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Effect of Water on Deep Eutectic Solvent/β-Cyclodextrin Systems

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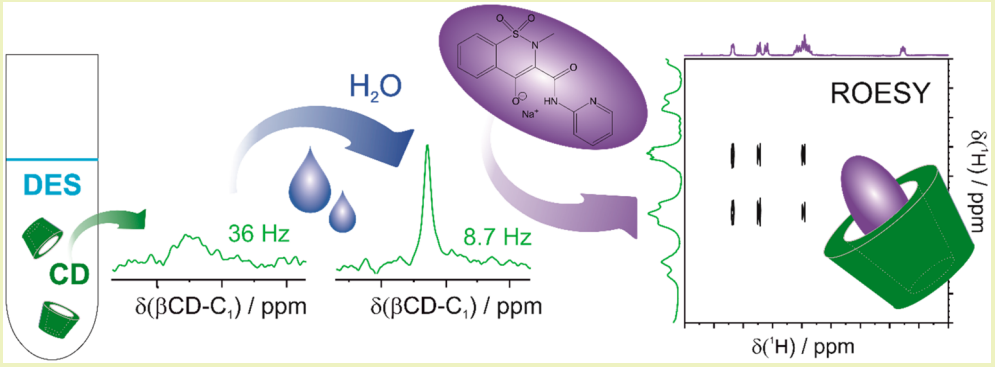
Supporting Information



ABSTRACT: The rotational dynamics of the mixture composed of the deep eutectic solvent (DES) reline, and the macrocyclic oligosaccharide β-cyclodextrin (βCD) is investigated at the molecular level by NMR relaxation spectroscopy with and without known amounts of water. The progressive addition of water affects the rotational motion of the DES and βCD in different ways. The findings are consistent with βCD interacting primarily with the added water and reline preserving some degree of residual molecular network. Combining in this way the features of the individual components in the mixture may be highly advantageous for future applications. As a proof-of-concept for the encapsulation capacity of βCD within the DES/βCD/H_2O mixture, the formation of an inclusion complex with the nonsteroidal anti-inflammatory drug piroxicam is demonstrated through NMR chemical shifts variation and intermolecular host−guest NOEs in the rotating frame.

KEYWORDS: *NMR spectroscopy, Relaxation, NADES, Cyclodextrin, Correlation time, Inclusion complex*

INTRODUCTION

Deep eutectic solvents (DES) are an innovative class of eco-friendly liquids containing large ions that have low lattice energy and hence low melting points.[1] They are usually obtained by mixing a hydrogen bond donor (HBD) and a hydrogen bond acceptor (HBA), which self-associate to form a new eutectic phase characterized by a melting point lower than that of the individual counterparts.[1−4] In 2011 a new term—"natural deep eutectic solvents" (NADES)—was introduced to describe a subset of DES obtained by mixing primary metabolites, namely amino acids, organic acids, sugars, or choline derivatives.[5,6] The first and most studied NADES reported in the literature is a mixture of choline chloride ([Ch]Cl) and urea at a 1:2 mole ratio, indicated as ChU and often referred to as reline.[7,8] Thanks to their huge chemical diversity, biodegradability, ease of handling, and acceptable toxicity profile, DES (and NADES) have attracted interest in a wide range of applications: they are used as solvents for enzymatic or chemical synthesis and biocatalysis, metal processing, electrochemistry, purification, and processing in biodiesel production, removal of environmental contaminants and pollutants, separation of azeotropes, or isolation and fractionation of compounds.[4,9−11] Indeed, DES display unique properties that make them ideal sustainable extracting media, and they are currently quite popular as extraction solvents for natural products.[12,13] Compared to conventional organic solvents and ionic liquids,[14] DES are usually less expensive, easy to produce, and biodegradable with very low toxicity.[15] Moreover, it is possible to tailor their physicochemical properties in order to enhance their extraction power by effectively regulating several parameters such as polarity, viscosity, or hydrogen bonding.[16]

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A way to improve DES's capabilities as extracting and/or sequestrating agents is to add another component with host properties. Cyclodextrins (CD) are doughnut-shaped macrocyclic oligosaccharides produced by the enzymatic modification of starch. They typically contain six, seven, or eight D-(+)-glucopyranose units (α-, β-, and γ-CD, respectively) linked by α-1,4 glycosidic bonds and differ in size (0.5−0.9 nm in diameter).[17] Thanks to their relatively hydrophobic inner walls and the hydrophilic outer surface, they are able to form inclusion complexes with a wide range of molecules of low hydrophilicity and suitable geometrical size through noncovalent forces. This made CD popular in a wide range of applications from detergency, pharmaceuticals, and cosmetics to analytical chemistry, catalysis, bioconversion, and environment protection.[12,17−29]

Given their complementary individual properties, a combination of DES and CD can potentially result in highly appealing materials. For instance, a recent work investigated the solubilization of native CD and cucurbit[n]urils in reline.[30] 50 wt % solutions of CD in the DES could be obtained. Other findings revealed that incorporation of CD into the DES may act as an extraction booster for polyphenol extraction, giving even higher extraction yield,[27,31] and highlighted the importance of CD as additives in increasing the stability of polyphenol-containing extracts in DES.[32] Such combination of DES and CD takes benefit from the complementary features of the two components, as CD encapsulate more hydrophobic moieties, whereas DES have a strong ability to dissolve protic molecules. Even though the current application and future prospects of DES/CD systems as extraction media seem to be at present a hot topic especially in the agri-food sector,[27,32] to the best of our knowledge there is no fine characterization at the molecular level of such materials. However, in order to fully exploit the synergy of DES and CD it is of paramount importance to study the physicochemical properties of the mixture, as this can unveil crucial information for their potential applications. In this paper, we investigate the mixture of the deep eutectic solvent reline (choline chloride/urea at 1:2 mole ratio, ChU) and β-cyclodextrin (βCD) at 10 wt % (Figure 1).

To get a deep understanding of DES/CD mixtures at the molecular level, one should first remember that DES owe their unique properties to the presence of an extensive hydrogen bond network between each component and, to a lesser extent, van der Waals and electrostatic interactions.[1] In a DES/CD mixture the free hydroxyls on the CD exterior act as competitors and interact with DES via the formation of numerous hydrogen bonds, then strongly affecting the hydrogen bond network.[15] Also water can dramatically change the structure of DES due to the rupture of hydrogen bonds between the initial constituents.[33] This latter point deserves particular attention, since water is the most abundant liquid on earth and most DES are highly hygroscopic. As a consequence, a DES absorbs moisture as soon as it is exposed to atmosphere and hence intrinsically includes water molecules to some extent in its structural network. Moreover, water is often suggested as an important environmentally friendly additive to enhance DES's properties in many applications.[16,33] It regulates viscosity downward and modifies polarity, and this turned out to affect DES's extraction capability and to help in industrial scale and in continuous-flow reactions.[27,34] Given the strong effect of water on the macroscopic behavior of the material, it is imperative to study how structure and molecular

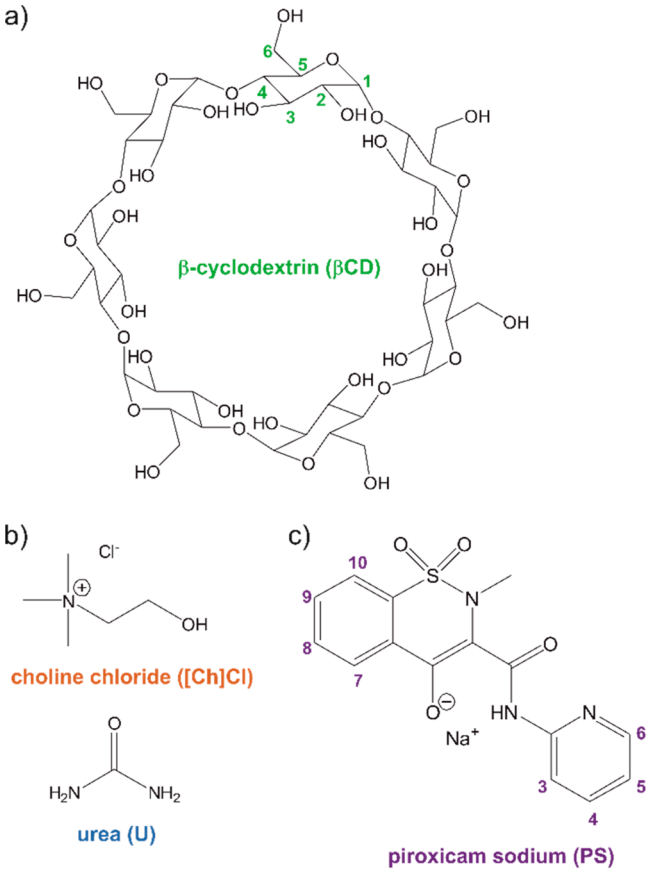

Figure 1. Structures of (a) β-cyclodextrin (βCD), (b) the deep eutectic solvent reline (ChU), composed of choline chloride and urea at 1:2 mole ratio, and (c) piroxicam sodium (PS).

interactions of (NA)DES change in the presence of water and an excellent review has been recently published.[33] Here, we investigated the impact of progressive addition of water not on a pure DES but on a more complex mixture composed of ChU and βCD. NMR spectroscopy was chosen as a technique, since it gives access to both dynamics and interactions at the molecular level. A remarkable effect of the addition of water on the line widths in carbon one-dimensional spectra was the first clear indication of a dramatic change in the dynamic properties of the system. NMR relaxation methods have been hence exploited to get insights into the dynamics and unveiled interesting differences in the motional regimes. Finally, as proof-of-concept for the encapsulation capacity of the system, the nonsteroidal anti-inflammatory drug piroxicam sodium (Figure 1) was added to the mixture and the inclusion complex was investigated in terms of chemical shifts variation and dipolar intermolecular interactions.

EXPERIMENTAL SECTION

Samples. Four representative samples (**1** to **4**) of the reline/βCD/water system were prepared. All the samples share the same basic composition: 10% w/w βCD in ChU. Considering that each βCD has 21 OH groups available for hydrogen bonding, we have considered 21 equiv of water as the amount sufficient for competing with ChU for the first solvation shell of βCD. Sample **5** was prepared from sample **4** by adding 1 equiv of piroxicam sodium with respect to βCD. The composition of the samples is summarized in Table 1, and more experimental details are given in the Supporting Information (SI).

NMR Experiments. ^{13}C T_1 and T_2 relaxation times were measured on samples **1** to **4** using the inversion recovery (IR)

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Table 1. Samples Used in This Work

Sample	Added water (eq)	Added water (wt %)	Piroxicam sodium (eq)
Sample **1**			
Sample **2**	21	2.9	
Sample **3**	63	8.3	
Sample **4**	210	23.2	
Sample **5**	210	23.2	1

pulse sequence with power gated proton decoupling and the Carr−Purcell−Meiboom−Gill (CPMG) pulse sequence with inverse gated proton decoupling, respectively. HSQC spectra were recorded for the assignment of βCD proton peaks in samples **4** and **5**. Dipolar intermolecular interactions in sample **5** were investigated through a ROESY spectrum. Acquisition and processing parameters for all NMR experiments are given in the SI.

Density and Viscosity Measurements. Density and viscosity measurements were carried out for samples **1** to **4** on a Stabinger viscometer (experimental details in the SI).

RESULTS AND DISCUSSION

1D ^{1}H and ^{13}C NMR Spectra. Proton spectra of the four ChU/βCD samples are unfortunately characterized by broad

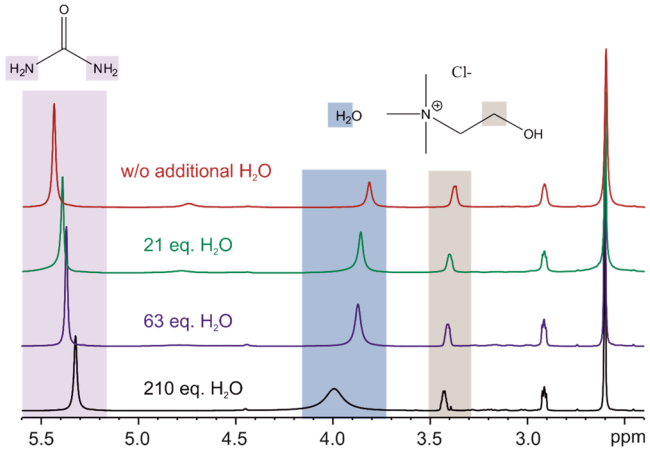

Figure 2. 1D ^{1}H NMR spectra measured at 305 K for the four samples ChU/βCD with different amount of water. The peak assignment is consistent with spectra previously reported.[35] A downfield shift of the methylene group vicinal to the hydroxyl group of choline (gray box) and of water (blue box) and an upfield shift of urea (violet box) are observed as dilution increases. Spectra were recorded with 16384 points and 1 scan.

lines due to the inherent viscosity of DES and partial overlap of cyclodextrin signals (see Figure 2 and S1). However, a qualitative idea of the supramolecular structure of the DES after dilution with water can be obtained simply by observing the chemical shift modifications in ^{1}H 1D spectra. A downfield shift of the choline signal corresponding to the methylene group vicinal to the hydroxyl group as well as of the water signal has been observed after addition of water in choline chloride-based DES.[16,35] This is reported to be indicative of the progressive rupture of the hydrogen bonds of DES during dilution with an increased hydration of choline and the formation of hydrogen bonds in water. A similar phenomenon is observed here with a downfield shift for CH_2−OH and H_2O after dilution. For urea, an upfield shift is observed with increasing water content and increasing temperature, which is

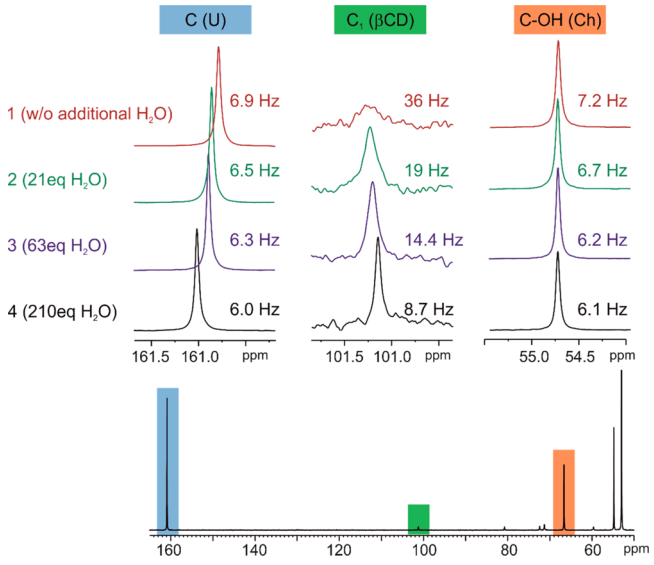

Figure 3. 1D proton-decoupled ^{13}C NMR spectrum and enlargements of signals corresponding to the carboxylic carbon of urea (left), the anomeric carbon of β-cyclodextrin (middle), and the methylene carbon vicinal to the hydroxyl group (right), measured for the four samples ChU/βCD with different amounts of water (**1**: red, **2**: green, **3**: blue, **4**: black). For each enlargement, the fwhm (in Hz) is reported. Spectra were recorded with 32768 points and 32 scans and processed using an exponential filter (LB = 5 Hz).

compatible with results found in aqueous solution of urea at different concentrations.[36]

What appears to be more informative are the proton-decoupled carbon spectra ^{13}C-{^{1}H}. A quick look at the spectra measured for samples **1** to **4** (Figure 3) immediately reveals that the effect of dilution on the line widths varies significantly from a component to the other. Indeed, as a consequence of the decrease in the viscosity of the sample with the addition of water, the Full Width at Half Maximum (fwhm) reported for three selected carbon signals gets smaller from the top (sample **1**) to the bottom (sample **4**). Interestingly, the line widths of urea and choline are only slightly affected, whereas the peak of β-cyclodextrin gets significantly sharper (by a factor of 4). The same holds also for spectra at 315 K. Actually, the fwhm (in Hz) for an absorption peak is given by[37]

$$\text{fwhm} = \frac{1}{\pi T_2^*}; \quad \text{with} \frac{1}{T_2^*} = \frac{1}{T_2^{\text{inh}}} + \frac{1}{T_2} \tag{1}$$

where T_2 is the transverse or spin−spin relaxation time constant, which takes into account the decay of the macroscopic transverse nuclear magnetization and is responsible for the homogeneous broadening of NMR peaks, whereas T_2^* contains also the inhomogeneous contribution to the line width T_2^{inh} due to the variation of the macroscopic magnetic field over the volume of the sample. T_2 is indeed a key dynamic NMR parameter as it depends on molecular motional processes. Broadly speaking, small rapidly rotating molecules have longer T_2 times and sharper NMR lines, while larger molecules that rotate slowly are characterized by shorter T_2 times and broader NMR lines. Hence, the significant difference in terms of line width observed between choline, urea, and β-cyclodextrin in Figure 3 is a first clear evidence of a marked difference in the rotational dynamics of the three components in the presence of water. Following this first evidence we

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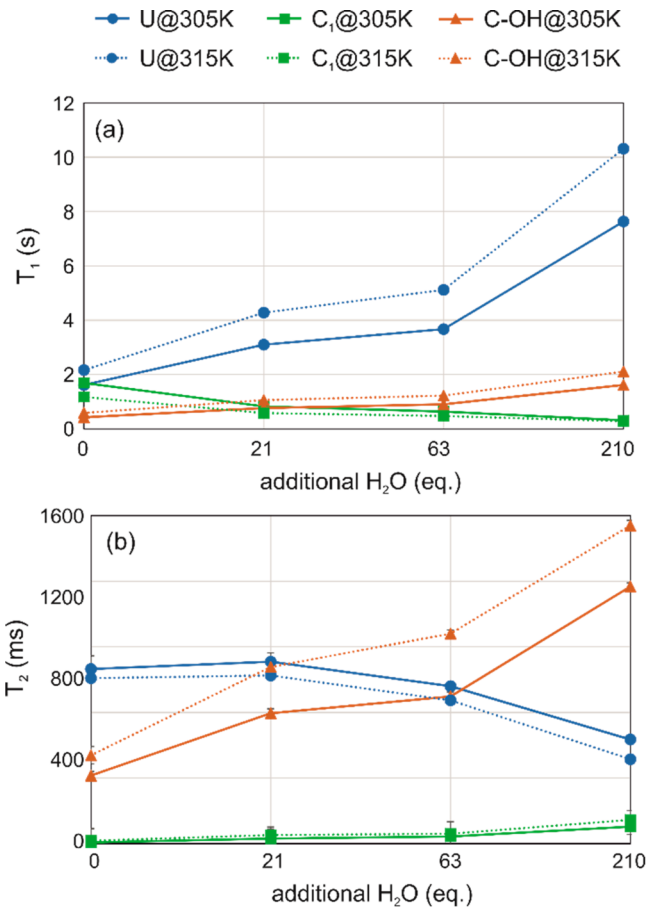

Figure 4. Plot of the ^{13}C T_1 (a) and T_2 (b) relaxation times measured at 305 K (solid lines) and 315 K (dashed lines) for urea (U, blue circles), the C_1 carbon of β-cyclodextrin (C_1, green squares), and the methylene carbon next to the hydroxyl group of choline (C−OH, orange triangles) in the four samples ChU/βCD with increasing water content. When not visible, error bars are smaller than the marker size.

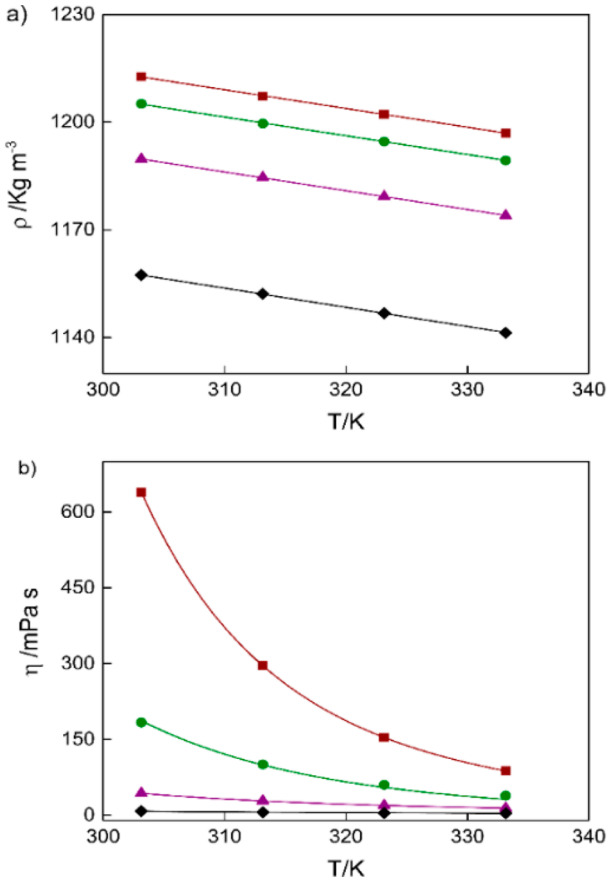

Figure 5. Experimental values for (a) the density and (b) the viscosity of samples **1** to **4**: ChU/βCD (**1**, red square), ChU/βCD/21eq H_2O (**2**, green circle), ChU/βCD/63eq H_2O (**3**, purple triangle), ChU/βCD/210eq H_2O (**4**, black diamond).

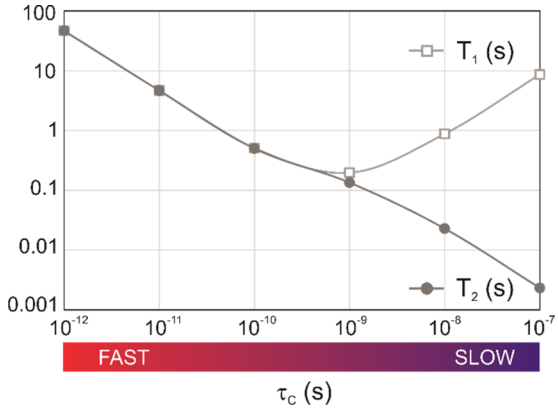

Figure 6. Behavior of T_1 and T_2 dipole−dipole relaxation times for a two-spin system consisting of a proton and a carbon at a magnetic field of 11.7 T (corresponding to 500 and 125 MHz for the proton and carbon Larmor frequency, respectively) at a distance of 1.09 Å as a function of the correlation time τ_c. The location of fast and slow regimes is also shown.

decided to investigate further the dynamics of the ChU/βCD/H_2O system, and to this end T_1 and T_2 relaxation time constants are particularly useful NMR parameters.

^{13}C T_1 and T_2 Measurements. Spin−lattice ($R_1 = 1/T_1$) and spin−spin ($R_2 = 1/T_2$) relaxation rates contain valuable information on the rotational dynamics of molecules in solution. In general, molecular relaxation may occur through several mechanisms, including direct dipole−dipole coupling, chemical shift anisotropy, spin rotation, quadrupole as well as cross-correlation effects,[37] but for spins 1/2 the dominant one is usually the through-space dipolar coupling between the spins. For protonated ^{13}C nuclei dipolar relaxation occurs mainly between carbon and nearby ^1H nuclei and this mechanism can be readily interpreted in terms of molecular dynamics.[38−40] The picture is more complicated for carbonyl carbons, as other interactions may have minor but measurable contributions to the relaxation mechanism.[41]

^{13}C T_1 and T_2 values were measured at 305 and 315 K for samples **1** to **4** and are reported in Tables S2. One representative peak was selected for each molecule and will be used in the following discussion, namely C_1 for βCD, C−OH for Ch, and clearly C═O for U (Figure 4). NMR experiments to measure ^1H relaxation time constants were also run, but due to severe signal overlap and broadness of NMR peaks, only T_1 and T_2 values for choline (methylene protons next to the hydroxyl group and methyl protons) and urea could be measured properly and are reported for comparison in Table S3.

The ^{13}C T_1 and T_2 trends of Figure 4 indicate that the three components show a different behavior under addition of water. Both ^{13}C T_1 and T_2 relaxation times of C−OH of choline increase moving from the sample without water to the sample

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Table 2. Rotational Correlation Times τ_c (in ns) Calculated at 305 and 315 K for the C_1 Carbon of β-Cyclodextrin and the Methylene Carbon Next to the Hydroxyl Group of Choline in the Four ChU/βCD Samples with Different Amounts of Water, following the Methodology of Carper *et al.* for Moderately Viscous Media[40] and the Extreme Narrowing Approximation,[39] Respectively (see SI for a detailed description)

	w/o H_2O	21 eq	63 eq	210 eq
	C_1 (βCD)[a]			
305 K	31	9.0	7.6/7.7[c]	3.1
315 K	14	5.6	4.4	1.7
	C–OH (Ch)[b]			
305 K	0.10	0.06	0.05	0.03
315 K	0.07	0.04	0.03	0.02

[a] τ_c calculated in the assumption of moderately viscous media. [b] τ_c calculated in the extreme narrowing condition. [c] Measured using the coefficients in two different ranges (see Table S8 of SI).

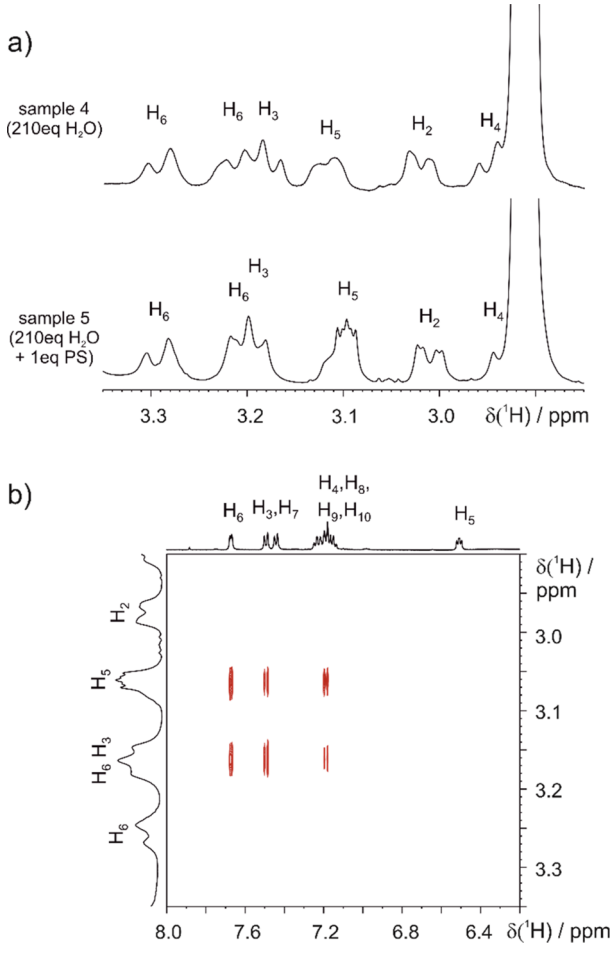

Figure 7. (a) Comparison of spectral regions between 2.85 and 3.35 ppm of ^{1}H spectra measured at 305 K for sample **4** (ChU/βCD/210 eq. H_2O) and sample **5** (ChU/βCD/210 eq. H_2O/1 eq. PS). (b) Enlargement of the ^{1}H–^{1}H ROESY spectrum showing intermolecular correlation peaks between signals corresponding to H_3 and H_5 protons of β-cyclodextrin (vertical dimension) and aromatic protons of piroxicam (horizontal dimension). The spectrum was measured for sample **5** using data matrices of 4096 (t_2) × 768 (t_1) complex data points and processed by applying a sine squared window function in both dimensions and zero-filling to 4096 × 1024 data points prior to the Fourier transform.

with 210 equiv of water by roughly a factor of 4. As expected for a small molecule, T_1 and T_2 are of the same order of magnitude, with a ratio T_1/T_2 close to 1. It is worth noting that ^{13}C relaxation times measured for choline in sample **4** at 305 K (T_1 = 1.8 s and T_2 = 1.3 s) are smaller than those measured in a standard aqueous solution (T_1 = 4.3 s and T_2 = 3.2 s). This might indicate that the excess of water added in sample **4** does not destroy entirely the hydrogen bond network within the DES, which still retains unique properties different from its individual components.

For C_1 of β-cyclodextrin the ^{13}C T_1 trend shown in Figure 4a seems to be out of place, as it decreases with the progressive addition of water by a factor of 6 and 4 at 305 and 315 K, respectively. When an excess of water is present (sample **4**), the T_1 relaxation times at 305 and 315 K (ca. 300 ms) are indeed close to ^{13}C T_1 values reported in the literature for 10 mM CD solutions in D_2O at 300.6K at 11.7 T (ca. 270–280 ms).[42] Contrary to T_1, the ^{13}C T_2 values measured for C_1 of βCD increase with the addition of water, and in sample **4** they are much higher than in the sample without water (by a factor of 12 at 305 K and a factor of 9 at 315 K). This means that with the progressive addition of water the ratio T_1/T_2 changes dramatically, going from 328 to 4.5 at 305 K and from 89.2 to 2.3 at 315 K.

The interpretation of ^{13}C relaxation data collected for urea might be tricky, since different mechanisms in addition to the dipole–dipole interaction can contribute to the relaxation for C=O carbons. Overall, the behavior of urea appears to be reversed with respect to β-cyclodextrin; *i.e.* its ^{13}C T_1 time increases (by a factor of 5) and T_2 value decreases (by a factor of 2) with the addition of water. Such unusual behavior of urea is also confirmed by proton relaxation times (Table S3). In sample **4**, where an excess of water is present, relaxation times are expected to get closer to those found in standard aqueous solution. Indeed, a T_1 time of 35 s has been measured in a 10 M solution in H_2O, and similar values of 37–40 s are reported in the literature for aqueous solutions of urea and correlated amides.[41,43]

Density and Viscosity. Densities and viscosities were measured for the different ChU/βCD samples as a function of temperature, without water and with additional amounts of water (Tables S4 and S6 of SI), and were fitted to appropriate polynomials and to VFT functions, respectively (Tables S5 and S7 of SI). Density decreases with increasing temperature and with the addition of water, starting at 1212.7 kg m^{-3} for ChU/βCD and reaching the lowest value of 1157.4 kg m^{-3} for ChU/βCD/210eq H_2O at 303 K. Similarly, viscosity decreases when water is added, as displayed in Figure 5. For the selected T = 303 K, sample **4** experiences the dramatic drop of viscosity of a factor 80 compared to pristine sample **1**.

Rotational Motion. A way to characterize molecular rotational motion in a liquid is by the rotational correlation time (τ_c), which may be thought of as the time required for the molecule to rotate by approximately 1 rad. Generally speaking, small molecules have short rotational correlation times (fast rotation), whereas large molecules or aggregates have long rotational correlation times (slow rotation). The equations relating τ_c to the longitudinal and transverse heteronuclear and homonuclear relaxation rates are well established,[37,44] and the theoretical treatment is described for the interested reader in the SI. To get a general idea, Figure 6 displays the behavior of T_1 and T_2 for an aliphatic CH carbon at a magnetic field of 11.7 T. At very short correlation times (fast motion regime)

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the values of T_1 and T_2 are equal. This is called the extreme narrowing limit ($\omega_0\tau_c \ll 1$). As the correlation time increases, T_1 passes through a minimum and then gets longer and longer. The transverse relaxation time constant T_2, on the other hand, continues to decrease, meaning the NMR peaks get progressively broader. Motion which gives rise to correlation times which are much longer than $1/\omega_0$ is described as being in the slow motion limit ($\omega_0\tau_c \gg 1$).[37]

In practice, the correlation time depends on the physical parameters of the system, such as temperature and viscosity. As shown in Figure 5, viscosity decreases after addition of water to the ChU/βCD system. Consequently, rotation is faster, meaning we are moving from higher τ_c values to smaller ones (*i.e.* from the right to the left side of Figure 6). The relaxation results observed for choline and β-cyclodextrin can be then qualitatively explained in terms of fast and slow regime. Both T_1 and T_2 of choline increase with addition of water; then its dynamic regime can be defined as fast motion (left side of Figure 6). For β-cyclodextrin a decrease of T_1 value and an increase of T_2 is observed moving from sample **1** to **4**, indicating hence that the molecule is in slow motion (right side of Figure 6). Urea apparently displays an odd behavior after addition of water, since an increase of T_1 value and a decrease of T_2 are observed moving from sample **1** to **4**. Indeed, the role of urea as HBD and HBA in solution as well as its ability to aggregate and/or form strong interactions has been long debated.[45−48] However, even though it is tempting to speculate in this direction, it should be remembered that the relaxation times of the carboxylic carbon of urea cannot be simply interpreted by means of dipole−dipole relaxation mechanism and trying to classify urea in terms of fast and slow regimes displayed in Figure 6 would be then a bit of a stretch.

The correlation time is also temperature dependent. In general, warming the sample speeds up the motion, reducing the correlation time, whereas cooling the sample slows down the fluctuations, lengthening the correlation time. The effect of temperature on T_1 depends on the location of τ_c with respect to the T_1 minimum. For systems with long correlation times (viscous solutions or large molecules), warming the sample generally reduces the spin−lattice time constant T_1. For systems with short correlation times (small molecules in nonviscous solutions), warming the sample generally increases the spin−lattice time constant T_1.[37] In this sense, results found for the ChU/βCD system at 315 K are coherent with previous observations at 305 K: both T_1 and T_2 increase for choline (fast regime), whereas T_1 decreases and T_2 increases for β-cyclodextrin (slow motion).

For molecules in the fast motion regime - that is for choline here - the rotational correlation times can be easily calculated from T_1 values using simplified equations.[39] Computations of τ_c are more complicated for molecules in the slow motion regime, namely βCD. We resorted to a method proposed in the late '90s by Carper et al. for the calculation of the correlation time in moderately viscous systems (*i.e.* outside of the region of extreme narrowing) for carbon nuclei undergoing dipolar relaxation with ^{1}H once the ratio T_1/T_2 is known.[40] The method holds in cases where dipolar relaxation is the major contributing relaxation mechanism, which is the case with protonated ^{13}C of βCD in our samples. As done above, we considered as representative of choline and β-cyclodextrin the ^{13}C relaxation rates of the methylene carbon next to the hydroxyl group and of the ring carbon C_1, respectively. The correlation times estimated with such approaches are listed in Table 2, and more details are given in the SI. It can be observed that τ_c of the anomeric carbon of βCD decreases under addition of water by a factor of 10 and 8.2 at 305 and 315 K, respectively. This clearly indicates a strong effect of water on the dynamics of βCD. As a simple consequence of the lower molecular weight, the τ_c values found for choline are significantly shorter than those for βCD. More interestingly, the decrease of τ_c of Ch under addition of water (by a factor of 3 roughly) is smaller than for βCD. Nicely, this last outcome gives an answer to the question raised at the beginning of the work: the effect of water on the line widths of Figure 3 is so different because the rotational correlation times of the molecules decrease with a different speed when the viscosity of the medium is decreased by addition of water.

Case Study: Inclusion Complex βCD/Piroxicam in DES. From the experimental data presented above a possible scenario at molecular level can be proposed. Apparently, water interacts primarily with the hydroxyl groups of β-cyclodextrin through hydrogen bonding, leading to a supramolecular network with progressive increase of its molecular rotational motion. This effect gets stronger with addition of water to the DES/βCD mixture, up to the sample **4** with 210 equiv of water, where βCD experiences an aqueous-like environment. A key point is that, even when an excess of water is present, the DES seems not to entirely lose its self-organization.

The 3D H-bonding network within reline has been elegantly described by Hammond et al.[8] According to this model, a choline moiety and two urea molecules interact strongly with a chloride anion via hydrogen bonds. In the binary mixture DES/H_2O, water is reported to interact strongly with the anion and dampen the urea-choline interactions, but various, somewhat controversial, conclusions are drawn in different theoretical and experimental studies.[33] Recent NMR studies report for instance on the impact of water on the molecular interactions of reline and other pure DES and conclude that the deep eutectic solvent tends to become a simple solution of its individual counterparts upon dilution.[49] On the other hand, molecular dynamics simulations as well as multiple experimental techniques indicate that even at high water mole fraction the components of reline are only partially hydrated, which means that the solution still retains the nature of pure DES.[50−54] In the mixture studied here, the description of intermolecular interactions is more complicated, since both water and β-cyclodextrin are available for hydrogen bonds, and the NMR results collected give some clues in this respect. With the addition of water choline gets more hydrated, as witnessed by the change in ^{1}H chemical shift and the decrease of τ_c, but the T_1 and T_2 values are still shorter than those observed in aqueous solutions. This would indicate that the structure stabilized by hydrogen bonds is not fully destroyed by the addition of water. Interestingly, this means that the strong hygroscopic attitude of DES is likely to be a minor problem when dealing with large-scale industrial applications. These conclusions are coherent with the results published by the group of Del Monte on reline−water mixtures observed via Brillouin and ^{1}H NMR spectroscopies.[55] The authors indeed reported two limiting states: isolated DES domains with a continuous network of H_2O for low molar fraction of DES (X_{reline}) and a continuous DES network for high values of X_{reline}. In the intermediate concentration range these domains are reported to coexist in the fashion of "co-continuous structure of H_2O and DES domains".

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This molecular scenario seems to be very promising when looking at possible applications of the DES/βCD/H_2O system, since it strengthens the individual features of the single components: β-cyclodextrin in water would have the benefit of enough mobility to form inclusion complexes with solutes, while reline preserves a residual molecular network. In order to get an insight into the encapsulation properties of βCD within this complex system, the molecule of piroxicam sodium (PS, see Figure 1 for structure and atom numbering) was used as proof-of-concept.

PS is a well-known nonsteroidal anti-inflammatory drug, and its attitude to form a 1:1 inclusion complex with β-cyclodextrin in bulk water has been demonstrated in 1992 via ^{1}H NMR in D_2O solution.[56] For the present work it may be considered as a reference standard for inclusion. The actual formation of PS/βCD inclusion complex in DES in the presence of water was studied by ^{1}H NMR. Figure 7 shows the spectral changes in the ^{1}H NMR spectral region where the glucose protons fall induced by the presence of PS (complexation-induced chemical shift variations). This is the first piece of evidence of the formation of an inclusion complex. The encapsulation of PS in the hydrophobic cavity of βCD is demonstrated by the selective change in the chemical shift of the inner protons H_3 and H_5 of βCD in sample **5** in comparison with the chemical shifts of the same protons in sample **4** (Figure 7a). The HSQC spectra reported in Figure S2 of the SI confirm the peak assignment of βCD. These data demonstrate that βCD retains its ability to encapsulate PS also in the ChU/βCD/H_2O mixture (sample **5**), in line with results obtained recently for volatile organic compounds.[57] Moreover, the intermolecular interactions between inner protons of βCD and aromatic protons of PS are evident in the ROESY spectrum of Figure 7b, corroborating further the formation of a genuine inclusion complex rather than a nonspecific PS/βCD aggregate.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssuschemeng.9b00315.

Experimental details, additional figures and tables, and theoretical treatment (PDF)

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■ ABBREVIATIONS

βCD, β-cyclodextrin; CD, cyclodextrin; CPMG, Carr−Purcell−Meiboom−Gill; DES, deep eutectic solvent; fwhm, Full Width at Half Maximum; HBA, hydrogen bond acceptor; HBD, hydrogen bond donor; HSQC, Heteronuclear single quantum coherence; IR, inversion recovery; LB, line broadening factor; NADES, natural deep eutectic solvents; NMR, nuclear magnetic resonance; NOE, nuclear Overhauser enhancement; PS, piroxicam sodium; ROESY, rotating frame Overhauser enhancement spectroscopy; VFT, Vogel−Fulcher−Tammann

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