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Dependence of ^{1}H-NMR T_1 relaxation time of trimethylglycine betaine deep eutectic solvents on the molar composition and on the presence of water†‡

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^{1}H-NMR spin lattice relaxation times (T_1), measured by inversion recovery technique, allowed to establish the stoichiometric coefficient (ratio between the H-bond acceptor and H-bond donor) of a series of trimethylglycine betaine/diol based deep eutectic solvents (DESs); ethylene glycol, triethylene glycol and 1,3-propandiol were selected as H-bond donors. The maximum amount of water tolerated by the DES, before its complete hydration, was determined as well. Finally, the method was validated comparing the eutectic composition of the betaine/glycol system with that determined by means of differential scanning calorimetry analysis; the stoichiometric coefficients were identical.

The term eutectic was coined by Guthrie in 1884 from the old Greek *eutecticos*, easy meltdown, to describe the melting point depression of a mixture with respect to its pure components.[1] The term deep eutectic solvent (DES) was used for the first time in the seminal work of Abbott in 2003,[2] where the adjective "deep" was meant to describe a much lower melting temperature than the ideal mixture of its components, typically two. Ever since many others DESs were discovered, and nowadays this new class of solvents has become extremely popular, especially in green chemistry. Later in 2011, Choi introduced the term natural DES (NADES), to describe the so called third liquid phase in microbial mammalian and plant cells, besides water phase and membrane lipids phase.[3,4]

Even though the scientific community is still debating on a full comprehension of the nature of these solvents,[5] during the last decades, the number of DES-based applications in a wide range of fields has rapidly grown.[6–9]

In addition to their melting/freezing point, these solvents are characterized by many other physicochemical properties such as viscosity, density, ionic conductivity, surface tension, vapour pressure and refractive index.[10] Some of these observables (ionic conductivity, solvent polarity and viscosity) vary significantly with the molar composition of the mixture.[10,11] Noteworthy, very recently, it was found that the presence of small amounts of water (embedded water) might play an important role in promoting the DES formation.[12]

At nanoscopic level, the combination of a hydrogen bond acceptor HBA (usually solid) with a hydrogen bond donor HBD (either liquid or solid) leads to the formation of an HBA···HBD supramolecule, self-assembled by means of a cluster of H-bonds, and accordingly the mixture becomes liquid at eutectic temperature only at a *definite* HBA/HBD molar ratio (eutectic composition). However, it is not rare to find in literature different stoichiometric coefficients for the same DES. For instance, the formation of trimethylglycine betaine/ethylene glycol DES has been reported with three different stoichiometries: the ratio of 1 : 2 was used for the biocatalyzed synthesis of phospholipids in water,[13] whereas for the purification of gasoline a ratio of 1 : 3 was preferred,[14] the same composition was later found by Paiva,[15] instead, for the extraction of palmitic acid from palm oil, a mixture with more glycol (1 : 4) was applied.[16] Lastly, it is not always clear which is the maximum

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† The trimethylglycine betaine and diols were used as received by the suppliers without any further treatment. Trimethylglycine betaine (1.17 g, 1.0 mmol) was mixed with diol (see the ESI‡ for the molar ratio) and stirred at 75 °C until the reaction mixture appeared completely homogeneous, then, it was left to stir for others 2 hours. The freshly prepared mixtures were submitted to NMR analysis. All NMR experiments were carried out on an Avance 400 Bruker instrument at 75 °C or at 29 °C, using an automation routine (IconNMR software). ^{1}H-NMR T_1 relaxation times were measured using the Bruker library inversion recovery pulse program (Topspin software, version 2.5). D_2O was used as external lock in coaxial tube (5 mm), and the chemical shift calibration was done on the HDO residual signal (δ = 4.71 ppm). Acquisition and processing parameters: number of scans = 1; relaxation delay $d1$ = 20 s; dummy scans ds = 2; variable delay list (s): 0.1, 0.2, 0.3, 0.5, 0.7, 1.0, 1.4, 1.8, 2.3, 2.8, 3.4, 4.1, 5.0, 7.0, 9.0, 12.0, 16.0, 20.0; line broadening lb = 2 Hz. T_1 fittings were obtained using Dynamic Center software, version 2.7.4 (see ESI‡).

‡ Electronic supplementary information (ESI) available: Copies of ^{1}H-NMR spectra and T_1 fitting. See DOI: https://doi.org/10.1039/d2ra08082f

amount of water tolerated before that the H-bond disruption of the supramolecule occurs, in any case, the water added, typically does not exceed the 30–50% in weight.[17]

Prompted by the above considerations, the following study aims: (i) to verify if and how the ^{1}H-NMR spin-lattice relaxation time (T_1) of a set of selected DESs changes with the HBD molar fraction (X), and (ii) to ascertain how such a suggested correlation might be modified by progressive additions of water.

Our choice of the longitudinal relaxation time constant T_1 as an observable potentially informative of DES-supramolecule formation is based on its well-known dependence on the molecular tumbling motion (random molecular rotations and diffusion movements) in a liquid.[18,19] Thus, it is reasonable to think that larger and more rigid is an organic molecule, slower is its tumbling in solution. Indeed, without going too much in details of the complex spin-lattice relaxation theory, T_1 depends, among many other variables, on the molecular size and therefore on the molecular weight. Now, such relationship hints our tentative conjecture that the DES-supramolecule formed at the eutectic point might be characterized by a lower tumbling rate than its HBD component and conceivably by a specific value of T_1.

In the frame of our ongoing research work[13] we focused our attention on the trimethylglycine betaine/diol based DESs. In Fig. 1 the selected HBD diols to be combined with the HBA trimethylglycine betaine (**Gb**) are shown: ethylene glycol (**D1**), triethylene glycol (**D2**) and 1,3-propandiol (**D3**), for more details on sample's preparation see the experimental part. However, the moisture content of diols was checked by Karl Fischer titration (<1.5% in weight) before mixing with Gb.

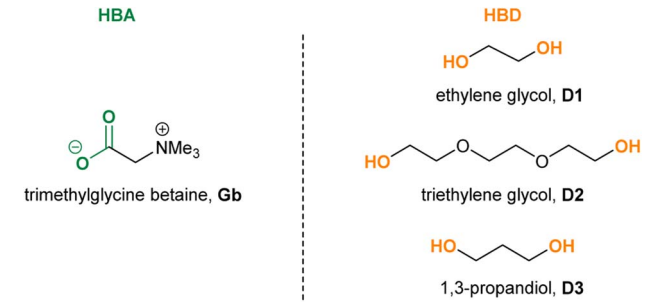

Fig. 1 Components of trimethylglycine betaine-based DESs.

The T_1 were measured by inversion recovery method at 75 °C, to avoid detrimental effects of viscosity on the linewidth (*i.e.*, spectral resolution) of the ^{1}H-NMR spectra. In addition, at this temperature we could widen the number of observable HBA/HBD mixtures, especially those with molar fraction X (X = [diol]/([diol] + [Gb])) lower than the eutectic mixture (*infra* eutectic region), which otherwise at room temperature would precipitate.

In Fig. 2a we show the ^{1}H-NMR spectra of **Gb**/**D1** DES (molar ratio 1 : 3, at 75 °C, external lock on D_2O in a coaxial tube). The OH resonance (broad singlet, chemical shift δ = 5.53 ppm) is

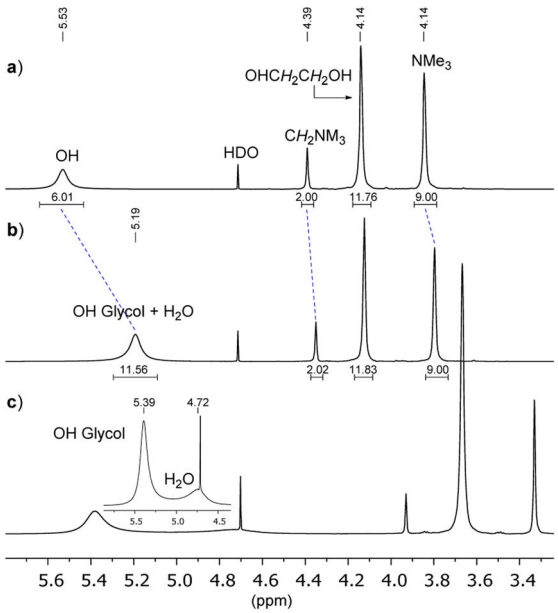

Fig. 2 ^{1}H-NMR spectra (400 MHz) of trimethylglycine betaine/ethylene glycol DES (1 : 3, $X_{D1} \approx 0.75$), the sample was externally locked using D_2O in a coaxial insert tube, the chemical shift calibration was done with respect to HDO (δ = 4.71 ppm, residual signal of D_2O), and a recovery delay d = 20 s was applied: (a) DES at 75 °C; (b) sample (a) plus 3 eq. of H_2O with respect to HBA betaine; (c) sample (a) plus few drops of water at 29 °C.

clearly well separated from the other signals, moreover, using a long relaxation delay (d = 20 s), the integrals result fully consistent with the ethylene glycol molar fraction of the mixture ($X_{D1} \approx 0.75$). Then, by adding 3 eq. of water to the freshly prepared DES, the OH signal appears significantly shielded (δ from 5.53 to 5.19 ppm) indicating a partial disruption of the H-bond self-assembled supramolecule (Fig. 2b), whereas the chemical shift of the other signals did not change so much.

Since both OH protons of DES and of water at 75 °C are under rapid chemical exchange, the observed chemical shift is the weighted average of the chemical shifts of the two species. Indeed, by lowering the temperature at 29 °C it was possible to discriminate the different nature of OHs (5.39 ppm *vs.* 4.72 ppm, Fig. 2c).

Now, to begin with aim (i), the measured T_1 of OH proton in **Gb**/**D1** mixtures appears to change with the molar fraction (X_{D1}) as shown in Fig. 3a. Indeed, the diagram T_1 *versus* X_{D1} exhibits a minimum (T_1 = 1.26 s) in correspondence of the molar composition **Gb**/**D1** = 1 : 3 ($X_{D1} \approx 0.75$), somehow reminding the customary solid–liquid phase diagram of binary eutectic mixtures. However, this point should correspond to the formation of the $Gb(D1)_3$ supramolecule, in which the lowest mobility of OH is most likely due to the formation of stronger H-bonds in the newly self-assembled supramolecule. Besides, in the *ultra*-eutectic region, the relaxation time increases linearly as the X_{D1} *increases* (linear regression equation: $T_1 = -2.46 + 5.0\ X_{D1}$, R^2 = 0.98) reaching the upper value of neat D1 (technical glycol T_1 = 2.63 s *vs.* T_1 = 2.11 s of pure and anhydrous glycol,[20]

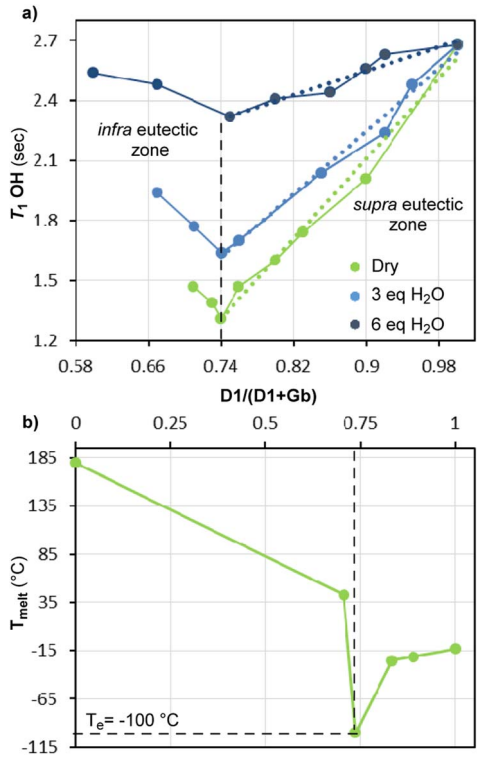

Fig. 3 (a) Plot of OH T_1 (measured with external lock) *vs.* X_{D1} at 75 °C for the **Gb**/**D1** system, using commercial ethylene glycol (H_2O < 1.5% w/w). Green solid line: mixtures without addition of water; blue solid line: addition of 3 eq. of H_2O with respect to **Gb**; blue-navy solid line: addition of 6 eq. of H_2O with respect to **Gb**. The dashed lines correspond to the linear regression fitting. (b) DSC analysis: plot of melting point T (°C) *vs.* X_{D1}, without addition of water.

purity ≥99.8%, the two values are different likely for the moisture, lit.[21] H_2O T_1 = 9.11 s at 75 °C).

As initial approximation, we reckon that the T_1 observed in this region of the diagram is the molar average of the eutectic point and neat glycol values. However, quite recently, it was shown by IR and Raman spectroscopy that ethylene glycol is in equilibrium with its H-bond self-assembled dimer,[22] thus it is not unreasonable to think that the measured T_1 might be arise also from other supramolecular species present in solution.

On the other hand, in the *infra*-eutectic region of the diagram, the relaxation time becomes longer as X_{D1} *decreases*. However, since the leftmost point of this region was determined from a mixture with $X_{D1} \approx 0.71$, not much different from that of the eutectic composition ($X_{D1} \approx 0.75$), the correlation between the small increments of T_1 (from 1.26 s to 1.42 s) and the progressive dissociation of DES supramolecule was not reliable.

Finally, the 1 : 3 eutectic stoichiometry found by OH T_1 measurements (data in agreement with that reported by Paiva[15]) was confirmed by differential scanning calorimetry (DSC) analysis (Fig. 3b), and a eutectic temperature (T_e) of −100 °C was determined.

Intrigued by the net influence of DES formation on the OH bonding donor mobility, the relaxation times of the remaining proton signals were analysed as well (see ESI‡); however, the variations of T_1 were not anymore significantly traceable to a neat formation of $Gb(D1)_3$, mainly for two reasons: (i) these hydrogens are not involved in strong non-covalent interactions and therefore their mobility is less influenced by the formation of the H-bond self-assembled supramolecule; (ii) the chemical shift of these signals may change with X, and in some mixtures partial overlap of signals occurred, making the T_1 measure less accurate and reliable, and consequentially not anymore diagnostic of DES formation.

Concerning aim (ii), the relaxation time measurements of the **Gb**/**D1** mixtures were repeated on samples containing three and then six equivalents of water with respect to the HBA Gb (Fig. 3a, blue and blue-navy solid lines, respectively). The T_1 diagrams of the wet mixtures have a similar shape to that of the "dry" system (H_2O < 1.5% in weight), but there are two discernible differences. First, by adding H_2O the T_1 value at the eutectic point becomes longer, in agreement with recently published studies,[23] secondly, the slopes of both regions of the diagram decrease remarkably (for instance in the *ultra*-eutectic region $T_1 = 1.35 + 3.95\ X_{D1}$ with 3 eq. of H_2O and $T_1 = 1.14 + 1.51\ X_{D1}$ with 6 eq. of H_2O, $R^2 = 0.98$ and $R^2 = 0.94$, respectively).

These observations suggest that the addition of water has the beneficial effect of reducing the viscosity, while the eutecticity of the system is partially conserved; however, it is conceivable that higher concentrations of water promote the complete dissociation of the eutectic supramolecule $Gb(D1)_3$ in the individual hydrated components.

Lastly, we repeated the T_1 measurements for the **Gb**/**D2** and **Gb**/**D3** mixtures (Fig. 4a and b), and analogously to that seen for the **Gb**/**D1** system, the OH mobility of each diol decreased linearly to a minimum value in the correspondence of the eutectic point. More precisely, for the **Gb**/**D2** system the T_1 of

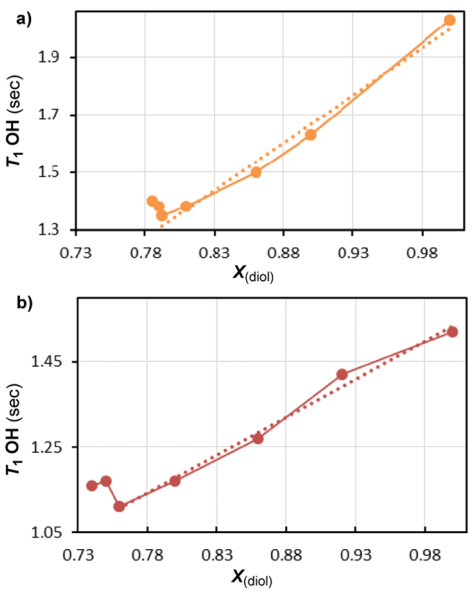

Fig. 4 Plot of OH T_1 (measured with external lock) *vs.* X_{diol} at 75 °C for the trimethylglycine betaine/diol system: (a) with triethylene glycol **D2**; (b) with the 1,3-propandiol **D3**. Dashed lines correspond to the linear regression fitting in the *ultra*-eutectic region.

triethylene glycol decreased from 2.03 s to 1.35 s, ($T_1 = 1.30 - 3.29\ X_{D2}$, $R^2 = 0.98$), suggesting that the formation of DES-supramolecule occurs most likely with the $Gb(D2)_4$ stoichiometry (ratio 1 : 4, $X_{D3} \approx 0.80$), such eutectic composition fully agreed with the one reported in literature.[24] While for **Gb**/**D3**, the formation of DES-supramolecule was achieved by mixing **Gb** with **D3** in a ratio of 1 : 3 ($X_{D3} \approx 0.75$, $Gb(D3)_3$), indeed at such molar composition the OH relaxation time reached the lowest value (*i.e.*, $T_1 = 1.11$ s with $T_1 = 0.25 - 1.78\ X_{D3}$, $R^2 = 0.99$). Even in this case, the eutectic composition determined by T_1 measurements resulted in full agreement with the literature data.[24]

In conclusion, we have shown that T_1 measurements allow to establish the appropriate stoichiometry to which the trimethylglycine betaine and the diol form the corresponding DES. The T_1 values were determined by inversion recovery method on a standard NMR instrumentation using an automated system; the eutectic compositions were validated by DSC analysis. All in all, the presented methodology compares well in terms of simplicity and time consuming with other analytical methods, especially if it will be updated with the new rapid T_1 estimation technique, recently reported.[25]

Lastly, our study shows that the trimethylglycine betaine/glycol DES can tolerate a maximum of 15% in weight of water at 75 °C, indeed, using a higher amount of water (26% in weight), the ethylene glycol seems no longer H-bonded to the glycine betaine.

Author contributions

P. D'Arrigo and F. G. Gatti conceived and designed the experiments. C. Allegretti, L. A. M. Rossato and E. Ruffini performed experiments and analysed data. P. D'Arrigo and F. G. Gatti prepared the manuscript.

Conflicts of interest

There are no conflicts to declare.

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