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ORIGINAL ARTICLE

Effect of β-cyclodextrin on the aggregation of the non-ionic surfactant Igepal CO-630 in water as studied by 1D and 2D NMR spectroscopy

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Abstract The micellization process of the non-ionic surfactant, Igepal CO-630, and its inclusion complexes with β-cyclodextrin (β-CD) have been investigated by NMR spectroscopy. The critical micelle concentration of Igepal was determined by measuring the chemical shifts of different resonances. The structure and binding of the inclusion complexes between the Igepal and the β-CD have been studied by 1D proton NMR and ROESY experiments. The stoichiometry of the inclusion complex is mainly 1:1, with a slight contribution of 2:1. At high concentrations of surfactant, the plots of the chemical shifts in the absence and presence of β-CD coalesce, which indicates that the complexes do not take part into the micelles. The ROESY spectrum displays strong correlations between the internal cavity protons of the CD and the aromatic and aliphatic regions of the Igepal, suggesting the formation of a 2:1 primary face-to-face inclusion complex at high concentrations of β-CD.

Keywords Cyclodextrins · Self-aggregation · Non-ionic surfactant · NMR · ROESY

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Introduction

Non-ionic surfactants have been very attractive candidates as micelle-forming agents that play a key role in many industrial and biological processes [1]. The modification of their micellization properties, such as the critical micelle concentration (cmc) can be attained by different approaches: (a) the change of the working temperature, (b) the addition of cosolutes or additives, and (c) the formation of polymolecular host/guest structures, such as CD complexes [2]. The latter approach involves the inclusion of the hydrophobic moiety of the surfactant into the cavity of the CD, resulting in an enhancement of its solubility in water. Thus, the presence of the CD introduces a new equilibrium, which competes with the micellization and modifies the self-organization process of the surfactant [3]. These amphiphilic molecules can also be used as probes in order to investigate the phenomenon of complexation, since their molecular structures can be easily modulated (e.g., polar nature of the head and charge, length of the hydrocarbon tail, etc.) [4].

In this study we have chosen the non-ionic surfactant Igepal CO-630 to investigate host–guest interactions with β-CD in conditions above the cmc of the surfactant in its binary system. This surfactant has an oligo(oxyethylene) group as the polar head and an alkylphenyl group as the lipophilic part of the molecule (Fig. 1). In particular, we have studied the effects of the complexation with β-CD in the micellization of this non-ionic surfactant by NMR experiments, such as 1D ^{1}H NMR and 2D ROESY [5]. This two-dimensional experiment yields correlation signals that are caused by dipolar cross-relaxation between spins in a close spatial

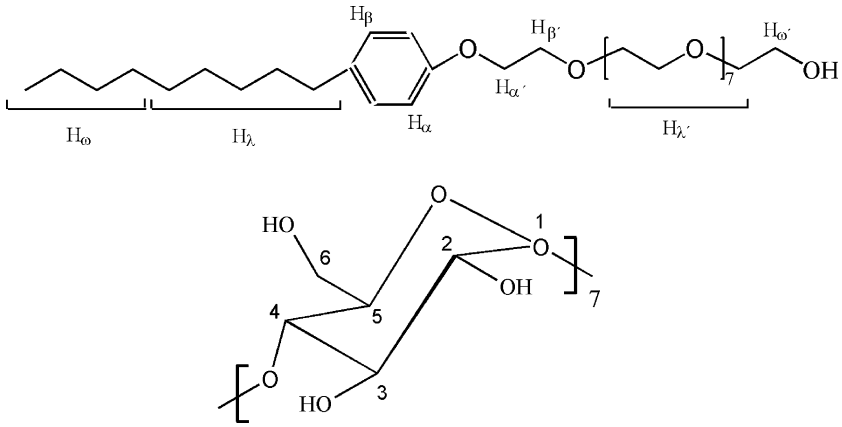

Fig. 1 Chemical structures of Igepal CO-630 and β-cyclodextrin (β-CD)

relationship as protons of host/guest molecules that constitute the complexes [6].

Experimental

Chemicals

β-CD was purchased from Aldrich, having water content of 13.5%, as determined by thermal analysis. Igepal CO-630 was purchased from Sigma and used as received. All the NMR samples were prepared in D_2O (Aldrich Chemical Co., 99.9% minimum in D). Mehtanol-d4 was obtained form CIL (99% D).

^{1}H NMR spectra

For the study of the binary system in water, a stock solution of Igepal CO-630 was added to vials containing different volumes of water. In the case of the ^{1}H NMR experiments of the ternary systems, different Igepal/CD molar ratios were prepared from stock solutions of cyclodextrin, by weighing increasing amounts of surfactant in suitable vials. The concentration of CD in these experiments was kept constant above the cmc at 6.0 mM. The proton spectra were recorded at 298 K in a Bruker Avance AV-500 spectrometer (11.7 T) by averaging 32 scans, with a digital resolution of 0.30 Hz. Deuterated methanol in the inner of a coaxial tube was used as external reference in all the samples [7]. The signal assignment of the surfactant was established by conventional NMR methods (COSY and TOCSY).

ROESY experiments

A Bruker Avance DPX-300 spectrometer (7.05 T) was used, by applying the pulse sequence defined in the literature [8]. 32 scans were collected in each spectrum. Different spin-lock mixing times ranging between 200 ms and 800 ms were applied to ensure the validity of the linear approximation for the ROE cross-peaks and to obtain the best signal-to-noise ratio, which was achieved with 600 ms. Linear prediction in F1 and cosine square apodization in both dimensions were applied to the FIDs before the Fourier transformation and 2D phase tuning. The temperature was kept constant in these experiments at 298 K.

Results and discussion

Chemical shifts

The ^{1}H NMR spectrum of the Igepal CO-630 in D_2O can be divided into three sets of signals according to its structure (Fig. 2). Regarding the hydrocarbon tail, it is

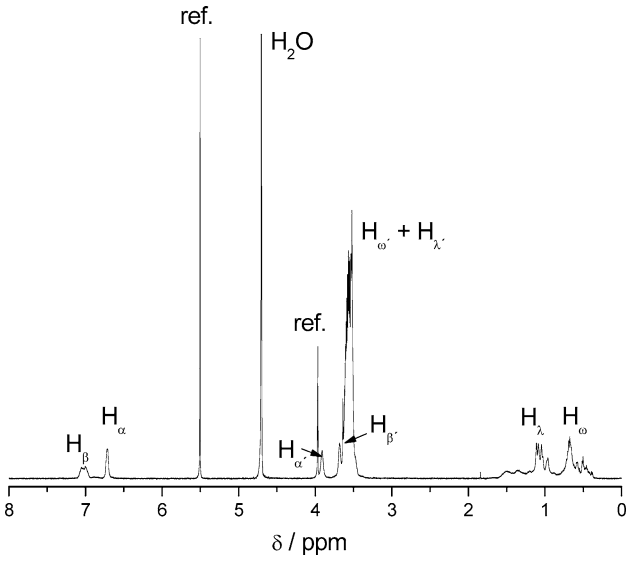

Fig. 2 ^{1}H NMR spectrum and proton assignment for Igepal CO-630 in D_2O at 10.0 mM, using deuterated methanol as external reference

observed the broad signals corresponding to H_ω (0.3–0.9 ppm, 9H) and H_λ (0.9–1.8 ppm, 9H). As for the oxyethylene region, the broad peak at 3.4–3.7 ppm can be identified as the corresponding non-equivalent ethylene protons $H_{\omega'}$ (2H) and $H_{\lambda'}$ (6H). In addition, the two multiplets centred at 3.716 and 3.945 ppm correspond to the protons $H_{\beta'}$ (2H) and $H_{\alpha'}$ (2H). The aromatic protons, H_α (2H) and H_β (2H), are assigned at 6.75, and 7.06 ppm, respectively.

The micellization of Igepal may be probed through any property that changes sharply around the critical concentration, in our case, the plots of chemical shift, δ, versus the concentration. Under fast exchange in the NMR time scale [9], the measured chemical shifts for the corresponding proton are the sum of the property of the free surfactant and the aggregated form, each one averaged with its respective molar fractions [10]. The cmc of the pure surfactant is observed at a concentration range of 0.05–0.08 mM, in good agreement with the literature value [11]. Below this concentration, the chemical shifts change smoothly with the surfactant concentration, and the extrapolation at infinite dilution provides the property corresponding to the monomer, δ_{S_F}. The chemical shifts of the surfactant in its micelle form, δ_{S_N}, can be obtained at high concentration of Igepal, in which δ, remains constant. The properties thus calculated for selected protons of the surfactant have been collected in Table 1.

The relative values of the chemical shifts, $\Delta\delta = \delta - \delta_{S_F}$, of aromatic protons, H_α and H_β, with respect to the monomer versus the surfactant concentration have been plotted in Fig. 3. At concentrations above the cmc, the chemical shifts decrease as a result of micelle formation up to reaching a constant value. All the resonances undergo remarkable upfield shifts, $\delta_{S_N} - \delta_{S_F}$ (Table 1). The resonances of the aromatic moiety and the oxyethylene and aliphatic chains close to the benzene ring of the surfactant suffer the largest changes, in a maximum extent of –0.341 ppm for H_β at 15.0 mM. In contrast, oxyethylene and aliphatic

Table 1 Chemical shifts upon micellization and complexation of Igepal CO-630 and non-linear fitting analysis of the H_3 chemical shift of β-CD

	δ_{S_F}/ppm	δ_{S_N}/ppm	$\delta_{S_N} - \delta_{S_F}$/ppm	δc/ppm
H_β	7.397	7.056	–0.341	–0.132
H_α	6.957	6.748	–0.209	0.021[a] –0.116[a]
$H_{\alpha'}$	4.182	3.945	–0.237	0.022
	δ_F/ppm	$\delta_{1:1}$/ppm		$\delta_{2:1}$/ppm
H_3	3.942	3.855		3.831

$K_1 = (2.5 \pm 0.7) \times 10^4$ L mol^{-1}; $K_2 = (1.0 \pm 0.3) \times 10^3$ L mol^{-1}

[a] At high r = [Igepal]/[β-CD], the two H_α protons are magnetically non-equivalent

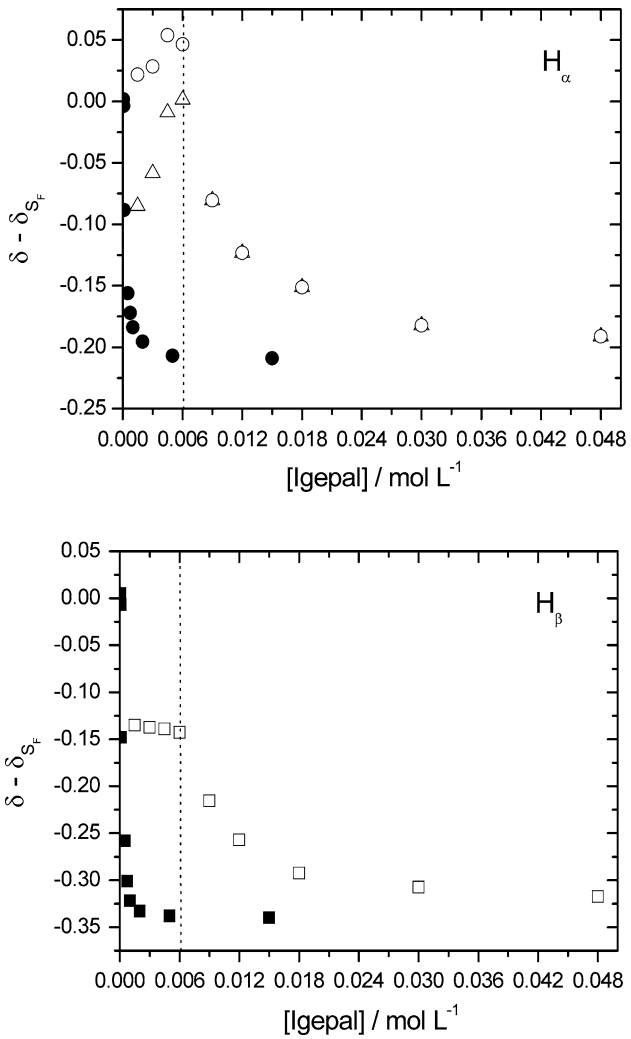

Fig. 3 Increment in the chemical shifts versus concentration of surfactant for selected protons of the Igepal at the binary system (close symbols) and in the presence of β-CD (open symbols). Dotted line indicates [β-CD] = 6.0 mM

protons distant from the aromatic region change scarcely. These evidences may be explained in terms of an approach between benzene rings of the surfactant in the self-aggregation that results in a strong interaction of the aromatic portion of Igepal [12].

The fresh solutions of the ternary system has been studied keeping the β-CD concentration constant in 6.0 mM, and increasing that of the surfactant. The ^{1}H NMR spectra of the Igepal CO-630 at r = [Igepal]/[β-CD] = 0.25 and 1.50 are depicted in Fig. 4 for the three selected regions of the surfactant. Upon addition of the amphiphile the resonances of all the guest protons undergo significant shifting, with the exception of the protons distant from the aromatic group, $H_{\omega'}$, $H_{\lambda'}$, and H_ω. Such changes are related with a direct interaction between the aromatic moiety of the Igepal with the CD cavities. The relative chemical shifts of H_α and

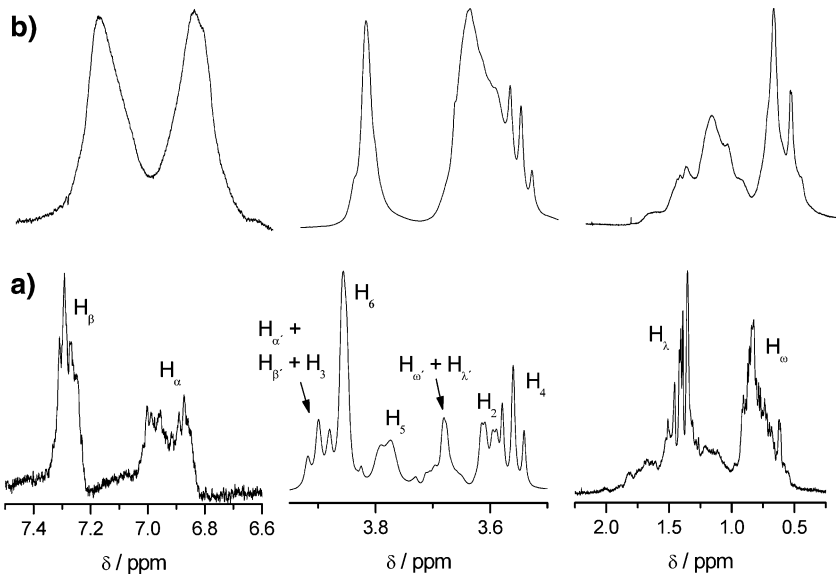

Fig. 4 Expansions of the ^{1}H NMR spectra of Igepal CO-630 + β-CD mixtures at (**a**) r = [Igepal]/[β-CD] = 0.25, and (**b**) r = 1.5 ([β-CD] = 6.0 mM)

H_β with respect to the monomer at the binary system are shown in Fig. 3. The observed break in the curves corresponds to the stoichiometric point in which the free monomer is in the sufficient amount to reach the new apparent cmc*. For both aromatic protons, the cmc* is observed around r = [Igepal]/[β-CD] = 1.0, which indicates that the main stoichiometry of the complexes is 1:1. The chemical shifts at zero concentration, δ_c, corresponds to the interaction between the CD and the surfactant in the complex of the highest stoichiometry (Table 1). It must be noted that below the molar ratio r = 1.0, the protons H_α are magnetically non-equivalent as a consequence of a different chemical environment caused by the complexation. These protons, close to the oxyethylene chain, move first downfield up to r = 1.0 and then converge and shift downfield when $r > 1$. Such changes suggest that the interaction between the Igepal and the CD undergoes a slight dependence in the stoichiometry upon addition of surfactant, indicating that, at high concentration of macrocycle, more than one molecule of β-CD per Igepal can form the complex. This result will be confirmed by the analysis of the ROESY experiment. A similar behavior between other surfactants and CDs has been reported previously, in which these changes are attributed to the formation of 2:1 channel type complexes in different single-chain surfactants [13]. By contrast, the chemical shift of H_β is practically constant below r = 1.0, which might reflect a similar interaction between the aromatic protons and the cavity of the macrocycles upon the effect of the different stoichiometries.

With respect to the micellization upon the cmc*, the curves of the ternary system are displaced, but the values of the chemical shifts at high concentration of Igepal are similar in the presence and absence of β-CD, proving that the complexes do not interact with micelles. This result implies that, in the case of the CD protons, the observed shift is not influenced by the micellization, but by the complexation. All the resonances are shielded upon addition of surfactant, with overall changes that reach values of –0.111, –0.026, and –0.033 ppm for H_3, H_6, and H_1, respectively (Fig. 5). A similar behavior is observed for the rest of the CD protons, although the superposition of their resonances with those of the oxyethyelenes of Igepal does not permit a reliable analysis.

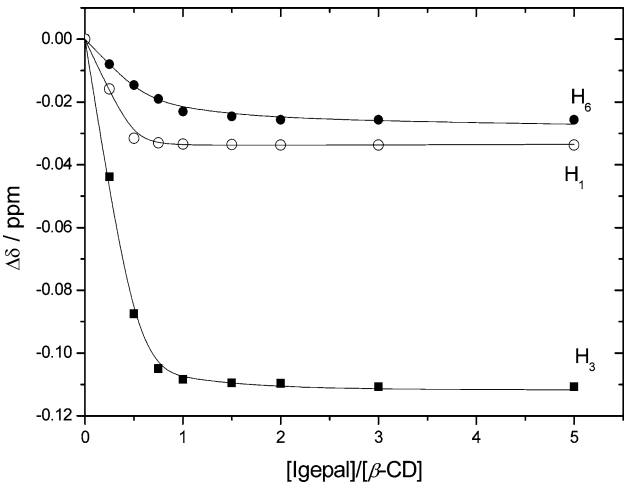

Fig. 5 Increments for the β-CD chemical shifts versus the molar ratio r = [Igepal]/[β-CD] ([β-CD] = 6.0 mM). Solid lines are the best fit to Eq. 1

On this basis, assuming 1:1 + 2:1 and a fast exchange on the NMR time scale [9], the chemical shifts of β-CD measured in the analysis of the ternary system can be expressed as the sum of the contributions of the chemical shifts, δ, due to the free macrocycle, δ_F, to the 1:1 complex, $\delta_{1.1}$, and to the 2:1 complex, $\delta_{2.1}$, each one weighted by its mole fraction, that is

$$\delta = \chi_F \delta_F + \chi_{1.1}\delta_{1.1} + 2\chi_{2.1}\delta_{2.1} \quad (1)$$

The concentrations of all the components in solution are connected by the corresponding mass action law, and the overall binding constants, K_1 and K_2, and the chemical shifts of the complexes have been estimated by using a non-linear fitting of the H_3 chemical shifts respect to r (Table 1) [14]. The result of the regression yields a value of the binding constants, $K_1 = (2.5 \pm 0.7) \times 10^4$ L mol^{-1} and $K_2 = (1.0 \pm 0.3) \times 10^3$ L mol^{-1}. We have excluded from the fitting analysis the external protons H_1 and H_6, which change scarcely with the concentration of surfactant. However, an inspection of the H_1 and H_6 chemical shifts fitting for the above mentioned binding constants confirms the goodness of the applied model (Fig. 5).

The dependence of the cmc* with respect the concentration of macrocycle ([β-CD] = 6.0 mM) can be quantitatively obtained when the concentration of free surfactant in the complexation reaches the cmc value [15]. For this purpose, K_1 and K_2 obtained from the H_3 chemical shifts have been used, and the resulting cmc* is around r = [Igepal]/[β-CD] = 1.0 in good agreement with experimental data. The concentrations of all the components in solution assuming a pseudophase separation model for micelle formation have been plotted versus the molar ratio in Fig. 6.

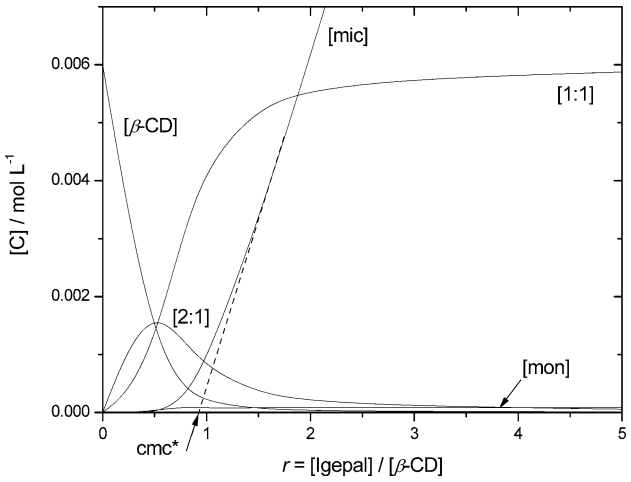

Fig. 6 Evolution of the concentration of the different species in solution versus the molar ratio r = [Igepal]/[β-CD] ([β-CD] = 6.0 mM). Dotted line points the cmc*. [mic], [mon], [1:1], [2:1], and [β-CD] are the concentrations of Igepal in the micelar and monomer form, the concentration of 1:1 and 2:1 complexes, and the concentration of free macrocycle, respectively

Structure of the complexes

Figure 7 shows expanded regions of the 2D ROESY spectrum of a mixture of 12.5 mM β-CD and 5.0 mM Igepal (r = [Igepal]/[β-CD] = 0.4). At 300 MHz the ^{1}H NMR region of the oxyethylene protons, $H_{\omega'}$ and $H_{\gamma'}$, and CD resonances is crowded and their ROE interactions cannot be determined. However, the absence of cross-peaks corresponding to the well-resolved proton $H_{\alpha'}$ suggests a lack of interaction between the hydrophylic chain of the surfactant and the CDs. These findings are consistent with the results of Harada and Kamachi, who found that β-CD forms complexes with polypropylene glycol and polyisobutylene but not with polyethylene glycol [16]. In the aromatic zone of the sepctrum, intense cross-peak arise between the cavity protons of the β-CD and the aromatic moiety of the Igepal. Following in intensity are the cross-peaks of the protons of the aliphatic chain, regardless of the higher number of resonances of this region with respect to the aromatic ring. This may be explained by the coexistence of a 1:1 complex, in which the CD interacts directly with the aromatic ring, and a 2:1 complex of relatively lower concentration (β-CD_2:Igepal), formed by the inclusion of a second macrocycle in the hydrocarbon chain of the surfactant.

The resonance corresponding to the non-equivalent aromatic protons, H_α, exhibits an intense ROE effect with the internal protons H_5 and the primary rim protons H_6. Their magnetic non-equivalence can be justified in terms of the different intensity of the cross-peaks of both resonances with the methylene protons of the CD. Also, H_β correlates strongly with H_3 suggesting the complete inclusion of the aromatic group, in which H_α must be found at the narrower rim of the CD, whereas H_β protons are directly interacting with the wider rim. In the aliphatic region of the Igepal, there are ROE enhancements for the pairs $H_\lambda\{H_3\}$, $H_\omega\{H_6\}$, and $H_\omega\{H_5\}$. We can associate this finding with the complexation of the free chain by a second CD at higher concentration of macrocycle, in which the wider rims of both included macrocycles are directly forming a head-to-head dimeric unit [17].

Conclusion

The changes in the aggregation behavior of the Igepal CO-630, produced by the complexation with β-CD,

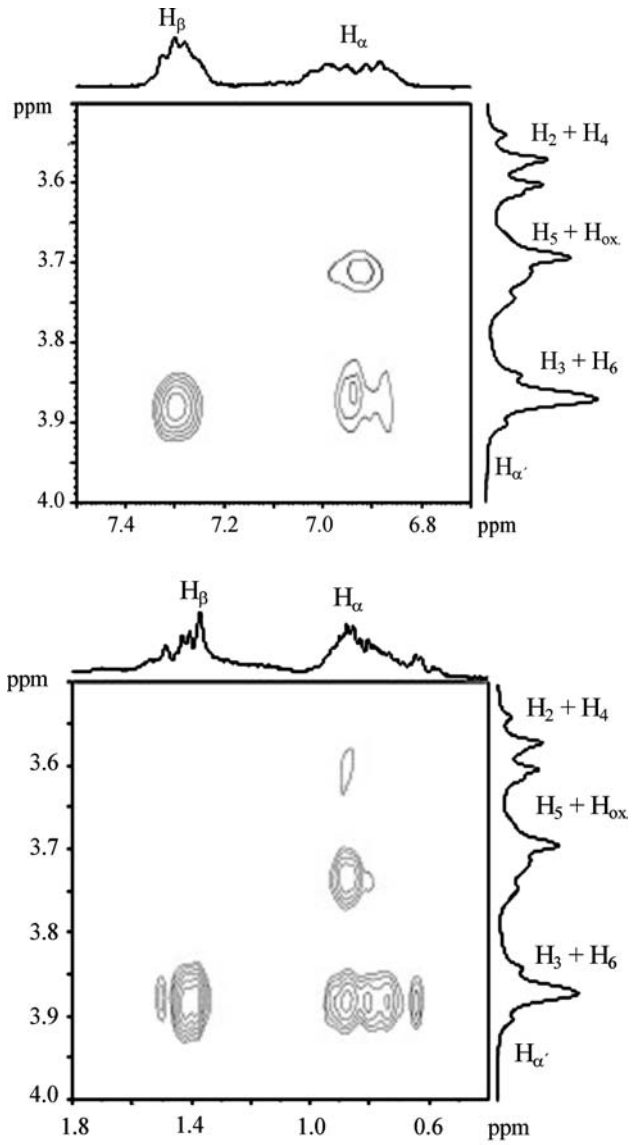

Fig. 7 Partial views of the 2D ROESY spectrum for the r = [Igepal]/[β-CD] = 0.4 ([β-CD] = 5.0 mM)

have been investigated by NMR spectroscopy. The cmc increases in the presence of β-CD as a consequence of the disruption of the aggregates, modifying the self-aggregation exhibited by Igepal. The CD binds mainly to the aromatic part of the surfactant in a 1:1 stoichiometry, and to the aliphatic tail in a 2:1 complex leaving uncomplexed the polyoxyethylene chain. The extent of formation of the aggregated species can be successfully controlled with CDs.

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