might even be facilitated by the bidentate OAc anion that could act as a bridge between the exchange sites. Under dilution, the signal corresponding to these exchangeable protons (H<sub>2</sub>O and OH) in OAc-based samples shows a marked upfield shift, in contrast to the more intuitive downfield shift of H<sub>2</sub>O in ChCl:U (Figure 6a,b). An upfield shift of water molecules has been observed also in [C<sub>2</sub>mim][OAc]/H<sub>2</sub>O mixtures.<sup>66</sup> According to the authors, such behavior indicates that the addition of water to the IL causes the water molecules to bond more strongly with the anion than with other water molecules. Note that in our ChOAc:U system, the signal of methyl protons of acetate does not change significantly, whereas in IL [C<sub>2</sub>mim][OAc] it

shows a downfield shift as large as 0.5 ppm due to the strong hydrogen bonds with the water molecules.<sup>66</sup>

Overall, from the  $^1\text{H}$  one-dimensional (1D) analysis it is observed that in ChCl:U H-bonds become weaker for U protons, and stronger for water and OH, suggesting a sort of rearrangement of the H-bond network. In ChOAc:U, no  $^1\text{H}$  upfield shift is observed, indicating that all protons, U, H<sub>2</sub>O, and OH, undergo a weakening of their H-bond interactions. Likely, the formation of strong water–acetate interactions in ChOAc:U might justify the different behaviors. On the other hand, the weakening of H-bonds formed by the urea NH groups upon hydration is common for both systems, as also revealed by UVRR data.

$^{13}\text{C}$  relative chemical shifts were also considered (Figure 6c,d) but they should be interpreted more carefully. Although the  $^{13}\text{C}$  signal in donor and acceptor nuclei are affected by H-bonding, with H-bond shifts of 1–2 ppm in C=O acceptors, other factors provide equally important contributions to the total chemical shift.<sup>67</sup> However, some considerations can be drawn. First, a downfield shift is observed for the C=O of urea in both DESs (less marked in ChOAc:U than in ChCl:U, with relative shifts of 0.6 and 1.2, respectively). This is again in line with Raman observations, supporting the formation of stronger C=O⋯H<sub>2</sub>O interactions in hydrated DESs. As seen for  $^1\text{H}$

chemical shifts, there are only slight changes of choline signals upon water addition in both DESs, indicating a minor role of the cation. Notably, the chemical shift variation of acetate carbons in ChOAc:U, with marked downfield and upfield shifts for the quaternary and the methyl carbons, respectively. Even if interpreted with care, this is indicative of the heavy involvement of the anion in H-bond formation and disruption with different species in the mixture upon dilution. In particular, the carbonyl carbon of acetate is the site showing the most intense downfield shift, suggesting a strong competition with urea C=O in the hydrated DES. Indeed, the oxygen acceptor of acetate was found to be involved in the shortest and strongest H-bond interactions in neat ChOAc:U,<sup>49</sup> and its major role is even strengthened upon the addition of water.

**Dynamic Scenario: Changes in Local and Long-Range Mobilities upon Dilution.** The structural picture mapped out above for the selected ChCl:U and ChOAc:U systems can be strengthened by the NMR investigation of short and long-range dynamics. Structure and dynamics are indeed two sides of the same coin, as the motion of a site/species depends on how strongly it is bound to the intermolecular network.

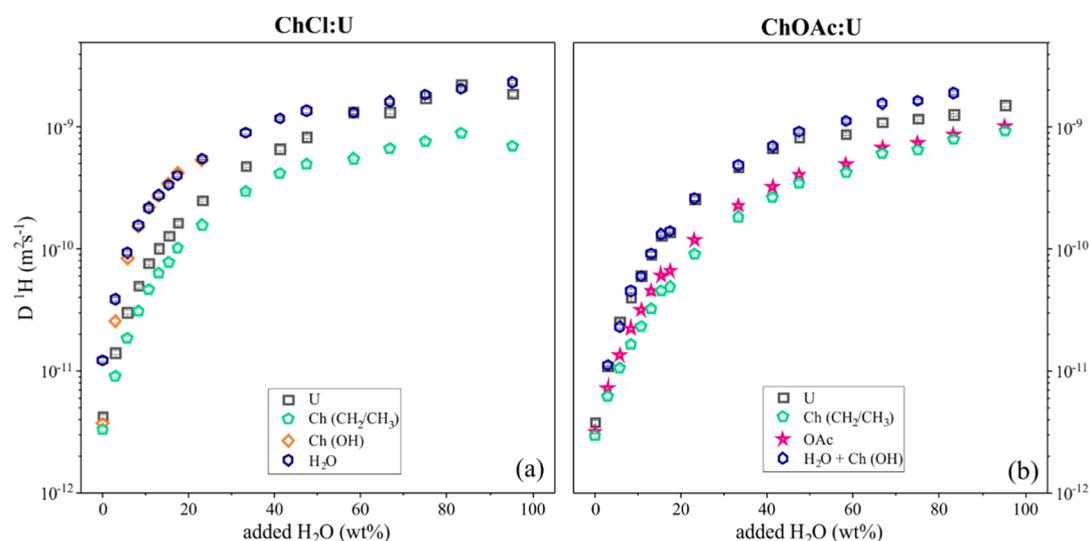
The longitudinal (spin–lattice,  $T_1$ ) and transverse (spin–spin,  $T_2$ ) relaxation times are indicators of rotational mobility and may provide information about the intra and intermolecular relaxation mechanisms of a particular spin (mainly dipolar interactions for 1/2 spins). Figure 7a,b shows the  $^1\text{H}$   $T_1$  curves as a function of the added  $\text{H}_2\text{O}$ . All signals but that for acetate pass through a minimum: in ChCl:U the  $\text{CH}_2$  and  $\text{CH}_3$  groups of choline as well as  $\text{H}_2\text{O}$  at 2.9 wt %, OH at 5.7 wt %, and U at 8.3 wt %; and in ChOAc:U the  $\text{CH}_2$  and  $\text{CH}_3$  groups of Ch at 5.7 wt %, urea and the other exchangeable protons at 10.7 wt %. Even if these  $T_1$  minima cannot be rigorously interpreted as the temperature-dependent relaxation data, they anyway give some hints on the rotational mobility of the species in the two systems. Overall the minima for ChOAc:U are shifted to higher water contents, and this may be related to the viscosity of the two DESs. It turns out from the literature that neat ChOAc:U is more viscous than neat ChCl:U,<sup>40,43,72</sup> hence species in ChOAc:U would tumble somehow more slowly than in ChCl:U, and their minima are then shifted to a higher  $\text{H}_2\text{O}$  mole fraction. This is confirmed by  $^{13}\text{C}$   $T_1$  relaxation times, which are plotted as a function of added  $\text{H}_2\text{O}$  in Figure 7c,d. In general, carbon relaxation data are easier to interpret due to the absence of the intermolecular and spin-diffusion contributions to relaxation rates.<sup>73</sup> As expected, all  $T_1$  values increase upon dilution in both DESs. However, while in ChCl:U no real minimum is observed, in ChOAc:U, the  $T_1$  values of acetate and urea pass through a minimum at around 2.9 wt %  $\text{H}_2\text{O}$ . This shift to a higher  $\text{H}_2\text{O}$  mole fraction indicates again that species in the mixture, at least urea, have a slower rotational motion in ChOAc:U than in ChCl:U.

Looking back at  $^1\text{H}$   $T_1$  relaxation data (Figure 7a,b), it can be seen that, in both Cl- and OAc-based systems, the dispersion behavior of the relaxation times are quite similar, with pretty close minima for all of the protons of Ch, U, and  $\text{H}_2\text{O}$ . As reported for the mixture of ChOAc and 30 wt %  $\text{H}_2\text{O}$ ,<sup>68</sup> this similarity indicates that relaxation is not only induced by the modulation of intramolecular dipolar interactions between nearest-neighboring protons but the rotational motions of the species in the mixtures are also correlated due to H-bonding interactions. In ChCl:U, water

shows a minimum overlapping Ch, while OH shows a distinct minimum which is intermediate between Ch/ $\text{H}_2\text{O}$  and U. In ChOAc:U, OH and  $\text{H}_2\text{O}$  are not distinguishable and the  $T_1$  minimum overlaps that of urea. Assuming spin-diffusion contributions to be negligible, the similarity of the data qualitatively suggests concerted motions of the species. This points toward a preferred location of at least some of the  $\text{H}_2\text{O}$  molecules closer to Ch in ChCl:U than in ChOAc:U and closer to U in ChOAc:U than in ChCl:U. This can be related to the different size, shape, and H-bonding capabilities of the two anions, which would affect the overall molecular structuring of the DESs and their hydration properties. Likely, the relatively small and spherical chloride can be effectively solvated by a larger number of urea molecules when compared to acetate, despite the capability of the latter to form strong H-bonds. As a result, choline will be relatively more exposed to water in ChCl:U with respect to ChOAc:U as also suggested by the Raman data. From a different perspective, the replacement of Cl by OAc would make urea molecules relatively more prone to interact/exchange with water in competition with choline and the anion. This is coherent with the larger downfield shift observed for  $\text{NH}_2$  protons of urea in ChOAc:U, indicating that in the latter a higher number of strong H-bonds in the neat DES are replaced with weaker interactions, probably with water. Also, the observation is in line with the slight upfield shift of the methylene  $\text{CH}_2\text{OH}$  protons in ChCl:U which is not observed in ChOAc:U. This would suggest that in ChCl:U the cation forms a considerable number of H-bonds with water which are stronger than the pre-existing ones, while in ChOAc:U, urea is probably dominating the interactions with water. These findings could explain the fact that the  $\nu(\text{NH}_2)$  and  $\rho(\text{NH}_2)$  Raman bands of urea show a larger concentration dependence at the beginning of the hydration process for ChOAc:U, while a larger dependence is observed for the  $\nu(\text{CH})$  Raman signal of choline for ChCl:U (see Figures 3–5).

In contrast to all other  $^1\text{H}$   $T_1$  curves, that corresponding to acetate does not pass through a minimum (Figure 7b), indicating that the methyl protons tumble faster, which is also correlated with the unchanged relative shift against the water content. A different picture emerges from  $^{13}\text{C}$   $T_1$  relaxation data, with both methyl and carbonyl carbons showing a minimum that overlaps that of urea C=O. Following the above speculations, this would indicate that rotational motions of these species in the mixtures are correlated due to—maybe  $\text{H}_2\text{O}$ -mediated—intermolecular interactions. Overall, this confirms the major role played by acetate, mainly through its quaternary carbon, in the H-bonding network, supporting chemical shift analysis.

$T_2$  relaxation times have also been measured for  $^1\text{H}$  and  $^{13}\text{C}$  in the two series of samples (Figure S5). The  $T_2$  values of the methyl and methylene protons and carbons of cholinium and acetate, as well as of carbonyl carbon of acetate, increase steadily as a function of the  $\text{H}_2\text{O}$  content in both DESs. Anomalous trends, probably related to the exchange process, are observed for all labile protons ( $\text{H}_2\text{O}$ , OH, and U) and for C=O of urea. The pattern followed by  $^1\text{H}$   $T_2$  of  $\text{H}_2\text{O}$  is particularly interesting as a sort of minimum can be distinguished at 33.3 and 8.3 wt % for ChCl:U and ChOAc:U, respectively. Minima in  $T_2$  have been observed in mixtures of water and the ternary DES composed of resorcinol and hexylresorcinol as HBDs and tetraethylammonium bromide as HBA.<sup>65</sup> This was ascribed to a scenario where hydroxyl



**Figure 8.**  $^1\text{H}$  diffusion coefficients measured for ChCl:U (a) and ChOAc:U (b) upon dilution.

protons fully exchanged for the DES dilution at which  $T_2$  reached a minimum value. This holds in our case for ChCl:U, confirming the results of chemical shift analysis. Full exchange between OH and  $\text{H}_2\text{O}$  over the whole concentration range was instead assumed for ChOAc:U based on the  $^1\text{H}$  1D spectra. However, the  $T_2$  minimum found for  $\text{H}_2\text{O}$  at 8.3 wt % still confirms the discrepancy between the two systems in terms of exchange, with the OAc-based DES more prone to allow the exchange of labile protons.

To complete the molecular description of the two systems, the change of the translational motion of the DES constituents upon hydration was also explored using PFG diffusion experiments. In both ChCl:U and ChOAc:U there is a steep increase of diffusion at the first dilutions (till ca. 20 wt %), then the increase is more gradual (Figure 8). The respective self-diffusion coefficients for U, cation, and acetate anion (in ChOAc:U) are close but still different, even in the neat systems (in ChCl:U  $4.2 \times 10^{-12}$  for U and  $3.3 \times 10^{-12}$  for Ch; in ChOAc:U  $3.8 \times 10^{-12}$  for U,  $3.0 \times 10^{-12}$  for Ch and  $3.2 \times 10^{-12}$  for OAc). This means that no contact ion pairs or stable aggregates are formed, which would imply ratios of self-diffusion coefficients equal to one.<sup>68</sup> However, all components must maintain a sizable mutual contact that slows down their individual motion ( $D$  in neat DESs in the order of  $10^{-12}$   $\text{m}^2/\text{s}$ ).<sup>65</sup>  $D_{\text{water}}$  starts also from the  $10^{-11}$   $\text{m}^2/\text{s}$  range, in agreement with the participation of  $\text{H}_2\text{O}$  in the DES-based H-bond network.<sup>65</sup> It approaches the diffusion of bulk water (ca.  $2 \times 10^{-9}$   $\text{m}^2/\text{s}$ ) only upon dilution above 75 wt %.

Comparing the two DESs, it can be seen that the diffusion coefficients of choline and urea are comparable.  $D_{\text{urea}}$  experienced an increase of ca. 2 orders of magnitude upon dilution with 25 wt % water, while a concentration of water equal to 30 wt % or even 40 wt % is needed for  $D_{\text{Ch}}$  for the increase of ca. 2 orders of magnitude in ChCl:U and ChOAc:U, respectively. In all cases, U moves faster than choline as one would expect, due to its smaller molecular size and lower molecular weight. The same relationship in motion–size has been reported for reline and other ChCl-based DESs, with ethylene glycol or glycerol as HBD.<sup>74,75</sup> However, translational motion correlates not only with the molecular weight but also with the extent of H-bonding, and this is evident when considering the self-diffusion coefficient of

acetate, which is still faster than choline, but quite close (Figure 8b). This again indicates a strong involvement of OAc in the H-bond network, which slows down the translational motion of the anion. Note that due to the fast relaxation of the  $^{35}\text{Cl}$  nucleus, the diffusion coefficient of chloride cannot be experimentally measured. However, all diffusion coefficients measured here for Ch, U, and  $\text{H}_2\text{O}$  in ChCl:U/ $\text{H}_2\text{O}$  mixtures are in excellent agreement with those calculated based on MD simulations.<sup>29</sup> Simulated diffusivities for chlorine are intermediate between  $\text{H}_2\text{O}$  and U, but closer to the latter, corroborating again the key role of  $\text{Cl}^-$  in the intermolecular network.

A final point to be investigated is the translational mobility of exchangeable protons. When  $\text{H}_2\text{O}$  is added to ChCl:U, the diffusion coefficients of the hydroxyl proton of Ch start deviating from the diffusivity of the rest of the cholinium molecules and approach the larger diffusivity values observed for water. The two diffusivities overlap above 8.3 wt % (up to 23.1 wt %, where a single peak is observed). This agrees with previous findings,<sup>75</sup> and clearly indicates a fast exchange between the water protons and the hydroxyl protons. Upon the addition of more than 60 wt %  $\text{H}_2\text{O}$ , the diffusion coefficient corresponding to OH and  $\text{H}_2\text{O}$  practically coincides within the experimental error with that of urea. A different situation is observed when chloride is replaced by acetate; in ChOAc:U diffusion coefficients of urea and exchangeable protons are practically not distinguishable in the whole range of dilution. This nicely matches the considerations drawn from  $T_1$  data, which pointed toward a higher number of  $\text{H}_2\text{O}$  molecules closer to urea in ChOAc:U with respect to ChCl:U.

## CONCLUSIONS

A multifaceted picture in terms of structure and dynamics of two DESs, ChCl:U and ChOAc:U, and their mixtures with water is outlined here using a combined UVRN and NMR approach. The comparative study of the two DESs under progressive dilution highlights, for the first time, the effect of the anion—chloride vs acetate—in the intermolecular interactions and mobilities of the different species over a wide compositional range. If ChCl:U and its mixtures with water have been largely investigated at the molecular level, this work represents the first attempt to unveil the structure and

dynamics of ChOAc:U/H<sub>2</sub>O mixtures and compare them with ChCl:U/H<sub>2</sub>O mixtures under the same experimental conditions.

The joint NMR and UVRR approach provides a consistent description of the systems from the atomic to the intermolecular scale and in a wide range of concentrations of water in DES. Some similarities are observed between the systems upon dilution, with the progressive insertion of water molecules among DES components and an overall reduction of the strength of mutual interactions. In both cases, water strongly binds to the urea C=O group and weakens H-bonding interactions around the urea NH<sub>2</sub> groups, while choline plays a minor role in the intermolecular network. As observed for chloride, the acetate anion is highly involved in the H-bond network. However, the replacement of chloride with acetate would make the system less “robust” and this is particularly evident at lower dilutions. Indeed, all proton-mediated H-bond interactions are weakened in ChOAc:U under dilution, while in ChCl:U, H-bonds get weaker for NH<sub>2</sub> protons of urea, but stronger for water and choline OH. Notably, the replacement of Cl with OAc would modulate the hydration properties of both choline and urea. In this respect, water molecules are relatively closer to Ch in ChCl:U than in ChOAc:U, but closer to U in ChOAc:U than in ChCl:U. In other words, the replacement of Cl with OAc would make urea molecules more prone to interact/exchange with water, competing with choline and the anion. Overall, ChOAc:U appears to be continuously affected by hydration and, even at low water concentrations, starts to undergo a break up of DES–DES interactions, with rapid solvation of the urea portion and full exchange of mobile protons. On the contrary, ChCl:U seems to maintain its structure as small interplays gradually occur between the urea of DES and the surrounding water molecules.

The different behaviors that emerge here for these two structurally related DESs underlines once more the crucial role of the anion in the DES formulation and yields valuable data toward the exploitation of DESs as tunable systems. Also, the dual character of U (HBD and HBA) is likely playing an important role. It can be foreseen that the replacement with a different HBD with no HBA capabilities will further alter the fine balance of intermolecular interactions and studies in this direction are currently under progress.

## ■ ASSOCIATED CONTENT

### SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acssuschemeng.1c03811>.

Experimental details, <sup>35</sup>Cl relative shifts, NMR spectra, and T<sub>2</sub> relaxation times (PDF)

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## Notes

The authors declare no competing financial interest.

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