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New Insight into the Structure of CTAB Micelles in the Presence of Cyclodextrins, Using Non-Steroidic Anti-Inflammatory Agents – *Nabumetone*, *Naproxen* – as Fluorescent Probes

MARGARITA VALERO, ANA DEL ARCO-GÓMEZ and LICESIO J. RODRÍGUEZ*
Departamento de Química física, Facultad de Farmacia, Universidad de Salamanca, Campus Unamuno, Apartado 449, 37080 Salamanca, Spain

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Abstract

The structure of CTAB micelles in the presence of α-, β- and hydroxypropyl-β-cyclodextrin has been investigated by means of conductivity and spectroscopic measurements – absorption and steady state fluorescence – using *Naproxen*, *Nabumetone* and pyrene as probes. In the presence of the three cyclodextrins, two types of micelles have been detected. The first type is a pure surfactant micelle, while the other is formed by surfactant monomers complexed with cyclodextrins. The presence of cyclodextrins produces a decrease in the cmc of the pure micelle. The second type of micelle is formed at higher cmc* values, 25.6×10^{-4} M, 27.8×10^{-4} M and 38.9×10^{-4} M for α-, β-, and HPβCD respectively. A significant increase in the ionisation degree has been detected. The aggregation number is strongly increased, being 92 ± 3 , 97 ± 3 and 80 ± 2 for for α-, β-, and HPβCD respectively. The polarity of this micelle decreases, indicating that it becomes tighter and its hydrophobic region becomes less polar.

Introduction

The great interest in the use of surfactants in several environmental and industrial related fields [1], or as model systems to partially mimic the behaviour of biological systems [2], among a wide variety of fields, is well known.

In particular, the analysis of the behaviour of the micellar aggregates in the presence of a third component, a cyclodextrin (CD), whose apolar cavity is capable of encapsulating the hydrophobic tail of the surfactant and have no toxic effects has received some attention [3–9]. Particularly, cyclodextrins are known to form inclusion complexes with surfactant molecules mainly with 1:1 stoichiometry [4, 6, 10–15], although other stoichiometries (1 : 2, 2 : 1, etc.) have also been found [6].

More recent studies have focused on the changes occurring within the micelle, due to the presence of the complex formed between the unmicellized surfactant and the CDs [5, 13]. In general, the critical micelle concentration has been found to shift to higher values in the presence of CD and/or complex [3–6].

However, whether the complex is incorporated into the micelle or not is the first and most important question still unanswered that these complicated ternary systems present. Some authors claim that neither the cyclodextrin nor the complex form part of the micelles [4, 16–17] whereas other research groups [6, 18–20] suggest that a 1 : 1 CD:surfactant complex is the hydrophobic entity that associates itself to form the micelle.

In view of this controversy, and in order to understand how the inclusional association affects the micellization process, conductometric and spectroscopic studies of 1 : 1 surfactant-CD complexes of a typical cationic surfactant, *n*-cetyl trimethyl ammonium bromide (CTAB), and cyclodextrins of different cavity size and hydrophobic character, have been carried out in aqueous solution. The results obtained are presented in this paper.

The cyclodextrins used were: α-cyclodextrin (α-CD), β-cyclodextrin (β-CD) and hydroxypropyl-β-cyclodextrin (HPβ-CD). Two different methods have been used. A conductivity study, which leads to the determination of the cmc and the dissociation degree of the micelles formed in these systems, is followed by a spectroscopic investigation of both the uv-vis absorption and the fluorescence emission of the systems. *Nabumetone* and *Naproxen* have been used as probes. The two probes were selected because, despite their structural similarity, *Nabumetone* has a non ionisable butanone group in the 2-position, whereas *Naproxen* has a carboxylate group which is totally in its ionic form. The difference will determine the region where each one will bind and give information about different regions in the micelle aggregate. Finally, a study of fluorescence static quenching of pyrene by *n*-cetylpyridinium chloride has been carried out to determine the aggregation number and polarity of the aggregates formed.

* Author for correspondence. Fax number: 34-923-294515; E-mail: LJR@gugu.usal.es

Materials and methods

Materials. *n*-Cetyltrimethylammonium bromide, CTAB, and *n*-cetylpyridinium chloride (monohydrate), CePyCl, were purchased from Merck; 4-(6-methoxy-2-naphthyl)-2-butanone, *Nabumetone*, 2-(6-methoxy-α-methyl-2-naphthyl) propionic acid sodium salt, *Naproxen*, α-cyclodextrin and β-cyclodextrin were from Sigma Chemical Co.; hydroxypropyl-β-cyclodextrin, containig an average molar substitution of 0.8 hydroxypropyl groups per glucopyranose unit was from Aldrich; pyrene was from Janssen Chimica (purity higher than 98%). These reagents were considered sufficiently well characterized by the manufacturer as to be used without further purification. Water was treated with a Milli-Q system from Millipore.

Methods. An aqueous solution of fixed concentration of 5.0 $\times 10^{-5}$ M of either *Nabumetone* or *Naproxen* were prepared by weight and stirring.

CTAB and CTAB : CD concentrated aqueous solutions were prepared, the former by weight of pure surfactant, and the latter by mixing equal molar amounts of CD and CTAB, both disolved in the corresponding aqueous drug solution. Solutions with variable CTAB or CTAB : CD concentration were obtained by sucessive dilution using the aqueous solution of the respective drug at the referred concentration. All measurements were carried out at 25.0 °C and at least 24 h after its preparation to ensure that equilibria was reached.

Conductivity Measurements. The electrical conductivity was measured using a CMD 83 (Radiometer) conductivimeter. The cell constant was calibrated with solutions of KCl of a known concentration [21]. The technique used for the determination of cmc by conductivity measurements has been described in previous works [22, 23]. All measurements were performed at 25.0 ± 0.1 °C.

Spectroscopy. Uv-vis absorption spectra were recorded with a Hitachi uv-vis spectrophotometer, model 150-20. Fluorescence emission spectra were recorded with a Perkin-Elmer LS 50B Spectrofluorometer. The instrumental response at each wavelength was corrected by means of a curve provided with the apparatus. The emission spectra have been obtained in the range λ_{em} = 325–450 nm, with excitation at λ_{exc} = 317.0 nm. The spectral slits used were: 2.5 nm and 2.5 nm for *Nabumetone*; 2.5 and <2 nm for *Naproxen* (this value corresponds to the minimum possible width, which remains constant for any particular instrument). The fluorescence quantum yield, Φ, has been determined using the following expression:

$$\Phi_f = \Phi_r \left(\frac{n_u^2}{n_r^2}\right)\left(\frac{A_r}{A_u}\right)\left(\frac{F_u}{F_r}\right), \qquad (1)$$

where r and u subscripts refer to reference and probe, respectively; n is the refractive index; A is the absorbance at the excitation wavelength (less than 0.1 in a 1.00 cm cuvette); F is the area under the corrected emission spectra, and Φ_r is the fluorescence quantum yield of the reference (Φ_r = 0.543 for quinine sulfate in aqueous 0.1 N sulfuric acid). The refractive index measurements have been carried out using an Abbè Refractometer Atago, model DR-A1.

Pyrene was used as the luminiscence probe and *n*-hexadecylpyridiniumm chloride was chosen as the static quencher for the aggregation number [24, 25] and polarity determinations. The excitation wavelength was selected at 340 nm, while the emission spectra were collected from 360 to 500 nm.

Solutions were prepared following the Infelta and Grätzel method [26]. An aliquot of a stock solution of pyrene in ethanol (for fluorescence spectroscopy) was transferred into a flask and the solvent was evapored. The surfactant:cyclodextrin solution was added, the probe being solubilized into the micelles after stirring the solution for 24 h. [CTAB] : [CD] concentrations were kept constant in every experiment. The pyrene concentration remained constant at a [quencher]/[micelles] ratio of 0.01. The quencher concentration was varied in a range that gave [pyrene]/[micelles] less than 0.8, assuring in both cases a Poisson distribution [26, 28].

Table 1. cmc* values of CTAB in the presence and absence of cyclodextrins obtained by conductometric measurements

	CMC × 10^4 (M)		
	Without probe	With *Nabumetone*	With *Naproxen*
CTAB : WATER	9.4 ± 0.08	10.2 ± 1.2	10.1 ± 0.7
CTAB : α-CD	24.3 ± 0.9	24.2 ± 0.8	34.4 ± 3.1
CTAB : β-CD	27.4 ± 2.1	26.8 ± 1.0	27.5 ± 4.0
CTAB : HPβ-CD	38.2 ± 1.7	37.8 ± 2.9	38.7 ± 2.1

Results and discussion

Electrical conductivity measurements

Most of the studies undertaken with different surfactant : cyclodextrin systems have shown that the inclusion complexes are mainly formed with 1 : 1 stoichiometry, and therefore the 1 : 1 CTAB : CD solutions used were prepared by mixing equal molar amounts of CTAB and that of each cyclodextrin. Figure 1 shows the plots of the specific condutivity κ vs [CTAB] and vs [CTAB] : [CDs] mixtures at the same molar concentration. The inflection observed in the curves at a certain concentration of CTAB or of CTAB : CD is generally accepted as the cmc of the formed micelles. Such an inflection appears in all the systems studied, showing that micellization of CTAB in the presence of the three cyclodextrins occurs. Table 1 presents the cmc* (critical micelle concentration of the aggregates formed in the presence of cyclodextrins) values of CTAB aqueous solutions determined from conductivity measurements, in the absence and presence of cyclodextrins. The presence of cyclodextrins produces an increase of cmc*, the trend being:

$$H_2O << \alpha\text{-CD} < \beta\text{-CD} \ll HP\beta CD.$$

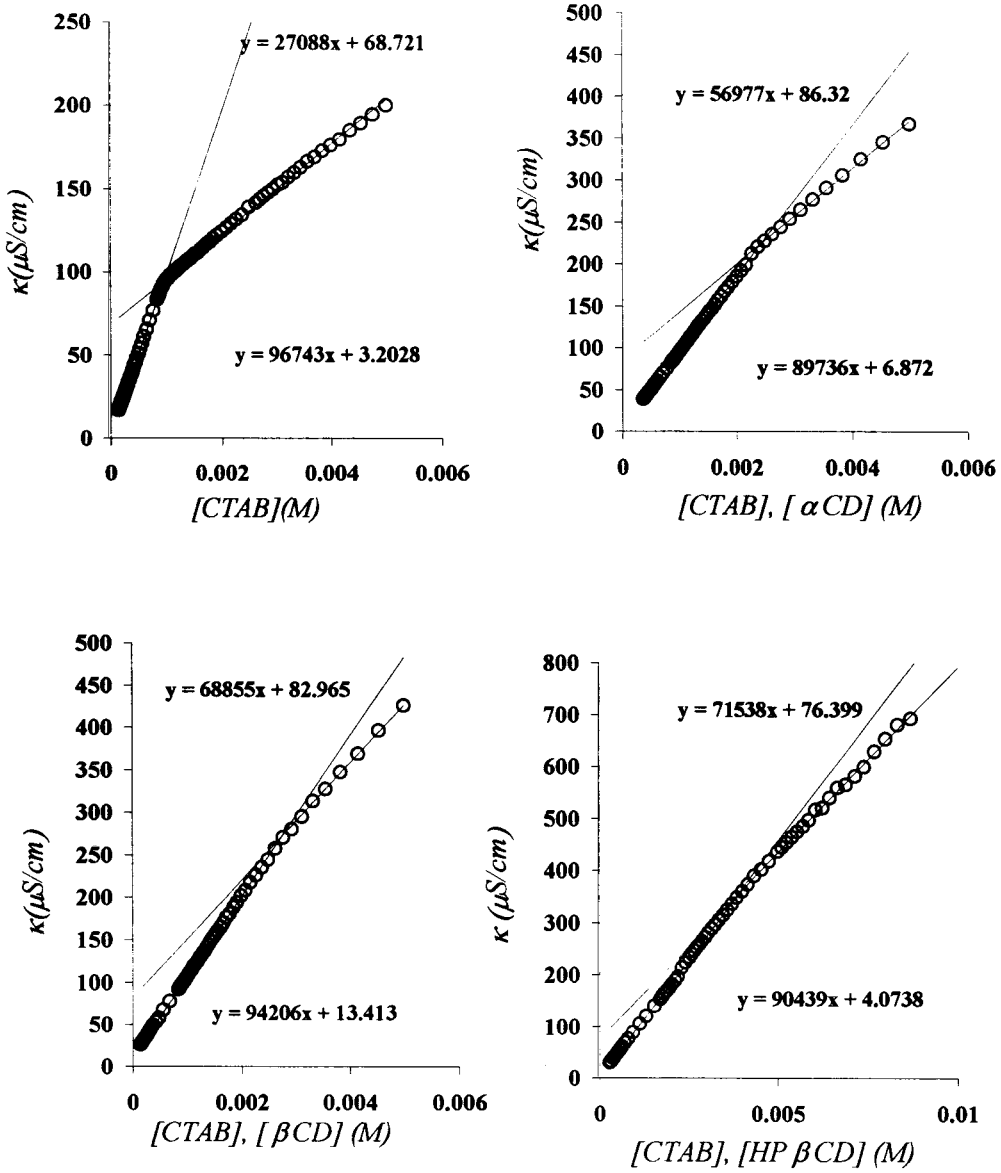


Figure 1. Conductivity curves of CTAB in water and in the presence of α-, β- and HPβCD.

As expected, the presence of all cyclodextrins produces an important increase in the cmc* of the CTAB. The results obtained for CTAB in water and in the presence of β-CD are in good agreement with those described in the literature [20, 29].

These data show that there is some effect of the CD structure on the cmc* which is not only due to its different inner size. The presence of hydroxyl substituents in the β-CD produces a greater effect in the micelle formation ability than the increase in the cavity size of the cyclodextrin. This increase may indicate that the micelle is different or that it begins to form at higher concentrations due to the monomer-cyclodextrin complexation.

The slopes of the two conductivity straight lines are different for the three cases. The micellar ionisation degree may be calculated from the ratio of the slopes after and before the aggregation [30].

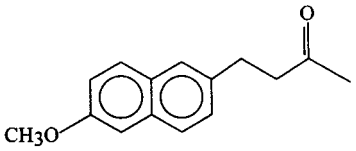
The results presented in Table 2 clearly show that the presence of cyclodextrins produces an important increase in the micelle ionisation. The increase in the ionisation degree indicates that the micelle formed in the presence of cylodextrins has the head groups in a looser contact, diminishing the repulsive forces among the positive charges of ammonia groups. As a result, the bromide ions remain further away from the micellar surface [31]. The change

Table 2. Ionisation degree (α) of the CTAB aggregates formed in the absence and presence of cyclodextrins

	Ionisation degree (α)		
	Without probe	With *Nabumetone*	With *Naproxen*
CTAB : WATER	0.28	0.32	0.32
CTAB : α-CD	0.63	0.59	0.72
CTAB : β-CD	0.73	0.75	0.82
CTAB : HPβ-CD	0.79	0.76	0.75

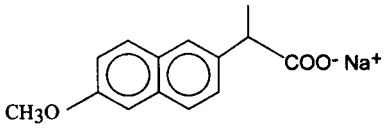
NABUMETONE

(4-(6-methoxy-2-naphthyl)butan-2-one)



NAPROXEN

(S) 6-methoxy-α-methyl)-2-naphthaleneacetic acid, sodium salt



Scheme I. Structures of the anti-inflammatory drugs *Nabumetone* and *Naproxen*.

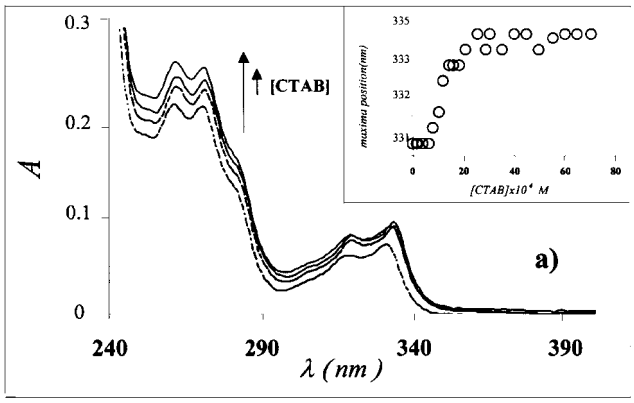
in this micellar parameter is similar in the presence of the two seven-membered-ring cyclodextrins, and larger than in α-CD, i.e., the ionisation degree does not follow the same trend as the cmc*. In these conditions, the bromide ion is not greatly complexed with the cyclodextrins, as the binding constants are 3.5 and 6.5 M^{-1} for α- and β-CD respectively [32]. Consequently, it may be eliminated as a possible cause for the increase in the ionisation degree detected.

The changes observed indicate that the presence of cyclodextrins produces some change in the micellar structure and not only changes in the amount of free surfactant monomer for micellization.

The conductivity measurements have been carried out in the presence and absence of the two probes, *Naproxen* and *Nabumetone*. The results presented in Tables 1 and 2 give evidence that none of the drugs modify significantly the structure of the micellar aggregate and therefore they may be used as probes in the study of these systems.

Spectroscopic study

The spectroscopic study – absorption and steady state emission – of the four systems, CTAB/H_2O, CTAB/α-CD/H_2O, CTAB/β-CD/H_2O and CTAB/HPβCD/H_2O was carried out in presence of *Naproxen*, in its sodium salt form, and *Nabumetone*. These anti-inflammatory drugs are naphthalene derivatives (Scheme I) with spectroscopic properties sensitive to the nature of the environment in which they are located [33, 34]. Their structural differences should provide a different location in the micellar aggregate, giving information of two different micellar regions. Their concentrations were kept constant at 5×10^{-5} M, while the concentration of CD and CTAB was changed, keeping the molar ratio [CD] : [CTAB] as 1 : 1.



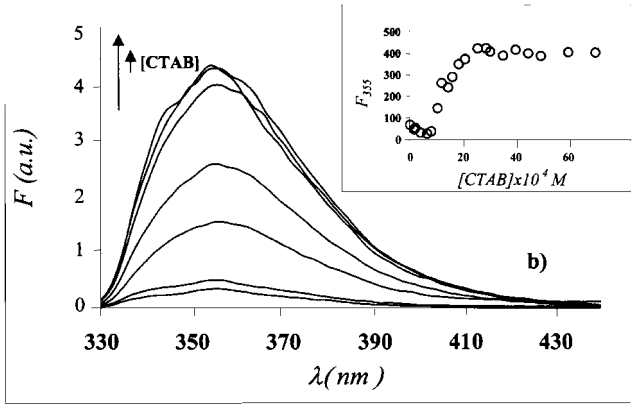


Figure 2. Absorption (a) and emission (b) spectra of *Nabumetone* in CTAB solutions. Inset, variation of the absorption maxima position and fluorescence intensity, respectively vs CTAB concentration.

Spectroscopic study for Nabumetone

The absorption and emission spectra in water and in the presence of variable amounts of CTAB and CTAB : CD were obtained in a concentration range of 0–5 mM.

Addition of CTAB, or CTAB : CD, produces a red shift (Figure 2a) in the absorption spectra that reaches a plateau value, indicating that most drug molecules are included inside the drug in a less polar environment [34] that is, into the micelle.

The *Nabumetone* binding constant to the formed micelles in the absence and presence of cyclodextrins has been determined by a new method proposed by Merino et al. [35]. This method relates the change of experimental wavelength, $\Delta\lambda$, observed at a fixed guest concentration, with the complexation degree, by the following expression:

$$\Delta\lambda = \Delta\lambda_{max} f = (\Delta\lambda_{max} K_{binding}[host])/(1 + K_{binding}[host]). \quad (2)$$

Where $\Delta\lambda_{max}$ is the maximum value of these shifts at a hypothetical 100% complexation degree. In this case, the concentration of the host micelle must be considered, instead of that of the surfactant. At a given *Nabumetone* concentration, the micelle concentration (host) has been determined taking as cmc the value of the surfactant concentration at which the shift begins: 6.4×10^{-4}, 20×10^{-4}, 20×10^{-4}, and 26.9×10^{-4} for CTAB, CTAB : α-CD, CTAB : β-

Table 3. Spectroscopic parameters of *Naproxen* and *Nabumetone* in water and in micelles formed in the absence and presence of cyclodextrins. In all cases the surfactant concentration was over the cmc*

	$\phi_{Naproxen}$	K_b (M^{-1}) *Nabumetone*	$\phi_{Nabumetone}$
WATER	0.289	–	0.030
CTAB : WATER	0.140	$(6.2 \pm 0.8)10^4$	0.079
CTAB : α-CD	0.173	$(12.4 \pm 3.2)10^4$	0.056
CTAB : β-CD	0.163*	$(24.0 \pm 7.0)10^4$	0.045
CTAB : HPβ-CD	0.159*	$(14.4 \pm 3.5)10^4$	0.065

* Mean quantum yield of data over the cmc*.

CD and CTAB : HP-β-CD, respectively, and as aggregation number, $n = 60$ [36].

From a NLR analysis (based on a Marquard algorithm) of the experimental data, the $\Delta\lambda_{max}$, and $K_{binding}$ values can be obtained as fitting parameters (Table 3). The uncertainties on the $K_{binding}$ values obtained from uv data are higher than those obtained from other methods as expected, since the variation observed in the experimental property is lower than that in the fluorescence data. Despite that, the value for the binding constant of *Nabumetone* to the CTAB pure micelle is in very good agreement with that determined by different techniques, for naphthalene, 7×10^4 [36]. The values obtained in the presence of the cyclodextrins are larger than in pure CTAB, which suggests some difference among the micelles formed in the presence of the three cyclodextrins.

A more complicated situation arises from fluorescence data. The surfactant addition (Figure 2b) produces a quenching of fluorescence below the cmc (free) while above the cmc (aggregated) the quantum yield increases (see Table 3). This result indicates that *Nabumetone* in the micelle is protected from the bromide effect, which means that is in a deep position far from the interface.

When CTAB : CD are added, the emission spectra present an irregular trend, but in all cases there are two points at which the fluoresence behaviour changes (Figure 3). This complex behaviour seems to indicate the existence of different equilibria among which the probe is being exchanged. If one compares the concentrations at which these changes occur (Figure 3) to the cmc values included in Table 1, it is very noticeable that the first break point is close to the cmc of the pure CTAB, while the second break point is sligthly lower than the cmc value in the presence of cyclodextrins.

The second break point is well known to correspond to the beginning of the micelle formation. The first increase of fluorescence may be the consequence of either one of the following two protective proccesses:

1. *Drug-cyclodextrin complexation.* Considering the binding constant values [37], this possibility may be discarded for the case of α-CD but not for the other two cyclodextrins.
2. *Formation of other type of micelle.* In this case the fluorescence behaviour (Figure 3) would be related to the following processes:
   (i) Formation of a pure surfactant micelle (sudden increase of the fluorescence)

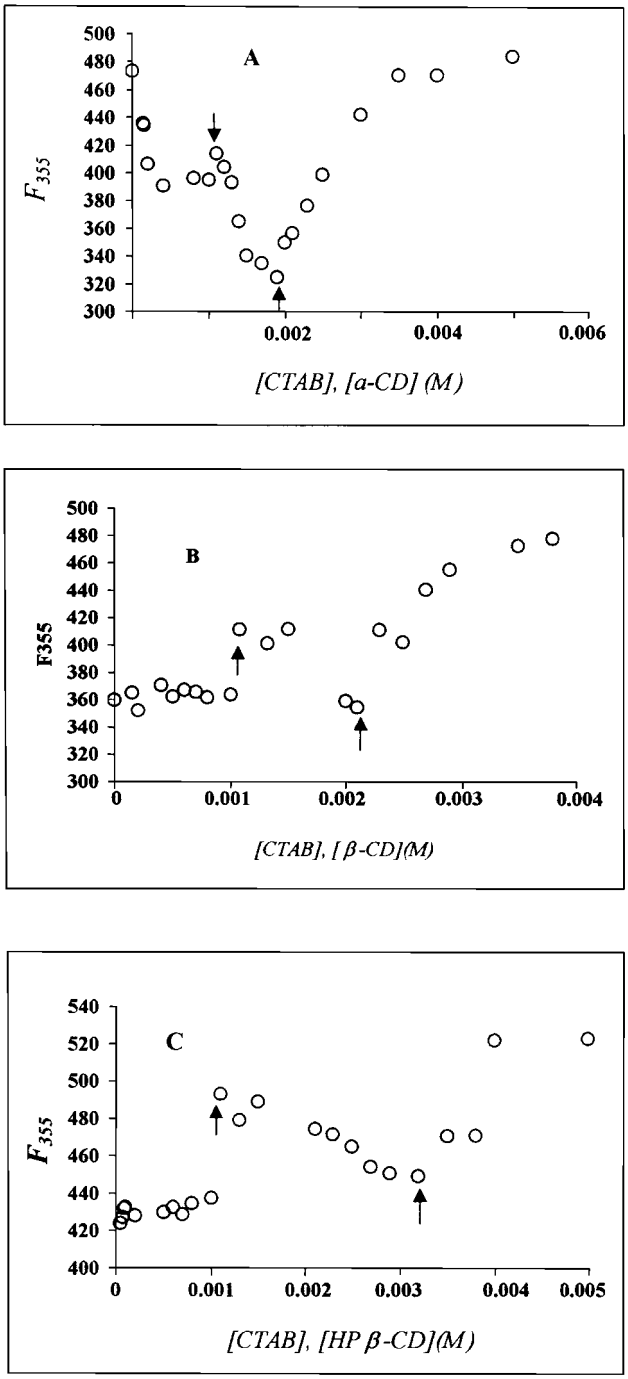


Figure 3. Fluorescence emission intensity of *Nabumetone* in: *A* : CTAB : α-CD solutions; *B* : CTAB : β-CD; *C* : CTAB : HPβCD.

   (ii) Stop of the micelle growth due to S-CD complexation (fluorescence decrease) and therefore a rapid increase of the free surfactant : CD complex concentration.
   (iii) Formation of a second type of micelle with the S-CD complex as monomeric units.

Considering the establishment of the following complexation equilibrium:

$$S + CD \rightleftharpoons S\text{-}CD \quad (3)$$

between the free surfactant, S, and the cyclodextrin, CD, to give a free surfactant-cyclodextrin complex, S-CD, with an equilibrium constant given by:

$$K = [\text{S-CD}]_{eq}/[\text{S}]_{eq}[\text{CD}]_{eq} \quad (4)$$

the following relations hold at equilibrium:

$$K\,[\text{S}]^2_{eq} = [\text{S-CD}]_{eq} \quad (5)$$

$$[\text{S}]_0 = [\text{S}]_{eq} + [\text{S-CD}]_{eq} \quad (6)$$

since $[\text{S}]_0 = [\text{CD}]_0$ and $[\text{S}]_{eq} = [\text{CD}]_{eq}$, the total and equilibrium concentrations of surfactant and cyclodextrin species, respectively. From the foregoing relations, it can be shown that there is a $[\text{S}]_0$ value at which $[\text{S}]_{eq} = [\text{S-CD}]_{eq}$ and above which $[\text{S-CD}]_{eq}$ increases much more quickly than $[\text{S}]_{eq}$, which depends greatly on the binding constant value. In the present case, the $[\text{S}]_0$ value is found around 10×10^{-4} M (the average value for the three CDs), which enables the calculation of a binding constant value for CTAB : cyclodextrin of approximately 2000 M^{-1}. This is in very good agreement with the value of 2240 M^{-1} [38] determined by other authors for CTAB : β-CD. According to this, the presence of cyclodextrins would decrease the cmc of the CTAB, which has also been shown elsewhere [19]. Therefore, the second option is theoretically possible in good agreement with our data and that existing in the literature and therefore must be taken into account.

On the other hand, the quantum yield of *Nabumetone* into the micelle formed in the presence of the cyclodextrins (Table 3) shows a different interaction between the drug and the bromide ions, which indicates a different site of the drug solubilization. This change in the probe location indicates a change in the structure of the aggregate formed either in the presence or absence of CDs, in good agreement with the previous results.

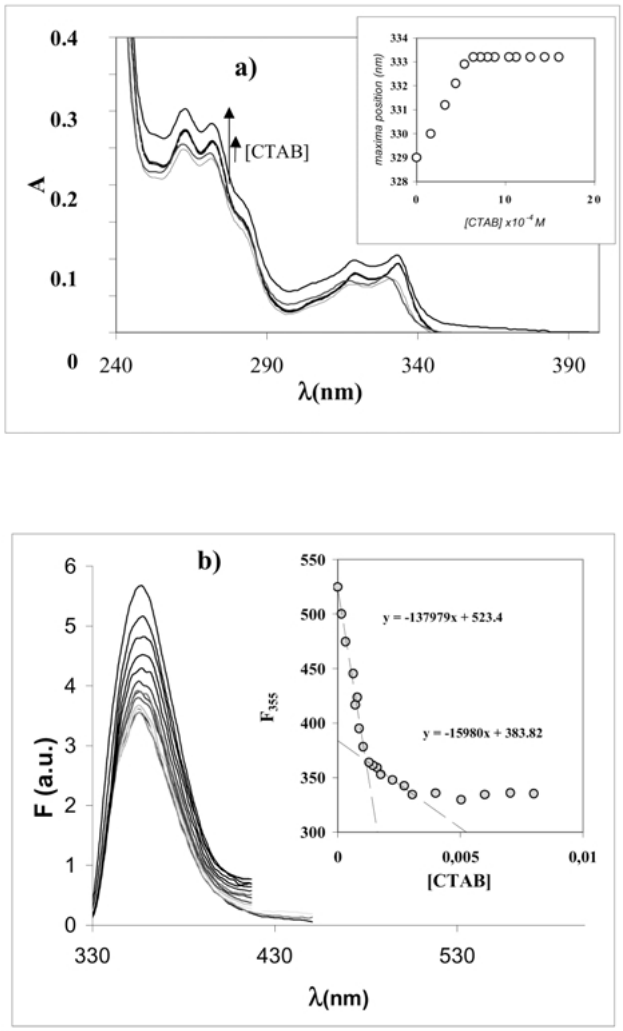


Figure 4. Absorption (a) and emission (b) spectra of *Naproxen* in CTAB solutions. Inset, variation of the absorption maxima position and fluorescence intensity, respectively vs CTAB concentration.

Spectroscopic study for Naproxen

A similar study was carried out using *Naproxen* as a probe. The absorption and emission spectra were obtained as in the previous case.

Addition of CTAB (Figure 4a) produces a red shift in the absorption maxima of the probe in water, showing that the drug is in a less polar microenvironment. The change in the maxima position occurs under the cmc value, and remains constant thereafter. This fact seems to indicate that the presence of the negative charge in *Naproxen* stimulates the monomer-drug interaction.

When CTAB : CD are added, a red shift is also produced, but only in the range between 10×10^{-4} and 20×10^{-4} M. Once again a change above the respective cmcs (in CTAB or CTAB:CD) is detected, and once again the same two possibilities must be considered as the cause for the first change: (1) probe : CD complexation or (2) CTAB pure micelle formation. *Naproxen* does not form an inclusion complex with α-CD ($K < 15$ M^{-1}) [39], and the binding constants of *Naproxen* to the two β-CDs are lower ($K_{\beta\text{-CD}} = 1100 \pm 66$ M^{-1} and $K_{\text{HP}\beta\text{CD}} = 1062 \pm 53$ M^{-1} [37]) than that of CTAB ($K_{\beta\text{-CD}} = 2240$ M^{-1} [38]), therefore the second option must be considered the most possible.

The addition of CTAB does not modify either the structure or the position of the emission bands of the fluorescence emission spectra (Figure 4b). The inclusion of *Naproxen* in the CTAB micelle produces a strong fluorescence quenching, cutting the quantum yield to half that in pure water (Table 3). The probe quenching in the micelle indicates the proximity of bromide ions to the naphthalene ring and gives clear proof that *Naproxen* is located near the micelle interface in close contact with such quenchers.

The addition of CTAB : CDs produces similar effects on the fluorescence behaviour (Table 3). The quantum yield of *Naproxen* in the aggregates formed in the presence of α-, β- and HPβ-CD show that the drug is located in the interface in the presence or absence of the cyclodextrins. On the other hand, the quantum yield experiences a small increase when the polymers are present, possibly due to the decrease in the quencher concentration in the interface produced by the increase in the ionisation degree.

Table 4. Break points in the fluorescence intensity at 355 nm vs [CTAB] curves for Naproxen

NAPROXEN	$C_1 \times 10^{-4}$ (M)	$C_2 \times 10^{-4}$ (M)
CTAB : WATER	11.4 ± 3.5	–
CTAB : α-CD	10.0 ± 4.6	26.9 ± 3.2
CTAB : β-CD	13.5 ± 4.2	28.3 ± 6.8
CTAB : HPβ-CD	15.6 ± 4.9	39.5 ± 9.3

Addition of cyclodextrins causes a change in the profile of the curves F_{355} vs concentration. When pure CTAB (Figure 4b) is added only one break point can be detected, which could be considered as the begining of a change, after which the studied parameter does not remain constant. From these data a cmc for CTAB of 1.14 mM results, in good agreement with the literature value (1.09 mM, [20]).

Comparing the curves in the presence of CDs with those in their absence, it seems evident that they present one additional break point. Taking into account that the break in the presence of pure CTAB corresponds to a change in the location of the drug, the slope changes observed in the presence of cyclodextrins must also correspond to changes in the environments where *Naproxen* is included.

A fit of the data of F_{355} vs. [CTAB] : [CD] to three straight lines (Figure 5) gives the concentrations corresponding to the two break points (Table 4). The data obtained are very close to the cmc of CTAB in the absence and presence of cyclodextrins.

The profile and the slopes of the different parts of the fluorescence curves give clear evidence that the changes detected for the drug are not related to its inclusion into the cyclodextrins based upon the two following reasons:

1. *Naproxen*: CDs complexation results in an increase of fluorescence,which is opposite to the observed behaviour.
2. The inclusion of *Naproxen* into the cyclodextrins protects the drug from quenchers such as iodide (data not shown). If the first break point was due to the drug : CD complexation, the slope of the second part of the curves would be lower than the first one, opposite to that observed in the present case.

Our results show that two types of micelles in the presence of cyclodextrins have been formed. The first one, with free surfactant monomers, and the second one may be formed by a "surfactant-cyclodextrin" complex as monomeric units, as reported for CTAB : β-CD [20] and other 1 : 1 surfactant : β-CD [7, 19] complexes. It can be assumed here that CTAB is the hydrophobic alkyl chain included in the CDs cavity with the major part of the chain protruding into the bulk phase [20], while the *Naproxen* molecule remains out of the CD ring, close to the micelle interphase. The *Nabumetone* molecule should be located in a deeper position inside the micelle, but it cannot be so clearly ascertained.

The pure surfactant micelle has been mainly detected in various cyclodextrin : surfactant systems by different methods, while just in a few studies, and only for CTAB : β-CD, the second micelle has been found. However, we presume

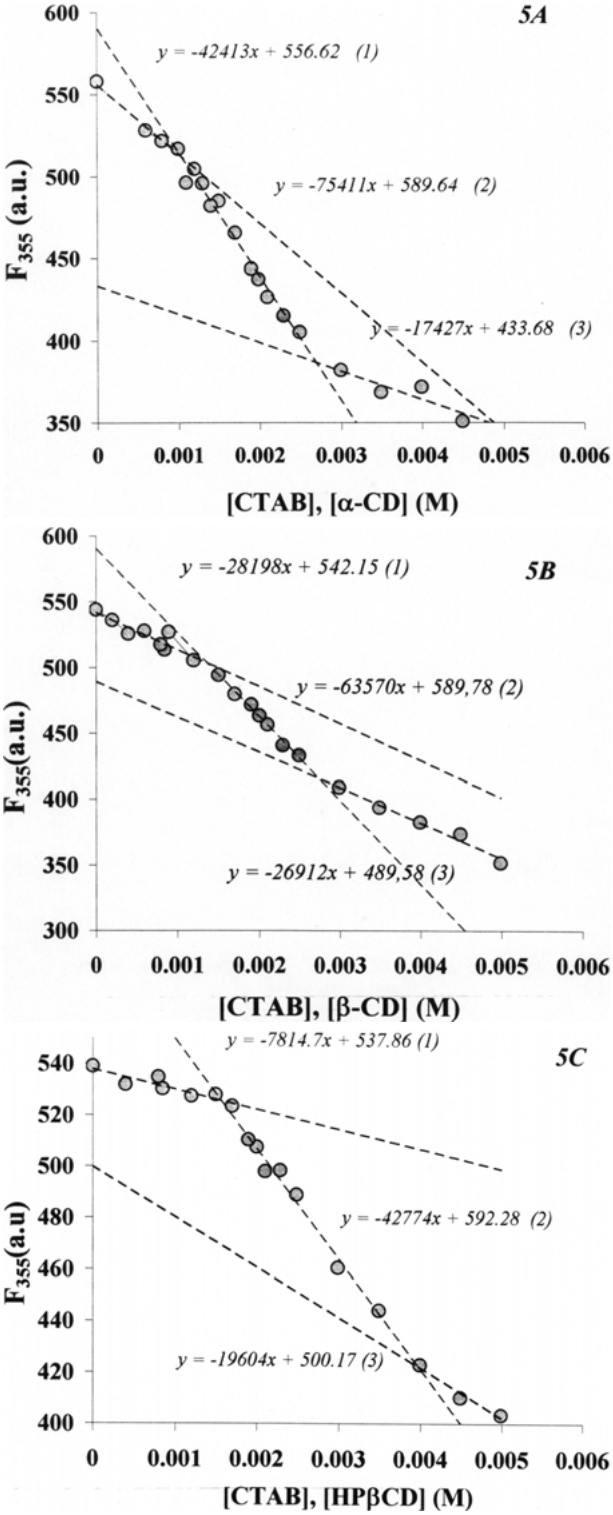


Figure 5. Fit of the *Naproxen* fluorescence intensity vs concentration of: (A): CTAB : α-CD ; (B): CTAB : β-CD; (C): CTAB : HPβ-CD.

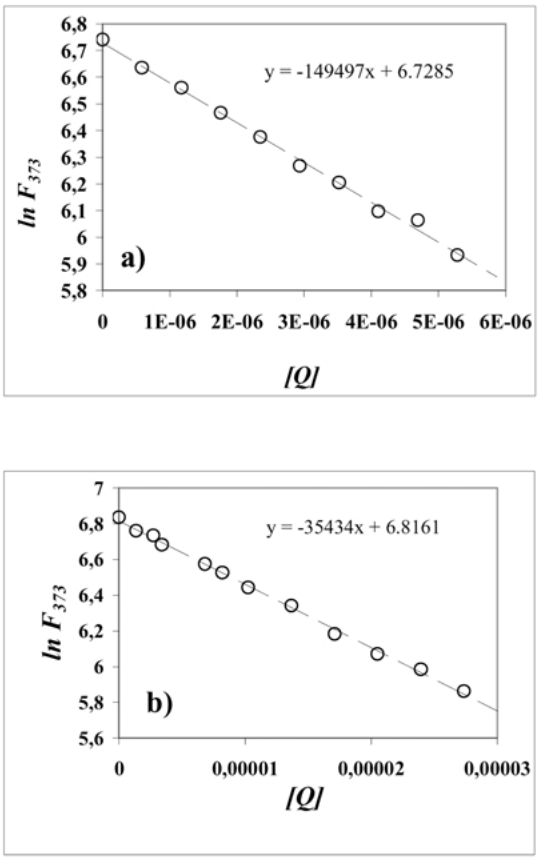


Figure 6. Plot of the logarithm of the fluorescence intensity (ln F_{373}) as a function of the CePyCl concentration for a solution of pyrene (a) in 20×10^{-4} M [CTAB] : [HPβ-CD] (after cmc) and (b) in 50×10^{-4} M [CTAB] : [α-CD] (after cmc*).

that either the conditions or the probes used were not sensitive enough to detect the simultaneous formation of both types of micelles.

Aggregation number and polarity of the new aggregates

The determination of the aggregation number of the micelles formed after each break point, as shown from fluorescence data, was used to confirm the formation of these two types of aggregates. Pyrene, as the luminiscent probe, and hexadecylpyridinium chloride, as static quencher, were chosen for the purpose. The aggregation number of the monomers in micelles, n, can be determined from the steady state fluorescence data, assuming a Poisson distribution as valid to describe the equilibrium distribution of solubilizates between aqueous and micellar phases. The equation to be applied is [28]:

$$\ln I = \ln I_0 - [Q]/C_m = \ln I_0 - n[Q]/(C_t\text{-cmc}) \qquad (7)$$

where $[Q]$, C_m, and C_t are the concentration of quencher, micelles, and total surfactant, respectively, while I_0 and I are the fluorescence intensities in the absence and presence of several quencher concentrations. This method had been succesfully used previously in other surfactant-cyclodextrins

Table 5. Agreggation number of the micelles formed after the first cmc (15 or 20×10^{-4} M) and after the second cmc* (50 or 70×10^{-4} M) for 1 : 1, [CTAB] : [CD] mixture

	N After cmc	N After cmc*
CTAB : WATER	60[a]	–
CTAB : α-CD	58 ± 2	92 ± 3
CTAB : β-CD	69 ± 6	97 ± 3
CTAB : HPβ-CD	72 ± 3	80 ± 2

[a] Data from ref. [36].

systems [16]. Figure 6 reports the values of ln I for the micellar solutions in the presence of several quencher concentrations for CTAB : HPβCD (Figure 6a) and CTAB : α-CD (Figure 6b) systems. From the slope of this plot and the average of the cmc values previously determined by conductometric and spectroscopic data, a value of n for the aggregates formed in the presence of the different cyclodextrins, can be determined. For each cyclodextrin the aggregation number was calculated at two concentrations, one corresponding to the concentration of surfactant and cyclodextrin above the first break point obtained in the curves of fluorescence vs CTAB : CD, and the other above the second break point obtained by absorption, fluorescence and conductivity. The sets of the two surfactant : cyclodextrin concentrations were: 15 $\times 10^{-4}$ M and 50 $\times 10^{-4}$ M for α- and β-CD; and 20 $\times 10^{-4}$ M and 70 $\times 10^{-4}$ M for HP-β-CD. The results obtained are included in Table 5. The aggregation number obtained for the two concentrations of all the systems studied are clearly different. The surfactant concentration is far from that at which the transition from spherical to rod micelles occurs. Therefore, at the two studied concentrations, the micelles are spherical. The mean size of spherical micelles is relatively insensitive to the surfactant concentration above the cmc, and the micelles are fairly monodisperse [40]. On this basis, the results indicate the existence of two types of aggregates. The n value obtained when the surfactant:cyclodextrin concentration is above the first cmc, is in good agreement with $n = 60$, reported for CTAB micelles in the absence of any additive [36].

After the second cmc*, a new aggregate with an aggregation number of 92 ± 3, 97 ± 3 and 80 ± 2 is formed for α-, β-, and HPβCD, respectively. That corresponds to micelles formed with the surfactant monomers included into the cyclodextrins. This large increase in the aggregation number in addition to the increase in the ionisation degree, detected by conductometric measurements for these aggregates, means a decrease in the inter-head group repulsion. Considering that a neutral additive is added, the only possible explanation is that the intramicellar Coulombic repulsive forces decrease. The same situation arises in the case of solubilization of n-octylamine in CTAB micelles [41], and in our case it confirms again the presence of the inclusion complex in the aggregates.

On the other hand, the relative intensities of the first and third vibronic peaks of pyrene are directly related with the apparent dielectric constant of the medium where the

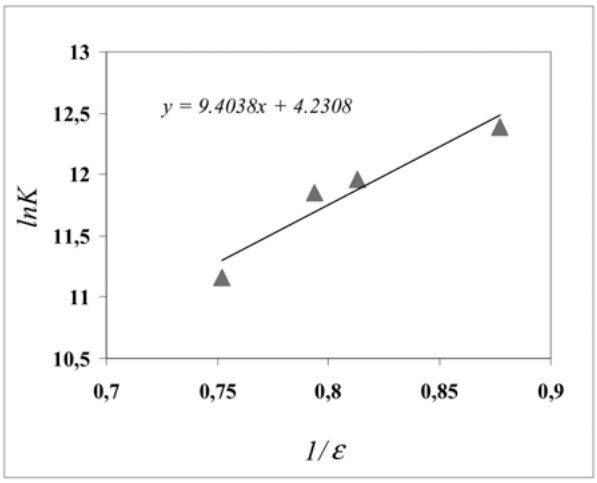


Figure 7. Binding constant of *Nabumetone* to the micelle formed by surfactant : cyclodextrins complexes vs micelle dielectric constant, determined by using pyrene as probe.

probe is housed. For pyrene, characteristic values of I_I/I_{III} are 1.70, 1.38, 1.15 and 0.60 in water, methanol, ethanol and hexane, respectively [41]. The value obtained in this work for I_I/I_{III} averaged over all the curves for each set of measurements, has been used to detect the polarity of the new aggregates formed. The values of I_I/I_{III} obtained in the presence of α-, β-, and HPβCD are 1.23, 1.14 and 1.26, respectively. It is evident that these values are close to those for polar solvents like ethanol. This indicates that CTAB : CDs micelles provide the solubilized pyrene with a microenvironment that is nearly as apolar as hydrocarbon solvents. For ordinary surfactant micelles, the solubilized site of aromatic hydrocarbons, such as pyrene, is the palisade layers of the micelles [42, 43]. Consequently, the solubilized site of pyrene in the mixed micelle has to be the palisade layers. The polarity detected inside the micelle is lower than that detemined for pure CTAB micelles (I_I/I_{III} =1.33 [29]), using pyrene as probe. The polarity change seems to indicate that the second micelle becomes tighter in the presence of cyclodextrins, and that the hydrophobic region is less polar than that of CTAB. The same situation has been described for the CATB : β-CD system [20] and for CTAB in the presence of other aditives [41].

When $\log K_{\text{binding}}$ of *Nabumetone* (Table 3) vs $1/\epsilon$ is plotted (Figure 7), a good linear correlation is obtained indicating that the change in the affinity between the drug and the micelles formed is related to the difference in their polarities. This relation gives a clear proof of the difference of the structure of the micelle formed in the presence of each cyclodextrin.

Conclusions

The properties of CTAB micelles formed in the presence of α-, β- and HPβCD have been studied. In the presence of the three cyclodextrins two types of micelles are clearly formed: one with the free monomers, and another one by micellization of the monomers complexed with the cyclodextrins.

The presence of cyclodextrins seems to produce a decrease in the cmc of the pure micelle but does not produce any change in the aggregation number.

The second type of micelle is formed at higher [CTAB] : [CD] values: 25.6×10^{-4} M, 27.8×10^{-4} M and 38.9×10^{-4} M for α-, β-, and HPβCD, respectively. A significant increase in the ionisation degree has been detected (0.65, 0.77, 0.77 for α-, β-, and HPβCD, respectively). The aggregation number is also strongly increased, in accordance with the higher ionisation degree, being 92 ± 3, 97 ± 3 and 80 ± 2 for for α-, β-, and HPβCD, respectively. The dielectric constant in the micelle detected by pyrene is decreased in the presence of cyclodextrins. The polarity change seems to indicate that in the presence of cyclodextrins, the micelle formed by surfactant : CD complexes becomes more tight and the hydrophobic region is less polar.

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