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The respective benefits of NMR and mass spectrometry in the investigation of inclusion complexes of cyclodextrins. Application to the inclusion of ionic surfactants in β- and γ-cyclodextrins

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The formation of inclusion complexes between model surfactants and natural cyclodextrins has been investigated by both nuclear magnetic resonance and mass spectrometry. Apparent discrepancies between results obtained using these two complementary techniques are explained in terms of differences in effective experimental conditions. The respective benefits of the two methods are evidenced as well as their complementarity in the investigation of such weakly interacting systems.

INTRODUCTION

The interaction of surfactants with cyclodextrins represents a very active and promising research field to get deeper insight into the understanding of the haemolytic properties of cyclodextrins. It has indeed been observed that cyclodextrins interact with components of red blood cells leading to haemolytic action, the latter being strongly dependent upon the nature and size of the cyclodextrin[1]. However, no clear mechanism for the molecular origin of this effect has ever been proposed. Interactions with various cell membrane components has been evidenced but no clear reason for the structure dependence of this property has ever been proposed. As a prerequisite for the synthesis of cyclodextrin (CD) derivatives exhibiting higher performance in terms of solubilization of hydrophobic drugs, the haemolytic character is of importance and, therefore, a clear picture of the molecular origin(s) of this undesired effect has to be drawn.

A large number of experimental data can be found in the literature concerning the thermodynamics of the interaction of cyclodextrins (mainly dealing with β-CD) and simple or complex surfactants. Many experimental techniques such as conductivity[2–5], competitive binding using UV, visible and fluorescent probes[6–8], surface tension[9,10], sound velocity[11,12] and electrochemical methods[13,14] have been used for these investigations and, as a consequence, a number of inconsistencies are observed in the conclusions, especially concerning the stoichiometry of the interaction process. We wish to report here on the comparison of two techniques, namely Nuclear Magnetic Resonance (NMR) and Mass Spectrometry (MS) for the investigation of the formation of inclusion complexes between cyclodextrins and surfactants. For the sake of simplicity, only a limited number of model surfactant molecules will be considered. As far as possible, the length of the aliphatic chain will be left constant to limit one variable parameter. NMR and MS present the specificity of observing both participating species as well as the inclusion complex, if present. Although, owing to the specificity of these two techniques to observe the molecular species under different conditions, especially in terms of effective concentrations, they can lead to apparently contradictory conclusions; they both belong to the general class of local techniques by opposition to global methods which are better dedicated to the description of the general properties without any possibility of defining molecular properties. The opposition between local and global tech-

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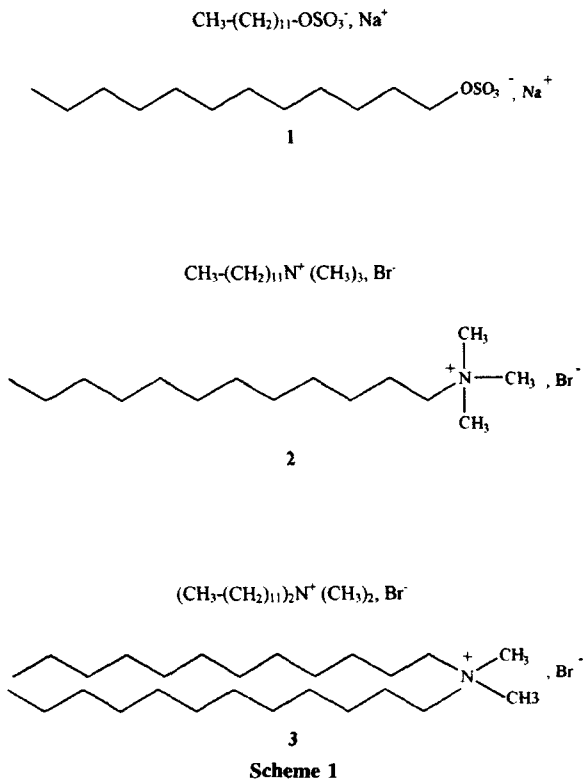
niques can be at the origin of considerable discrepancies found in the literature, concerning for example association constants[6]. Moreover, the two proposed methods require small quantities and allow an economical investigation of a variety of host-guest pairs.

RESULTS AND DISCUSSION

Selection of surfactant molecules

In order to investigate the respective effects of the charge and of the number of aliphatic chains on the interaction behavior with cyclodextrins, the three following surfactants have been selected: sodium dodecyl-sulfate **1** (SDS), dodecyl-trimethyl-ammonium bromide **2** (DTAB) and didodecyl-dimethyl-ammonium bromide **3** (DDAB).

Comparison of the inclusion behavior of **1** and **2** in cyclodextrins (β- and γ-CD's) will allow a direct estimation of the charge effect and of the nature of the polar head. These two surfactants have indeed very similar critical micellar concentrations (cmc) (8 mM and 15 mM for SDS and DTAB, respectively)[15,16] and identical aliphatic chain lengths. In addition, comparison of **2** and **3** will allow a direct investigation of the effect of the number of chains on the inclusion behavior, the lengths and charge being left invariant in both surfactants.



Scheme 1

NMR evidence of the formation of inclusion complexes

In the first step, only single chain surfactants will be considered. NMR is a very useful technique to evidence the formation of inclusion complexes, since the latter process will affect the environment of protons of both the host and guest and will hence be reflected by chemical shift variations of protons from both species.

Figure 1 shows the variation of the proton NMR spectra of β-cyclodextrin upon addition of SDS. A very similar variation is observed with DTAB. It should be emphasized here, that, in all cases, the concentration of the surfactant was kept under the cmc to avoid competition effects related to the formation of micelles.

In both cases, large variations of the chemical shifts of both H-3 and H-5 protons are observed. This observation is the first clue of the formation of an inclusion complex since these protons are located in the hydrophobic cavity of the CD and are hence expected to be the most prone to variations of chemical shifts if an inclusion complex is formed[17].

Similar observations can be derived with γ-CD indicating that an inclusion complex can also be obtained between this cyclodextrin and the two single chain surfactants considered here.

Concerning the interaction of cyclodextrins with DDAB (double chain surfactants), a quite different situation is encountered. DDAB gives vesicles at low concentration since its cmc is lower than 0.1 mM[18]. Figure 2 shows that the addition of β-CD leads to a complete destruction of these vesicules by formation of an inclusion complex between β-cyclodextrin and DDAB as evidenced by the considerable sharpening of the NMR lines and important variations of the chemical shifts of the signals of both partners.

More direct evidence for the formation of inclusion complexes can be derived from the observation of dipolar interactions between protons of the surfactant and of the cyclodextrin. This has been presented in

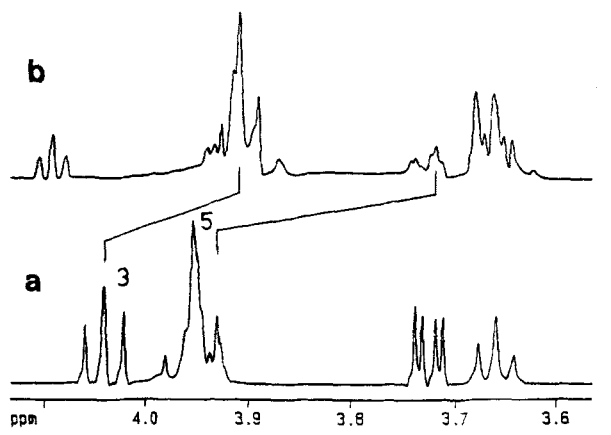


Figure 1 Partial 500 MHz ^{1}H NMR spectra of 5 mM solutions of (a) free β-CD and (b) β-CD/SDS complex in D_2O at 298K.

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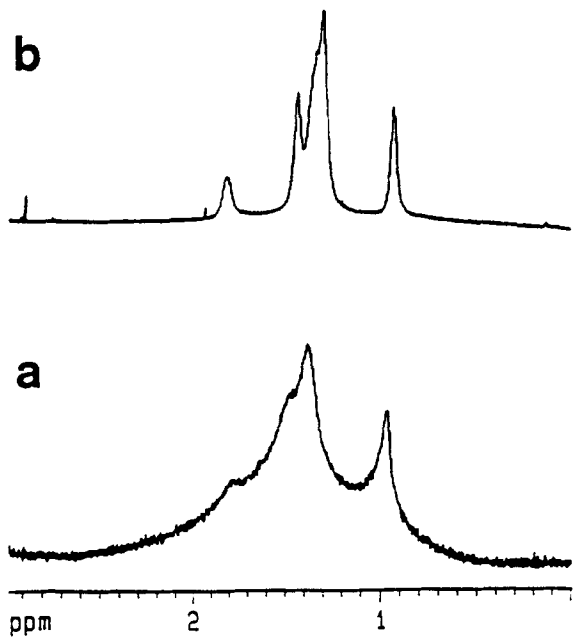

Figure 2 Partial NMR spectra (^{1}H, 500 MHz, 298K, D_2O) of 5 mM solutions of DDAB in the absence (a) and in the presence of 5 mM solution of β-CD (b).

details elsewhere[19] and can be derived from Noesy (or Roesy)[20] experiments dedicated to evidence nuclear Overhauser effects. In many cases, the Roesy experiment is preferred owing to a better intrinsic sensitivity to spatial proximities in this type of molecules.

Figure 3 displays a partial contour plot of a Roesy experiment obtained with a mixture of β-CD and SDS. The presence of strong cross peaks between the aliphatic protons of the surfactant and protons from the cavity of

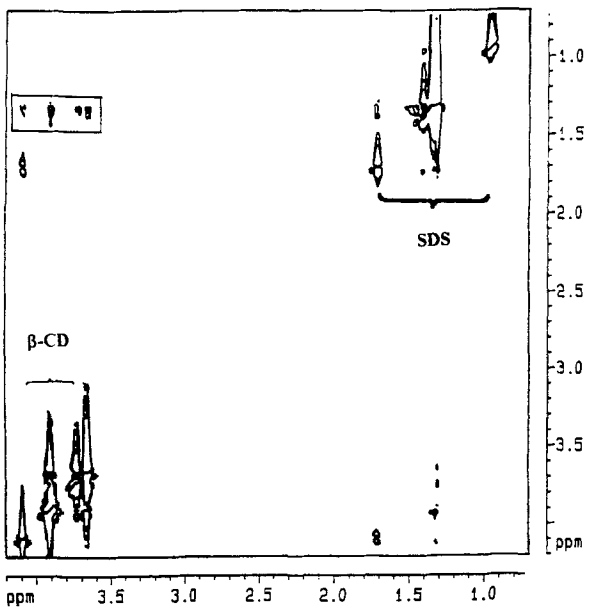

Figure 3 Partial contour plot of a ROESY experiment (350 ms spin-lock time, attenuation 22 dB, 512 scans per increment) performed at 500 MHz on a 5 mM solution of β-CD/SDS complex in D_2O at 298K.

the CD fully supports the formation of an inclusion complex. Similar observations can be derived with γ-cyclodextrin.

As a general conclusion to this section, it is observed that β- and γ-CD give inclusion complexes with all surfactants (**1**, **2** and **3**) considered here as evidenced by the induction of strong variations of the chemical shifts of protons of the cavity of cyclodextrins and by the presence of intense dipolar interactions between chain and cavity protons. In the case of DDAB, a complete destruction of the vesicular system is clearly observed. However, this preliminary investigation is not sufficient to derive the two important parameters which will define the equilibrium between free and bound species i.e. the stoichiometry and association constant[21]. In a first attempt to estimate the strength of the association constant (overall binding constant), simple competition experiments were performed.

Competition experiments as observed by NMR

A very efficient, although approximative, way to estimate the strength of an inclusion complex is to use competing guests for which the binding constant for the CD cavities is well documented. In the present case, owing to the expected strength of the considered inclusion complexes, a competing molecule, the sodium salt of 2-anthraquinone sulfonic acid (ASANa)[22] was considered. It indeed leads to inclusion complexes with β- and γ-CD's with quite similar association constants (K = 300 M^{-1}). This competition experiment cannot be observed easily by 1D NMR but is more clearly evidenced by a dipolar correlation experiment (Roesy in the present case) in which evidence for the presence of inclusion complexes between the CD and the added competitor can be shown unambiguously.

Figure 4 displays Roesy contour plots obtained with βCD-SDS-ASANa and γ-CD-SDS-ASANa mixtures showing that the latter guest cannot compete with SDS in the case of β-CD (no dipolar interaction between the aromatic protons of ASANa and the CD cavity is observed), but that it can partially expell the surfactant from the γ-CD cavity as evidenced by dipolar interactions between ASANa and SDS with γ-CD. Attempts to perform similar experiments with DTAB failed because of the precipitation of mixtures of ASANa and DTAB. It should be noted that no competition experiment was performed on cyclodextrin/DTAB inclusion complexes since no competing molecule has shown the required qualities i.e to form inclusion complexe with β- and γ-CD with similar association constant and to be non-interacting with DTAB.

Hence, a preliminary conclusion concerning the strength of inclusion complexes is that β-CD is a stronger complexing agent that γ-CD for SDS. Owing to the single chain nature of this surfactant, the effect of the

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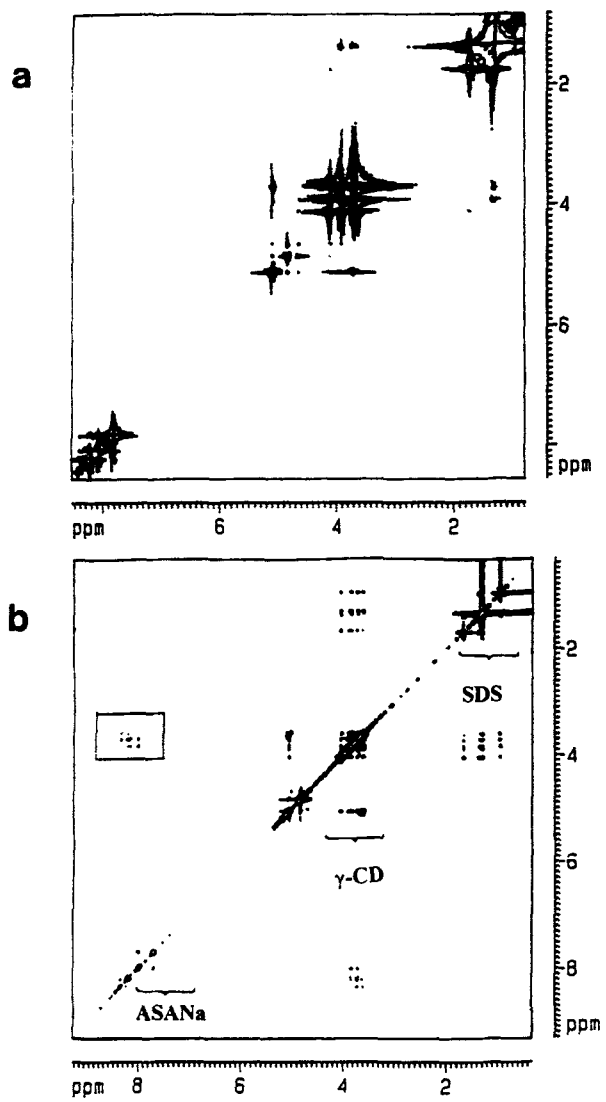

Figure 4 Complete contour plot of a ROESY experiment (350 ms spin-lock time, attenuation 22 dB, 512 scans per increment) performed at 500 MHz on a 5 mM solution of mixture of (a) β-CD/SDS/ASANA and (b) γ-CD/SDS/ASANA in D_2O at 298K.

size of the cavity appears to be determinant for an improved fitting between host and guest molecules and this result is quite reasonable in terms of simple steric considerations.

Evaluation of the stoichiometry of the inclusion process by NMR

The data presented above provides a quite rough evaluation of the inclusion constant. It should be kept in mind that any precise determination of the association constant implies that the stoichiometry of the interaction process is unambiguously determined. As described elsewhere[23], this parameter can be derived or estimated from the *continuous* variation analysis leading to the so-called Job plots. In this procedure, the sum of both participating species is kept constant (in terms of molar concentrations), the molar ratio r of each component being varied from 0 to 1. The observation of any parameter varying in a linear fashion with the concentration of bound species (chemical shifts of the host or guest in the present case) is obtained and affords the corresponding Job Plots as described elsewhere.

Figure 5 displays the corresponding plots obtained for various cyclodextrin/surfactant pairs. They show maxima at r = 0.66 and 0.33 for the inclusion complex β-CD/SDS, at r = 0.33 and r = 0.66 for γ-CD/DTAB and in both cases at r = 0.5 for both β-CD/DTAB and γ-CD/SDS. This implies that the stoichiometry is 2:1 for the inclusion complex β-CD/SDS, 1:2 for γ-CD/DTAB and 1:1 for both γ-CD/DTAB and γ-CD/SDS, respectively.

It should be noted that for a same surfactant (SDS or DTAB), the stoichiometry is different if the size of the cavity of the cyclodextrin changes. For example one molecule of DTAB is sufficient to fill the cavity of β-CD but two molecules of DTAB are required for γ-CD. Moreover the nature of the polar head seems to be very important since, for the same cyclodextrin, the stoichiometry changes when the nature of the polar head is modified.

This shows the advantages of NMR over other local or global techniques in the sense that both species can be

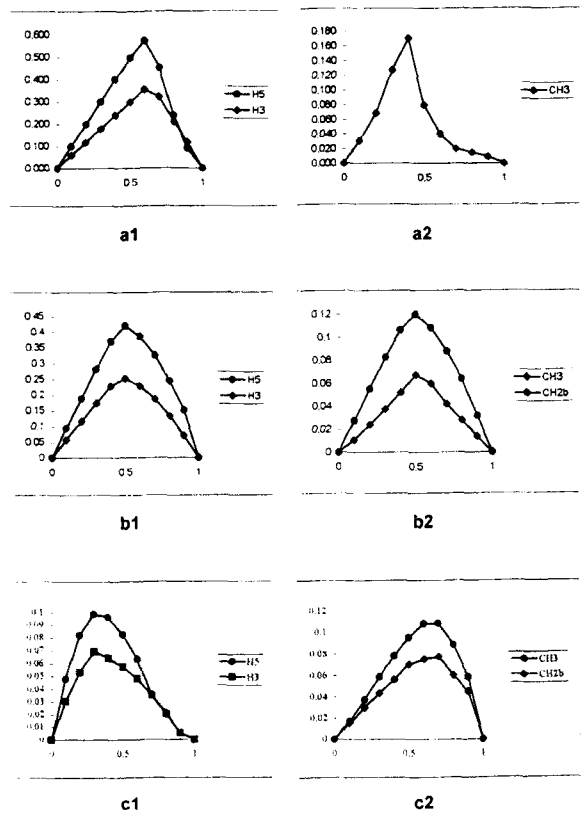

Figure 5 Continuous variation plots of $\Delta\delta_{obs}[CD]_t$ vs r_{CD} and $\Delta\delta_{obs}[S]_t$ vs r_S obtained from experimental data for selected protons (a_1) of β-CD and (a_2) of SDS in the case of β-CD/SDS complex, (b_1) of β-CD and (b_2) of DTAB in the case of β-CD/DTAB complex and (c_1) of γ-CD and (c_2) of DTAB in the case of γ-CD/DTAB complex.

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observed simultaneously leading to a safer analysis of experimental data. As the surfactant concentration is always lower than the cmc, dimerization or micellization effects normally should not affect the results. However, the observation speaks for a contribution of condensed surfactant species even below its cmc in the presence of cyclodextrin. The presence of surfactant oligomers also evidenced in other observations[24] indicates that the micellization process is not a fully cooperative process under theses conditions.

The data presented indeed raises a number of questions. In situations where very clear 1:1 inclusion complexes are evidenced, an effective association constant should be derived using a numerical analysis of the induced shifts[25]. However, for the two systems (β-CD/DTAB and γ-CD/SDS) a determination of the effective association constant is possible only in the domain where the concentration of surfactants is lower than that of cyclodextrins; under these conditions, the presence of surfactant dimers or multimers which should induce small variation of the NMR data could be neglected. In fact, this implies that NMR data reflects the major components, but that the presence of other minor complexes cannot be excluded as they are evidenced by deviations of the expected behavior in the Job plot approach. In these conditions, we have found in both cases an average associate constant of $3 \cdot 10^3$ M^{-1}. Conversely, in the case where non 1:1 stoichiometries are observed, the question of the dependency of multiple association constants has to be raised. On the basis of the experimental data, no direct choice between a direct formation of a 1:n complex (corresponding to one single overall constant) or the stepwise process involving two or more independent constants can be safely made. Obviously, a direct determination of the respective proportions of the possible complexes (i.e. 1:1 and 1:2) should help in a detailed investigation of the elementary processes leading to non 1:1 complexes. This question calls for the use of techniques which are expected to be sensitive to the molecular weight of inclusion complexes. A quite obvious answer to this question is to use Mass Spectrometry (MS) data.

Data obtained from MS experiments

Although the existence of inclusion complexes with cyclodextrins has been demonstrated either in solution or in the solid state by spectroscopic and physical-chemical methods, only recently mass spectrometry has been assayed for their direct detection. Soft mass spectrometry methods, like fast atomic bombardment[26], plasma desorption[27] and electrospray ionization[28], were employed in order to preserve the integrity of such weak supramolecular associations in the gas phase. In this paper, we report the use of electrospray ionization mass spectrometry (ESI-MAS) to investigate the interactions of β- and γ-cyclodextrin with SDS and DTAB. The interest of this relatively soft ionization technique for this type of inclusion complex will be demonstrated. The problem associated with the use of MS is related to the technique used for ionization and to the potential matrix effects. Electrospray methods combines all advantages in the present case since it strongly limits the formation of molecular adducts allowing the use of inactive matrices which can strongly interfere with the inclusion complexes. The samples used in the NMR section were thus investigated by ESI-MS using the electrospray infusion technique. In all cases, the concentration of the surfactant molecule in the original solution used for injection was lower than cmc of the relevant component. However, it should be emphasized that, owing to the intrinsic process of ionization used in the electrospray method (as well as in others), this condition is probably not fulfilled in the separation and detection regions of the spectrometer.

Figure 6 shows the results obtained in the case of the γ-CD/SDS system and of SDS alone. The 1:1 γ-CD:SDS system is, indeed, observed as a main component at m/z = 1607.6 corresponding to $[\gamma\text{-CD} + \text{SDS} + \text{Na}]^+$. However, a significant proportion of a γ-CD:SDS 1:2 complex is also observed (m/z = 1895.9; $[\gamma\text{-CD} + 2\text{SDS} + \text{Na}]^+$) along with a number of peaks corresponding to SDS aggregates (containing 1, 2...6 monomer units) in

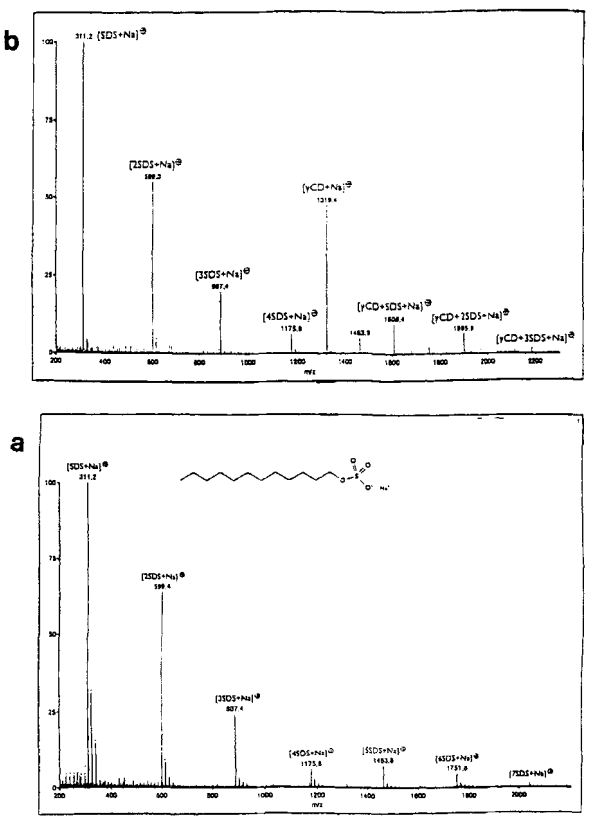

Figure 6 Electrospray mass spectra obtained for SDS alone *(a) and for* the γ-CD/SDS complex (b).

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the case of inclusion complexes and for SDS alone although the SDS concentration in the infusing solution was far below the cmc. The presence of aggregation could be due to the ionization process. In reality, the SDS concentration is higher than the cmc during the progressive evaporation of the matrix.

In the case of β-CD, the evidence for the formation of an inclusion complex of 1:1 stoichiometry is derived from the presence of a weak signal at m/z = 1445.5, $[\beta\text{-CD} + \text{SDS} + \text{Na}]^+$. Moreover the β-CD/SDS inclusion complex does not exhibit, by mass spectrometry, the 2:1 stoichiometry observed by NMR. This result can be explained by the very low value of K_2 (200 M^{-1}) as claimed in literature[6,29]. However mass spectroscopy allows the selection between the two possible routes of formation of inclusion complexes with 2:1 stoichiometry.

$$2\ \beta\text{-CD} + \text{SDS} \rightleftharpoons \beta\text{-CD}_2/\text{SDS}$$

or

$$\beta\text{-CD} + \text{SDS} \rightleftharpoons \beta\text{-CD/SDS}$$

$$\beta\text{-CD/SDS} + \beta\text{-CD} \rightleftharpoons \gamma\text{-CD}_2/\text{SDS}$$

The presence of a signal at m/z = 1445.5 corresponding to a 1:1 stoichiometry for β-CD/SDS complex confirms the second route. This result is in agreement with literature data[6,29]. It should be emphasized that this complex was observed more easily than γ-CD/SDS under the same experimental conditions. It should be concluded that β-CD/SDS complex is stronger than γ-CD/SDS complex.

In the case of DTAB surfactants, the direct observation of inclusion complexes at m/z = 1362.8 corresponding to the ion $[\beta\text{-CD} + \text{DTA}]^+$ by ESI-MS was more difficult and required smooth experimental conditions. In the case of γ-CD, no complex formation with DTAB could be observed. Conversely, dimers of the surfactant are clearly evidenced as explained for SDS. These results confirm the weaker affinity of this surfactant for the CD cavities. This information is of interest since no competition experiment could be performed by NMR on this type of inclusion complexes.

From the previously presented data, it appears that although ESI-MS experiments are in partial agreement with NMR data, discrepancies are still present. A quite intuitive explanation to this is related to the observation process used in the electrospray technique involving the formation of "concentrated" charged micro-droplets. This artificial concentration effect can be sufficient to explain the observation of surfactant agregates since the concentration condition (lower than cmc) is clearly not retained in the microparticle subjected to mass selection. This point is clearly evidenced in the observation of surfactant multimers in the case of SDS although the initial concentration of the solution is lower than cmc. The local concentration effect induced by the spraying process can account as well for the observation of inclusion complexes containing more than one guest molecule.

MATERIALS AND METHODS

β- and γ-cyclodextrins obtained from Roquette Frères SA and Wacker GmbH, respectively, were freeze-dried before use. SDS, DTAB and DDAB were purchased from Aldrich and used as received. All NMR experiments were performed using Bruker AMX500 spectrometer operating at 500.13 MHz, using standard pulse programs from the Bruker library. In all cases, the length of the 90° pulse was ca 6.5 μs. 1D NMR spectra were collected using 16K data points. All two-dimensional experiments were acquired using 2K data points and 256 time increments. In all cases, a total relaxation delay of 1.5s (taking the acquisition time into account) was allowed. For dipolar correlations (ROESY experiment) the phase sensitive (TPPI) sequence was used and processing resulted in a 1K*1K (real-real) matrix. The probe temperature was carefully controlled within 0.1° by means of a Haake exchange device. Chemical shifts are given in ppm downfield from external TetraMethylSilane (TMS). D_2O was obtained from Euriso-Top (France).

ESI-MS analysis was performed on a Sciex API I (Q) mass spectrometer. The inclusion complexes previously used for NMR experiments were freeze-dried in water and dissolved in water for CD/SDS or in water/methanol 1:1 v:v for CD/DTAB at a final concentration of 0.1 mg/mL. The pure surfactants (SDS or DTAB) were dissolved in pure water at a concentration of 0.3 mg/mL. Samples (20 μl) of CD/DTAB mixtures and CD/SDS mixtures were injected into a flow (5 μL/mn) of water/methanol in the 1:1 ratio (v:v) or in pure water, respectively, and directed to the electrospray source.

REFERENCES

1 Duchêne, D.; Wouessidjewe, D.; Poelman, M. C. in *New trends in cyclodextrins and derivatives*, ed. D. Duchêne, Edition de Santé, Paris, **1991**, Chap 13.
2 Palepu, R.; Reinsborough, V. C. *Can. J. Chem.* **1989**, **67**, 1550.
3 Palepu, R.; Richardson, J. E.; Reinsborough, V. C. *Langmuir* **1989**, **5**, 218.
4 Satake, I.; Yoshida, S.; Hayakawa, K.; Maeda, T.; Kusumoto, Y. *Bull. Chem. Soc. Jpn.* **1986**, **59**, 3991.
5 Satake, I.; Ikenoue, T.; Takeshita, T.; Hayakawa, K.; Maeda, T. *Bull. Chem. Soc. Jpn.* **1985**, **58**, 2746.
6 Park, J. W.; Song, J. H. *J. Phys. Chem.* **1989**, **93**, 6454.
7 Sasaki, K. J.; Christian, S. D.; Tucker, E. E. *J. Colloid Interface Sci.* **1989**, **134**, 412.
8 Turro, N. J.; Okubo, T.; Chung, C. J. *J. Am. Chem. Soc.* **1982**, **104**, 3954.
9 Tunçay, M.; Christian, S. D. *J. Colloid Interface Sci.* **1994**, **167**, 181.
10 Dharmawardana, U. R.; Christian, S. D.; Tucker, E. E.; Taylor, R. W.; Scamehorn, J. F. *Langmuir* **1993**, **9**, 2258.

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11 Junquera, E.; Conzalez Benito, J.; Pena, L.; Aicart, E. *J. Colloid Interface Sci.* **1994**, **163**, 355.
12 Jobe, D. J.; Verrall, R. E.; Junquera, E.; Aicart, E. *J. Phys. Chem.* **1994**, **98**, 10814.
13 Mwakibete, H.; Cristantino, R.; Bloor, D. M.; Wyn-Jones, E.; Holzwarth, J. F. *Langmuir* **1995**, **11**, 57 and references herein.
14 Jezequel, D.; Mayaffre, A.; Letellier, P. *Can. J. Chem.* **1991**, **69**, 1865.
15 Muller, N.; Simsohn, H. *J. Phys. Chem.* **1971**, **75**, 942.
16 Zielinski, R.; Ikeda, S. *J. Colloid Interface Sci* **1987**, **11**, 398.
17 Djedaïni, F.; Perly, B. *J. Mol. Struct.* **1990**, **239**, 161.
18 Dubois, M.; Zemb, T. *Langmuir* **1991**, **7**, 1352.
19 Lin, J.; Djedaïni-Pilard, F.; Azaroual-Bellanger, N.; Perly, B.; Moysan, A.; Guénot, P.; Drifford, M.; in *Proceedings of the 7th International Cyclodextrins symposium* Tokyo, **1994**, p. 508.
20 Berthault, P.; Djedaïni, F.; Perly, B. in *New trends in cyclodextrins and derivatives*, ed D. Duchêne, Edition de Santé, Paris, **1991**, Chap 5.
21 Djedaïni, F.; Lin, S. Z.; Perly, B.; Wouessidjewe, D. *J. Pharm. Sci.* **1990**, **79**, 643.
22 Djedaïni, F.; Perly, B. *Magn. Resonan. Chem.* **1990**, **28**, 372.
23 Djedaïni, F.; Perly, B. in *New trends in cyclodextrins and derivatives* ed D. Duchêne, Edition de Santé, Paris, **1991**, Chap 6.
24 Jiang, Y. B.; Wang, X. J. *Applied Spectroscopy* **1994**, **48**, 1428.
25 Djedaïni, F. *Ph. D. Dissertation, University of Paris-Sud, France* **1991**.
26 Voyksner, R. D.; Williams, F. P.; Smith, C. S.; Koble, D. L.; Seltzman, H. H. *Biomed. Environ. Mass Spectrom.* **1989**, **18**, 1071.
27 Haegele, K.; Born, M.; Ritter, H.; Svoboda, M.; Przybylski, M.; in *12th International Mass Spectrometry conference* Amsterdam, **1991**, book of abstracts, p 196.
28 Haskins, N. J.; Saunders, M. R.; Camilleri, P. *Rapid Comm. Mass Spectrom.* **1994**, **8**, 423 and references therein.
29 Wan Yunus, W. M. Z.; Taylor, J.; Bloor, D. M.; Hall, D. G.; Wyn-Jones, E. *J. Phys. Chem.* **1992**, **96**, 8979.

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