Do Cyclodextrins Encapsulate Volatiles in Deep Eutectic Systems?

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ABSTRACT

Efficient renewable and non-toxic absorbents can now be designed to eliminate air pollutants such as volatile organic compounds (VOCs) from confined atmospheres. New hybrid materials result from the combination of Deep Eutectic Systems (DES) with well-known VOCs capture agents like β-cyclodextrin (βCD). Yet, a question arises: does βCD retain its encapsulation ability in DES? Multiple NMR techniques are used here to demonstrate the formation of inclusion complexes of βCD with two VOCs, aniline and toluene, in the pure DES reline and in reline/water mixtures. Complexation-induced chemical shift changes and intermolecular host-guest NOEs in the rotating frame give evidence of a genuine encapsulation in the βCD cavity, and complementary information on the dynamics of the VOC is gathered *via* relaxation and diffusion experiments. This work shows how different NMR techniques can contribute to the design of task-specific sustainable materials for absorption/extraction processes.

INTRODUCTION

Volatile Organic Compounds (VOCs) are among the most common air pollutants emitted from chemical and petrochemical industries, transportation vehicles and commercial products such as solvents, paints, cleaners, and lubricants.[1–3] It is today compulsory in many countries to limit and control VOCs emissions both in terms of environment, since they affect the climate change and the growth and decay of plants, and in terms of human and animal health, since VOC exposure causes respiratory distress, eye and throat irritation, neurological toxicity, and cancer, among other effects.[1–3] In the presence of sunlight, VOCs may react with nitrogen oxides and other

airborne chemicals to form ozone, leading to various environmental hazards.[4] VOCs removal is hence a major concern of the society's commitment towards the ecosystem.[1]

Among the materials used as VOC capture agents, β-cyclodextrin (βCD) is one of the most attractive.[2,5–8] βCD is a torus-shaped cyclic oligosaccharide made up of seven α-1,4-linked D-glucopyranose units. It can form inclusion complexes by entrapping small hydrophobic molecules (guests) in the hydrophobic cavity of the macrocyclic sugar (host) through non-covalent host–guest interactions. This is especially true if the target molecules contain hydrophobic groups, such as phenyl groups that are present in many VOCs.[9] βCD-based materials have been reported to be efficient in inclusion complex formation towards several organic pollutants.[2,3,5,6,10–14]

Deep Eutectic Systems (DES) have been recently suggested as innovative materials with potential in gas absorption.[15–18] Practically speaking, a DES is the result of the combination in the proper ratio of an opportune hydrogen-bond donor (HBD) and a hydrogen-bond acceptor (HBA).[19–21] Self-association of the HBD and HBA lowers the entropic difference of the phase transition, so that basically a eutectic is formed with a depressed freezing point that lies well below that of the individual components. DES show many beneficial characteristics: among other, they can be prepared from cheap, readily available, and toxicologically well characterized starting materials, which also implies low cost. A large number of different combinations of HBA and HBD has been reported and their use proposed in many fields of application.[22–25] Interestingly, studies using static headspace gas chromatography (SH-GC) on choline chloride- or tetrabutylphosphonium bromide-based DES showed they can be good candidates for VOC absorption processes.[15]

In this context, DES/βCD mixtures are likewise of interest as new hybrid materials, since in principle their individual absorbing/sequestrating properties may be mutually reinforced. Indeed, the complexation ability of several native and substituted cyclodextrins in the DES reline (choline chloride:urea at 1:2 molar ratio, ChU) towards four VOCs (dichloromethane, toluene, *tert*-butylcyclohexane and limonene) has been recently investigated *via* SH-GC.[26] The authors showed a decrease of the chromatographic peak area, which indicates a higher solubilization of the VOC in the mixture. No further information on the retention mechanism - whether genuine inclusion or aggregation or other - is unfortunately accessible by means of SH-GC. Yet, to concretely exploit reline/βCD mixtures for capturing toxic contaminants, a better characterization of the guest-host inclusion complex at the molecular level is crucial.When searching for an alternative and/or complementary technique to study interactions of small molecules with supramolecular hosts and in particular for investigating the formation of inclusion complexes, Nuclear Magnetic Resonance (NMR) spectroscopy is probably the best option. It is not only possible to directly probe the genuine inclusion of the guest in the CD cavity, but the large number of spectral parameters that can be measured and analysed give also access to unique qualitative and quantitative information.[7,9,27–30] In the present work, we demonstrate that the measurement of different NMR parameters is extremely beneficial to investigate the sorption behaviour of βCD towards selected VOC in the DES reline (see Fig. 1 for structures). First, complexation-induced chemical shift changes and intermolecular host-guest NOEs in the rotating frame allow us to assess whether or not a genuine encapsulation occurs in ChU/βCD mixtures. Moreover, Diffusion Ordered SpectroscopY (DOSY) experiments and measurements of non-selective and selective spin-lattice relaxation times (T_1^{NS} and T_1^{SE}) are applied to glean additional insights into the dynamics of the guest molecule.

A practical limitation in ChU/βCD systems is the relatively high density and viscosity of the mixtures, which strongly reduces the resolution in NMR spectra. Luckily, DES readily lend themselves to be added with a considerable amount of water without losing their unique properties. The effect of water on the hydrogen-bonding network of a DES is still debated and it is generally accepted that reline keeps its nanostructure up to water concentrations of about 35 wt% and a transition from a "water-in-DES" to a "DES-in-water" regime only occurs at ca. 50 wt%.[31,32] The possibility to reduce DES viscosity while preserving its molecular interactions and structure is an extremely advantageous feature, if one considers that DESs are highly hygroscopic and latent absorbed water is unavoidable, especially in the context of industrial applications. This holds true also in the presence of βCD. Indeed, some of us have recently demonstrated that in reline/βCD/H_2O systems, β-cyclodextrin interacts primarily with the added water, while reline preserves a residual molecular network.[33] In this mixture, βCD benefits of enough mobility to retain its encapsulation properties, as proved by selective variation of the chemical shifts and ROESY host-guest intermolecular correlations in the case of a model drug, namely the anti-inflammatory drug piroxicam.

Here we intend to apply multiple NMR experiments to investigate more deeply the potential of reline/βCD mixtures for VOC absorption in the absence and presence of water. As model compounds, we selected two major volatile organic compounds in the environment, aniline and toluene (Figure 1), which are listed as hazardous air pollutants in the Clean Air Act by the US Environmental Protection Agency (https://www3.epa.gov/ttn/atw/orig189.html). The formation of genuine inclusion complexes is evaluated and the dynamic properties of the guest molecules investigated.

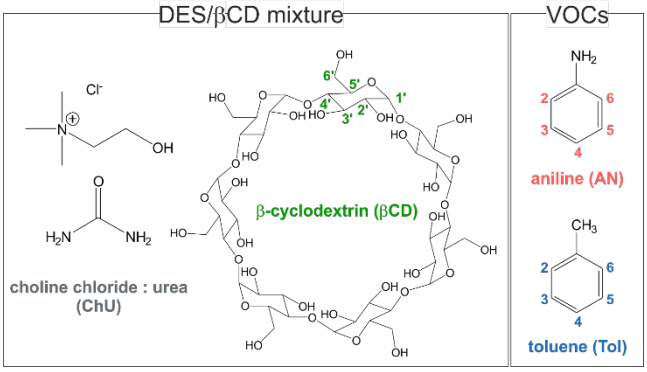

Figure 1. Structure and numbering of compounds used in this work.

EXPERIMENTAL SECTION

A summary of all samples used is reported in Table 1. The deep eutectic solvent reline (ChU) was prepared by the heating method, which consists in mixing the two components [Ch]Cl and U at molar ratio 1:2 and then heating at 80 °C under constant stirring until a homogeneous liquid is formed. In all samples containing β-cyclodextrin, 10% in weight of βCD was added to the reline under stirring at room temperature, until the formation of a homogenous liquid. In all hydrated samples, 210 equivalents of water with respect to βCD were added under stirring. As there are 3 hydroxyl groups for each of the 7 glucose units in a single βCD molecule, this corresponds to a mole ratio βCD:added H_2O equal to 1:10. As reline was not dried prior to utilization, also not hydrated samples contain traces of water due to DES's hygroscopic nature. The water content of the freshly prepared solutions was determined using a KF coulometric titrator from Mettler Toledo and was found equal to 2.1% for ChU/βCD and 0.5% for ChU. Overall this translates into a total water content in hydrated samples between 25 and 27 wt%, which is below the expected transition to an aqueous solution. Samples **1** to **4** were prepared by adding either aniline or toluene to the ChU/βCD and ChU solvents. Samples **5** to **8** were prepared by adding the same

VOCs to the corresponding hydrated systems, ChU/βCD/H_2O and ChU/H_2O. All samples containing βCD (**1**, **3**, **5** and **7**) were prepared to have a final host/guest molar ratio of 1:1 and the same weight percentage of VOC was also used in samples without βCD (**2**, **4**, **6** and **8**). Samples **9** and **10** without VOCs were used as references. The samples for NMR analysis were placed in standard 5 mm tubes and multiple NMR experiments were performed (details are given in the SI).

Table 1. Composition of samples used in this work.

Sample	**Short name**	**DES**	**βCD**	**Added H_2O**	**Guest molecule**
1	ChU/βCD/AN	ChU	10%	-	AN (1 eq.)
2	ChU/AN	ChU	-	-	AN (1 eq.)
3	ChU/βCD/Tol	ChU	10%	-	Tol (1 eq.)
4	ChU/Tol	ChU	-	-	Tol (1 eq.)
5	ChU/βCD/H_2O/AN	ChU	10%	210 eq.	AN (1 eq.)
6	ChU/H_2O/AN	ChU	-	210 eq.	AN (1 eq.)
7	ChU/βCD/H_2O/Tol	ChU	10%	210 eq.	Tol (1 eq.)
8	ChU/H_2O/Tol	ChU	-	210 eq.	Tol (1 eq.)
9	ChU/βCD	ChU	10%	-	-
10	ChU/βCD/H_2O	ChU	10%	210 eq.	-

RESULTS AND DISCUSSION

Does β-cyclodextrin form inclusion complexes with VOCs in pure (non-hydrated) reline?

It is well known that NMR chemical shift is sensitive to changes in electronic environment. Hence, comparing the observed chemical shifts of guest protons as well as of protons located in the CD cavity ($H_{3'}$ and H_{5}') in the ^{1}H 1D spectra of their mixture compared to pure components give a first clue on the formation of an inclusion complex. Figure 2 shows selected regions of ^{1}H spectra corresponding to VOC's signals for the case of aniline (AN) and toluene (Tol) in reline, with or without βCD. In the presence of βCD, it is observed the expected downfield shift of the peaks of the guest molecule, which suggests the formation of βCD/VOC inclusion complexes (see Table S1 for complexation-induced chemical shifts).[30] Moreover, a considerable line broadening of the aromatic peaks is observed after the addition of βCD, which might indicate an exchange regime between free and encapsulated species. Overall, the variation of the chemical shifts as well as the line broadening are more prominent in the case of toluene than aniline, which might point towards a stronger interaction of the former with βCD. Unfortunately, non-hydrated reline mixtures are highly viscous, which causes short relaxation times, broad lines and overall poorly resolved spectra. The effect is even more relevant of βCD's signals, which also suffer from severe overlap with peaks of choline (see Figs. S1-S2). Therefore, any further analysis of ^{1}H-NMR spectra is not possible. Aiming at confirming the formation of βCD/VOC inclusion complexes, we performed rotating-frame Overhauser (ROESY) experiments (Fig. S3). Again, the signal loss due to the short relaxation time in the highly viscous DES solutions made it impossible to distinguish any intermolecular host-guest cross peaks.

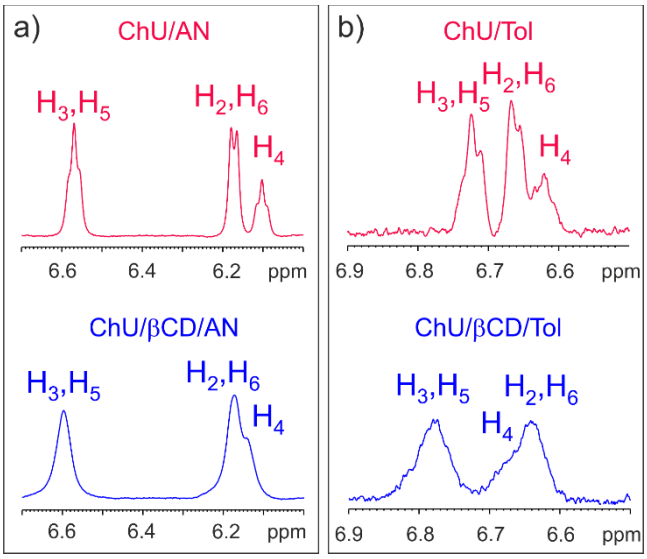

Figure 2. Selected regions of ^{1}H NMR spectra corresponding to aromatic protons of (a) AN samples ChU/βCD/AN (bottom, in blue) and ChU/AN (top, in red) and (b) Tol in samples ChU/βCD/Tol (bottom, in blue) and ChU/Tol (top, in red).

Given the poor quality of βCD signals, we focused on aromatic peaks of VOCs, which are relatively far from DES's signals and give detectable signals. Well-established NMR methods to study inclusion complexes from the guest's viewpoint are relaxation and diffusion experiments. The use of relaxation NMR spectroscopy to study aggregation and/or complexation is well known and relies mostly on the use of inversion recovery experiments for the measurement of proton non-selective and selective spin-lattice relaxation rates, R_1^{NS} and R_1^{SE}.[27,29,34] Indeed, assuming that the bound and free states interconvert quickly with respect to both chemical shift difference and proton relaxation rate, the formation of inclusion complexes affects R_1^{NS} and R_1^{SE} at different extents. In particular, it has been shown that R_1^{SE} is more sensitive to the slower rotational tumbling of the complex than R_1^{NS}.[28,35–39] Therefore, the combination of non-selective and selective inversion recovery measurements is a rich source of information in dynamic

studies. It has been used extensively to measure chemical exchange rate constants separated from the spin−lattice relaxation rates in slow exchanging systems,[40] to describe molecular conformational motion in solution,[41,42] as well as to study interactions between small molecules with macromolecules,[28,29,37,38] and estimating the binding affinities of ligands with protein.[39,43,44] In the case of inclusion complexes with cyclodextrins, the application of ^{1}H nonselective, selective, and bi-selective spin−lattice relaxation rates can be used to address intermolecular interactions and motional dynamics of drugs encapsulated within the βCD cavity.[45] A theoretical description is provided for the interested reader in the SI. Basically, measuring R_1^{NS} and R_1^{SE} within the initial rate approximation it is possible to infer the molecular rotational correlation time τ_C of the encapsulated drug, which may be thought of as the average time required for the molecule to rotate by approximately 1 radian.[36,38,46] Both the R_1^{NS}/R_1^{SE} ratio and τ_C are related to the motional regime of the guest, which in turns reflects its equilibrium between the free and bound states.[28,35,45]

Here we applied this method to get information about the dynamics of the selected VOCs in reline and on how it changes in the presence and absence of β-cyclodextrin. As overlapping peaks are not suitable for selective inversion, after investigation of ^{1}H NMR spectrum, the most isolated peaks were chosen for observation, namely protons H_3,H_5 for aniline and methyl protons for toluene (see Figs. 2 and S1-S2). Figure 3 shows as an example the stack plots obtained with conventional nonselective pulses in the 180°-t-90° IR sequence (a) and with a selective IR experiment with the selective 180° centered on protons H_3,H_5 of aniline (b). In Fig. 3(b), the signals of unselected protons remain essentially unperturbed, but the resonance of the selected protons relaxes faster. The values of the R_1^{NS}/R_1^{SE} ratio obtained for samples **1** to **4** are summarized in Table 2.

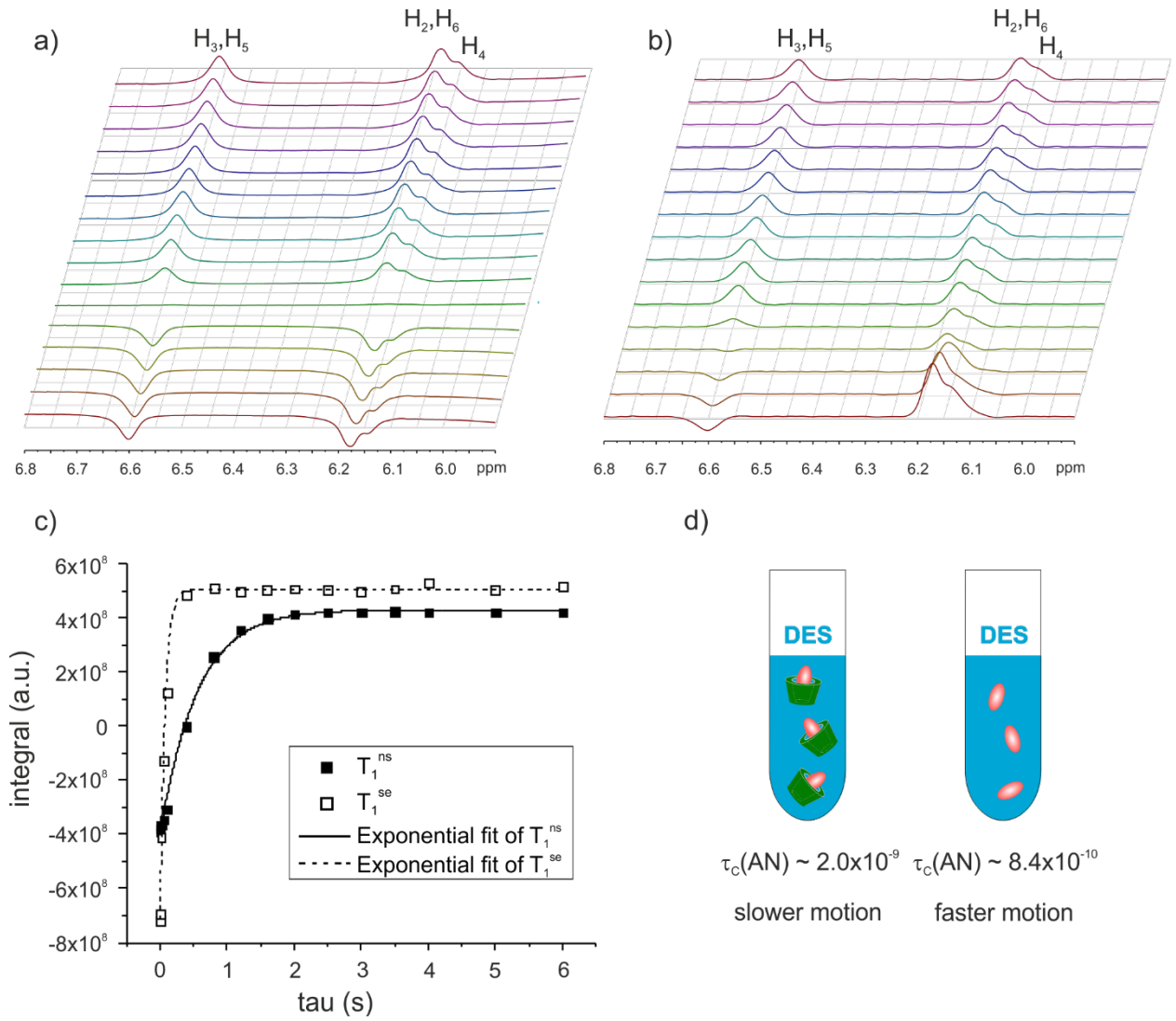

Figure 3. Stack plots of (a) nonselective and (b) selective for H_3, H_5 protons of AN inversion recovery experiments of sample ChU/βCD/AN. (c) T_1 relaxation decays of H_3, H_5 protons of AN measured with nonselective and selective inversion recovery experiments and corresponding mono-exponential fits. (d) Comparison of correlation times estimated for AN in the samples ChU/βCD/AN and ChU/AN. Maximum errors are estimated to be about 20%.

Table 2. R_1^{NS}/R_1^{SE} ratio and τ_C values obtained for aniline (AN) and toluene (Tol) dissolved in reline mixtures. Maximum errors are estimated to be 10% and 20% for the hydrated and non-hydrated samples, respectively.

Sample	**Short name**	**Guest**	**Selected proton(s)**	$\mathbf{R_1^{NS}/R_1^{SE}}$	$\boldsymbol{\tau_C}$ **(s)**
1	ChU/βCD/AN	AN	H_3,H_5	0.13	$2.0x10^{-9}$
2	ChU/AN	AN	H_3,H_5	0.50	$8.4x10^{-10}$
3	ChU/βCD/Tol	Tol	CH_3	0.04	$3.9x10^{-9}$
4	ChU/Tol	Tol	CH_3	0.39	$1.0x10^{-9}$
5	ChU/βCD/H_2O/AN	AN	H_3,H_5	0.83	$4.8x10^{-10}$
6	ChU/H_2O/AN	AN	H_3,H_5	1.37	$1.2x10^{-10}$
7	ChU/βCD/H_2O/Tol	Tol	CH_3	0.74	$5.7x10^{-10}$
8	ChU/H_2O/Tol	Tol	CH_3	0.93	$4.1x10^{-10}$

In all four samples the R_1^{NS}/R_1^{SE} ratio is far smaller than 1. This is symptomatic of slow motion regime, which was expected for such viscous systems. For both aniline and toluene the R_1^{NS}/R_1^{SE} ratio decreases from the pure reline to the reline/βCD mixture. This indicates that in the presence of βCD the rotational motion of the guest gets even smaller, which is compatible with the formation of an inclusion complex. Even if it is tempting to compare the two VOCs, it should be remembered that different protons were selected for the two molecules (namely aromatic protons for aniline and methyl protons for toluene) and a direct comparison might not be fair.

From the ratio of the nonselective and selective relaxation rates, it was possible to get an estimate of the correlation times of VOCs in the ChU/βCD mixtures and to compare them with the systems without βCD. Results are reported in Table 2 and Fig 4. For both AN and Tol, τ_C in the ChU/βCD mixture is bigger than the corresponding value in the pure reline. Since the

correlation time is inversely proportional to the degree of molecular mobility, that is the smaller values of τ_C correspond to higher molecular mobility,[38] this is again compatible with a slowdown in dynamics of the guest molecule because of its encapsulation in the βCD cavity.

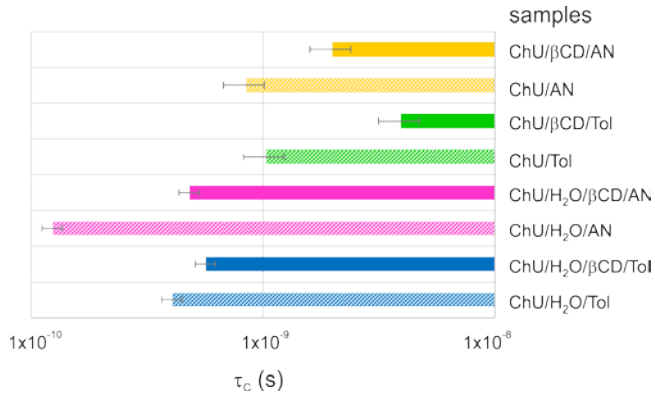

Figure 4. Summary of τ_C values obtained for aniline (AN) and toluene (Tol) dissolved in reline mixtures. Maximum errors are estimated to be 10% and 20% for the hydrated and non-hydrated samples, respectively.

Diffusion experiments have received attention since the mid-1990s thanks to the highly representative processing called Diffusion Ordered SpectroscopY (DOSY).[39,47,48] In a DOSY map, the diffusion experiment is processed in a 2D spectrum in which one dimension is related to the chemical shift information, while the other represents the diffusion coefficient.[49,50] Since the translational diffusion coefficients (D) of molecular species reflect their effective sizes and shapes, DOSY NMR allows both the identification and separation of the chemical entities in multicomponent systems, and provides information on their intermolecular interactions as well as on the dynamics of the system.[49,51,52] In other words, a small molecule diffuses faster than a large one and the binding of a freely diffusing molecule to another species leads to a decrease of

D. Indeed, in the fast exchange limit, the observed diffusion coefficient of the guest is the mole fraction weighted average of the diffusion coefficient of the free and bound states. This means that, in the presence of a complex, the diffusion of the encapsulated molecule will be slower than that measured for the free compound. DOSY maps are well suited to study aggregation and quantify molecular interactions in chemical systems,[49,52–54] and many examples can be found in the literature where DOSY NMR has been used to investigate structure, stoichiometry, host bound and unbound fractions to guest, association binding constant, and host/guest relative positioning in CD complexes.[34,49,55–58] DOSY maps were acquired for samples **1** to **4** (Fig. S4). Unfortunately, signals of βCD were not detectable in any samples and VOC's signals were observed only in pure reline. This indirectly suggest that in the presence of βCD, the guest (AN or Tol) are probably encapsulated, so that their lines get broader and not visible anymore in the 2D spectra. However, no additional considerations can be drawn.

Does β-cyclodextrin form inclusion complexes with VOCs in hydrated reline mixtures?

In the previous section, practical limitations to the NMR investigation of reline/βCD emerged because of the inherent viscosity of the samples. Even though both the changes in ^{1}H pattern of the guest as well as the modification of its dynamic behaviour suggest that the formation of βCD/VOC complexes really takes place, we do not have a direct proof of a genuine encapsulation of the VOC in the βCD cavity. To overcome this problem, we decided to add 210 equivalents of water with respect to the β-cyclodextrin in the mixture. The same NMR methodology was then applied to hydrated reline samples.

Fig. 5 shows selected regions of ^{1}H spectra of samples **5** to **8**. Both VOC's and βCD's signals are clearly visible. In the presence of βCD, a modification of the aromatic spectral pattern of the

guest molecule is observed, more relevant for toluene than for aniline. As for βCD, if a guest molecule is incorporated into the CD cavity, one should observe modification of the chemical shift of protons $H_{3'}$ and $H_{5'}$, which are located in the hydrophobic cavity of the host molecule. Here slight changes are visible, more prominent on $H_{3'}$ than on $H_{5'}$.

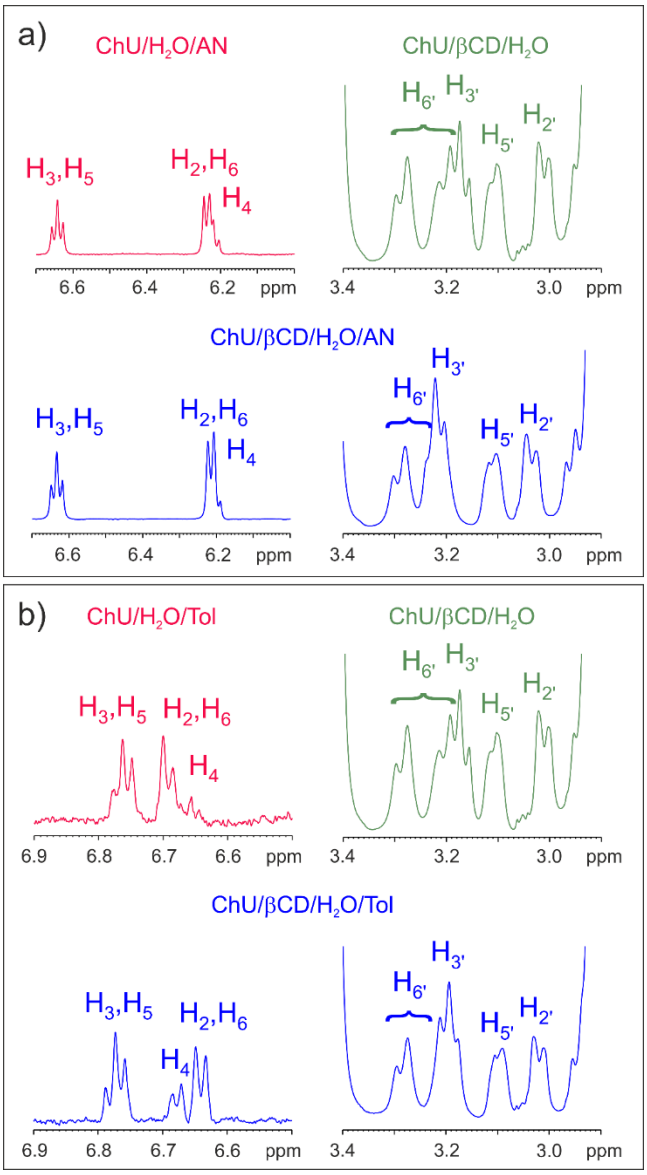

Figure 5. Comparison of ^{1}H NMR signals of the guests aniline (AN) and toluene (Tol) and the host macromolecule β-cyclodextrin (βCD) in different samples: (a) ChU/βCD/H_2O/AN (bottom spectrum, in blue) *vs* ChU/H_2O/AN (top spectrum, left, in red) and ChU/βCD/H_2O (top

spectrum, right, in green); (b) ChU/βCD/H_2O/Tol (bottom spectrum, in blue) *vs* ChU/H_2O/Tol (top spectrum, left, in red) and ChU/βCD/H_2O (top spectrum, right in green).

To unambiguously confirm the encapsulation, rotating-frame Overhauser spectroscopy (ROESY) is the preferred NMR tool. ROESY studies have been extensively used to get information on inclusion complexes with βCD through analysis of intermolecular peaks between cavity protons and part of the guest involved in complexation.[9,27,53–55,59,60] Fig. 6 shows representative ROESY spectra obtained for the sample ChU/βCD/H_2O/AN and ChU/βCD/H_2O/Tol. The host-guest interactions are displayed as intermolecular correlation peaks between aromatic protons of aniline and toluene and protons $H_{3'}$ and $H_{5'}$ of βCD. This unequivocally confirms the formation of the inclusion complex of the VOC in βCD. Both AN and Tol produce larger NOEs on the $H_{3'}$ proton, located in the larger diameter internal part of βCD, than they did on the other internal $H_{5'}$ proton nearer the smaller diameter rim. This would indicate they are included through the larger diameter cavity βCD.

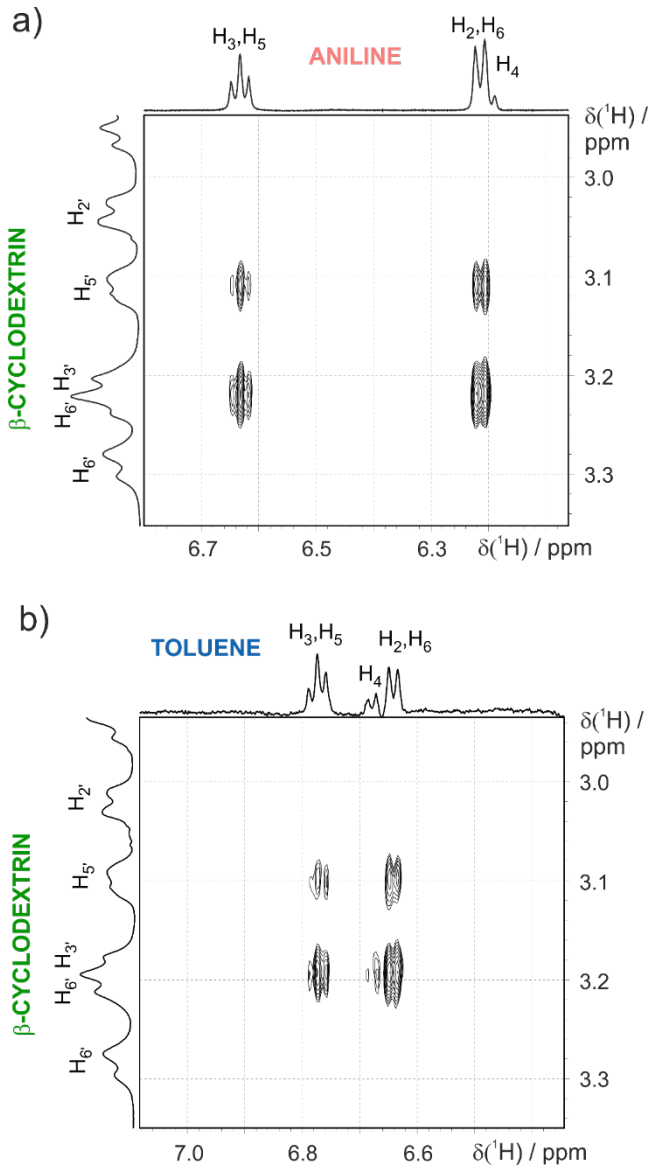

Figure 6. Selected regions of ^{1}H-^{1}H ROESY spectra showing intermolecular correlation peaks between signals corresponding to $H_{3'}$ and $H_{5'}$ protons of β-cyclodextrin (vertical dimension) and aromatic protons of (a) aniline and (b) toluene (horizontal dimension).

Nonselective and selective relaxation experiments were performed on samples **5** to **8** to get dynamic information on the encapsulated VOC. Results are summarized in Table 2 and Fig. 4. Clearly, all R_1^{NS}/R_1^{SE} ratios and τ_C values for hydrated reline mixtures are bigger than the corresponding values in non-hydrated samples. More in detail, for aniline in ChU/βCD/H_2O, the R_1^{NS}/R_1^{SE} ratio is around 1.4. This value is expected when the molecule is in the extreme narrowing region ($\omega_0\tau_C \ll 1$).[38,45] For toluene in ChU/βCD/H_2O, the value of the R_1^{NS}/R_1^{SE} ratio is just close to 1, meaning that it is in the intermediate motion limit. This would indicate that in the mixture composed of reline and water, aniline rotates more freely than toluene. Nevertheless, as different protons were selected for the two VOCs (aromatic protons for AN vs methyl protons for toluene) any further comparison would sound forced. When β-cyclodextrin is added to the mixture, the R_1^{NS}/R_1^{SE} ratio and the τ_C values for both AN and Tol get smaller. Since the correlation time is inversely proportional to the degree of molecular mobility (the smaller values of τ_C correspond to higher molecular mobility), this is compatible with a slowdown in dynamics of the guest molecule (Tol or AN) because of its encapsulation in the βCD cavity.

Finally, diffusion experiments were performed on samples **5** to **8**. DOSY maps obtained for AN and Tol in reline/water mixtures, with or without βCD, are reported in Figure 7. It can be seen that the diffusion of the VOC in the samples with βCD (left column) is in both cases slower that in the corresponding samples without βCD (right column). This is a consequence of formation of inclusion complexes with the βCD. A graphical summary of diffusion coefficients for the different species is reported in Fig. S5.

To confirm that the reduced diffusion of the guest in the presence of βCD is not due to viscosity changes caused by the addition of the macrocyclic oligosaccharide, the self-diffusion coefficient of H_2O was used as an internal reference.[61] A look at Fig. S5 and Table S2 reveals

that the diffusion coefficient of water remains constant upon addition of βCD in the reline system. This means that the variations in solution viscosity are minimal and do not account for the change in diffusion of the guest molecules, toluene or aniline.

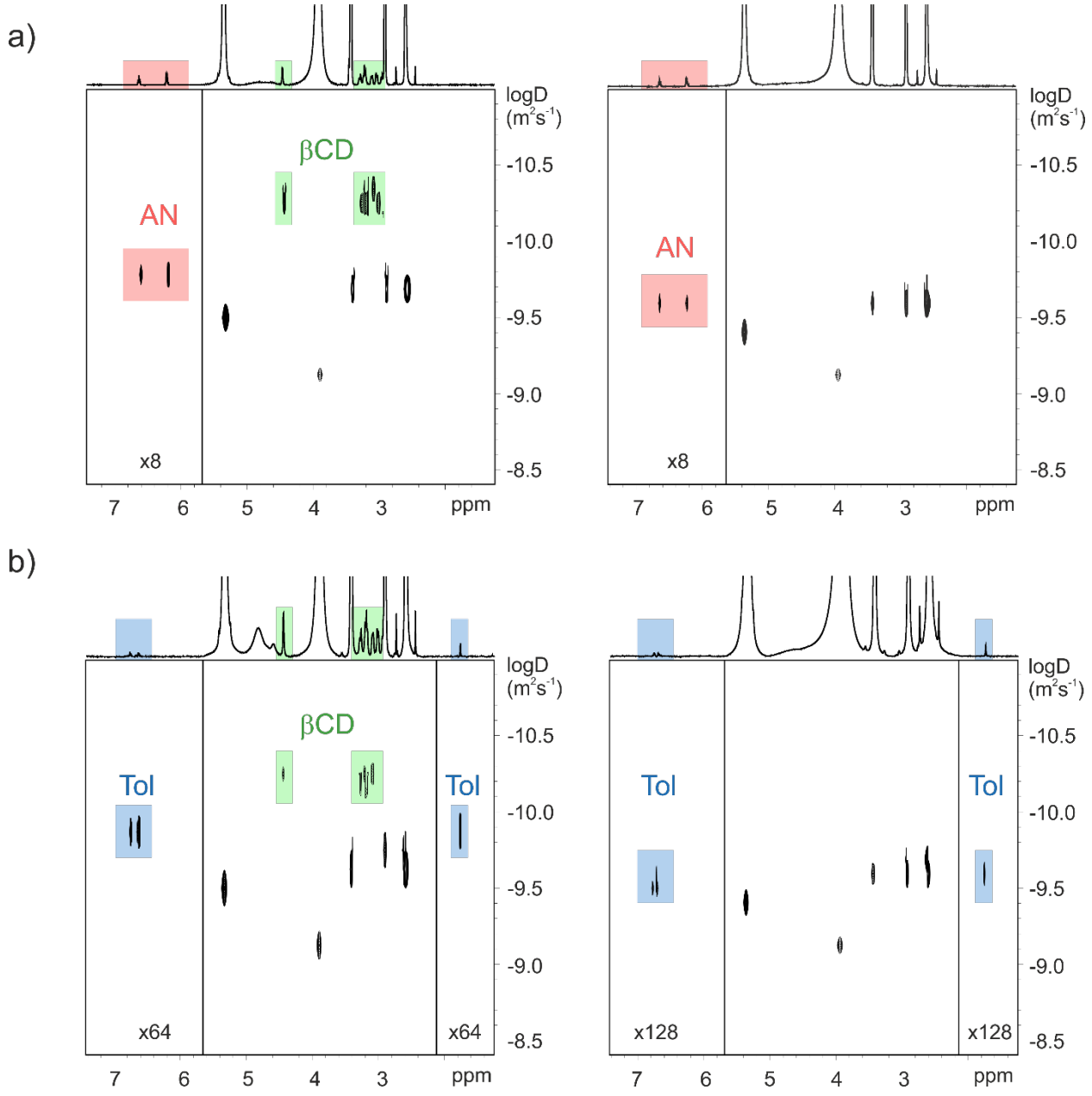

Figure 7. DOSY maps acquired for hydrated reline mixtures with (left column) and without (right column) βCD for (a) aniline and (b) toluene.

Assuming a rapidly equilibrating system, the diffusion coefficients are weight-averaged NMR values between free and bound species. In this condition diffusion coefficients measured using DOSY experiments for the guest molecule, D_G^{obs}, can be used to calculate the molar fraction of the bound guest x_G^{bound} and apparent association constants K_a of host–guest complexes (see SI for a theoretical treatment).[50,53,55–57] Briefly, the observed diffusion coefficients (D_G^{obs}) of AN and Tol measured in the equimolar host-guest mixtures (samples **5** and **7**) are the averaged values weighted by mole fractions of their bound and free molecules. The diffusion coefficients of free guests, D_G^{free}, can be measured directly in the absence of the host (samples **6** and **8**). As for the diffusion coefficients of bound guests, D_G^{bound}, it is typically assumed that when a small guest molecule binds to a large host molecule, the diffusion coefficient of the host is only insignificantly affected by the complexation. Therefore, the diffusion coefficient of the complex and the observed diffusion coefficient of the CD host, D_H, are assumed to be equal. This approximation allows to determine K_a from a single DOSY experiment. Diffusion coefficients for AN and Tol are reported together with the calculated x_G^{bound} and K_a in Table 3. Taking into account the inaccuracy in the calculations coming from the different approximations and the experimental errors both in sample preparation and in the acquisition and treatment of the experimental data, the K_a values are given with an error equal to 20% of the value. Reported values of association constant in water are in the range 140-287 M^{-1} for βCD/toluene and about 50 M^{-1} βCD/aniline,[7] which are higher than the K_a values measured here. It has been recently observed using static headspace gas chromatography (SH-GC), that association constant for the

VOC/βCD of in reline are lower than the corresponding values in water.[26] However, it should be noted that also in reline/H_2O mixtures studied here, toluene binds more strongly to βCD than aniline, which is in agreement with data from the literature. Moreover, the K_a value found here for βCD/toluene complex is higher than the one calculated by SH-GC (79 M^{-1} *vs* 11 M^{-1}). This could be ascribed to the presence of water in the systems used in this work.

Table 3. Diffusion coefficients measured in hydrated reline mixtures and corresponding molar fraction of the bound guest x_G^{bound} and apparent association constant K_a estimated for AN and Tol

Guest	$\mathbf{D_G^{obs}}$ **(m^2s^{-1})**	$\mathbf{D_G^{free}}$ **(m^2s^{-1})**	$\mathbf{D_H}$ **(m^2s^{-1})**	$\mathbf{x_G^{bound}}$	$\boldsymbol{K_a}$ **(M^{-1})**
AN	$1.7x10^{-10}$ $\pm0.1x10^{-10}$	$2.6x10^{-10}$ $\pm0.1x10^{-10}$	$5.6x10^{-11}$ $\pm0.6x10^{-11}$	0.46±0.05	19±4
Tol	$1.4x10^{-10}$ $\pm0.1x10^{-10}$	$3.2x10^{-10}$ $\pm0.2x10^{-10}$	$5.6x10^{-11}$ $\pm0.6x10^{-11}$	0.70±0.07	79±16

Conclusions

DES/βCD mixtures are interesting as new hybrid materials for VOC removal. To design the most performant DES-based absorbents, it is imperative to probe the formation of inclusion complexes and to have access to the dynamics of the system at the molecular level. Here we have illustrated the complementarity of the structural and dynamic information obtained from the 1D ^{1}H and ROESY spectra with diffusion and relaxation NMR experiments, and shown that their combination provides unique information to understand the formation of inclusion complexes with β-cyclodextrin in complex media such as deep eutectic systems.

Interestingly, we have proved that a certain amount of water is beneficial in reline systems, since the viscosity of the medium is reduced, while preserving both the DES network and the complexing ability of β-cyclodextrin. In non-hydrated systems, NMR results suggest the formation of inclusion complexes but given the impossibility to obtain correlation peaks in the ROESY spectra, a direct evidence is not available. When water is added to the mixture, intermolecular peaks are clearly visible in the ROESY spectra, confirming unequivocally the inclusion of the VOC.

In both hydrated and non-hydrated samples, selective T_1 measurements turned out to be very sensitive and convenient for the investigation of the complexation of VOCs into the β-cyclodextrin, allowing the calculation of the correlation times. The R_1^{NS}/R_1^{SE} ratio is a marker for the transition between dynamic states of solutes in DES systems: the border value 1.5 for molecules in the extreme narrowing limit can be exploited to monitor the transition between fast and slow tumbling solutes. The data of Table 2 related to samples **5** and **6** clearly point out that the formation of an inclusion complex between βCD and AN causes the transition between fast and slow regime, not observed in the other examples of Table 2. Additionally, DOSY experiments allowed a rough but simple estimate of the molar fraction of the bound guest and the association constant of the complex in reline/water mixtures.

ASSOCIATED CONTENT

Supporting Information.

The following files are available free of charge.

Experimental details, additional NMR spectra, graphical summary of diffusion coefficients and

theoretical background for the treatment of relaxation and diffusion data (PDF)

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SYNOPSIS

The combination of the 1D ^{1}H and ROESY spectra with diffusion and relaxation NMR experiments provides unique structural and dynamic information on inclusion complexes in DES/βCD mixtures, crucial for the design of task-specific sustainable absorbents.