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A fluorimetric, potentiometric and conductimetric study of the aqueous solutions of naproxen and its association with hydroxypropyl-β-cyclodextrin

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Abstract

The dissociation constant, K_a, of naproxen, a nonsteroidal antiinflammatory drug of the family of arylpropionic acids, has been determined in aqueous solutions at 25°C by using a potentiometric and a conductimetric techniques. The solubility limit of the drug in water, a controversial point in the literature, has been found to be less than 3×10^{-5} M. The interaction of naproxen with hydroxypropyl-β-cyclodextrin (HPBCD), in terms of the binding constants of the complexes formed by the CD and the nonionic (HNAP) and ionic (NAP^-) species of the drug, has been evaluated at 25°C as well by means of steady-state fluorescence enhancement studies. A discussion of the results, $K_{HPBCD:HNAP} = 6500 \pm 400\ M^{-1}$ and $K_{HPBCD:NAP^-} = 1400 \pm 80\ M^{-1}$, emphasizing the crucial importance of the choice of the pH at a value that $pH \geq pK_a + 2$ or $pH \leq pK_a - 2$, is also included.

Keywords: Association constant; Conductimetry; Fluorescence; Hydroxypropyl-β-cyclodextrin; Inclusion complex; Naproxen; Potentiometry

1. Introduction

Inflammation, pain and pyresis are distinct physiological responses which can occur independently. (*S*)-6-methoxy-β-methyl-2-naphthalene acetic acid, commonly named (+)-Naproxen, is a nonsteroidal antiinflammatory (NSAID) agent of the family of the arylpropionic acids (i.e. ketoprophen, ibuprophen, flurbiprophen, etc.), with antiinflammatory, analgesic and antipyretic properties, often used in the treatment of rheumatic and arthritis diseases (Mahler et al., 1976; Calvo et al., 1987). All of these drugs act by inhibiting the cyclooxygenase enzyme. In those containing a chiral center, as is the case of naproxen, the

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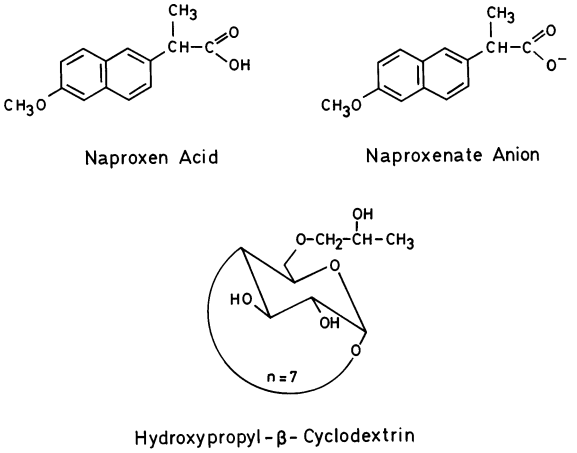
biological activity is associated with the $S(+)$ isomer. However, the very low aqueous solubility of naproxen in water causes undesirable effects on the gastric mucosa, when it is orally administered.

Cyclodextrins (CD's), cyclic oligosaccharides consisting of $\alpha(1-4)$ linked α-D-glucopyranose units, have been widely used (Szejtli and Osa, 1996) to enhance solubility, chemical stability, and bioavailability of poorly soluble drugs, in general, and of arylpropionic acid derivative anti-inflammatory drugs, in particular. From a pharmaceutical point of view, CD:Drug inclusion complexes turned out to be very convenient to reduce the local concentration of free drug in the gastro-intestinal tract, thus ameliorating the deleterious ulceration effects of NSAID's. However, inclusion of the drug within a cyclodextrin will only be effective for these purposes if the association constant of the complex falls within the proper range. Complexes with low association constants will rapidly release the drug, reducing the effect of the complexation on its bioavailability. On the other hand, if the association constant is very high, the release rate of the drug may be so slow that, not only the side effects, but also the therapeutic ones are reduced. Consequently, it is extremely important to choose the proper CD for each drug, in such a manner that the association constant of the CD:Drug inclusion complex will be moderately high.

In previous papers, we have investigated the association of β-CD and/or its methylated (DIMEB) and hydroxypropylated (HPBCD) derivatives with a number of antiinflammatory and/or analgesic agents of the families of: (i) salicylic acid-salicylates (Junquera and Aicart, 1997a; Junquera et al., 1998); and (ii) NSAID's, i.e. ketoprophen (Junquera and Aicart, 1997b), ibuprophen and flurbiprophen (Junquera and Aicart, 1998). We have insisted, among other precautions widely explained elsewhere (Junquera and Aicart, 1997a), on how important is the choice of the pH of the experiment when the drug is a weak acid, to assure that only the ionic or the nonionic form of the drug is present in the solution, and therefore that only one of the two possible encapsulations by the CD is analyzed. However, unfortunately, the association constant of complexes formed by a CD and a weak acid are continuously reported in the literature from experiments on unbuffered solutions, or at pH's which do not guarantee the above mentioned condition. In those cases, the reported binding constant is an average over the association constants of the complexes formed by the CD and the two species of the drug. Consequently, they are apparent constants without any physical meaning.

Particularly, naproxen is subjected to a great controversy (Chowhan, 1978; Herzfeldt and Kuemmel, 1983; McNamara and Amidon, 1986; Erden and Çelebi, 1988; Zecchi et al., 1988; Bettinetti et al., 1989; Valsami et al., 1990; Bettinetti et al., 1991; Brown et al., 1991; Orienti et al., 1991; Otero-Espinar et al., 1992; De Guidi et al., 1993; Melani et al., 1995; Bettinetti et al., 1996; Vélaz et al., 1997) with respect to its solubility limit in aqueous solution, its dissociation constant K_a, and the association constants of the CD complexes with both the nonionized (HNAP) and the ionized (NAP^-) forms of the drug (see Scheme 1). In fact, the association of naproxen with the parent α-, β-, and γ-CD's is well documented, while very few studies have been reported on the interaction of this antiinflammatory agent with CD's derivatives. Many of these studies were done either with unbuffered solutions (Erden and Çelebi, 1988; Bettinetti et al., 1989, 1991; Melani et al., 1995; Bettinetti et al., 1996), or buffered at not appropriate pH's (Brown et al., 1991; Orienti



Scheme 1.

et al., 1991; Otero-Espinar et al., 1992; Vélaz et al., 1997), or at drug concentration above its solubility limit (Bettinetti et al., 1989; Brown et al., 1991; Orienti et al., 1991; De Guidi et al., 1993). The aim of the present study is to clarify the behavior of naproxen in aqueous and in CD solutions. For this purpose, a potentiometric, a conductimetric and a steady-state fluorescence techniques have been used, at 25°C, to determine the K_a for the acid, and the association constant of the complexes HPBCD:HNAP and HP-BCD:NAP^-.

2. Experimental section

2.1. Materials

(*S*)-6-methoxy-α-methyl-2-naphthalene acetic acid, commonly named as (+)-naproxen (NAP), with 99% purity or greater, was purchased from Sigma (St. Louis, MO, USA). Hydroxypropyl-β-cyclodextrin (HPBCD), containing an average of 0.6 hydroxypropyl groups per glucopyranose unit (molar degree of substitution, MDS = 4) was purchased from Janssen Biotech. A termogravimetric analysis (TG) of HPBCD showed a water contents of 2.8% mass, which was considered in the calculations of solute concentrations. All were used as purchased without further purification. The buffer solutions used in the fluorescence experiments were: hydrochloride acid/potasium chloride at pH = 1, monobasic sodium phosphate/dibasic sodium phosphate at pH = 7, and boric acid/borax at pH = 9, all from Metrohm. Distilled water was purified by using a Super Q Millipore System. The homogeneity of the initial solutions, freshly prepared, was assured by sonicating them for 1 h in an ultrasonic bath.

2.2. Potentiometric method

pH potentiometric data were carried out at 25°C, with a Metrohm 713 Ion Meter using a combined glass electrode containing 3 M KCl as the reference electrolyte solution. The equipment, the temperature control system, and the experimental computerized procedure were widely described previously (Junquera and Aicart, 1997b). The pH data are obtained as a statistical average of 250 measurements for each concentration, with an accuracy better than 0.0003 units.

2.3. Conductimetric method

Conductivity data were collected, at 25°C, with a Hewlett-Packard 4263 LCR Meter, using a Metrohm electrode with a constant cell of 0.8129 cm^{-1}. The experimental procedure, fully computerized, and the temperature control system were widely described elsewhere (Junquera and Aicart, 1994). The accuracy on the specific conductivity κ, obtained as an average of 2400 measurements for each concentration, is believed to be better than 0.03%.

Both, potentiometric and conductivity measurements, were made in aqueous solutions of the drug, as a function of naproxen concentration, always below its solubility limit. Mixtures were prepared volumetrically by a digital burette with an accuracy better than 0.002 ml. The control of the temperature in the conductimetric or potentiometric cell and in burette cylinder was better than ± 1 mK. As a consequence, the accuracy in the molarity of the solutions was better than 0.1%.

2.4. Fluorescence method

Steady-state fluorescence experiments were performed with a Perkin-Elmer LS-50B Luminiscence Spectrometer. Data acquisitions and analysis of fluorescence spectra were carried out with the FLDM software supported by the manufacturer. Details of the experimental procedure was fully described earlier (Junquera and Aicart, 1997a). A 10 mm stoppered rectangular silica cell was placed in a stirred cuvette holder whose temperature was kept constant at 25.00 ± 0.01°C. During the experiments, the excitation and emission slits were fixed at 5 and 2.5 nm, respectively, the excitation wavelength was set at 320 nm and the emission spectra were collected from 330 to 500 nm. In all the measurements the scan rate was selected at 240 nm/min. The titrations were made at constant drug concentration, varying the HP-BCD concentration in such a range that a proper

saturation degree for the inclusion process is reached (Deranleau, 1969).

3. Results and discussion

One of the highest controversies of naproxen reported data is its aqueous solubility. Most of these data, extrapolated to zero ligand concentration on phase solubility diagrams, fall in the range of 10^{-4} M. In order to address this point, several aqueous solutions of the drug, with concentrations covering the range 10^{-5}–10^{-3} M, were prepared by weight, and shaken on a thermostated ultrasonic bath at 25°C for 2 days, to allow them reach equilibrium. Only those solutions with concentration below 3×10^{-5} M were transparent and homogeneous. More concentrated solutions did not show turbidity, but solid particles of naproxen could be clearly seen, either forming a suspension or on the surface of the liquid, just by visual observation. To avoid any solubility problem (although it is known that the solubility increases with CD concentration), the maximum concentration of NAP reached during the experiments was 2.5×10^{-5} M.

Since NAP is a weak acid, both nonionized (HNAP) and ionized (NAP^-) species are present in the solution, and both can be included within the CD cavity, when this host molecule is added. Assuming a 1:1 stoichiometry for the complexes, the following equations show the processes taking place in the solution:

$$HNAP \rightleftarrows H^+ + NAP^-$$

$$K_a = a_{H^+} a_{NAP^-} / a_{HNAP} \quad (1)$$

$$CD + HNAP \rightleftarrows CD{:}HNAP$$

$$K_{CD:HNAP} = a_{CD:HNAP} / (a_{CD} a_{HNAP}) \quad (2)$$

$$CD + NAP^- \rightleftarrows CD{:}NAP^-$$

$$K_{CD:NAP^-} = a_{CD:NAP^-} / (a_{CD} a_{NAP^-}) \quad (3)$$

$$CD{:}HNAP \rightleftarrows CD{:}NAP^- + H^+$$

$$K_{a'} = (a_{CD:NAP^-} a_{H^+}) / (a_{CD:HNAP}) \quad (4)$$

where K_a is the dissociation constant of the HNAP acid, and $K_{CD:HNAP}$ and $K_{CD:NAP^-}$ are the association constant of the 1:1 complexes formed by the HPBCD and the acid and base forms of NAP, respectively. The constant K'_a is easily related to the previous ones through a simple expression.

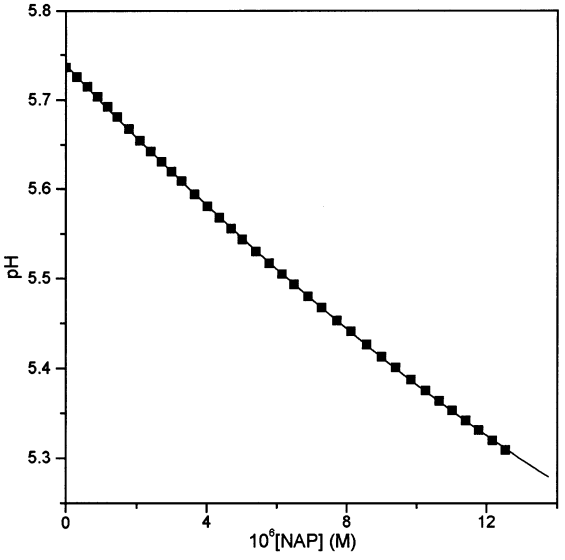


Fig. 1. Values of pH as a function of total naproxen concentration, at 25°C.

The knowledge of K_a is extremely important when the association of these acidic drugs with CD's is studied at fixed pH. We have recently proved (Junquera and Aicart, 1997a,b) that only if the pH is fixed at values differing from the pK_a in more than 2 units, just equilibrium 2 ($pH \leq pK_a - 2$), or equilibrium 3 ($pH \geq pK_a + 2$) occur when the CD is added. In such conditions, the equilibria 1 and 4 are almost totally shifted towards the acid forms of the drug and the CD:HDrug complex, or to their ionized counterparts, respectively.

Thus, the first parameter to determine is the dissociation constant of naproxen in water solution. In this work, it has been obtained from both pH potentiometric and conductimetric experiments. Fig. 1 shows the variation of the pH of an aqueous solution of NAP as a long as $[NAP]_{tot}$ increases. Eq. (1), those for the activity coefficients using the Debye–Hückel theory, and the mass and charge balances, permits us to obtain K_a, from the pH experimental data with a nonlinear regression method (NLR) based on a Marquardt algorithm ($K_a = (3.0 \pm 0.2) \times 10^{-6}$, i.e. $pK_a = 5.52$). In order

to confirm this value from another property, conductivities of aqueous solutions of naproxen were measured in a similar experiment where a NAP aqueous solution was titrated over pure water. From this data (see Fig. 2), a value of $K_a = (2.8 \pm 0.4) \times 10^{-6}$, i.e. $pK_a = 5.55$, was obtained, in very good agreement with that one determined from pH data. If the pK_a is 5.5, the nonionized (HNAP) and the ionized (NAP^-) species of naproxen will be predominant in the medium, with negligible presence of the conjugated species, at $pH < 3.5$ and at $pH > 7.5$, respectively.

The associations of HNAP and NAP^- with HPBCD were studied fluorimetrically at $pH = 1$ and 9, to assure the above mentioned condition. It is well known that the intensification of luminescent processes of lumiphores partially or totally encapsulated by the CD cavity is a result of the better protection from quenching and other processes occurring in the bulk solvent. The CD cavity behaves similarly to an organic solvent; it affords an apolar environment and a nonhydrated state for the included probe. Figs. 3 and 4 show the fluorescence emission spectra of naproxen in the absence and in the presence of different HPBCD concentrations, at $pH = 1$ and 9, respectively. The total drug concentration is kept constant at 1.25×10^{-5} M at $pH = 1$, and at 2.48×10^{-6} M at $pH = 9$, and the HPBCD concentration was varied. Table 1 reports the maximum fluorescence intensities and emission wavelengths for the pure substrates at the working conditions. From the data on Table 1, it can be deduced that naproxenate is much more fluorescent than its protonated counterpart, as

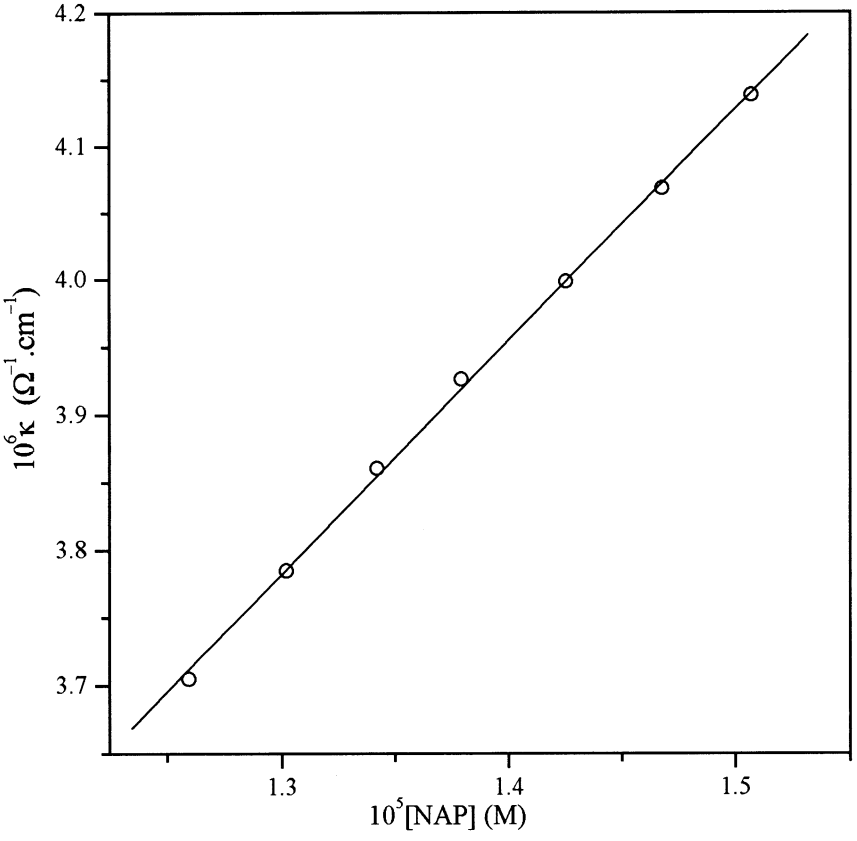


Fig. 2. Values of specific conductivity, κ, as a function of total naproxen concentration, at 25°C.

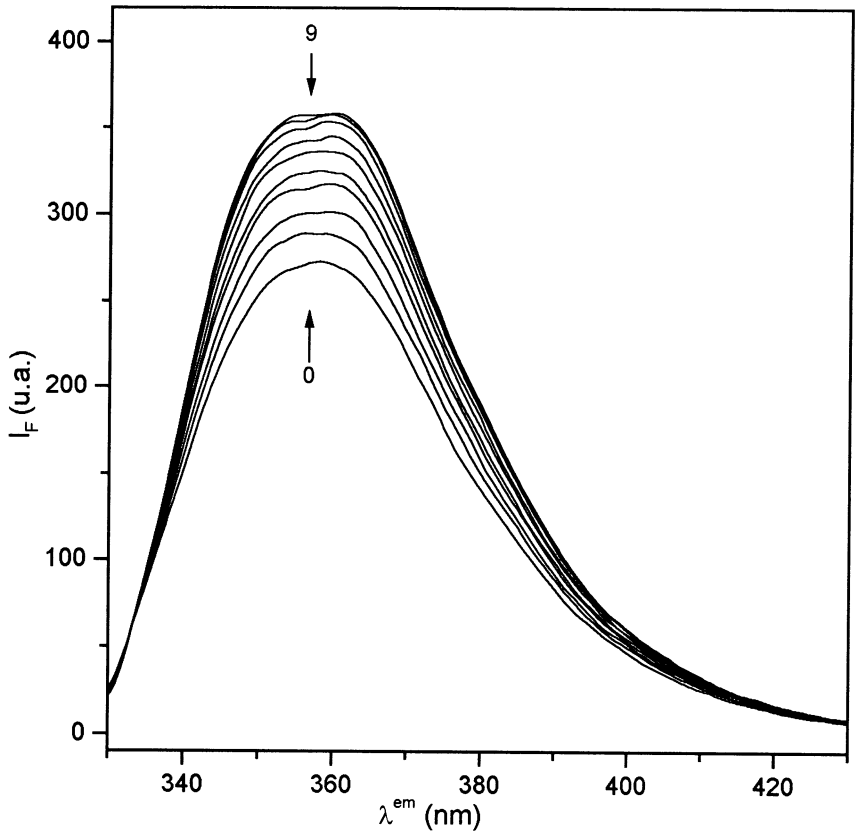


Fig. 3. Fluorescence emission spectra of HNAP ([HNAP] = 1.25×10^{-5} M) at pH = 1 at different HPBCD concentrations ($\times 10^{-4}$ M): **0**, 0.00 mM; **1**, 0.35 mM; **2**, 0.69 mM; **3**, 1.04 mM; **4**, 1.38 mM; **5**, 2.06 mM; **6**, 2.72 mM; **7**, 4.03 mM; **8**, 5.31 mM; **9**, 6.55 mM. λ_{ex} = 320 nm, ex./em. slitwidths = 5/2.5 nm, scan rate = 240 nm/min, buffer: KCl/HCl solution.

previously found for other drugs (Junquera and Aicart, 1997a). In fact, at pH = 9, it was necessary to decrease the [NAP^-] one order of magnitude to avoid saturation. Emission intensity enhancements, and neither hipsochromic nor batochromic shifts were observed at both pH's as long as [CD] increases. Fig. 5 shows, as an example, the plot of the ratio I/I_0 values (I and I_0 being the fluorescence intensity of the solution in the presence and absence of cyclodextrin, respectively) as a function of [HPBCD], at three different λ^{em} values for the experiment run at pH = 1. The experimental I/I_0 values can be fitted to the wellknown binding isotherm (Connors, 1987, 1997):

$$\frac{I}{I_0} = \frac{1 + (k_{CD:Drug}/k_{Drug})K_{CD:Drug}[CD]}{1 + K_{CD:Drug}[CD]} \quad (5)$$

by using a NLR analysis based on a Marquardt algorithm. The ratio $k_{CD:Drug}/k_{Drug}$, where k_i represents the proportionality constants connecting the intensities and concentrations, and the association constant of the CD:Drug inclusion complex $K_{CD:Drug}$, can be obtained as fitting parameters. Table 2 reports these values obtained at three different λ^{em} values, at pH = 1 and 9.

We have previously pointed out that, besides a proper and cautious choice of the buffer (pH), it is also important: (i) to check that the determination of $k_{CD:Drug}/k_{Drug}$ and $K_{CD:Drug}$ is not affected by the λ^{em} chosen to analyze the I/I_0 data; and (ii) to assure that a proper range of the saturation degree, f, for the inclusion process is covered. The constancy of the fitting parameters, is already seen in Fig. 5 and definitively confirmed with the re-

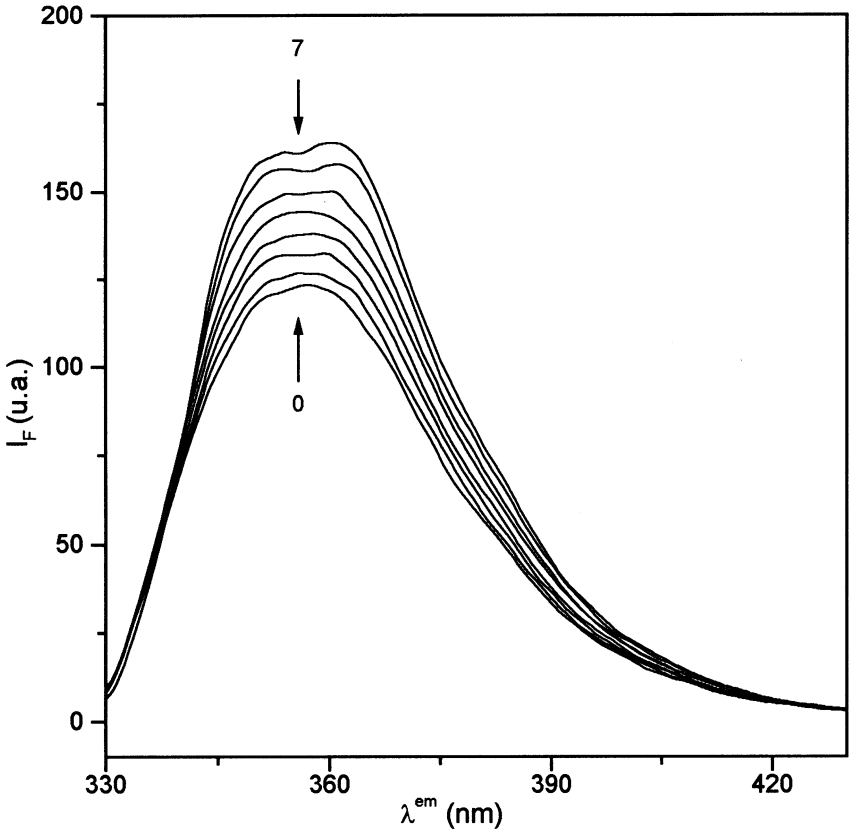


Fig. 4. Fluorescence emission spectra of HNAP ([HNAP] = 2.48×10^{-6} M) at pH = 9 at different HPBCD concentrations ($\times 10^{-4}$ M): **0**, 0.00 mM; **1**, 1.17 mM; **2**, 2.32 mM; **3**, 3.81 mM; **4**, 5.99 mM; **5**, 8.76 mM; **6**, 37.6 mM; **7**, 52.5 mM. λ_{ex} = 320 nm, ex./em. slitwidths = 5/2.5 nm, scan rate = 240 nm/min, buffer: boric acid/borax solution. Other curves have been omitted for the sake of clarity.

sults reported in Table 2, the goodness of the method and the accuracy of the results being thus guaranteed.

Fig. 6 shows the plot of f vs [HPBCD] at the two experimental pH's studied. As can be seen in the figure, 80% of the saturation curve is covered in both experiments, as recommended (Deranleau, 1969) for an accurate determination of association constants. Due to the highest value of KCD:HNAP with respect to KCD:NAP^-, the final CD concentration necessary to reach this 80% of saturation is $\sim 5 \times 10^{-5}$ M at pH = 9 while 0.9×10^{-5} M is sufficient at pH = 1.

A third experiment at pH = 7 ($[NAP]_{tot} = [HNAP] + [NAP^-] = 2.16 \times 10^{-6}$) was done to verify how crucial the difference $pK_a - pH_{buffer}$ is. Values of 2400 M^{-1} and 1.28 were obtained for $K_{CD:Drug}$ and $k_{CD:Drug}/k_{Drug}$ in this case. It means that 3% of the contribution of HNAP at pH = 7 (only 1.5 units above pK_a), is enough to drive to an apparent association constant, with an intermediate value between those for HPBCD:HNAP and HPBCD:NAP^-.

Table 1
Fluorescence intensities ($I_{0,max}$) and wavelength at the maximun peak of the fluorescence emission spectra for pure naproxen at 25°C, and at pH = 1 and 9

pH	Species	[Drug] (M)	λ^{em}_{max} (nm)	$I_{0,max}$ (a.u.)
1	HNAP	1.26×10^{-5}	358.0	273
9	NAP^-	2.48×10^{-6}	357.5	123

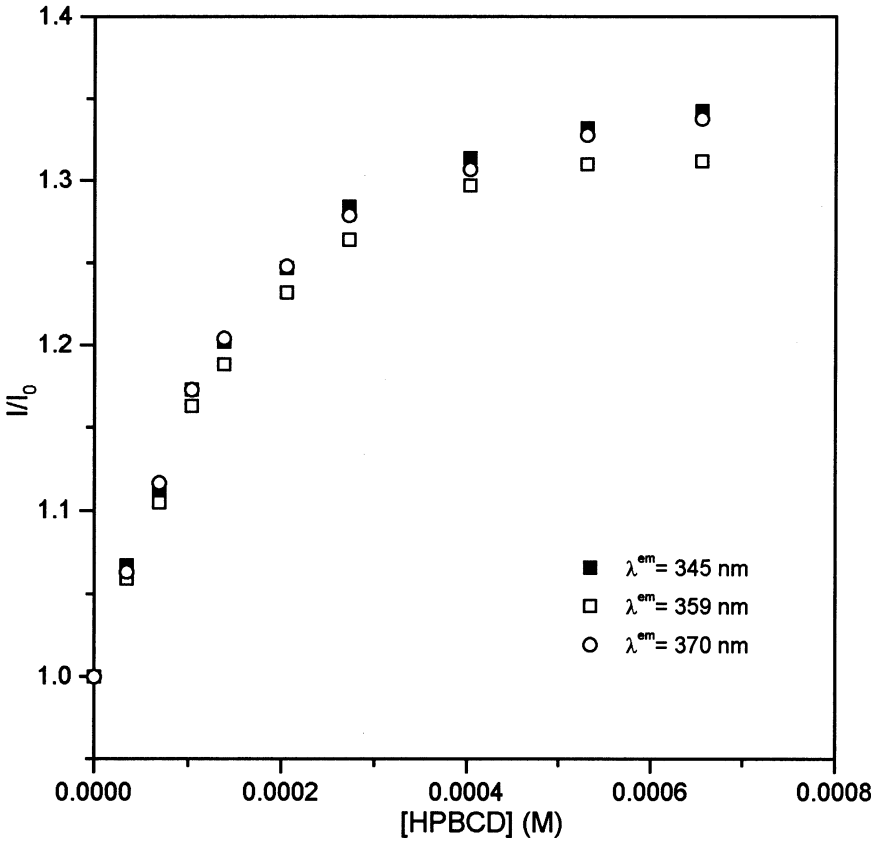


Fig. 5. Values of the fluorescence intensity enhancement (I/I_0) due to the encapsulation by the CD cavity of HNAP at pH = 1, as a function of [HPBCD] at three different emission wavelengths (λ^{em}).

Table 3 records the results obtained in this work for the system HPBCD + NAP with those previously reported by us for other HP-BCD:NSAID systems (Junquera and Aicart, 1997b, 1998). The highest affinity of the carboxylic species by cyclodextrin with respect to the carboxylate one, usually found in these systems, is observed as well in the system studied herein. In fact, a ratio $K_{CD:HDrug}/K_{CD:Drug-} = 4.0 \pm 0.7$ is obtained. This behavior has been previously reported for other carboxylic derivatives and it has been widely discussed elsewhere in terms of the contribution of different factors (Bergeron et al., 1978; Gelb et al., 1979, 1981; Cromwell et al., 1985; Martin-Davies and Savage, 1994).

It is worth noticing in Table 3 that the solubility of the drug increases in the same order as its dissociation constant in water: naproxen < ibuprophen < flurbiprophen < ketoprophen.

Table 2
Association constants for the HPBCD:HNAP and HP-BCD:NAP$^-$ inclusion complexes at 25°C from fluorescence data

pH	Drug	λ^{em} (nm)	$K_{CD:Drug}$ (M^{-1})	$k_{CD:Drug}/k_{Drug}$
1	HNAP	345	6350	1.44
		359	6590	1.40
		370	6670	1.42
9	NAP$^-$	350	1398	1.38
		360	1436	1.38
		370	1370	1.42

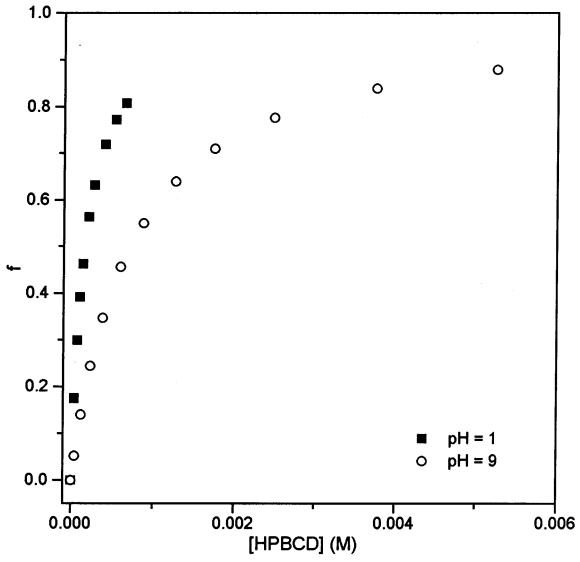


Fig. 6. Plot of the saturation degree f as a function of HPBCD concentration at pH = 1 and 9, considering the association constants $K_{CD:HNAP}$ (pH = 1) and $K_{CD:NAP-}$ (pH = 9) reported in Table 2.

However, it looks like the hydrophobic effect is not the main driving force of the inclusion of naproxen, ibuprophen and flurbiprophen within he apolar cavity of the cyclodextrin, since as long as the solubility of these drugs increases, the association of either the nonionized or ionized species becomes tighter. Ketoprophen is, nevertheless, an exception; it is the more soluble and its association constants are the lowest ones.

The spare information reported in the literature about the system HPBCD + Naproxen is obtained from unbuffered solutions. Values of 2582 M^{-1} (Bettinetti et al., 1991) or 2083 M^{-1} (Melani et al., 1995) for the apparent constant of the complex reveal the contribution of both HNAP and NAP^- to the association with HP-BCD. However, the association of naproxen with the parents α-, β- and γ-CD has been widely studied. The binding constants determined on unbuffered solutions (Erden and Çelebi, 1988; Bettinetti et al., 1989, 1991; Melani et al., 1995; Bettinetti et al., 1996) or at pH's ranging from 3.5 to 7.5 (Brown et al., 1991; Orienti et al., 1991; Otero-Espinar et al., 1992; Vélaz et al., 1997), will be not commented here, since its meaning is very limited. It is known that for a given guest, the inclusion within the HPBCD cavity is more favored than that within β-CD cavity. This fact explains the value of 5730 ± 90 M^{-1} obtained for the system β-CD + NAP at pH = 2 from fluorescence measurements as well (Vélaz et al., 1997), in concordance with the value for HPBCD + NAP at pH = 1, reported herein. Discrepancies on other reported values (Orienti et al., 1991; De Guidi et al., 1993), may be due to the high concentration of free naproxen (above the solubility limit) used in most of those experiments.

Acknowledgements

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Table 3
Values of the dissociation constant K_a, and the association constants of the 1:1 inclusion complexes formed by HPBCD and the acid and base forms of several NSAID's at 25°C

Guest	$10^5[Drug]_{max}$ (M)	$10^5 K_a$	$K_{CD:HDrug}$ (M^{-1})	$K_{CD:Drug-}$ (M^{-1})	$K_{CD:HDrug}/K_{CD:Drug-}$
Naproxen	2.5	0.30 ± 0.02	6500 ± 400	1400 ± 80	4.6
Ketoprophen[a]	50	6.3 ± 0.6	1430 ± 140	460 ± 50	3.1
Flurbiprophen[b]	5.0	4.5 ± 0.4	9600 ± 1000	2530 ± 250	3.8
Ibuprophen[b]	1.0	2.3 ± 0.2	15700 ± 1600	4700 ± 500	3.3

[a] Junquera and Aicart (1997b).
[b] Junquera and Aicart (1998).

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