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Determination of Binding Strengths of Host–Guest Complexes in Deep Eutectic Solvents Using Spin Probe Methodology

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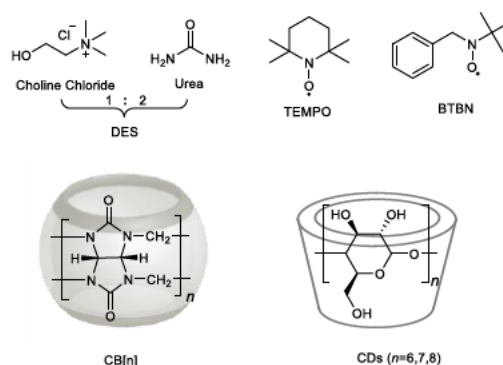
Abstract: EPR and spin probe methodologies have been employed to study the complexation properties of cyclodextrins (CDs) and cucurbit[n]urils (CB[n]s) in the deep eutectic solvent (DES) choline chloride–urea. In the presence of γ -CD an affinity constant very similar to that measured in water was measured in DES with benzyl-*tert*-butyl nitroxide (BTBN). With β -CD, complexation of BTBN is significantly depressed, although still maintained. Complexation of TEMPO radical probe by CB[7] or CB[8] was instead almost entirely cancelled in DES. In addition, this methodology enabled for the first time to measure the single rate constants for the association and dissociation processes with CDs in DES.

Supramolecular chemistry of cyclodextrins (CDs)^[1] and cucurbit[n]urils (CB[n]s)^[2] macrocyclic hosts is traditionally performed in aqueous media. Quite recently, however, it has been reported the dissolution of CDs^[3–5] and CB[n]^[3] macrocycles in choline chloride–urea (ChCl–Ur) mixture, an example of deep eutectic solvents (DES).^[6] This term refers to low melting point liquids generally formed by organic salts and hydrogen-bond donor components characterized by physicochemical properties similar to those of ionic liquids. The solubilities of CDs (α -, β - and γ -) and CB[n]s ($n=6–8$) in ChCl–Ur were found to be significantly enhanced if compared to aqueous environments, in some cases even by a factor of 1000.⁴ The enhanced solubility of CB[n]s and CDs was attributed to the ability of DES to include the macrocycles within the liquid structure and stabilize such high amounts through further hydrogen-bonding interactions.^[3,6] The ability to dissolve both CDs and CB[n] macrocycles in this new solvent at higher concentrations opens up many interesting possibilities and, in principle, it could significantly enhance the industrial exploitation of such macrocycles.^[3–5] However, in order to be useful, the dissolution of CDs and CB[n]s in DES should still maintain the host–guest properties of the macrocycles. Probing the binding properties of macrocycles in DES is not as straightforward as in aqueous environments or traditional organic solvents. The combination of high ionic strength and viscosity of the solvent does not allow an easy use of traditional ¹H NMR and isothermal titration calorimetry (ITC) for quantitative measure of binding and very few data on complexation in DES are available in literature.^[7–9] In particular, affinity constants for the complexation of methyl orange or volatile organic compounds (VOC) by different CDs were measured by UV–Vis,^[7] static headspace gas chromatography (SH–GC)^[7] and DOSY NMR experiments.^[8–9] All the measurements were conducted in pure DES with the exception of the NMR analysis which required some water to be performed. These studies showed that CDs retained

their inclusion ability in DES, even though a significant decrease of guest affinity for the CDs cavities was generally observed if compared to water.

On the other hand, with CB[n]s only qualitative proof on the formation of a host/guest complex has been reported by using UV/Vis spectroscopy and methylviologen as chromophoric reporter.^[3]

EPR and spin probe methodologies have been proven to be very powerful for studying complexation properties of CDs and CB[n]s.^[10] Due to their fast time scale, EPR spectra of radical probes, in presence of host systems, are generally characterized by different signals for the free and included species, and their ratio easily provides the equilibrium constant value for the inclusion complex formation. With CB[7] common spin probes like 2,2,6,6-tetramethylpiperidine-N-oxyl (TEMPO) have been employed for the determination of the binding constant by EPR.^[11] Because of the spectral parameters similarity between the free and complexed species, that impede a clear differentiation of their EPR signals, TEMPO probe cannot instead be used in the presence of CDs, and host–guest complexation with these hosts has been accurately investigated using more sensitive spin probes like diphenylmethyl-^[12] or benzyl-*tert*-butyl nitroxides (BTBN).^[13] In most cases, the EPR spectra of BTBN show also selective line width broadening. This effect indicates that the lifetimes of the free and included radicals are comparable to the time scale of EPR spectroscopy making possible to obtain information on the kinetics of association and dissociation of the inclusion complex.^[13]



Scheme 1.

Thus, in order to extend our knowledge on the host-guest processes in this novel medium we decided to employ for the first time EPR spectroscopy by using BTBN and TEMPO as paramagnetic guests (Scheme 1) in the presence of CDs and CB[n]s in ChCl-Ur mixture.

Good EPR spectra of BTBN were obtained in the temperature range between 298 K and 383 K by reaction of the magnesium salt of monoperoxyphthalic acid (1.0 mM) with benzyl *tert*-butyl amine (1.0 mM) in pure DES. As expected, all the spectra were straightforwardly interpreted on the basis of the coupling of the unpaired electron with the nitrogen nucleus and with the two equivalent benzylic protons. As an example, in Figure 1a is reported the spectrum obtained in DES at 323 K, while the corresponding spectroscopic parameters are reported in Table 1. The value of nitrogen splitting, $a_N = 15.97$ G, which depends on the polarity of the environment, is significantly smaller than that measured in water ($a_N = 16.69$ G), as expected on the basis of the normalized empirical scale of solvent polarity E^T_N , which has been reported to be 0.81 for ChCl-Ur.^[14] This E^T_N value corresponds to that measured in a methanol/water 80:20 (v:v) mixture.^[15] As a confirm, EPR spectrum of BTBN in methanol/water mixture showed a value of a_N (16.00 G) very close to that measured in DES (see Table 1).

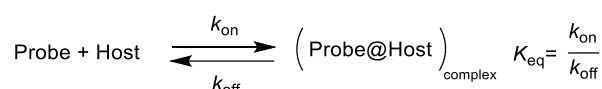


Figure 1. EPR spectra of BTBN recorded in DES at 323 K (a) and in the same solvent in the presence of γ -CD 50 mM at 323 K (b), 343 K (d), 363 K (e). Spectrum (c) was obtained in water at 323 K in the presence of 50 mM γ -CD. As an example, in red is reported the theoretical simulation of spectrum (e) obtained by using $k_{on} = 8.8 \times 10^7 \text{ s}^{-1} \text{ M}^{-1}$, $k_{off} = 3.8 \times 10^6 \text{ s}^{-1}$. Asterisks in spectrum (b) correspond to clean EPR lines deriving from the included radical.

The value of benzylic protons coupling, a_{2H} , which depends on the geometry adopted around the $\text{PhCH}_2\text{-N}$ bond is instead signi-

ficantly different when comparing the spectra recorded in DES and MeOH/H₂O mixture, suggesting a sizeable variation in the mean conformation adopted by the radical when dissolved in DES. The effect of a macrocyclic host on the EPR spectra was then investigated. We initially analysed the effect of the larger member of CD, γ -CD, containing 8 glucose units. When large amounts of γ -CD are added to DES solution, the increased viscosity of the solution is responsible of severe EPR line broadening at room temperature (see Supporting Information). Thus, to improve signal separations, the EPR analysis was accomplished in the range between 323 K and 383 K.

In Figure 1b it is reported the EPR spectrum of BTBN recorded at 323 K in the presence of 50 mM γ -CD in DES. It clearly shows additional signals that were assigned to the radical included in the cyclodextrin, in equilibrium with the free nitroxide (Scheme 2).



Scheme 2.

Because of the slower motion of the BTBN@ γ -CD complex in viscous DES solution, the EPR lines of this new species were characterized by a larger width in comparison to those of the free nitroxide. The ratio between the signals due to the complexed and free radicals increased linearly with increasing concentration of the dissolved host and with a concentration of γ -CD 300 mM the spectrum of the included radical became dominant (see Supporting information) allowing the determination of its spectroscopic parameters (see Table 1).

Table 1. EPR, thermodynamic and kinetic parameters for the inclusion of BTBN in CDs.

Solvent	Host	T	a_N / G	a_{2H} / G	K_{eq} / M^{-1}	k_{on} / $\text{M}^{-1} \text{ s}^{-1}$	k_{off} / s^{-1}
H ₂ O	-	298	16.69	10.57			
DES	-	298	15.97	9.98			
MeOH/H ₂ O ^a	-	298	16:00	8.66			
H ₂ O	γ -CD	303			41	4.1×10^8	1.0×10^7
H ₂ O	γ -CD	323	15.97 ^b	8.01 ^b	30	5.7×10^8	2.0×10^7
H ₂ O	γ -CD	343			21	9.3×10^8	4.4×10^7
DES	γ -CD	323	15.71 ^b	8.13 ^b	34		
DES	γ -CD	343			27	2.7×10^7	1.0×10^6
DES	γ -CD	363			23	8.8×10^7	3.8×10^6
DES	γ -CD	383			20	1.8×10^8	8.0×10^6
H ₂ O ^c	β -CD	323	15.84 ^b	8.02 ^b	613		
H ₂ O ^c	β -CD	343			395		
H ₂ O ^c	β -CD	363			277	2.5×10^9	9.0×10^6
DES	β -CD	323	15.51 ^b	8.33 ^b	22		
DES	β -CD	343			17		
DES	β -CD	363			11	2.3×10^7	2.1×10^6

[a] 80:20 v:v. [b] These values refer to the radical included inside the host cavity. [c] Data from ref. 13.

Inspection of Table 1 shows that the value of a_N decreases only slightly upon inclusion of BTBN into the γ -CD host cavity, while the value of $a_{2H\beta}$ decreases considerably on inclusion giving rise to significant differences in the resonance fields for the $M(2H\beta) = \pm 1$ lines of the included and free species. Because of this favourable spectroscopic feature, the two species can be easily differentiated by EPR and the determination of the association constants (K_{eq}) was straightforward. The same analysis was repeated in water in the temperature range 303–363 K and the data were compared to those measured in DES (see Table 1).

Inspection of the values reported in Table 1 shows that the binding constant, K_{eq} , in ChCl-Ur and in water are quite similar. In marked contrast to this insensitivity of K_{eq} to the solvent nature, the single rate constants, k_{on} and k_{off} , varied significantly. In water, selective line-broadening of the EPR lines of the probe corresponding to $M(2H\beta) = \pm 1$ was already evident at room temperature (see Supporting Information) and a relatively small increase of the temperature (323 K) is sufficient to observe averaged EPR signals for the free and complexed species (see Figure 1c). In DES alternating line width effect starts to appear from 343 K (see Figure 1d) and collapsed lines are observed above 383 K. These qualitative observations clearly suggest that the rates of exchange are slower in DES than in water. Accurate determination of the rate constants k_{on} and k_{off} for association and dissociation, respectively, were determined at different temperatures (see Table 1) by simulating the exchange-broadened EPR spectra using well established procedures based on the density matrix theory and assuming a two-jump model as illustrated in Scheme 2.^[16] Figures 1e shows, as an example, the excellent agreement between simulated (red line) and experimental spectrum recorded at 343 K in DES in the presence of 50 mM γ -CD. At 343 K, in DES, k_{on} is ca. 34 times and k_{off} is 44 times smaller than the corresponding values measured in water at the same temperature.

Very fast rates for CD complex formation can be observed in water, but diffusion limit is not generally reached.^[17] On the other hand, in highly viscous solvent like DES, diffusion control for the association process cannot be excluded and simultaneous chemical activation and diffusion could contribute to the observed kinetic as already observed, for example, in the binding of carbon monoxide by ferroprotoporphyrin IX in glycerol-water mixtures.^[18] In this regime, $1/k = 1/k_0 + 1/k_D$ where k is the observed second order rate constant and k_0 is the rate constant that would be measured if diffusion effects were absent.^[18] If this holds, plots of $\text{Log}(k)$ vs. T^{-1} should show increasing deviations from linearity when moving to lower temperature limit as a consequence of the increasing weight of viscosity term. In the range of temperatures investigated by our EPR kinetic measurements (ΔT 40 K), we do not see substantial deviation of $\text{Log}(k_{on})$ vs. T^{-1} from linearity (see Supporting Information) concluding that above 343 K the association kinetic is largely controlled by chemical activation.^[19] As mentioned in the introduction the enhancement of the overall solubility derives from the increased interaction of DES molecules with the host and, more precisely, to the ability of the hydroxyl groups of the CD to interact with the hydrogen-bonded network of the DES. Thus, we attributed the slower rate of association in DES to the increased energy required for the desolvation of the host in the proximity of its entry surface, which is necessary for the inclusion of the probe. After threading of the nitroxide onto the CD cavity resolution occurs and a stabilization of the complex is observed since k_{off} will then also be reduced by desolvation.

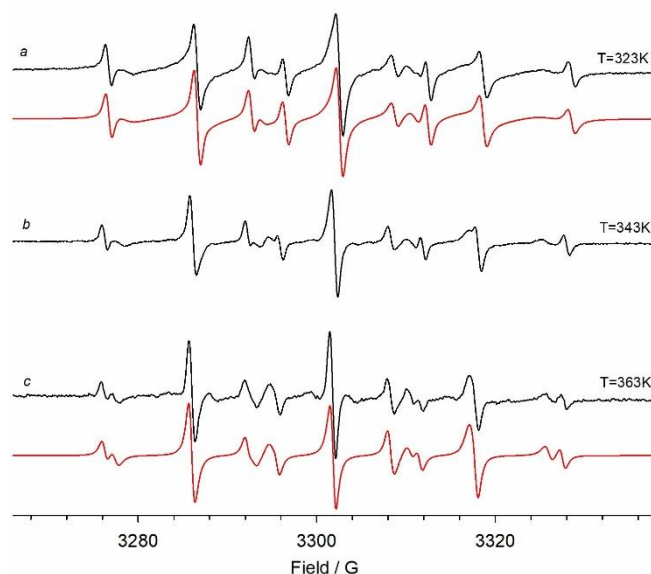


Figure 2. EPR spectra of BTBN recorded in DES in the presence of β -CD 100 mM at 323 K (a), 343 K (b) and 363 K (c). As examples, in red are reported the theoretical simulations of spectra (a) and (c) obtained by using the corresponding parameters reported in Table 1.

We then investigated the behaviour of β -CD in DES in the 323–363 K range. In the presence of β -CD 100 mM the EPR spectra (see Figure 2) clearly shows new signals due to the BTBN radical included inside the cavity. Thus, complexation of BTBN is preserved also with β -CD in DES. Compared to γ -CD, however, the association rate constants, k_{ON} , at 363 K reduces further of 4 times while the dissociation rate, k_{OFF} , is ca. half of that measured in the same condition with γ -CD (see Table 1). This give rise to an overall equilibrium constant which is even smaller than that measured with γ -CD ($K_{eq} = 22 \text{ M}^{-1}$ at 323 K). If we compare these data with that measured in water the affinity of the probe for the β -CD cavity reduces of ca. 25 times when passing in DES. It is evident that the increased steric requirements associated with the smaller cavity of β -CD increases the energy required for the desolvation process resulting in a dramatic inhibition of the host properties in DES as evidenced by the significant decrease of k_{ON} . We finally investigated the complexation properties of CB[n] in DES. Because the spectral resolution of the signals due to the free and included radical in CB[7] is much higher than that generally observed with cyclodextrins, common TEMPO spin probe can be suitably employed in this case.^[11] In DES at 298 K the EPR spectrum of TEMPO was characterized by $a_N = 16.60 \text{ G}$ (in water $a_N = 17.30 \text{ G}$, inside CB[7] cavity $a_N = 16.20 \text{ G}$)¹¹ and the high-field EPR lines showed larger width, due to the slower motion of the radical probe in DES at this temperature (see Supporting information).

When the possible maximum amount^[3] of CB[7] (17 mg/mL) is added to a DES solution of TEMPO the EPR spectrum remains identical to that measured in pure DES, suggesting that the probe is present only in the uncomplexed form still in the presence of a relatively large amount of host. This inhibitive effect by ChCl-Ur was so strong that even a small amount of DES in water ($\text{H}_2\text{O}:\text{DES}$ 20:1, v:v) is already sufficient to suppress completely the inclusion of the probe (see Figure 3). The absence of any signal due to the included radical in the condition described above suggest that K_{eq} must be lower than 10 M^{-1} , that is a value at least

three order of magnitude smaller than that measured in water for the same probe ($K_{eq}=25000\text{ M}^{-1}$).^[11] It is evident that in the case of CB[7] the increased interaction of DES molecules with the host should occur not only at the external surface but also inside the host cavity leading to a strong competition for the cavity itself, in agreement with previous data reporting the inclusion of choline chloride inside the CB[7] cavity.^[20]

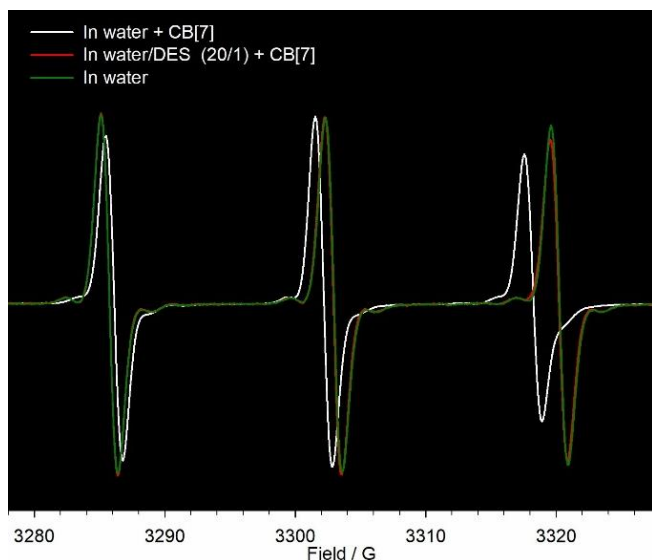


Figure 3. EPR spectra of TEMPO recorded at 298 K in the absence and in the presence of CB[7] 10 mM in water and water:DES (20:1) mixture.

Because of the very low solubility of CB[8] in water, a comparison of complexation behaviour in the two solvents with this host was not possible. Nevertheless, spectra of TEMPO recorded in DES, both in the absence and in the presence of the maximum concentration of CB[8] achievable, show the same value of a_N (16.42 G) suggesting that the complex is not formed under these conditions.^[21]

In conclusion, EPR and spin probe methodologies have been proven to be very powerful for studying complexation properties of CDs and CB[n]s also in DES medium. This methodology, enabled the rate constants for the association and dissociation processes with CDs to be measured for the first time in this medium. With large cavity of γ -CD, in DES, desolvation effects on both rate constants account for a K_{eq} very similar to that measured in water when using BTBN as spin probe. With small cavity of β -CD, complexation of BTBN in DES is significantly depressed although still maintained, as previously observed with VOC.^[7-9] With CB[7] or CB[8] complexation of TEMPO radical probe, is almost completely cancelled in DES. We cannot exclude that the host complex formation with guest molecules of higher affinity than TEMPO could be possible, as reported by Shermann and co-workers^[3] for the CBs binding of methylviologen.^[22] However, our data clearly suggests that all the advantages deriving from the increased solubility achieved in DES can be largely counterbalanced by a significant decrease in the host properties of macrocycles, at least in the case of cucurbit[n]urils.

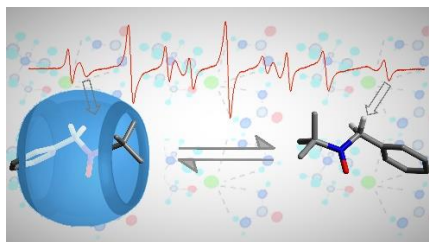
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Keywords: host-guest chemistry • deep eutectic solvents • electron paramagnetic resonance • spin probes • cyclodextrins

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Entry for the Table of Contents



Insert text for Table of Contents here. Host-guest complexes between cyclodextrins, cucurbit[n]urils and nitroxide spin probes have been investigated in choline chloride–urea mixture (DES) by EPR.

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