

NMR study of choline chloride-based deep eutectic solvents

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Abstract

Nowadays, the eutectic liquids (DESs) are considered an adequate alternative to the traditional solvents. Therefore, there is an increasing interest in the study of the structure, properties and applications of these mixtures. In this paper, several NMR spectroscopic techniques have been used in order to evaluate the molecular structure of three ternary DESs, composed of choline chloride, urea or glycerol or ethylene glycol, and water. The use of routine and non-routine NMR techniques, such as ¹H, ¹³C, correlation experiments, NOESY, ROESY, and diffusion experiments gave us some important evidences pointing to a supramolecular structure of the DES, which may be the origin of their particular behaviour. The mixture containing ethylene glycol shows the feeblest structure. In these ternary solvents, the water is found within the structure that is still retained when DESs are diluted at 10% wt.

22 Keywords: Deep Eutectic Solvents; NMR spectroscopy; Self-diffusion coefficients;
23 Choline chloride; Urea; Glycerol; Ethylene Glycol.

24 **1. Introduction**

25 The full solvents elimination in the laboratory or industry is an unreasonable
26 objective due to the different roles exerted by them in the physicochemical procedures.
27 The environmental problems derived by use of traditional ones, usually volatile organic
28 compounds (VOCs), have turned the development of new eco-friendly products a priority
29 line in the *Green Chemistry*. According to the IUPAC [1], this chemistry area “deals with
30 the design of chemical products and processes that reduce or eliminate the use or
31 generation of substances hazardous to humans, animals, plants, and the environment”.
32 Because of this, the synthesis processes should include: (i) non-toxic and renewable
33 substances; (ii) a maximum economy of materials avoiding by-products and wastes; (iii)
34 minimum requirements of energy (temperature and pressure); and (iv) minimum risks
35 respect to possible accidents. In addition, the products formed should be able to
36 decompose into non-harmful ones when their function has finished.

37 Several VOCs substitutes have been proposed such as supercritical fluids, biomass
38 based solvents, or low temperature transition liquids (LTTMs) [2–5]. In the latter group,
39 the ionic liquids (ILs) and the deep eutectic solvents (DESs) are included. Both type of
40 fluids are used for applications in similar fields. However, there are important differences
41 between them. The high toxicity and economic cost of some of ILs have caused the ionic
42 liquids are not currently catalogued as green solvents [6,7]. By contrast, the composition
43 and the synthesis procedure of the DESs make them eco-friendly solvents. The term DES
44 was proposed by Abbott et al. [8] to refer to mixtures (two or more substances) with a
45 melting temperature, T_m , lower to that of the individual components. It is necessary to

46 clarify that the composition of the eutectic mixtures studied in the literature does not
47 always correspond to that of the eutectic point. In bibliography, ionic and non-ionic
48 eutectic solvents have been reported; some of them are hydrophilic liquids and others
49 have hydrophobic behavior [9–13]. However, the common feature is the ability of the
50 components of the mixture to establish an important hydrogen-bond network. So, DESs
51 contain both hydrogen bond acceptors (HBA) and donors (HBD) substances. Natural
52 deep eutectic solvent (NADES) is a term frequently used in the literature when the
53 eutectic is composed by metabolites (sugars, organic acids, amines,...) [14]. Moreover,
54 if the components are active pharmaceutical ingredients (APIs), the term therapeutic deep
55 eutectic solvent (THEDES) is applied by some authors [15]. The issue of the
56 nomenclature is being a controversial subject [5]. In this paper, the acronym DES will be
57 used to refer to the studied mixtures.

58 Two methods are mostly used for the DESs synthesis from the adequate molar ratios
59 of HBA and HBD components: heating with agitation at soft temperature (about 323 K),
60 and dissolution of the components in water and subsequent evaporation [16]. In these
61 processes, no chemical reaction is involved so the efficiency is total, no waste is produced,
62 and the energetic cost is low; three basic issues in the Green Chemistry. In relation to the
63 environmental analysis, these compounds are usually more biodegradable than other
64 solvents but the toxicity profiles are varied. Caution must be applied in their classification
65 because both the toxicity of the individual components and possible synergistic effects
66 between them must be evaluated [17]. For instance, choline chloride-based DESs are
67 moderately toxic, relatively harmless or practically harmless depending of the HBD
68 compound [18–20]. It has also been observed that certain DESs can be used as
69 antibacterial, antifungal or anticancer agents; therefore, their toxicity become into an
70 advantage [3,21]. One major drawback for the implementation of these solvents in the

71 industrial processes is the high viscosity values of the mostly of DESs [9]. However, this
72 property can be modulated using the plasticizer effect of the water; the addition of small
73 amounts increases substantially the fluidity of the liquid [22,23]. The hygroscopic
74 capacity of several ChCl-based DESs has been studied by Chen et al [24].

75 The research to date has tended to focus on applications of these liquids rather than
76 on their chemical characterization. As a result, aspects related to the structure of the DESs
77 and the role of the water in the aqueous eutectics are not well elucidated. Nevertheless,
78 some papers have been published. For similar mixtures to those included herein, the self-
79 diffusion coefficients at several temperatures were determined by D'Agostino et al.
80 [25,26] from PFG-NMR spectroscopy. Recently, the micro-segregation in the diluted
81 eutectic has been observed using Brillouin and ^1H NMR techniques by Posada et al. [27]
82 The effect of the water in the nanostructure has been studied with neutron total scattering
83 by Hammond et al. [28] They have evidenced the existence of clusters even at high
84 dilution. Other authors [29–31] have performed quantum calculations and molecular
85 dynamics simulations in order to analyse the hydrogen bonding network. Finally, for
86 eutectics different from those studied in this work, a supramolecular structure has been
87 detected from several spectroscopic experiments [14,16,32,33].

88 The aim of this paper is to examine the molecular structure of three deep eutectic
89 solvents whose composition includes choline chloride, urea or glycerol or ethylene
90 glycol, and water. We evaluate the interactions established between the three components
91 of each mixture from several NMR spectroscopic studies performed at 298 K. In addition,
92 we calculate the self-diffusion coefficients of the different species and compare with those
93 of the literature. From these results, we analyse the role of the water in these aqueous
94 eutectics.

95 2. Material and methods

96 2.1 Materials

97 Choline chloride, [Ch]Cl, (purity $\geq 98\%$) provided by Sigma-Aldrich was used as
98 hydrogen bond accept (HBA) for all DESs studied in this work. This compound was dried
99 under vacuum during several hours. The several hydrogen bond donors (HBDs) were:
100 urea, U, (purity ≥ 99.5) acquired from Panreac, and glycerol, Gly, (purity $\geq 99.5\%$) and
101 ethylene glycol, EG, (purity $\geq 99\%$) from Sigma-Aldrich. Moreover, distilled deionized
102 water, W, with resistivity less than $18.2 \mu\text{S}\cdot\text{cm}^{-1}$ was utilized. A Sartorius balance
103 BP210S (± 0.1 mg) was used to weigh the compounds in adequately proportion: 1:2
104 ([Ch]Cl:HBD) mole ratio and a water content $< 10.5\%$ wt. The liquids were obtained by
105 heating to 50°C under magnetic agitation until a homogeneous liquid is formed. The
106 characteristics of the ternary eutectic mixtures are listed in Table S1.

107 2.2 Methods

108 NMR measurements were performed on a Bruker AVANCE operating at 400 MHz
109 for ^1H using a direct 5-mm broadband probe with z-axis gradients. The sample
110 temperature was established at 298 K. The chemical shifts were referenced to TMS as
111 external standard. ^1H -NMR spectra were recorded with a standard one-pulse sequence
112 with 90° flip angle for excitation (Bruker pulse program *zg*), with a spectral width of 16
113 ppm, centred at 5 ppm, 16 K data points and relaxation time fixed to 30 s. 8 scans were
114 acquired for each spectrum. ^{13}C -NMR spectra were recorded with an APT sequence
115 (Bruker pulse program *jmod*), with a spectral width of 240 ppm, centred at 110 ppm, 64
116 K data points and relaxation time fixed to 2 s. 256 scans were acquired for each spectrum.
117 Signal assignation was performed with routine gradient selected DQF-COSY, ^1H - ^{13}C
118 HSQC and ^1H - ^{13}C HMBC (Bruker pulse programs *cosygpmf*, *hsqcedetgp* and

119 hmbclpndqf respectively) employing optimized 90° angle pulses. NOESY experiments
120 were carried out using a gradient selected NOESY sequence (Bruker pulse program
121 *noesygpph*) with a spectral width of 4 ppm centred at 4 ppm, with 128 t1 increments and
122 8 scans for each increment. Mixing time (t_{mix}) was adjusted as described for each case.
123 ROESY experiments were measured using a non-gradient selected sequence (Bruker
124 pulse program *roesyph*) with the suitable spectral widths and centers, with 128 t1
125 increments and 16 scans for each increment. Diffusion experiments were performed by
126 using a stimulated echo sequence with bipolar gradient pulses (Bruker pulse program
127 *stebpgpls*) with a spectral width of 16 ppm centred at 5 ppm, and 16 K data points. Big
128 delta was set to 200 ms and 16 μ s for small delta. Gradient pulses strength were varied
129 over 32 steps (8 transients each) to obtain a 95% decrease in the resonance intensity at
130 the largest gradient amplitudes. The self-diffusion coefficients, D , were calculated by
131 fitting the intensity of each signal to the exponential function:

$$I(g) = I_0 \exp[-D\gamma_H^2 g^2 \delta^2 (\Delta - \delta/3)] \quad (1)$$

132 where $I(g)$ is the resonance intensity measured for a given gradient strength, g ; I_0 is the
133 NMR signal in the absence of the gradient pulse; γ_H is the gyromagnetic ratio of the
134 hydrogen nucleus; δ is the duration of the bipolar gradient pulse; and Δ is the observation
135 time.

136 **3. Results and discussion**

137 In this section, the chemical structure of each eutectic is analysed. First, we present
138 the ^1H and ^{13}C spectra that allow to identify the different atoms present in the mixtures.
139 Second, we used the Nuclear Overhauser Effect Spectroscopy (NOESY) technique in
140 order to examine the interactions that may be associated to the formation of the liquid
141 phase and the high stability of the DESs, supported by other experiments such as ROESY.

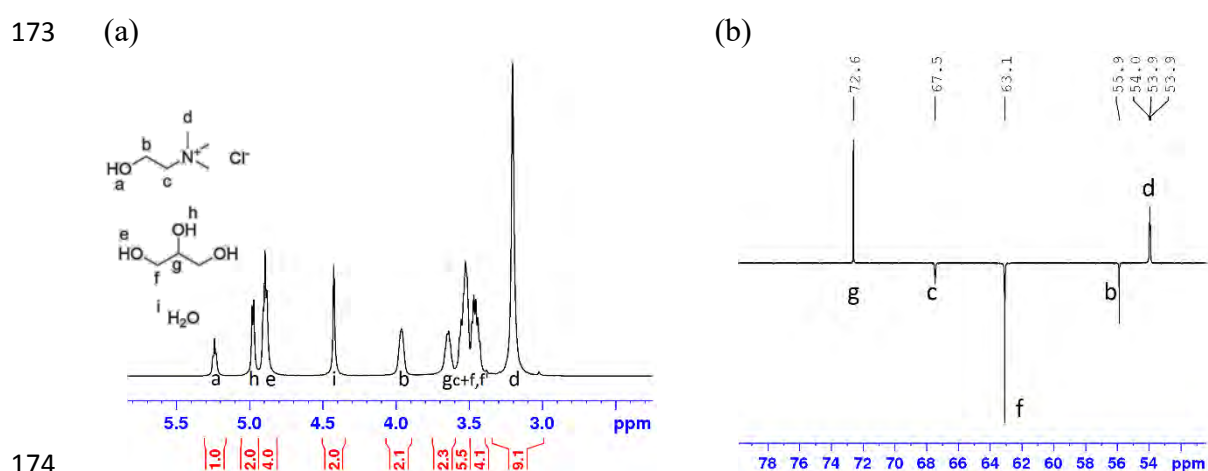
142 Third, we calculate the mobility of the species from Diffusion-Ordered Spectroscopy
143 (DOSY) spectra. Last, to evaluate the effect of addition of water on the eutectic structure,
144 NMR experiments for two dilutions of each DESs (10 % and 90 % D₂O) are included.
145 Throughout this section, we will use the following abbreviations: aliphatic hydrogens of
146 the choline, Ch⁺; hydroxyl hydrogens of the choline, OH – Ch⁺; aliphatic hydrogens of
147 the HBD compound, Gly, or EG; mobile hydrogens of the HBD compound, such as
148 hydroxyl, OH – Gly or OH – EG or aminic hydrogens from urea NH₂ – U; water, W; and
149 partially deuterated water, HDO. In addition, in each section a particular notation will be
150 used for each individual hydrogen or carbon. We have chosen the choline
151 chloride:glycerol:water as a model for a more detailed study, due to the higher number of
152 hydroxyl and aliphatic hydrogens.

153 3.1 [Ch]Cl:Gly:W

154 In the ¹H-NMR spectrum (Fig. 1a), the signals of the methylene group bounded to
155 the choline nitrogen, CH₂ – N⁺ (c), and those of two hydroxylmethylenes of the glycerol
156 (f), are overlapping; the rest are perfectly separated: δ = 3.04 (s, 9 H, H_d), 3.23-3.33 (m,
157 4 H, H_f), 3.33-3.43 (m, 6 H, H_f, H_c), 3.44-3.53 (m, 2 H, H_g), 3.76-3.85 (m, 2 H, H_b), 4.26
158 (s, 2.0 H, H_i), 4.73 (t, J = 5.0 Hz, 4 H, H_e), 4.82 (d, J = 4.8 Hz, 2 H, H_h), 5.08 (t, J = 4.7
159 Hz, 1 H, H_a). The ¹³C spectrum was the expected (Fig. 1b) with: δ = 53.9 (t, J = 3.0 Hz,
160 C_d), 55.9 (C_b), 63.1 (C_f), 67.5 (C_c), 72.6 (C_g). From the ¹H-NMR spectrum integration,
161 the calculated composition ($u(x)=0.05$) is choline chloride/glycerol/ water in molar ratio
162 1:1.96:0.97. The calculated water content is: 5.3%wt.

163 The addition of 10% of deuterated water, D₂O, in the ternary eutectic does not
164 causes significant changes in the ¹H-NMR signals of the aliphatic hydrogens but those of
165 the hydroxyl ones widen and decrease the value of their integral (Fig. S1a). Adding 90%

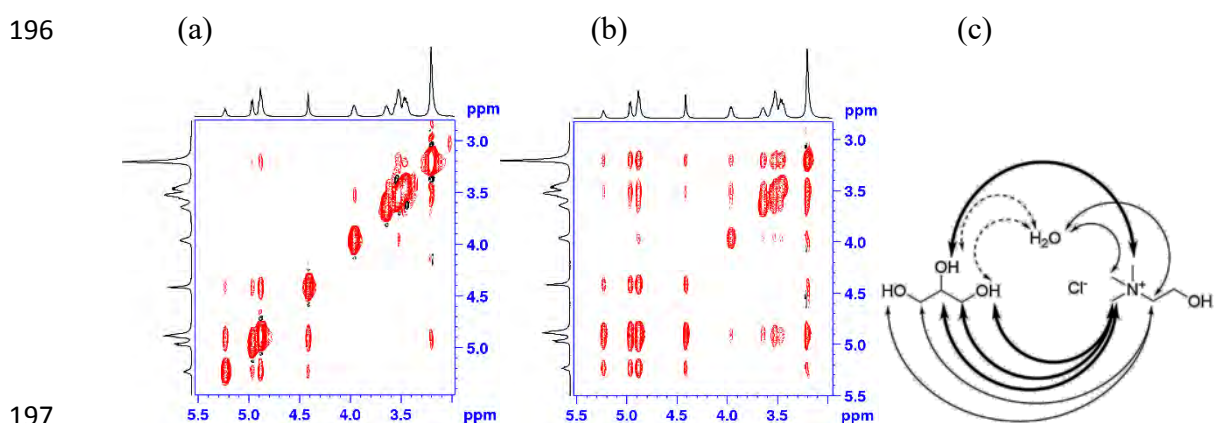
166 D₂O, the resolution of the spectrum improves and a single signal corresponding to HDO
 167 is observed. The pronounced displacement of aliphatic signals may indicate a substantial
 168 variation of the chemical environment showing the loss of the H-bond network. On the
 169 other hand, the 10% dilution causes small variations in the ¹³C spectrum being the signals
 170 of glycerol the most modified. Higher differences of chemical shift between neat and 90
 171 % diluted samples were observed: $\Delta\delta$ (neat – diluted) = +0.3 C_b, +0.1 C_c, +0.6 C_f, +0.4
 172 C_g (Fig. S1b).



175 Figure 1. [Ch]Cl:Gly:W spectra at 298 K: (a) ¹H-NMR (b) ¹³C-NMR.

176 Figure 2 shows the NOESY spectra for $t_{mix}=50$ (Fig. 2a) and $t_{mix}=800$ ms (Fig.
 177 2b); spectra for intermediated times are reported in Fig. S2. With the longer mixing time
 178 appear crosspeaks among almost all the proton signals (mobile and aliphatic hydrogens),
 179 what evidences the existence of interaction between the protons from the different species
 180 throughout space. It can be observed strong nOe couplings between choline and glycerol,
 181 both aliphatic and hydroxyl hydrogens, as well as choline with water (being the most
 182 intense ones coming from the methyl groups). No nOe signals were observed between
 183 glycerol aliphatic hydrogens and water. In the case of mobile protons, in these spectra, *a*
 184 *priori*, it is not possible to distinguish whether the signals are coming from chemical
 185 exchange or nuclear Overhauser effect. However, the increasing intensity of these

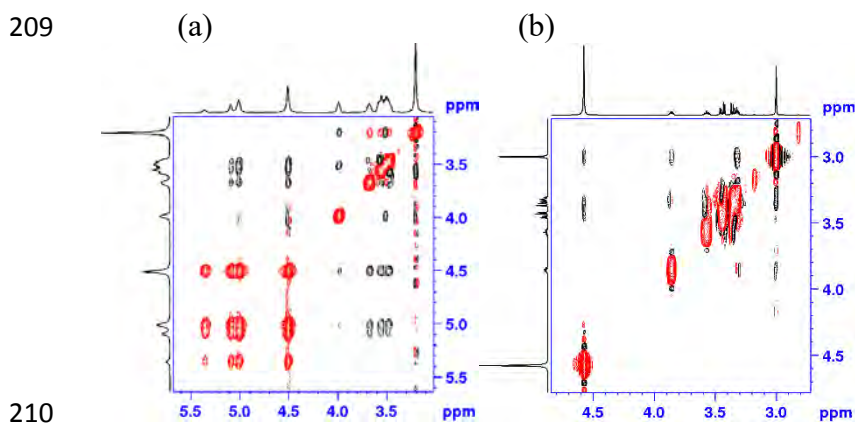
186 crosspeaks with the mixing time suggests an important contribution of nOe to signals,
 187 besides the chemical exchange. Spin diffusion effects were discarded when a ROESY
 188 experiments were measured, showing strong negative ROE crosspeaks between choline
 189 methyl groups and all the proton signals from the other species, as well as less intense
 190 signals from the glycerol hydrogens (Fig S3). Through the study of the enhancement of
 191 nOe signals with mixing time a tentative model of interaction between the hydrogens of
 192 the three species can be represented, as shown in Fig. 2c; the thicker the line the more
 193 intense the interaction. Moreover, the spectrum obtained after six months (Fig. S2c) was
 194 similar to that performed just after synthesis (Fig. S2a); so, we can conclude that the
 195 structure is stable.



198 Figure 2. NOESY spectra of [Ch]Cl:Gly:W at 298 K and at different mixing times: (a)
 199 $t_{mix}=50$ ms, and (b) $t_{mix}=800$ ms; (c) H-H interactions representation.

200 In the NOESY experiment of the 10 % diluted sample (Fig. 3a), the negative nOes
 201 previously observed between the methyl groups of the choline and the Gly continue. In
 202 addition, signals of exchange for OH – Ch⁺/W and OH – Gly/W appear. On the other
 203 hand, the interactions OH – Ch⁺/Gly, OH – Gly/Gly, and OH – Ch⁺/Ch⁺ show nOes of
 204 sign positive. These results suggest that the eutectic maintains its properties even though
 205 the viscosity has decreased. By increasing the dilution to 90%, the negative nOes

206 disappear and positive signals for Gly/HDO and Gly/methyls of the choline are observed
207 (Fig. 3b). The interaction between the compounds remains but as aqueous solution of
208 them.

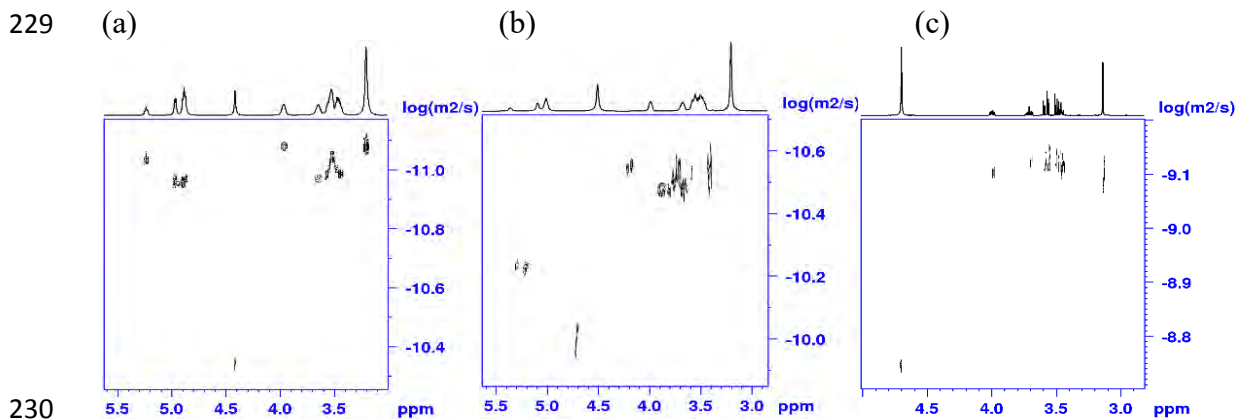


211 Figure 3. NOESY spectra of [Ch]Cl:Gly:W at 298 K, at $t_{mix}=800$ ms, and at several
212 dilutions: (a) 10 % D₂O, and (b) 90 % D₂O.

213 DOSY spectrum for the ternary DES (Fig. 4a) shows five clearly differentiated
214 traces whose numeric analysis allows calculating the self-diffusion coefficients (Table 1).
215 These traces correspond to (from higher to lower D): W, OH – Gly, Gly, OH – Ch⁺ and
216 Ch⁺. Obviously, water has the highest D value but is much lower than those obtained for
217 the eutectic containing urea (as seen below) or for free water: $D_{H_2O}(T=298.15\text{ K})=230\cdot 10^{-11}$
218 $\text{m}^2\cdot\text{s}^{-1}$ [34]. Highlight that the diffusivity for the rest of species (especially in the case
219 of hydroxyl hydrogens) is very similar indicating the presence of a very low mobility due
220 to a strong interaction between glycerol and choline, and with their mobile hydrogens.
221 This fact has already been reported in the literature [26] and our calculated values of D
222 agree with those. Again, we can suggest that the eutectic has an orderly and stable
223 supramolecular structure. When D₂O diluted samples were measured, very similar
224 behaviour was observed for the 10% diluted sample (Fig. 4b), with similar D values for
225 all type of hydrogens, except those from water. This result shows that the supramolecular

226 structure is conserved. However, for the 90% diluted sample (Fig. 4c), the self-diffusion
 227 coefficients correspond to those of individual and solvated molecules.

228



231 Figure 4. DOSY experiments of [Ch]Cl:Gly:W at 298 K. (a) neat eutectic; (b) 10% of
 232 D₂O; and (c) 90% of D₂O.

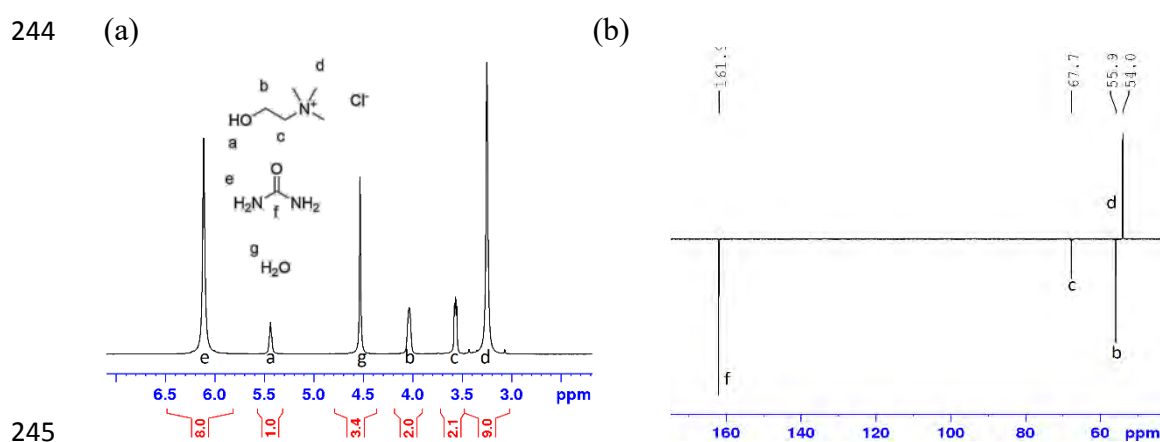
233 Table 1. The self-diffusion coefficients, D , at 298 K of the several species in the non-
 234 aqueous and aqueous DES with glycerol.

| DES | $10^{11}D_{\text{Ch}^+}$ / $\text{m}^2\cdot\text{s}^{-1}$ | $10^{11}D_{\text{OH}-\text{Ch}^+}$ / $\text{m}^2\cdot\text{s}^{-1}$ | $10^{11}D_{\text{Gly}}$ / $\text{m}^2\cdot\text{s}^{-1}$ | $10^{11}D_{\text{OH}-\text{Gly}}$ / $\text{m}^2\cdot\text{s}^{-1}$ | $10^{11}D_{\text{H}_2\text{O}}$ / $\text{m}^2\cdot\text{s}^{-1}$ |
|--|--|--|---|---|---|
| [Ch]Cl:Gly | 0.38 ^a | 0.38 ^a | 0.52 ^a | 0.52 ^a | --- |
| [Ch]Cl:Gly:W | 0.80 | 0.86 | 0.97 | 1.03 | 3.55 |
| | 0.7 ^b | 0.7 ^b | 0.7 ^b | 0.7 ^b | 4 ^b |
| [Ch]Cl:Gly:W (10% D ₂ O) | 2.72 | 5.10 | 3.18 | 5.71 | 9.65 |
| [Ch]Cl:Gly:W (90% D ₂ O) | 78.0 | --- | 74.6 | --- | 173.5 |

235 ^aD'Agostino et al. [25]; ^b D'Agostino et al. [26], $T=293$ K.

236 3.2 [Ch]Cl:U:W

237 All signals of the ^1H and ^{13}C -NMR spectra (Fig. 5a and 5b) were assigned. For ^1H -
 238 **NMR** 400 MHz at 298 K, the chemical shifts were: δ 3.25 (s, 9 H, H_d), 3.53-3.60 (m, 2
 239 H, H_c), 4.04 (s, 2 H, H_b), 4.53 (s, 3 H, H_f), 5.40-5.48 (m, 1 H, H_a), 6.12 (s, 8 H, H_e). The
 240 ^{13}C -**NMR** 100 MHz signals were: δ 54.0 (t, $J = 3.7$ Hz, C_d), 55.9 (C_b), 67.7 (t, $J = 2.7$ Hz,
 241 C_c), 161.9 (C_f). From the integration of each signal in ^1H spectrum, we have obtained the
 242 following composition: choline chloride/urea/water in molar ratio 1:1.95:1.67 (water
 243 content: 10.5% wt.) with a standard uncertainty, $u(x) = 0.05$.



246 Figure 5. [Ch]Cl:U:W spectra at 298 K: (a) ^1H -NMR; (b) ^{13}C -NMR.

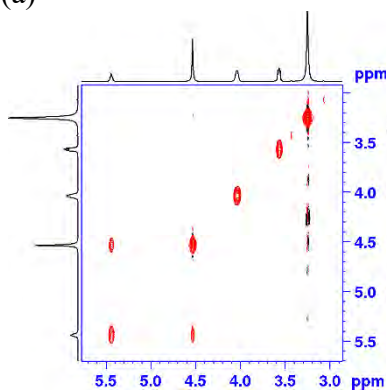
247 In the ^1H -NMR for the ternary eutectic diluted with 10% or 90% deuterated water,
 248 HDO, the OH – Ch^+ signal appears with HDO (Fig. S4a). For the amine groups of the
 249 urea, the peak decreases in intensity by exchange with deuterium of the solvent. In the
 250 ^{13}C -NMR (Fig. S4b), a drastic reduction of the urea carbonyl signal due to the exchange
 251 and coupling with deuterium atoms is observed. Also, the chemical shift signals changed
 252 in the following quantities: $\Delta\delta$ (neat – diluted) = +0.3 C_b , +0.3 C_c , +0.1 C_d , -0.8 C_f . In the
 253 NOESY experiments with short time of mixing (Fig. 6a), only a negative peak of
 254 correlation indicating chemical exchange between OH – Ch^+ and W is observed.
 255 Increasing t_{mix} (Fig. 6b and S5), an intermolecular positive nOe corresponding to the
 256 W/ Ch^+ interaction appears. Moreover, negative nOe signals for U/ Ch^+ correlation,

257 corresponding to choline methyl groups are detected. No U/W correlation is observed.

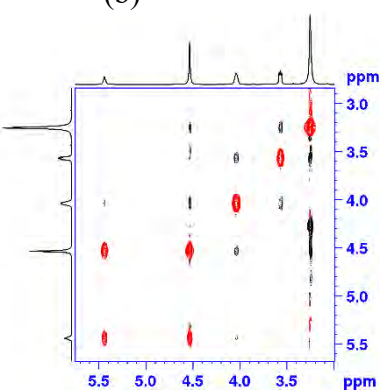
258 Fig. 6c shows a tentative drawing of the H-H interactions deduced from above results.

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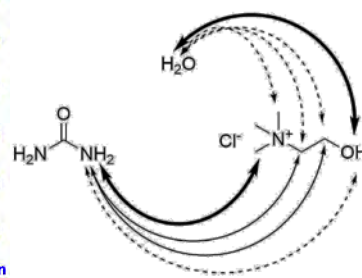
260 (a)



(b)



(c)

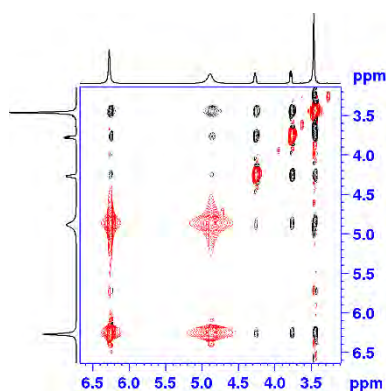


261

262 **Fig. 6** NOESY spectra of [Ch]Cl:U:W at 298 K and at several mixing times: (a) $t_{mix}=50$
263 ms, and (b) $t_{mix}=800$ ms; (c) H-H interactions representation.

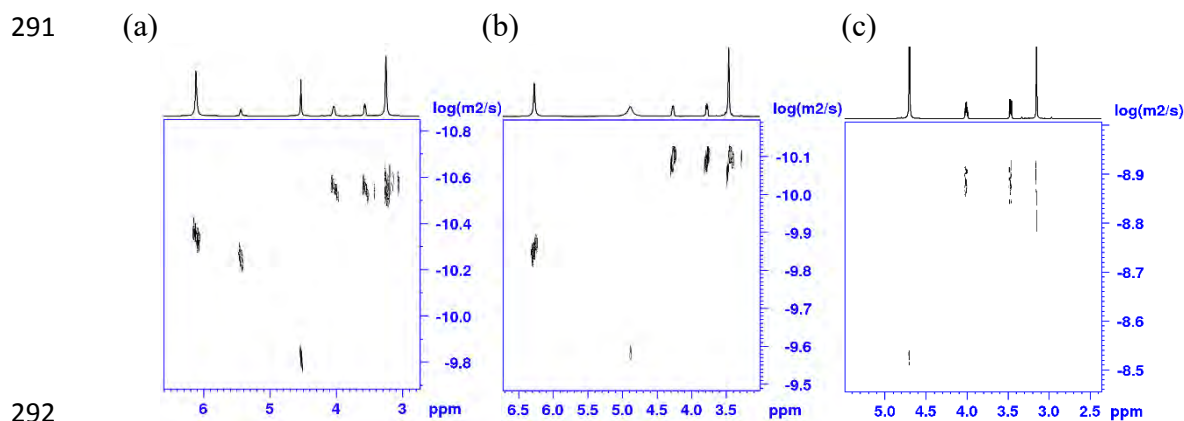
264 Sample diluted with 10% of D₂O showed a NOESY spectrum (Fig 7) very similar
265 to the pure sample, with positive nOe crosspeaks among all present signals (OH – Ch⁺
266 signal appears with HDO), indicating that remains the close contact between the species.
267 However, 90% diluted sample did not show any remarkable interaction apart those from
268 intramolecular protons.

269



270 Figure 7. NOESY spectrum of [Ch]Cl:U:W with 10% of D₂O at 298 K and with $t_{mix}=800$
271 ms.

272 Fig. 8 shows the results obtained with the DOSY technique. For this DES, four
273 different traces can be identified: Ch^+ , $\text{OH} - \text{Ch}^+$, U, and W (Fig. 8a). Again, in order to
274 calculate the self-diffusion coefficients, D , of the several species, we have fitted the
275 logarithm of the spin echo attenuation versus the gradient using the eq. (1). The values
276 obtained for $[\text{Ch}]\text{Cl}:\text{U}:\text{W}$ and those reported in the literature for the aqueous and non-
277 aqueous eutectics [25,26] are listed in Table 2. The addition of water to the binary eutectic
278 causes a difference of diffusivity between both type of hydrogens of the choline. Unlike
279 the study previously published by D'Agostino et al. [26], we have not detected
280 coalescence of the two peaks $\text{OH} - \text{Ch}^+$ and W. Moreover, the D coefficient of U is
281 slightly lower than that of $\text{OH} - \text{Ch}^+$ indicating a lower mobility due to a stronger
282 interaction with the neighboring molecules. The D coefficients calculated herein, except
283 for the $\text{OH} - \text{Ch}^+$, are in good agreement with those. Our values are slightly higher but it
284 is a logical result since the temperature is also slightly higher. Diffusion measurements
285 with 10% D_2O diluted sample (Fig. 8b) gave very similar results in self-diffusion
286 coefficients than the pure NADES, in the same order of magnitude. The unique value that
287 increases the most is the one corresponding to the amine hydrogens from urea, possible
288 due to the exchange with water protons. When sample diluted with 90% of D_2O , similar
289 data were obtained to $[\text{Ch}]\text{Cl}:\text{Gly}:\text{W}$ (90% D_2O) sample (Fig. 8c), consistent with a water
290 solution system.



293 Figure 8. DOSY experiments of [Ch]Cl:U:W at 298 K. (a) neat eutectic; (b) 10% of D₂O;
 294 and (c) 90% of D₂O.

295 Table 2. The self-diffusion coefficients, D , at 298 K of the several species in the non-
 296 aqueous and aqueous DES with urea.

| DES | $10^{11}D_{\text{Ch}^+}$ /m ² ·s ⁻¹ | $10^{11}D_{\text{OH}-\text{Ch}^+}$ /m ² ·s ⁻¹ | $10^{11}D_{\text{NH}_2}$ /m ² ·s ⁻¹ | $10^{11}D_{\text{H}_2\text{O}}$ /m ² ·s ⁻¹ |
|--------------------------------------|--|--|--|---|
| [Ch]Cl:U | 0.35 ^a | 0.35 ^a | 0.66 ^a | --- |
| [Ch]Cl:U:W | 2.50 | 4.49 | 4.07 | 10.2 |
| | 2 ^b | 10 ^b | 3 ^b | 10 ^b |
| [Ch]Cl:U:W (10% D ₂ O) | 7.57 | --- | 13.2 | 23.9 |
| [Ch]Cl:U:W (90% D ₂ O) | 82.35 | --- | 159.1 | 90.3 |

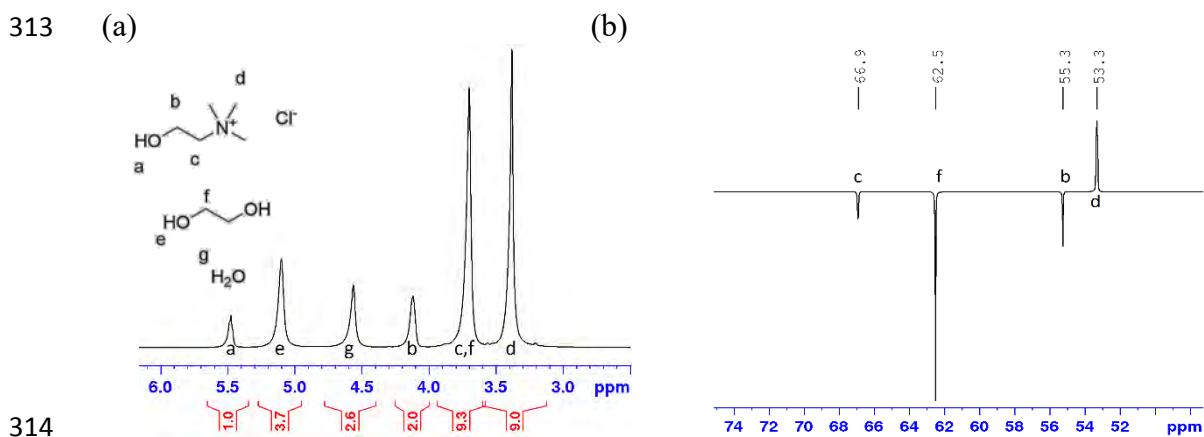
297 ^aD'Agostino et al. [25]; ^b D'Agostino et al. [26], $T=293$ K.

298 3.3 [Ch]Cl:EG:W

299 Figure 9a shows the ¹H-NMR spectrum of this DES. We can see that the peaks of
 300 the CH₂-N⁺ of the choline and the aliphatic hydrogens of the ethylene glycol are
 301 overlapped. The rest are separated and can be identified: δ 3.38 (s, 9 H, H_d), 3.70 (s, 10
 302 H, H_c, H_f), 4.12 (s, 2 H, H_b), 4.56 (s, 2 H, H_g), 5.10 (s, 4 H, H_e), 5.44-5.55 (m, 1 H, H_a).
 303 In the ¹³C-NMR spectrum (Fig. 9b), the signals are the expected: δ 54.1 (t, $J = 4.0$ Hz,
 304 C_d), 56.1 (C_b), 63.3 (C_f), 67.7 (t, $J = 3.0$ Hz, C_e). The molar ratio of the several
 305 components, choline chloride/ethylene glycol/water, is: 1:1.89:1.33 ($u(x)=0.05$). The
 306 calculated water content is 8.5% wt.

307 With 10% dilution of D₂O, a slight displacement of the ethylene signal respect to
 308 that of the CH₂-N⁺ is produced (Fig. S6a). Also, all mobile hydrogens (OH – Ch⁺, OH –

309 EG,W) are collapsed to a unique signal. With 90% dilution, the signals previously
 310 overlapped appear full separated and the mobile hydrogens coalesce to the HDO signal.
 311 Respect to the ^{13}C , the signals of the carbons attached to the oxygen are the most changing
 312 (Fig. S6b): $\Delta\delta$ (neat – diluted) = -0.3 C_b, -0.0 C_d, -0.1 C_c, -0.5 C_f.

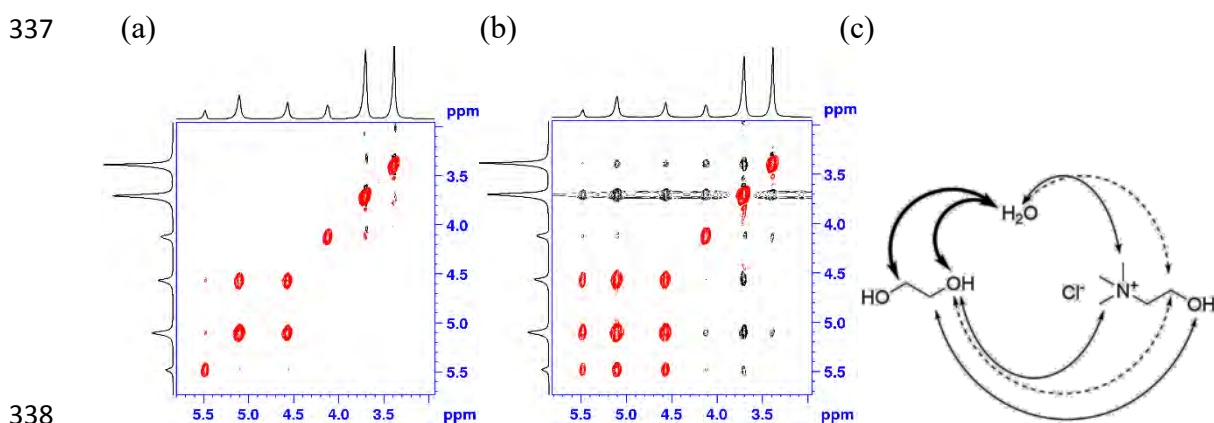


315 Figure 9. [Ch]Cl:EG:W spectra at 298 K: (a) ^1H -NMR (b) ^{13}C -NMR.

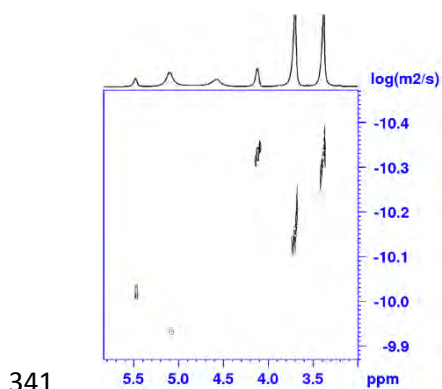
316 The NOESY spectrum at short t_{mix} (Fig. 10a) exhibits only exchange signals
 317 between the water and the hydroxyls of both choline and ethylene glycol; these latter are
 318 more intense. Increasing t_{mix} (Fig. S7), in addition to the exchange signals, we obtain
 319 positive nOes corresponding to both the intramolecular signals of the choline and the
 320 strong EG/W interaction. For $t_{mix} > 500$ ms (Fig. 10b), crossing peaks between all ^1H
 321 signals are observed. The intensity of hydrogen interactions is represented in Fig. 10c. To
 322 emphasize that contrary to the other studied eutectics, the donor specie in this mixture
 323 (ethylene glycol) is the one that presents stronger interactions, especially with water.
 324 NOESY spectra for the diluted samples did not afford any significant information, mainly
 325 due to the coalescence of the hydroxyl signals with HDO.

326 Fig. 11 shows the measured DOSY experiment for this DES. The self-diffusion
 327 constants calculated with eq. 1 (Table 3) show a clearly difference of mobility between

328 the several species, to except OH – EG and W which have similar values. This result is in
 329 accordance with the above conclusions and with those previously published [26]. From
 330 the overlapped signal for CH₂-N⁺ and EG, we have obtained an apparent coefficient,
 331 $D_{app}=6.83 \cdot 10^{-11}$ m²/s. Taking into account the calculated value of D_{Ch^+} in this DES
 332 (Table 3), we can deduce that D_{EG} will be significantly higher. Therefore, it can be
 333 concluded that a weak eutectic structure is established. As for the diluted samples the
 334 aliphatic hydrogens appear separated, it is possible to calculate the self-diffusion constant
 335 for each specie, however, all hydroxyl protons collapse to HDO signal, so no information
 336 can be obtained from them.



339 Figure 10. NOESY spectra of [Ch]Cl:EG:W at 298 K and at several mixing times: (a)
 340 $t_{mix}=50$ ms, and (b) $t_{mix}=800$ ms; (c) H-H interactions representation.



342 Figure 11. DOSY experiment of [Ch]Cl:EG:W at 298 K.

343 Table 3. The self-diffusion coefficients, D , at 298 K of the several species in the non-
 344 aqueous and aqueous DES with ethylene glycol.

| DES | $10^{11}D_{\text{Ch}^+}$ /m ² ·s ⁻¹ | $10^{11}D_{\text{OH-}\text{Ch}^+}$ /m ² ·s ⁻¹ | $10^{11}D_{\text{EG}}$ /m ² ·s ⁻¹ | $10^{11}D_{\text{OH-EG}}$ /m ² ·s ⁻¹ | $10^{11}D_{\text{H}_2\text{O}}$ /m ² ·s ⁻¹ |
|--|--|--|--|---|---|
| [Ch]Cl:Gly | 2.62 ^a | 2.62 ^a | 4.77 ^a | 4.77 ^a | --- |
| [Ch]Cl:Gly:W | 4.56 | 9.00 | --- | 10.7 | 10.3 |
| | 4 ^b | 7 ^b | 5.8 ^b | 9 ^b | 9 ^b |
| [Ch]Cl:Gly:W (10% D ₂ O) | 8.45 | --- | 12.6 | --- | 25.9 ^c |
| [Ch]Cl:Gly:W (90% D ₂ O) | 70.5 | --- | 85.4 | --- | 168.6 |

345 ^aD'Agostino et al. [25]; ^b D'Agostino et al. [26], $T= 293$ K. ^cApparent self-diffusion
 346 constant.

347 4. Conclusions

348 This paper has investigated the structure of three aqueous eutectics from several
 349 NMR experiments. The purpose of the current study was to assess the interactions
 350 between the species within the liquid as well as the role of the water in the mixtures. Also,
 351 we have calculated self-diffusion coefficients from DOSY spectra.

352 The composition of the studied eutectics has been: choline chloride, urea or glycerol
 353 or ethylene glycol, and water with a water content less to 10.5% wt. The results of this
 354 research support the idea that a supramolecular structure in these eutectic fluids is
 355 presented. For the eutectic with urea, the stronger interactions appear between the
 356 hydroxyl protons of the choline and the water followed by those between the methyl
 357 groups of the choline and the urea. No correlation urea/water is observed. For the second
 358 eutectic, interactions among all species are detected. Both the aliphatic and hydroxyl

359 hydrogens of the glycerol exhibit a significant correlation with the methyl groups of the
360 choline. The glycerol/water shows the weakest signal. Contrary to the previous mixtures,
361 the strongest interactions in the eutectic formed by ethylene glycol are those established
362 between the donor component and water. This eutectic has the feeblest structure. Finally,
363 we have found that these ternary solvents still retain the eutectic character when are
364 diluted at 10% wt.

365 **Supplementary data**

366 Supplementary data to this article can be found in version online.

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371 **References**

- 372 [1] M. Vert, Y. Doi, K. Hellwich, M. Hess, P. Hodge, P. Kubisa, M. Rinaudo, F.
373 Schué, Terminology for biorelated polymers and applications (IUPAC
374 Recommendations 2012)*, 84 (2012) 377–410.
- 375 [2] F.G. Calvo-Flores, M.J. Monteagudo-Arrebola, J.A. Dobado, J. Isac-García,
376 Green and Bio-Based Solvents, Top. Curr. Chem. 376 (2018) 1–40.
377 doi:10.1007/s41061-018-0191-6.
- 378 [3] C.J. Clarke, W.C. Tu, O. Levers, A. Bröhl, J.P. Hallett, Green and Sustainable
379 Solvents in Chemical Processes, Chem. Rev. 118 (2018) 747–800.
380 doi:10.1021/acs.chemrev.7b00571.

- 381 [4] M. Francisco, A. Van Den Bruinhorst, M.C. Kroon, Low-transition-temperature
382 mixtures (LTTMs): A new generation of designer solvents, *Angew. Chemie - Int.*
383 *Ed.* 52 (2013) 3074–3085. doi:10.1002/anie.201207548.
- 384 [5] E. Durand, J. Lecomte, P. Villeneuve, From green chemistry to nature: The
385 versatile role of low transition temperature mixtures, *Biochimie.* 120 (2016) 119–
386 123. doi:10.1016/j.biochi.2015.09.019.
- 387 [6] B. Kudłak, K. Owczarek, J. Namieśnik, Selected issues related to the toxicity of
388 ionic liquids and deep eutectic solvents—a review, *Environ. Sci. Pollut. Res.* 22
389 (2015) 11975–11992. doi:10.1007/s11356-015-4794-y.
- 390 [7] S.P.F. Costa, A.M.O. Azevedo, P.C.A.G. Pinto, M.L.M.F.S. Saraiva,
391 Environmental Impact of Ionic Liquids: Recent Advances in (Eco)toxicology and
392 (Bio)degradability, *ChemSusChem.* 10 (2017) 2321–2347.
393 doi:10.1002/cssc.201700261.
- 394 [8] A.P. Abbott, D. Boothby, G. Capper, D.L. Davies, R.K. Rasheed, Deep Eutectic
395 Solvents formed between choline chloride and carboxylic acids: Versatile
396 alternatives to ionic liquids, *J. Am. Chem. Soc.* 126 (2004) 9142–9147.
397 doi:10.1021/ja048266j.
- 398 [9] F. Pena-Pereira, A. Kloskowski, J. Namieśnik, Perspectives on the replacement
399 of harmful organic solvents in analytical methodologies: A framework toward the
400 implementation of a generation of eco-friendly alternatives, *Green Chem.* 17
401 (2015) 3687–3705. doi:10.1039/c5gc00611b.
- 402 [10] M. Espino, M. de los Ángeles Fernández, F.J.V. Gomez, M.F. Silva, Natural
403 designer solvents for greening analytical chemistry, *TrAC - Trends Anal. Chem.*

- 404 76 (2016) 126–136. doi:10.1016/j.trac.2015.11.006.
- 405 [11] D.J.G.P. Van Osch, L.F. Zubeir, A. Van Den Bruinhorst, M.A.A. Rocha, M.C.
406 Kroon, Hydrophobic Deep Eutectic Solvents : Water- Immiscible Extractants,
407 (2015) 1–36. doi:10.1039/C5GC01451D.
- 408 [12] B.D. Ribeiro, C. Florindo, L.C. Iff, M.A.Z. Coelho, I.M. Marrucho, Menthol-
409 based eutectic mixtures: Hydrophobic low viscosity solvents, ACS Sustain.
410 Chem. Eng. 3 (2015) 2469–2477. doi:10.1021/acssuschemeng.5b00532.
- 411 [13] M.A.R. Martins, E.A. Crespo, P.V.A. Pontes, L.P. Silva, M. Bülow, G.J.
412 Maximo, E.A.C. Batista, C. Held, S.P. Pinho, J.A.P. Coutinho, Tunable
413 Hydrophobic Eutectic Solvents Based on Terpenes and Monocarboxylic Acids,
414 ACS Sustain. Chem. Eng. 6 (2018) 8836–8846.
415 doi:10.1021/acssuschemeng.8b01203.
- 416 [14] Y.H. Choi, J. van Spronsen, Y. Dai, M. Verberne, F. Hollmann, I.W.C.E. Arends,
417 G.-J. Witkamp, R. Verpoorte, Are Natural Deep Eutectic Solvents the Missing
418 Link in Understanding Cellular Metabolism and Physiology?, Plant Physiol. 156
419 (2011) 1701–1705. doi:10.1104/pp.111.178426.
- 420 [15] I.M. Aroso, R. Craveiro, Â. Rocha, M. Dionísio, S. Barreiros, R.L. Reis, A.
421 Paiva, A.R.C. Duarte, Design of controlled release systems for THEDES—
422 Therapeutic deep eutectic solvents, using supercritical fluid technology, Int. J.
423 Pharm. 492 (2015) 73–79. doi:10.1016/j.ijpharm.2015.06.038.
- 424 [16] Y. Dai, J. van Spronsen, G.J. Witkamp, R. Verpoorte, Y.H. Choi, Natural deep
425 eutectic solvents as new potential media for green technology, Anal. Chim. Acta.
426 766 (2013) 61–68. doi:10.1016/j.aca.2012.12.019.

- 427 [17] M. Hayyan, M.A. Hashim, A. Hayyan, M.A. Al-Saadi, I.M. AlNashef, M.E.S.
428 Mirghani, O.K. Saheed, Are deep eutectic solvents benign or toxic?,
429 Chemosphere. 90 (2013) 2193–2195. doi:10.1016/j.chemosphere.2012.11.004.
- 430 [18] B. Zhao, P. Xu, F. Yang, H. Wu, M. Zong, W. Lou, Biocompatible Deep Eutectic
431 Solvents Based on Choline Chloride: Characterization and Application to the
432 Extraction of Rutin from *Sophora japonica*, (2015).
433 doi:10.1021/acssuschemeng.5b00619.
- 434 [19] P. De Moraes, F. Gonçalves, J.A.P. Coutinho, S.P.M. Ventura, Ecotoxicity of
435 Cholinium-Based Deep Eutectic Solvents, ACS Sustain. Chem. Eng. 3 (2015)
436 3398–3404. doi:10.1021/acssuschemeng.5b01124.
- 437 [20] K.S. Egorova, V.P. Ananikov, Fundamental importance of ionic interactions in
438 the liquid phase: A review of recent studies of ionic liquids in biomedical and
439 pharmaceutical applications, J. Mol. Liq. 272 (2018) 271–300.
440 doi:10.1016/j.molliq.2018.09.025.
- 441 [21] Y.P. Mbous, M. Hayyan, W.F. Wong, C.Y. Looi, M.A. Hashim, Unraveling the
442 cytotoxicity and metabolic pathways of binary natural deep eutectic solvent
443 systems, Sci. Rep. 7 (2017) 1–14. doi:10.1038/srep41257.
- 444 [22] H. Shekaari, M.T. Zafarani-Moattar, B. Mohammadi, Thermophysical
445 characterization of aqueous deep eutectic solvent (choline chloride/urea)
446 solutions in full ranges of concentration at $T = (293.15–323.15)$ K, J. Mol. Liq.
447 243 (2017) 451–461. doi:10.1016/j.molliq.2017.08.051.
- 448 [23] D. Lapeña, L. Lomba, M. Artal, C. Lafuente, B. Giner, The NADES glyceline as
449 a potential Green Solvent: A comprehensive study of its thermophysical

- 450 properties and effect of water inclusion, *J. Chem. Thermodyn.* 128 (2019) 164–
451 172. doi:10.1016/j.jct.2018.07.031.
- 452 [24] Y. Chen, D. Yu, W. Chen, L. Fu, T. Mu, Water absorption by deep eutectic
453 solvents, *Phys. Chem. Chem. Phys.* 21 (2019) 2601–2610.
454 doi:10.1039/C8CP07383J.
- 455 [25] C. D’Agostino, R.C. Harris, A.P. Abbott, L.F. Gladden, M.D. Mantle, Molecular
456 motion and ion diffusion in choline chloride based deep eutectic solvents studied
457 by ¹H pulsed field gradient NMR spectroscopy, *Phys. Chem. Chem. Phys.* 13
458 (2011) 21383–21391. doi:10.1039/c1cp22554e.
- 459 [26] C. D’Agostino, L.F. Gladden, M.D. Mantle, A.P. Abbott, E.I. Ahmed, A.Y.M.
460 Al-Murshedi, R.C. Harris, Molecular and ionic diffusion in aqueous-deep
461 eutectic solvent mixtures: Probing inter-molecular interactions using PFG NMR,
462 *Phys. Chem. Chem. Phys.* 17 (2015) 15297–15304. doi:10.1039/c5cp01493j.
- 463 [27] E. Posada, N. López-Salas, R.J. Jiménez Riobóo, M.L. Ferrer, M.C. Gutiérrez, F.
464 del Monte, Reline aqueous solutions behaving as liquid mixtures of H-bonded
465 co-solvents: microphase segregation and formation of co-continuous structures as
466 indicated by Brillouin and ¹H NMR spectroscopies, *Phys. Chem. Chem. Phys.*
467 19 (2017) 17103–17110. doi:10.1039/C7CP02180A.
- 468 [28] O.S. Hammond, D.T. Bowron, K.J. Edler, The Effect of Water upon Deep
469 Eutectic Solvent Nanostructure: An Unusual Transition from Ionic Mixture to
470 Aqueous Solution, *Angew. Chemie - Int. Ed.* 56 (2017) 9782–9785.
471 doi:10.1002/anie.201702486.
- 472 [29] D. Shah, F.S. Mjalli, Effect of water on the thermo-physical properties of Reline:

473 An experimental and molecular simulation based approach, *Phys. Chem. Chem.*
474 *Phys.* 16 (2014) 23900–23907. doi:10.1039/c4cp02600d.

475 [30] T. Zhekenov, N. Toksanbayev, Z. Kazakbayeva, D. Shah, F.S. Mjalli, Formation
476 of type III Deep Eutectic Solvents and effect of water on their intermolecular
477 interactions, *Fluid Phase Equilib.* 441 (2017) 43–48.
478 doi:10.1016/j.fluid.2017.01.022.

479 [31] R. Stefanovic, M. Ludwig, G.B. Webber, R. Atkin, A.J. Page, Nanostructure,
480 hydrogen bonding and rheology in choline chloride deep eutectic solvents as a
481 function of the hydrogen bond donor, *Phys. Chem. Chem. Phys.* 19 (2017) 3297–
482 3306. doi:10.1039/C6CP07932F.

483 [32] M. Francisco, A. Van Den Bruinhorst, M.C. Kroon, New natural and renewable
484 low transition temperature mixtures (LTTMs): Screening as solvents for
485 lignocellulosic biomass processing, *Green Chem.* 14 (2012) 2153–2157.
486 doi:10.1039/c2gc35660k.

487 [33] C. Florindo, F.S. Oliveira, L.P.N. Rebelo, A.M. Fernandes, I.M. Marrucho,
488 Insights into the synthesis and properties of deep eutectic solvents based on
489 cholinium chloride and carboxylic acids, *ACS Sustain. Chem. Eng.* 2 (2014)
490 2416–2425. doi:10.1021/sc500439w.

491 [34] J. Andanson, M. Traïkia, P. Husson, Ionic association and interactions in aqueous
492 methylsulfate alkyl-imidazolium-based ionic liquids, *J. Chem. Thermodyn.* 77
493 (2014) 214–221. doi:10.1016/j.jct.2014.01.031.

494

SUPPLEMENTARY MATERIAL

NMR study of choline chloride-based deep eutectic solvents

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Table S1. Characteristics of the studied DESs. Nomenclature used in this paper, composition, molar ratio, and calculated water content (mass percentage).

| DES abbreviation | Component 1(HBA) | Component 2(HBD) | Component 3 | Molar ratio ^a | Water content /(wt%) |
|------------------|------------------|------------------|-------------|--------------------------|----------------------|
| [Ch]Cl:Gly:W | Choline chloride | Glycerol | Water | 1:1.96:0.97 | 5.3 |
| [Ch]Cl:U:W | Choline chloride | Urea | Water | 1:1.95:1.67 | 10.5 |
| [Ch]Cl:EG:W | Choline chloride | Ethylene Glycol | Water | 1:1.89:1.33 | 8.5 |

^a NMR characterization; standard uncertainty, $u(x) = 0.05$. The compositions are in agreement with those calculated from the quantities weighed in and from Karl-Fischer method.

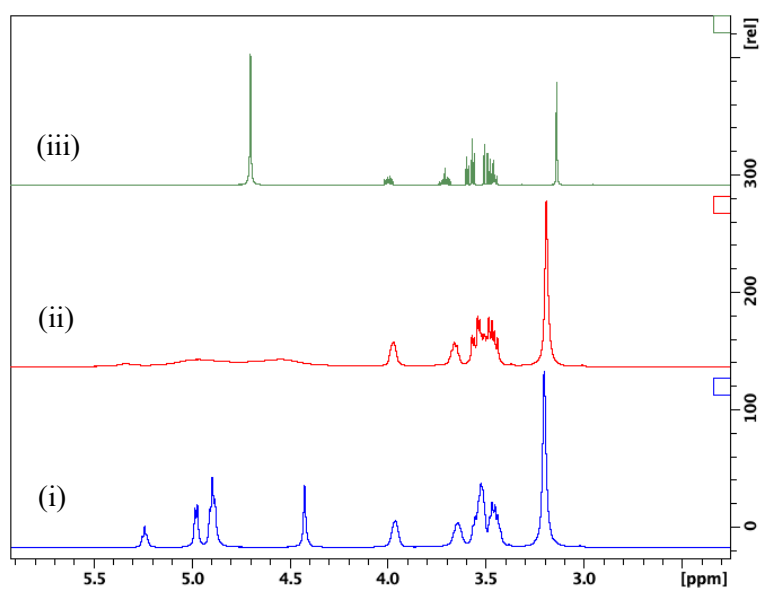


Figure S1a. ^1H -NMR 400 MHz spectra of [Ch]Cl:Gly:W at 298 K. i) neat eutectic; ii) 10 % D_2O ; iii) 90 % D_2O .

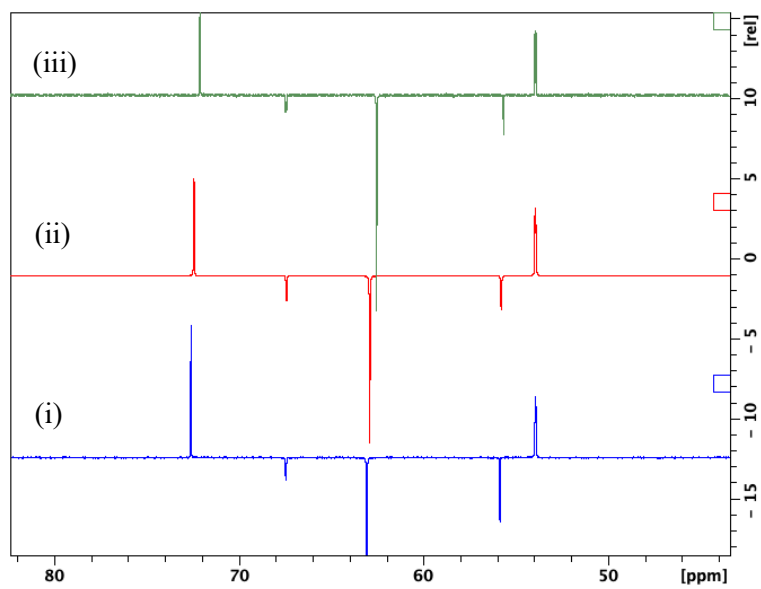


Figure S1b. ^{13}C spectra of [Ch]Cl:Gly:W at 298 K. i) neat eutectic; ii) 10 % D_2O ; iii) 90 % D_2O .

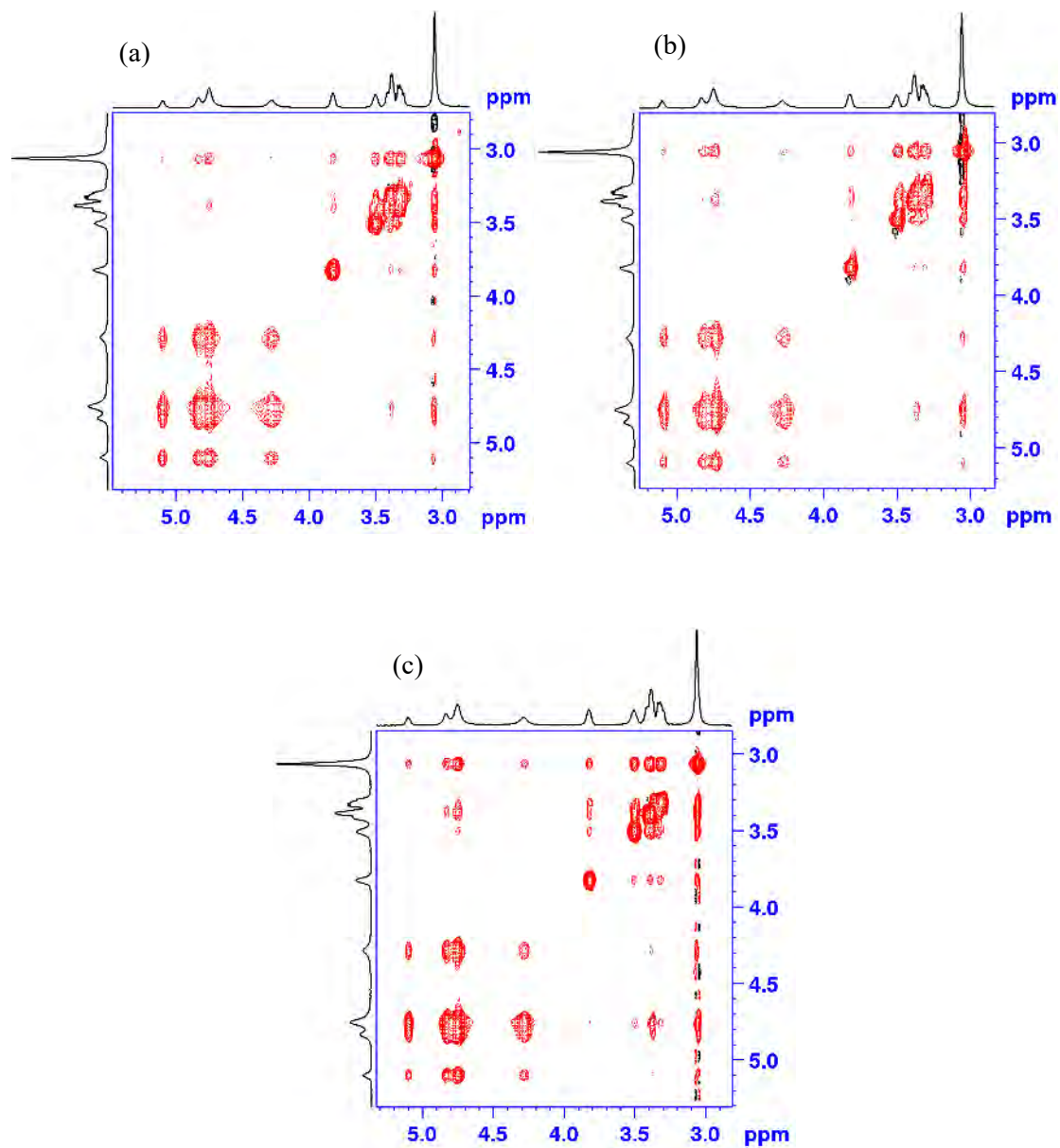


Figure S2. NOESY spectra of [Ch]Cl:Gly:W at 298 K and at several mixing times: (a) t_{mix} = 200 ms; (b) t_{mix} = 500 ms; (c) t_{mix} = 800 ms, spectrum made six months after the previous.

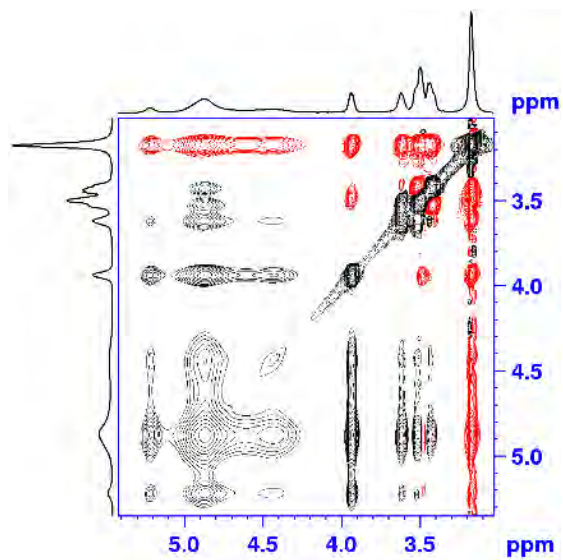


Figure S3. ROESY spectra of [Ch]Cl:Gly:W at 298 K.

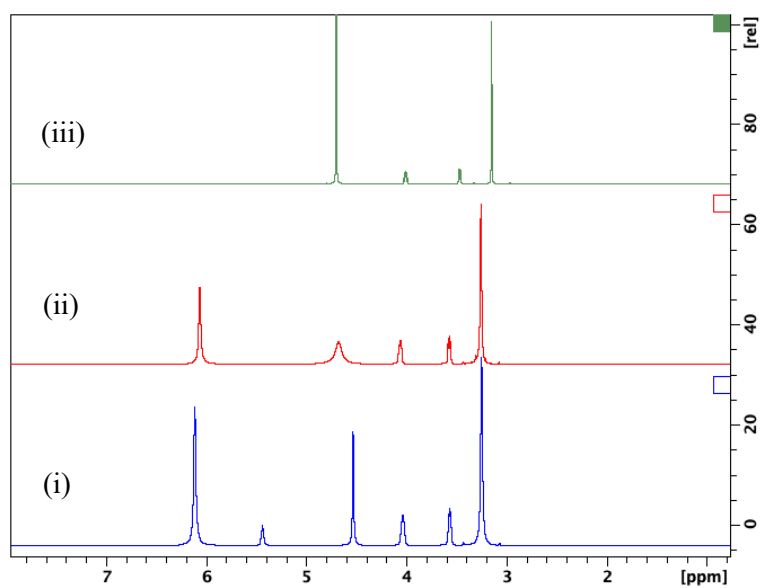


Figure S4a. ^1H -NMR 400 MHz spectra of [Ch]Cl:U:W at 298 K. i) neat eutectic; ii) 10 % D_2O ; iii) 90 % D_2O .

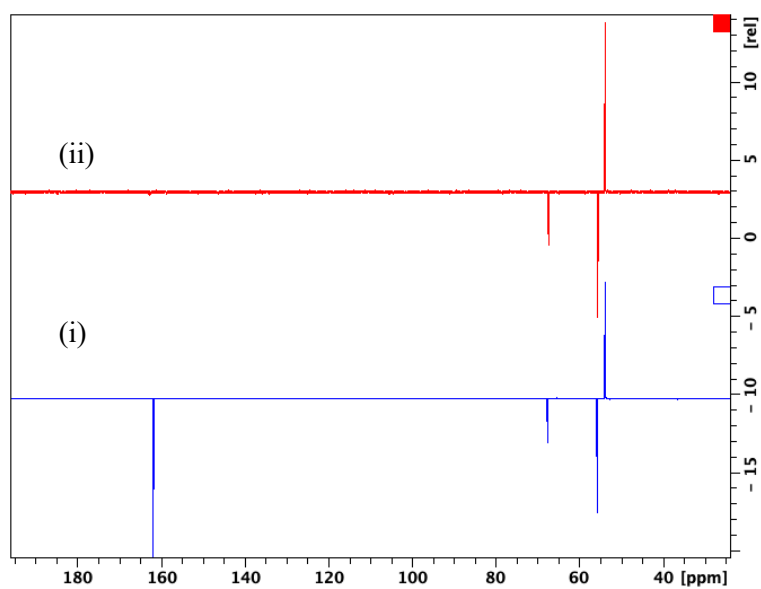


Figure S4b. ^{13}C spectra of [Ch]Cl:U:W at 298 K. i) neat eutectic; ii) 90 % D_2O .

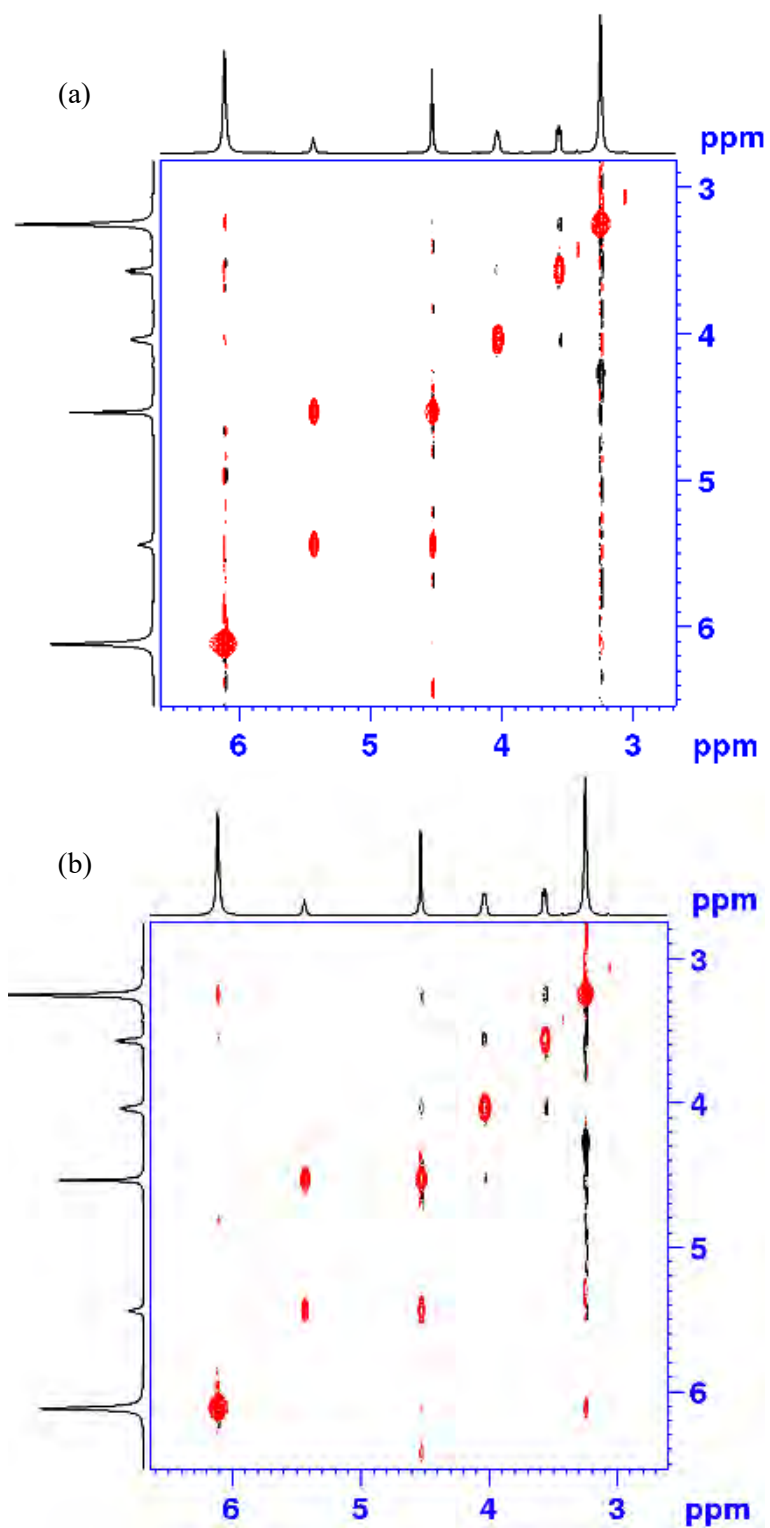


Figure S5. NOESY spectra of [Ch]Cl:U:W at 298 K and at several mixing times: (a) t_{mix} = 200 ms; and (b) t_{mix} = 500 ms.

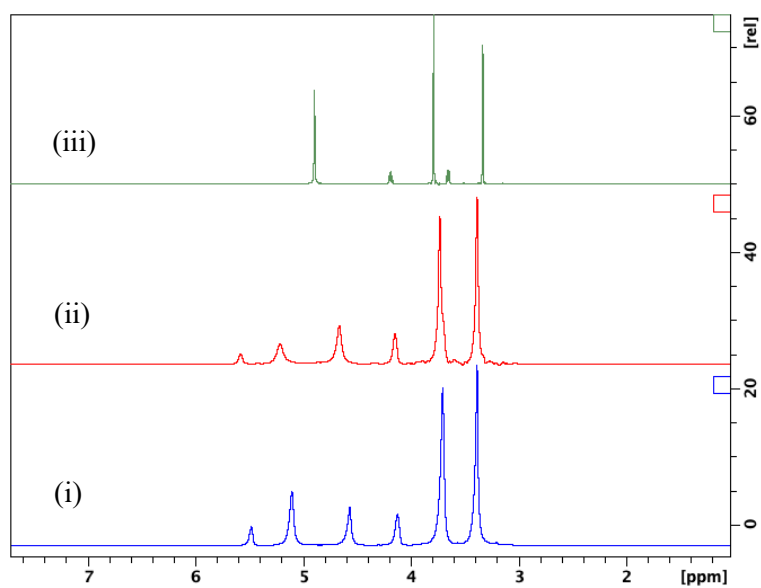


Figure S6a. ^1H -NMR 400 MHz spectra of [Ch]Cl:EG:W at 298 K. i) neat eutectic; ii) 10 % D_2O ; iii) 90 % D_2O .

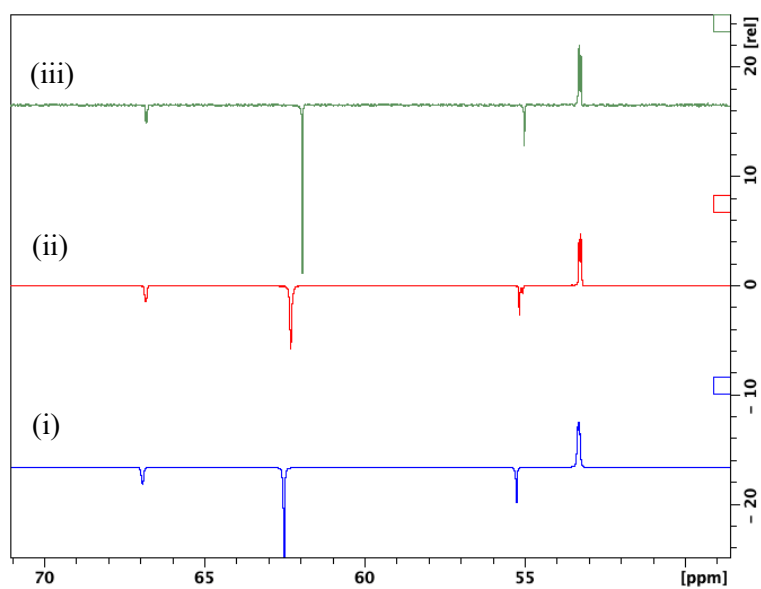


Figure S6b. ^{13}C spectra of [Ch]Cl:EG:W at 298 K. a) neat eutectic; b) 10 % D_2O ; c) 90 % D_2O .

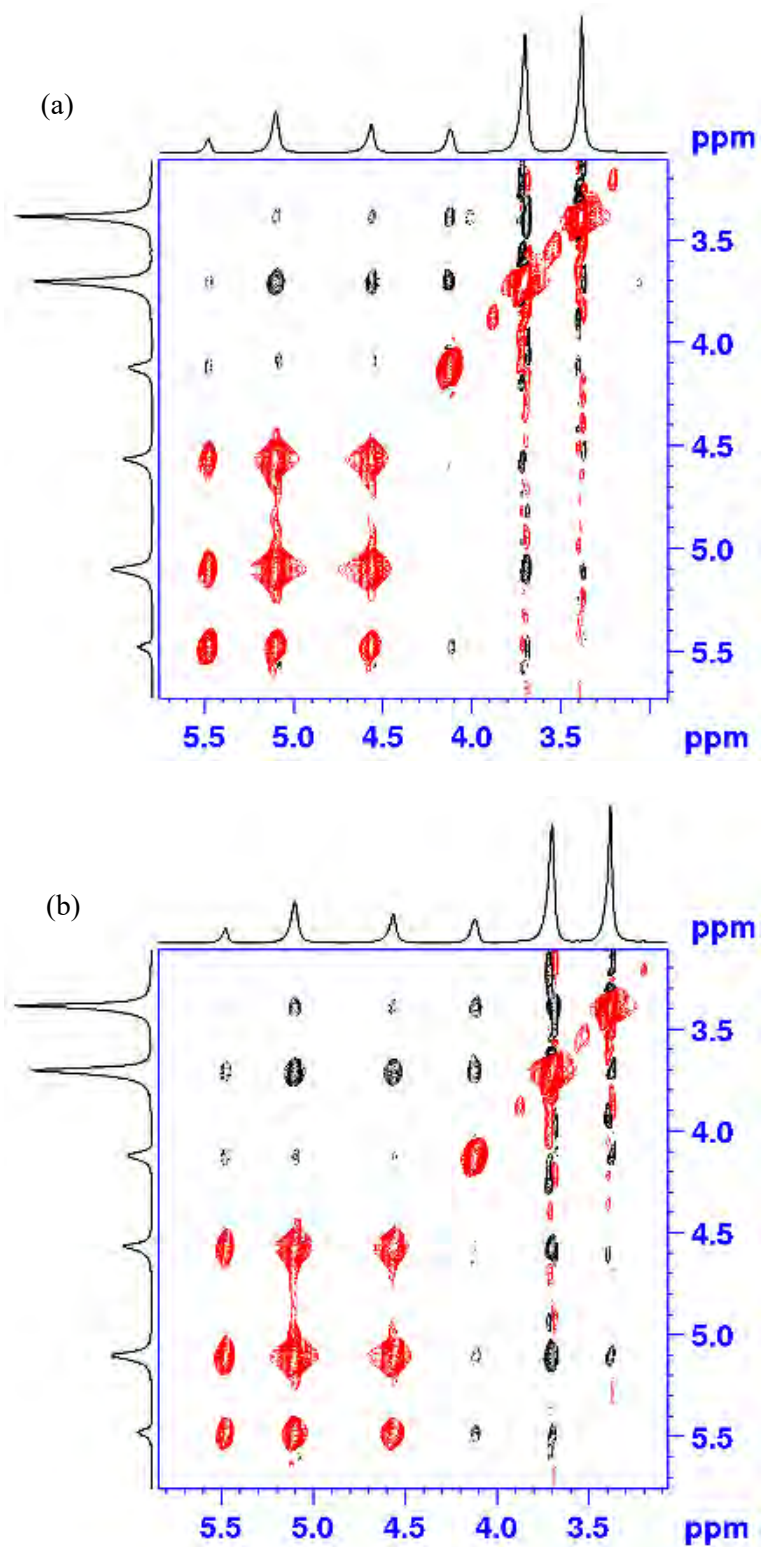


Figure S7. NOESY spectra of [Ch]Cl:EG:W at 298 K and at several mixing times: (a)

t_{mix} = 200 ms; and (b) t_{mix} = 500 ms.