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Article

Structural Features of β-Cyclodextrin Solvation in the Deep Eutectic Solvent, Reline

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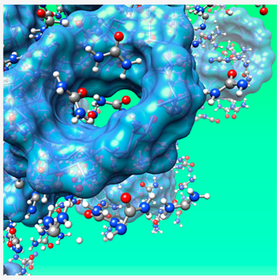

ABSTRACT: The inherently amphiphilic nature of native cyclodextrins (CDs) determines their peculiar molecular encapsulation features, enabling applications such as targeted drug nanodelivery, aroma protection, etc. On the contrary, it may also lead to poor solubility in water and other organic solvents and to potentially detrimental flocking in these media, thus posing limitations to more extensive usage. Here we use small angle X-ray scattering to show that deep eutectic solvent reline (1:2 choline chloride:urea) succeeds in dissolving large amounts of β-CD (≤800 mg/mL, compared with the solubility in water of 18 mg/mL), without aggregation phenomena occurring. At the microscopic level, molecular dynamics simulations highlight the complex interplay of hydrogen bonding-mediated hydrophilic interactions and hydrophobic force mitigation occurring between β-CD and reline components, leading to energetically favorable β-CD solvation. The possibility of achieving very high concentration conditions for unaggregated β-CD in an environmentally responsible media, such as reline, can open the way to new, so far unpredictable applications, addressing multiple societal challenges.

■ INTRODUCTION

Cyclodextrins (CDs) are natural products from enzymatic degradation of starch; they are shaped as hollow truncated cones formed from cyclized glucose moieties (most commonly six, seven, or eight such units, termed α-, β-, or γ-CDs, respectively) with primary and secondary hydroxyl groups at the narrow and wide rims, respectively, and alkyl/ether moieties in the internal part of the doughnut.[1−3] Such a chemical composition leads to amphiphilically behaving compounds with polar rims surrounding a rather hydrophobic cavity that can host small/medium-sized apolar molecules, forming noncovalent host−guest complexes.[4] This opportunity finds a huge number of applications in the fields of pharmaceutical, food, aroma, analytical chemistry, catalysis, etc. However, extended exploitation of such spectacular properties is hindered by the generally limited CD solubility in water or other organic solvents that can be as low as 18 mg/mL for β-CD in water[5] (although we mention that α- and γ-CDs are much more soluble in water[5]). Recent reports indicate that ionic liquids[6−11] (ILs) as well as deep eutectic solvents[12] (DESs) can perform as very good solvents for CDs, with β-CD levels as high as 1000 mg/mL being soluble in reline, a representative DES.[13−17] DESs make up a recently developed class of compounds that are obtained by the complexation of a quaternary ammonium salt (acting as a hydrogen bonding acceptor) with a metal salt or a hydrogen bond donor (HBD); they typically contain large, nonsymmetric ions that have low lattice energy and hence low melting points.[18] Reline is an archetypal example of a DES;[13] it is formed by a 1:2 molar ratio mixture of choline chloride and urea and shows a melting point of ∼305 K.[17] Recently, McCune et al. reported that reline succeeds in dissolving exceptionally large quantities of α-, β-, and γ-CDs [as well as other hollow macrocyclic compounds, such as cucurbit[*n*]urils (with *n* = 6−8)], as compared to the water solubility of these compounds, still maintaining their important host−guest capability.[12] Such an observation could represent a game changer for a large variety of applicative fields, addressing several societal requests. At present, there is little information about the solubilization of carbohydrates by DES media.[19−27] Especially from the point of view of the structural correlations responsible for the formation of stable mixtures, the field is still unexplored. To acquire such information, one needs to confront issues that might potentially hinder wide-ranging exploitations of CD−DES combinations. How are CDs solvated in these media? Do CDs tend to cluster in these solvents, as is the case, e.g., of water? Is reline a notable exception among DESs, concerning its ability to dissolve CDs? Previous studies reported the role of different CD concentrations and temperatures on macroscopic properties, such as the density and viscosity of reline mixtures; furthermore, dissolution calorimetric experiments have been conducted on β-CD in reline, revealing that such a process is an exotermic one, thus implying that the interactions between β-CD and reline moieties are energetically favorable. On the

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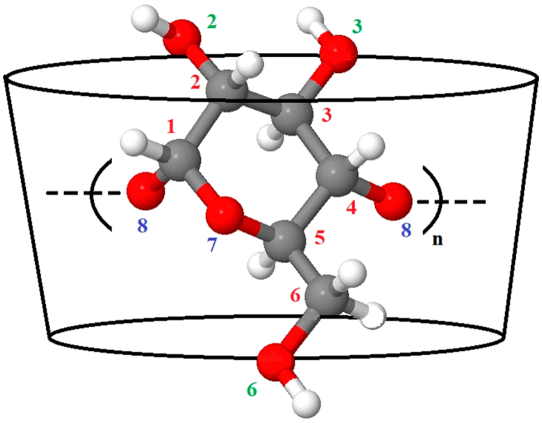
other hand, an increase in temperature is not a thermodynamically viable way to increase β-CD solubility in this solvent.[25]

In this work, we provide an atomistic level rationalization of structural features of the solvation and aggregation states of β-CD when dissolved in reline. After empirical observation of native and methylated CD solubility features in reline and other related DESs, we exploit the small angle X-ray scattering (SAXS) technique to address the issue of potential β-CD aggregation under highly concentrated conditions in reline. Finally, we use molecular dynamics simulations to account for atomistic details of structural organization of the different reline moieties surrounding β-CD, highlighting the important role played by both hydrogen bonding interactions[15,28] and urea's amphiphilicity[29] in efficiently coordinating β-CD in reline.

■ METHODS

Experimental Section. *Samples.* α-CD (99.3%), β-CD (98.6%), heptakis(2,6-di-*O*-methyl)-β-cyclodextrin (DIMEB95) (purity of >99%; isomer purity of 93.4%; average degree of substitution of 14.5), and heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin (TRIMEB) (purity of >99.5%) were CYCLOLAB products (see Scheme 1). These samples have been used as received.

Scheme 1. Schematic Representation of α-CD ($n = 6$) and β-CD ($n = 7$)[a]



[a]Heptakis(2,6-di-*O*-methyl)-β-cyclodextrin (DIMEB95) and heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin (TRIMEB) are β-CD derivatives, in which hydroxyl H atoms at positions 2 and 6 and positions 2, 3, and 6 are replaced by methyl groups, respectively. Indexes referring to the nomenclature of carbon atoms (red), hydroxyl groups (green), and ether oxygen atoms (blue) are shown.

Reline was prepared by accurately mixing choline chloride (TCI product; purity of 99.5%; drying loss of 0.0%) and urea (Sigma-Aldrich; purity of >99%) in a 1:2 molar ratio under an inert atmosphere. The two components were kept under vacuum at 60 °C for 24 h, before preparation. Subsequently, the mixture was kept at 50 °C under vigorous mixing for approximately 1 h. Reline appears as a solid compound under ambient conditions (20 °C at the time of preparation) (in agreement with ref 17 that reports a melting point of ∼32 °C for dry reline). Ethaline and glyceline were analogously prepared by mixing choline chloride with ethylene glycol (Sigma-Aldrich; purity of 99.91%; water content of 0.001%, used as received) and glycerol (Sigma-Aldrich; purity of >99%) (at a 1:2 ratio), respectively. These DESs appear as transparent liquid under ambient conditions.

Mixtures with CDs were prepared by weight, under an inert atmosphere. After being weighted, mixtures were heated at 50 °C for ∼2 h, under constant agitation.

SAXS capillaries were filled under an inert atmosphere.

SAXS Measurements. The SAXS measurements were performed at the SAXSLab Sapienza with a Xeuss 2.0 Q-Xoom system (Xenocs SA, Sassenage, France), equipped with a microfocus Genix 3D X-ray source ($\lambda = 0.1542$ nm) and a two-dimensional Pilatus3 R 300K detector that can be placed variable distances from the sample. Calibration of the scattering vector Q range, where $Q = (4\pi \sin \theta)/\lambda$, where 2θ is the scattering angle, was performed using a silver behenate standard.

Measurements with different sample−detector distances were performed so that the overall explored Q region was from 0.1 to 1 Å^{-1}. The samples were loaded into disposable quartz capillaries with a nominal thickness of 1.0 mm and sealed with hot glue before being placed in the instrument sample chamber at reduced pressure (∼0.2 mbar). The beam size was defined through the two-pinhole collimation system equipped with scatterless slits to be 0.25 mm × 0.25 mm.

The two-dimensional scattering patterns were subtracted for the dark counting and then masked, azimuthally averaged, and normalized for transmitted beam intensity, exposure time, and subtended solid angle per pixel, by using the FoxTrot software developed at SOLEIL. The one-dimensional $S(Q)$ versus Q profiles were then subtracted for the solvent and capillary.

Measurements were conducted at ambient temperature (∼20 °C), and samples were kept in liquid form and homogeneous during the whole length of the experiment.

Fitting of the SAXS data was performed using the SASView software (M. Doucet et al., SasView version 4.2, Zenodo, 10.5281/zenodo.1412041). Solvent and instrumental background-corrected $S(Q)$ data were modeled assuming a core−shell sphere model to account for isolated, isotropically oriented β-CD molecules. Interactions between non-aggregated β-CDs were described in terms of a hard sphere potential. In this model, the fitting parameters were β-CD sizes (that were kept constant across the explored concentration range), scaling factor, β-CD volume fraction, and background. After separate modeling of each data set, constant values for β-CD size have been used, leading to a sphere radius of 5.8 Å and a shell thickness of 1 Å. The hard sphere interaction was modeled using the Percus−Yevick closure,[30,31] using an effective radius of the hard sphere equal to the β-CD outer radius and the volume fraction of hard spheres that nicely agrees with the calculated value from macroscopic concentration determination. We stress that the same structural model described very well the digitized SAXS data set from Kusmin et al. on β-CD dissolved in water, under dilute conditions.[32]

Computational Details. Molecular dynamics (MD) simulations were performed using the GROMACS 5.1.1 package.[33,34] Bonded and nonbonded parameters for β-cyclodextrin (β-CD) and a deep eutectic solvent, namely reline, were described using an all-atom potential. In particular, reline was described using the all-atom OPLS force field developed by the group of Acevedo.[35] β-CD was described using the q4-MD force field.[36−38] A Gromacs-compatible version of the force field was kindly provided by Prof. Zhang [University of Science and Technology Beijing (PRC)]. The TIP3P potential was used for water for β-CD solutions.[39]

Simulations for reline solutions were performed using a cubic box of 500 reline moieties (500 cholines, 500 chlorides, and 1000 ureas) and 10 β-CD units (c = 2 mol %, equivalent to ~100 $mg_{\beta\text{-CD}}/mL_{reline}$); scaled partial charges (80% of the original ones) were used for the choline chloride, in agreement with ref 35. Simulations were conducted at 323 K, to enhance solvent fluidity, with respect to ambient conditions.

An additional simulation has been conducted on an isolated β-CD dissolved in water at 300 K. An isolated β-CD was placed in a box with 20000 water molecules (c = 0.005 mol %, equivalent to ~3 $mg_{\beta\text{-CD}}/mL_{water}$).

Periodic boundary conditions were applied to all of the simulations. Initial configurations were created by Packmol software,[40] and the starting densities were fixed at ~10% higher than the experimental ones. The equilibration procedure was performed in several steps, starting from an *NVT* simulation at 400 K, followed by a series of *NPT* runs progressively decreasing the temperature to their final values at 323 and 300 K for reline and water solutions, respectively; the pressure was fixed for both systems at 1 bar, and a total of 12 nanosecond equilibration runs were performed.

After the equilibration phase, the system was run for a total of 200 ns for a production run, and then the trajectory of the last 30 ns was saved at a frequency of 1 ps. Such a trajectory has been used for the calculation of the structural properties.

Simulations were always checked versus the energy profile, during their evolution. During the production runs for temperature coupling, we used a velocity rescaling thermostat[41] (with a time coupling constant of 0.1 ps), while for pressure coupling, we used a Parrinello−Rahman barostat[42] (1 ps for the relaxation constant). The Leap-Frog algorithm with a 1 fs time step was used for integrating the equations of motion. Cutoffs for the Lennard-Jones and real space part of the Coulombic interactions were set to 16 Å for the reline systems, while a value of 12 Å was chosen for the water solution. For the electrostatic interactions, the particle mesh Ewald (PME) summation method[43,44] was used, with an interpolation order of 6 and 0.08 nm of FFT grid spacing. Pair correlation functions and spatial distribution function were obtained by TRAVIS.[45,46] Selected graphs were made using the VMD software.[47] The *gmx energy* routine of Gromacs was used to calculate two types of short-range potential: Lennard−Jones short-range (LJ-SR) and Coulombic short-range (Coul-SR). This utility routine was used on the final equilibrated trajectory where the different groups (β-CD, water, choline, chloride, and urea) were selected to obtain the partial energy contributions.

■ RESULTS AND DISCUSSION

We preliminarily explored the solubility of native and selectively methylated CDs in reline and other common DESs. The solubility of β-CD in three different DESs {namely, reline[16,48,49] [1:2 choline chloride (ChCl):urea], ethaline[49] (1:2 ChCl:ethylene glycol), and glyceline[49,50] (1:2 ChCl:glycerol)} at 2.5 mol % (corresponding to several tens of milligrams per milliliter and to ~10 wt % for the different mixtures) was tested at ambient temperature (~20 °C). Under these conditions, β-CD is perfectly soluble in reline but turns out to be insoluble in ethaline and glyceline. In the past, β-CD solubility limits in water have been partially overcome by replacing hydroxyl groups with hydrophobic methyl ones;[51] in these cases, solubility in water is higher than in the case of native β-CD. Reline mixtures with α-CD, heptakis(2,6-di-*O*-methyl)-β-cyclodextrin, and heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin (indicated as DIMEB95 and TRIMEB, respectively) were also prepared at 1 mol % (~5 wt %), and while native α- and β-CD are soluble, the methylated β-CDs turn out to be insoluble at the chosen concentration. This behavior is to be compared with that reported for CRYSMEB (a low-level methylated β-CD; DS = 4.9) in reline, for which a solubility in reline of ≤10 wt % has been reported.[25] The methylated β-CDs studied here [DIMEB95 (with DS = 14) and TRIMEB (permethylated)] show a higher level of methylation and were not soluble at the chosen concentration (~5 wt %) in reline. The apparent disagreement with results proposed in ref 25 is presumably related to the different degree of methylation in the considered β-CDs. These observations are presently subject to further exploration and will be a topic for subsequent reports; accordingly, on the basis of this preliminary information, we limited our subsequent study to studies of native β-CD dissolved in reline.

The clustering of CDs in water and other media has long been observed[52−54] and acknowledged as a potentially detrimental effect that might lead to poorly soluble aggregates and complex drug−CD interactions.[55,56] The aggregation of CDs in water and other media is a debated issue. NMR studies[57,58] do not provide an indication of the existence of CD aggregates in water. Small angle X-ray and neutron scattering experiments conducted on β- and γ-CDs in dilute solutions in water did not show evidence of aggregates of ≤6 and ≤42.9 mg/mL, respectively.[32] On the other hand, photon correlation spectroscopy detected the existence of large polydisperse aggregates of α-, β-, and γ-CDs even in dilute solutions in water (12 mM).[59,60]

In this scenario, it is important to assess whether β-CD tends to cluster when dissolved in DES, at concentration levels comparable to or above the one accessible in aqueous media. SAXS is commonly used to probe the existence of aggregates over the mesoscopic scale between angstroms and hundreds of nanometers. Accordingly, SAXS data from mixtures of β-CD in reline at concentrations ranging from 70 to 800 mg/mL at 20 °C have been collected and are shown in Figure 1. The scattering patterns have been modeled considering a model of identical spherical shells (accounting for the isotropically oriented structure of monomer β-CD) embedded in a

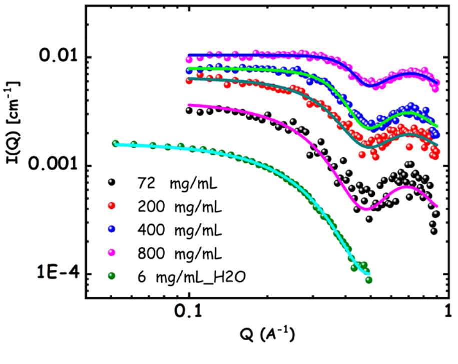


Figure 1. SAXS data from binary mixtures of β-CD dissolved in reline at 20 °C, at the following concentrations: 70, 200, 400, and 800 mg/mL (from bottom to top, respectively). Apart from the first data set, the others have been arbitrarily shifted for ease of comparison. The bottom data set refers to SAXS data collected by Kusmin et al. on β-CD dissolved in reline at 20 °C and 6 mg/mL (see the text). Data have been digitized, arbitrarily scaled, and fitted, using the same model used to account for the mixtures studied here.

homogeneous matrix and interacting through a hard sphere potential, thus using the relationship

$$I(Q) = A \times P(Q) \times S(Q) + \text{bkg}$$

where A is a scaling factor, bkg is a constant background level, and $P(Q)$ and $S(Q)$ are the form factor and structure factor, respectively.

We used $P(Q)$ for a hollow spherical shell, with parameters R, t, η_1, and η_2, where R is the inner radius, t is the shell thickness, and η_1 and η_2 are the contrast between the solvent and the inner part of the shell and the shell, respectively.[61] We defined η_1 as zero, assuming that the solvent can uniformly access the interior of β-CD, and η_2 as the electron density difference between reline and β-CD. $S(Q)$ was a hard sphere (HS) structure factor that depends only on the HS radius (that we took to equal $R + t$) and β-CD's volume fraction.[31,62] Calculations were performed using the SASVIEW 5.0 code (Doucet et al., SasView version 5.0.0, http://doi.org/10.5281/zenodo.3011184). After preliminary independent fits of experimental data sets, a constraint has been applied to the hollow sphere geometric parameters (R and t) that were fixed (but their common values have been optimized) across the different data sets. This analysis led to the fits reported in Figure 1 that accurately describe the experimental data. Such a fit leads to an R of 5.8 ± 0.5 Å and a t of 1.0 ± 0.2 Å, to describe the monomer β-CD geometry; also, the fitted value for the β-CD's volume fraction is in very good agreement with the composition value.

In Figure 1, a SAXS data set obtained by digitization from the report by Kusmin et al. dealing with a dilute solution of β-CD in water at 20 °C is also reported.[32] This data set was modeled using the same model used for the SAXS data of β-CD in reline; we stress here that the agreement is excellent, thus further supporting the quality of the modeling in this work.

The very good fit of the SAXS data unequivocally demonstrates the absence of β-CD aggregates in a reline solution, thus supporting the view that this solvent succeeds in maintaining β-CDs as isolated entities, even at the highest probed concentration.

Having assessed that β-CD does not tend to aggregate when dissolved in reline, we next explored several structural features of the solvation in such a system. A 2 mol % β-CD in reline system (corresponding to a concentration of ~100 mg/mL) was equilibrated and probed using MD simulation at 323 K. The probed concentration value lies well inside the concentration range where SAXS indicates that β-CD does not aggregate in reline. In agreement with this observation, the chosen interatomic potential leads to a homogeneous system, where no evidence of clustering occurs, during the whole course of the simulation. On the other hand, we mention that an exploratory simulation of β-CD in water at 0.05 mol % [corresponding to ~30 mg/mL, i.e., above the experimental solubility limit (~18 mg/mL)] indicates evidence of β-CD clustering during a few nanoseconds of simulation, thus providing some support to the quality of the potential used for β-CDs and the ability of MD simulations to detect β-CD segregation.

The most direct inspection into structural correlations between β-CD and solvating reline moieties was obtained through the pair distribution functions describing the normalized density of choline, chloride, and urea centers of mass (CoM) with respect to the geometrical center (GC) of a reference β-CD (hereafter indicated as the β-CD center), as shown in Figure 2. For ease of understanding, the shadowed area therein describes the intramolecular atomic distribution of the reference β-CD and the dashed line describes the corresponding distribution of water oxygen atoms around β-CD, obtained from an independent simulation of an isolated β-CD dissolved in water.

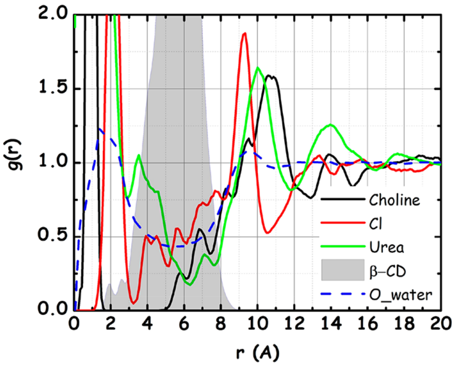


Figure 2. Pdfs of choline, chloride, and urea center of mass (CoM) correlations with respect to the geometrical center of a reference β-CD dissolved in reline at 323 K. The shadowed area indicates the intra-CD atomic distribution, while the dashed line refers to the distribution of water oxygen atoms around β-CD, obtained from an independent simulation of β-CD dissolved in water.

All of the reline components are found inside the reference β-CD (i.e., for $r \leq 4$ Å), with an average number of 0.1, 0.5, and 2 cations, anions, and urea species, respectively (to be compared with approximately seven water molecules by the same distance from the β-CD center). While urea has been reported not to form inclusion complexes with α- and γ-CD in aqueous solutions,[63] here we find a net preference for such a neutral molecule to occupy the hydrophobic β-CD interior, as compared to the other charged moieties.

Further inspection of Figure 2 shows us that the hydrophobic β-CD's exterior walls are surrounded by well-defined solvation shells, between 8 and 12 Å from the reference β-CD center. Chloride anions interact with β-CD already at distances as short as 4 Å; their complex solvation shell between 4 and 8 Å is formed by anions that are hydrogen bonded with the primary and secondary hydroxyl groups at the β-CD's rims. At a larger distance (~9 Å), a clear and sharp anion solvation shell develops, containing ≤11 chloride anions. Cation CoM form a more relaxed solvation shell at larger distances, containing ~15 members, presumably due to their larger sizes. Urea forms a first solvation layer at ~10 Å (containing ~34 molecules), and it is the only species that efficiently develops a well-defined second solvation layer at larger distances (14 Å). We also show the pdf related to water solvation around β-CD. This is consistent with previous related reports[64−66] and shows a single weak coordination layer (centered at ~9 Å) and no indication of a second solvation shell.

The presence of a large number of hydroxyl groups in β-CD and the chemical nature of reline, which is characterized by a large number of hydrogen bonding donor and acceptor moieties, make hydrogen bonding a very important interaction in these mixtures. In Figure 3, we report selected HB-related pdfs. In particular, we show the correlations between the chloride anion and the three hydroxyl groups belonging to each of the glucose units of β-CD. The strong peaks centered

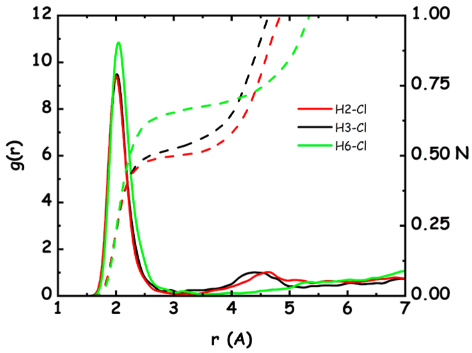


Figure 3. Selected pdfs (solid lines) and corresponding coordination numbers (dashed lines) related to HB interactions in β-CD dissolved in reline. The correlations between chloride anions and the three hydroxyl H's are shown (see Scheme 1 for the atomic nomenclature).

at ~2 Å correspond to the establishment of hydrogen bonding between chloride and the various hydrogen atoms. These are the most recurrent HB-mediated interactions involving β-CD in this system; on average, hydroxyl H atoms are coordinated by 0.5−0.7 chloride ion, as obtained from the integration of the peak in the corresponding pdf. In Tables 1−4, we report the calculated coordination numbers associated with the hydrogen bonding interactions between β-CD and the HB acceptor/donor solvent moieties, for both reline (Tables 1 and 2) and water (Tables 3 and 4). With regard to reline, one can appreciate the major coordination role played by the chloride anion, while urea and choline cation play a secondary role in this respect. The hydrophobic walls of β-CD, however, cannot efficiently interact with the solvent through HB or, more generally, Coulombic interactions. In Table 4, a comparison of calculated coordination numbers associated with the hydrogen bonding interactions between oxygen atoms belonging to β-CD and hydrogen atoms belonging either to water or urea, for the two different mixtures, is presented. It shows that the number of coordinating hydrogens around the different oxygen atoms changes following the same trend for the two solvents and the two acetalic oxygens (O7 and O8) are poorly solvated by both, as compared to hydroxyl oxygen atoms. On the other hand, Hammond et al. described a neat reline structure in terms of an intimate intertwining of charged species and urea, due to balanced HBs leading to sandwiching a chloride ion between a cation and urea.[16,48,49] Such an intricate blending of charged and neutral species can efficiently solvate β-CD's hydrophobic walls. Figure 2 shows already that the first outer β-CD solvation shell (i.e., between 8 and 12 Å from β-CD center) is built up by essentially all species (cation, anion, and urea). This is further confirmed by spatial distribution functions (sdfs) of the different reline moieties around a reference β-CD, as reported in Figure 4 (short distance HB correlations have been excluded). Therein, it is clear that this solvation shell is built up by all of the different reline components that are mutually distributed around the reference β-CD, to maintain local electroneutrality as well as to solvate β-CD's hydrophobic portions. Moreover, the second solvation layer of urea clearly emerges around the reference β-CD.

Table 1. Coordination Numbers Calculated from the Main Peak Pdfs Related to the Atomic Pair in the First Column and the First Row, Referring to β-CD Dissolved in Reline, and Peak Positions, r_{pea}k, and Minimum Positions, r_{min}, Up to Which Integration Was Calculated

	Cl		O_urea		O_choline	
	r_{peak} (r_{min}) (Å)	Nc	r_{peak} (r_{min}) (Å)	Nc	r_{peak} (r_{min}) (Å)	Nc
H3	2.0 (3.0)	0.53	2.1 (2.6)	0.04	2.1 (2.5)	0.017
H2	2.0 (3.0)	0.49	2.1 (2.8)	0.08	2.1 (2.5)	0.025
H6	2.0 (3.0)	0.7	2.1 (3.2)	0.216	2.1 (2.5)	0.028

Table 3. Coordination Numbers Calculated from the Main Peak Pdfs Related to the Atomic Pair in the First Column and the First Row, Referring to β-CD Dissolved in Water, and Peak Positions, r_{peak}, and Minimum Positions, r_{min}, Up to Which Integration Was Calculated

	O_water	
	r_{peak} (r_{min}) (Å)	Nc
H3	1.85 (2.5)	0.76
H2	1.85 (2.5)	0.42
H6	1.85 (2.5)	0.88

Table 4. Coordination Numbers Calculated from the Main Peak Pdfs Related to the Atomic Pair in the First Column and the First Row, Referring to β-CD Dissolved in Water (first column) and in Reline (second column), and Peak Positions, r_{peak}, and Minimum Positions, r_{min}, Up to Which Integration Was Calculated

	H_water		H_urea	
	r_{peak} (r_{min}) (Å)	Nc	r_{peak} (r_{min}) (Å)	Nc
O2	1.95 (2.5)	1.13	2.4 (2.9)	0.88
O3	1.95 (2.5)	0.8	2.4 (2.8)[a]	0.66
O6	1.95 (2.5)	1.4	2.4 (2.9)	1.0
O7	1.95 (2.5)	0.5	2.3 (2.8)[a]	0.46
O8	1.95 (2.5)	0.1	2.5 (3.0)[a]	0.25

[a] Weak or ill-defined O···H pdf peak.

Table 2. Coordination Numbers Calculated from the Main Peak Pdfs Related to the Atomic Pair in the First Column and the First Row, Referring to β-CD Dissolved in Reline, and Peak Positions, r_{peak}, and Minimum Positions, r_{min}, Up to Which Integration Was Calculated

	Cl		O_urea		O_choline		O_CD (all)	
	r_{peak} (r_{min}) (Å)	Nc	r_{peak} (r_{min}) (Å)	Nc	r_{peak} (r_{min}) (Å)	Nc	r_{peak} (r_{min}) (Å)	Nc
H_choline	2.2 (3.4)	0.63	2.1 (2.8)	0.23	2.1 (2.4)	0.04	[a]	0.44
H_urea	2.4 (3.3)	0.5	2.3 (3.1)	0.39	2.4 (2.7)	0.07	[a]	0.06

[a] This voice refers to the average coordination number of H_choline (the H atom connected to choline oxygen) and H_urea (the H atoms belonging to urea) toward all β-CD oxygen atoms. These correlations are characterized by slightly different peak positions and shell limits, depending on the different O atoms. Here we report the average coordination numbers.

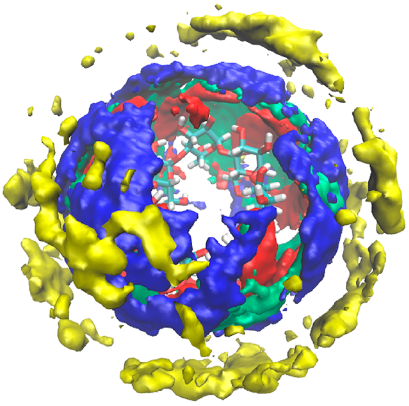


Figure 4. Spatial distribution functions of reline moiety CoM with reference to a reference β-CD dissolved in reline. The color code is as follows: red for chloride, blue for choline, and light green and yellow for the first and second shells of urea, respectively.

In this scenario, urea, due to its amphiphilic nature,[29] implements favorable dispersive interactions with β-CD's hydrophobic regions, thus mitigating β-CD hydrophobic clustering. This behavior emerges further from Figure 5, where the pdf between a carbon atom belonging to the hydrophobic β-CD walls [C4 (see Scheme 1)] and specific urea or choline sites are shown. It can be clearly seen that only urea's nitrogen atoms can efficiently access β-CD's hydrophobic locations, while all other species are largely depleted in that local environment. For comparison, the corresponding C4−O_{water} pdf is shown for the case of β-CD dissolved in water; also in this case, a distinct solvent depletion is observed in this hydrophobic environment, reflecting the difficulty water faces in solvating β-CD hydrophobic moieties.

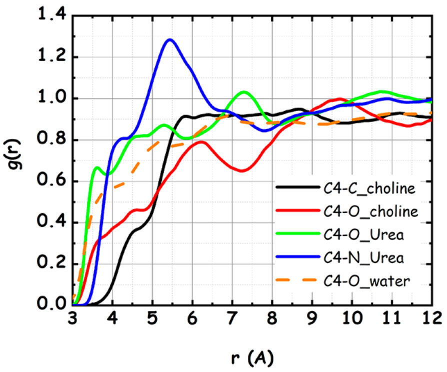


Figure 5. Pdfs of hydrophobic atom C4 of β-CD and different reline sites, namely, choline's methyl C, choline's O, and urea's O and N in β-CD dissolved in reline. The dashed line refers to the pdf between the same carbon and the water O in β-CD dissolved in water.

To further rationalize the role of Coulombic and dispersive interactions in solubilizing β-CD, we evaluated the energy of interaction between β-CD and the different reline moieties (namely, cation, anion, and urea), as well as water molecules, in terms of their Coulombic and dispersive contributions. Table 5 reports such results from the application of the Gromacs function gmx_energy to the two trajectories.

It can be noticed that in the case of water, both Coulombic and dispersive interactions are stabilizing ones. On the other hand, we also notice that the latter contribution amounts to one-third of the former; therefore, Coulombic interactions are dominant, and dispersive interactions have more limited relevance in energetically stabilizing β-CD in water. On the other hand, the reline solution is characterized by a more complex behavior. All reline moieties (choline, chloride, and urea) favorably interact with β-CD through Coulombic interactions, with a large contribution due to chloride and more modest ones due to urea and choline. Overall, the Coulombic contributions to energy of interaction between β-CD and either reline or water are comparable. The difference with respect to the water solution concerning the dispersive interactions is noteworthy; while the Chloride ions show a somehow destabilizing interaction with β-CD, urea and choline cations show an energetically favorable interaction with β-CD, and overall, the energy stabilization due to dispersive interactions in the case of reline is double the corresponding quantity with water as a solvent. These findings support the understanding that in reline solutions, anions develop strong HB with β-CD with strong Coulombic interactions; on the other hand, urea and choline cations also develop very favorable dispersive interactions with β-CD.

Table 5. Decomposition of Interaction Energy between β-CD and Different Solvent (both water and reline) Moieties in Terms of Coulombic and Dispersive Contributions

interacting species	contribution	average energy (estimated error) (kJ/mol)
water−β-CD	Coulombic	−786 (2)
water−β-CD	dispersive	−238 (0.2)
choline−β-CD	Coulombic	−20 (8)
chloride−β-CD	Coulombic	−715 (30)
urea−β-CD	Coulombic	−73 (9)
choline−β-CD	dispersive	−325 (2)
chloride−β-CD	dispersive	40 (4)
urea−β-CD	dispersive	−270 (2)

The results presented here highlight the important role played by all reline components in efficiently coordinating β-CD. Strong, direct HB correlations exist between β-CD's hydrophilic portions and different reline moieties. The large availability of HB donor and acceptor sites in reline (the "alphabet soup of HB" mentioned by Ashworth et al.[28]) enables this solvent to develop stabilizing hydrophilic interactions with β-CD. On the other hand, urea develops favorable dispersive interactions with β-CD hydrophobic portions, thus mitigating inter-CD hydrophobic interactions, while still maintaining the capability to develop strong HB with both the ionic species and β-CD itself. In many respects, we can then anticipate that this behavior is very similar to that encountered when dealing with cellulose dissolution[67−70] and protein denaturation[71−73] by using urea as a co-solute.

Such a multifaceted scenario can then help in rationalizing the substantially different solvation behavior detected when comparing reline with other common DESs, such as ethaline and glyceline. While the favorable hydrophilic interactions of chloride anions with CD hydroxyl groups are maintained across the whole DES series, due to the common presence of chloride anions in these DESs, the difference between these DESs manifests in their neutral species: urea, ethylene glycol (EG), and glycerol (GLY) in reline, ethaline, and glyceline, respectively. While urea is a well-established co-solute for enhancing CD dissolution,[63] the other neutral species in ethaline or glyceline are not. EG is not a good solvent for β-CD (solubility of ∼20 mg/mL), and only water/EG (20:80) mixtures reach a β-CD solubility of 29 mg/mL[74] (α-CD shows a solubility in EG of ~70 mg/mL[75]); analogously, GLY has been reported to be a poor solvent for both α- and β-CD.[76] On the other hand, the addition of urea to water (a poor β-CD solvent) allows the β-CD solubility to reach 250 mg/mL (solubility in neat water of 18 mg/mL[5])[77] and disrupts CD's aggregates.[63] This plausible explanation deserves to be studied further, e.g., by simulating dilute mixtures of β-CD in ethaline and glyceline, to better address the role of the neutral species EG and GLY therein. Such studies are under development and will be reported in the near future. Nevertheless, the results presented here provide useful information about the complex structural features of β-CD solvation in reline.

CONCLUSION

Here we report the first joint experimental and computational structural study of β-CD dissolved in the most common deep eutectic solvent, reline. Solubility tests on native and non-native CDs in reline and other DESs highlighted the peculiar behavior of reline with respect to other common DESs, such as ethaline and glyceline.

Binary mixtures of β-CD and reline were probed for the existence of β-CD aggregates by means of the SAXS technique, leading to the conclusion that no such aggregates can be detected at concentrations of ≤800 mg/mL.

Molecular dynamics simulations were used to explore the atomistic details of the structural correlations between β-CD and the different reline moieties. Overall, the study arrives at the conclusion that reline's exceptional solvation organization surrounding β-CD is a consequence of its peculiar chemical composition. While HB interactions mediated prevalently by chloride ions are crucial, their presence is a necessary but not sufficient condition for the solvent to favorably solvate β-CD. A fundamental structural feature is represented by urea's ability to mitigate hydrophobic interactions and establish favorable dispersive interactions with β-CD's hydrophobic portions. Reline's specific composition allows record solvation features to be achieved, preventing β-CD entities from coalescing into detrimental clusters, as outlined by SAXS measurements. Accordingly, while this study does not address entropic considerations involved in the solvation of DESs toward β-CD, it highlights the important, synergic role of the different reline moieties in efficiently solvating β-CD. Hydrophilic interactions develop between the solute and solvent, and the detrimental hydrophobic interactions between neighboring β-CD monomers are adequately attenuated by urea.

This encouraging scenario opens the way to the development of new smart applications involving concentrated CD solutions as well as new solvent media to widen the range of CD applications.

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Notes

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