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Cyclodextrins modify the proton transfer photoreactions of norharmane

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Abstract

Excited state proton transfer reactions of norharmane have been investigated in the presence of cyclodextrins (CDs): β-CD and modified β-CDs at pH values of 4.0, 7.8, 10.0 and 13.0. The spectral shape and the species present in the equilibria depend on the CDs and the pH of the media. Thus at pH 4.0 the emission corresponds to the cationic form. At pH 7.8 the emission spectra show the bands corresponding to the cationic and neutral forms in the case of the complexes with β-CD and HPβ-CD, and only the neutral band for the complexes of DMβ-CD and TMβ̃CD. At pH 13.0 all the complexes exhibit the neutral form together with the zwitterionic one. The low polarity environment afforded by CDs significantly alters the proton transfer photoreaction of norharmane. The titration curves obtained for the complexes with NaOH or HCl are related to the shifts in the acid–base equilibria in excited states.

Keywords: Cyclodextrins; Norharmane; Proton transfer photoreactions

1. Introduction

Natural and chemically modified cyclodextrins (CDs) are molecules capable to molecularly recognize an extensive variety of compounds. The inclusion of molecules into the cyclodextrin cavities alters their photophysical and photochemical behaviour. Thus one of the most relevant photophysical changes after inclusion complex formation is the possibility to observe room temperature phosphorescence (RTP) [1]. The radiationless deactivation processes and the luminescence quenching by oxygen are notably decreased. CDs also modify the chemical behaviour of the guest molecules including the reaction rates of hydrolysis, condensation and substitution reactions are modified and therefore CDs have been employed in catalysis to increase the reaction rates. The role of CDs in the organic reactions, including photochemical reactions, has been reviewed [2,3]. An increase in the stabilization of labile molecules against photodegradation has been described for the inclusion complexes promazine/β-CD [4], and for the cyclodextrin complexes of sunscreen agents [5] and also polyenes [6]. CDs also limit the photoisomerization process of retinoids, an extremely labile class of compounds, and the inclusion also modifies the relative proportion of the photoisomers [7]. Photoisomerization and controlled dimerization in the presence of β-CD have been described in the case of aromatic derivatives of norbornadiene [8]. β-CD reduces the photodecomposition of several drugs, i.e. nifedipine, furosemide and clofibrate among others [9].

β-Carboline alkaloids are a family of compounds with peculiar photoluminescence properties [10]. They possess a notable native fluorescence at room temperature as well as at 77 K [11]. Besides the proton transfer equilibrium in the excited state is faster than the deactivation of excited state and therefore the excitation of neutral species induces the cationic species emission due to protonation of neutral species in excited state [12,13]. Owing to the presence of two different nitrogens on the heterocyclic ring (pyridinic and pyrrolic nitrogens) the existence of four different species (cationic, neutral, zwitterionic and anionic species, see Scheme 1) is possible depending on the nature of the solvent and, in the case of aqueous solutions depending also on the pH range

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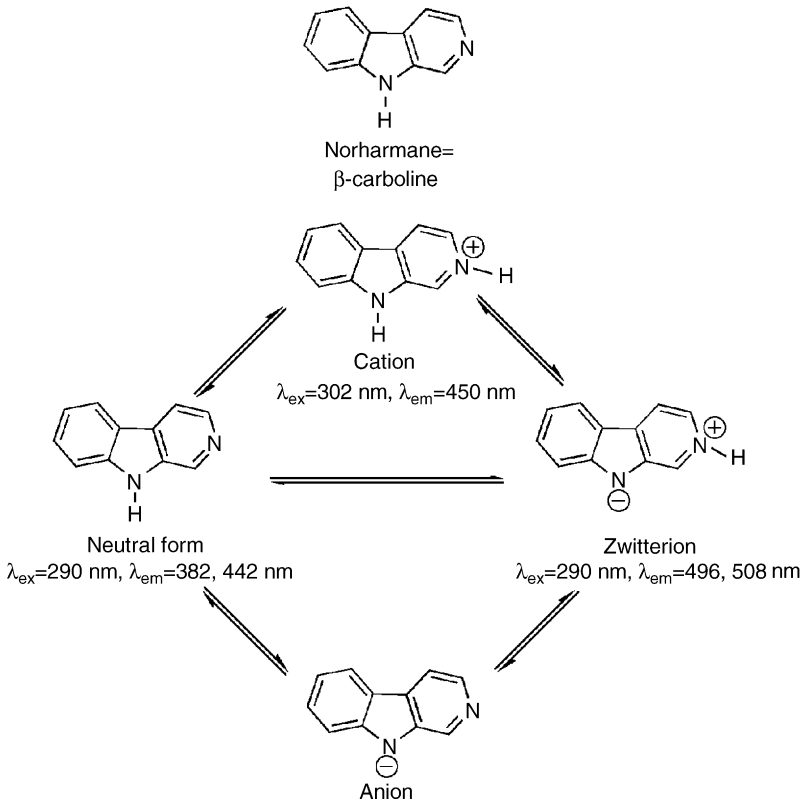


Scheme 1. Chemical structure of norharmane and different species involved in proton transfer reaction of norharmane.

selected. Anionic forms can only be detected in highly concentrated hydroxide solutions and outside the pH scale range [14,15]. Recently the fluorescent behaviour and the acid–base equilibria of 3-substituted β-carbolines in aqueous solutions have been reported [16,17]. Moreover the proton transfer reactions in organic solvents vary with the solvent and differ from the aqueous solutions [18]. The influence of the addition of acids on the proton transfer processes of harmine and 2-methylharmine [19] and norharmane [20] in organic solvents have been described.

The formation of inclusion complexes with CDs alters the acid–base equilibria and modifies the pK_a values as a consequence of the different solvation of the included molecule with regard to aqueous environment. In general, inclusion increases the pK_a values for acidic compounds and decreases pK_a for basic compounds [21]. These effects have been described for 1-naphthol [22], 2-naphthol [23] and carbazole [24,25]. Considering the peculiar behaviour of the proton transfer reaction of β-carbolines we decided to study the influence of the complexation process with CDs on the prototropic equilibria of harmane and harmine [26] demonstrating that the environment afforded by CDs is partially resemblant of organic solvents (ethanol). However different behaviours were observed for the two alkaloids regarding their association constants and stoichiometry of the complexes because of their different chemical structure and solubility properties [27]. Although norharmane is the simplest of the β-carboline alkaloids, not having any substituent, and therefore its inclusion behaviour merits study, it has been overlooked in the past. In the present paper we describe the fluorescence behaviour of the inclusion complexes of norharmane with native β-CD and the chemically modified HPβ-CD, DMβ-CD and TMβ-CD. The fluorescence spectral shape of the inclusion complexes norharmane/CDs at different pH values differs significantly from the corresponding aqueous solutions of norharmane. Changes in the proton transfer reaction as a consequence of the inclusion are also considered.

2. Experimental

2.1. Apparatus and reagents

Absorption UV–vis spectra were obtained with a Kontron Uvikon 810 double beam spectrophotometer. Uncorrected excitation and fluorescence emission spectra were measured with a Perkin-Elmer MPF 2A fluorimeter (xenon lamp, 150 W). In both cases quartz cells with 1 cm of path of length were employed. A thermostated water bath with multimagnetic stirring was used to prepare the inclusion complexes. For the pH measurements a Crison 2000 micro-pH pH-meter equipped with a combined glass electrode was employed. All reagents and solvents were of analytical reagent grade

and they were used without further purification. Norharmane (free base) was purchased from Aldrich. Regarding the cyclodextrins, β-CD was from Merck, heptakis(2,3-di-*O*-methyl)-β-cyclodextrin (DMβ-CD) and heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin (TMβ-CD) from Sigma and (2-hydroxypropyl)-β-cyclodextrin (HPβ-CD) was a generous gift from Rhône-Poulenc (France). Water was doubly distilled prior to its use.

2.2. Procedures

2.2.1. Inclusion complexes formation

Freshly prepared ethanolic solutions of norharmane were prepared at 1.0×10^{-3} M concentration. Aliquots of these solutions were taken and dissolved in HCl 0.1 M to obtain solutions 1.0×10^{-5} M and then these solutions were spectrophotometrically measured and the concentration of the stock solutions were corrected according to these values. Thus appropriate aliquots of the stock solutions were taken and placed in a round bottomed flask. The solvent was evaporated under reduced pressure at room temperature led to the formation of a thin film of norharmane. Then 10 mL of buffered aqueous solutions or buffered aqueous solutions of CDs in concentration 1.0×10^{-2} M were added to the thin film of norharmane. The solutions norharmane-CDs were magnetically stirred for 24 h in order to assure the inclusion of norharmane in the different cavities of the CDs studied. The final concentrations of norharmane and CDs in the complexes were 1.0×10^{-6} and 1.0×10^{-2} M, respectively. The same procedure was followed for the norharmane in buffered solutions without CDs.

2.2.2. Study of the influence of CDs on the acid–base equilibria of norharmane

The solutions of CDs were prepared in different buffered aqueous solutions at pH values of 4.0, 7.8, 10.0 and 13.0. These CDs solutions were left to stabilize for 24 h prior to their use and in order to ensure complete dissolution. At pH 4.0 solutions of CDs were prepared in buffered aqueous solutions (Walpole buffered solution, pH range 0.65–5.20; 1.0 M sodium acetate with the adequate volume of 1.0 M HCl to obtain the desired pH value). For pH values 7.8, 10.0 and 13.0 modified Britton-Welford titrated solutions were employed (0.2 M NaH_2PO_4 with the adequate volume of 0.2 M NaOH up to the desired pH value). The inclusion complex solutions were prepared as described in 2.2.1 at the different pH values studied and then the absorption UV–vis spectra and fluorescence emission spectra were taken. Blank experiments were prepared according to the above-described procedure in the different buffered solutions without CDs. All the experiments were carried out in duplicate or triplicate sets.

With the aim of studying the influence of CDs on the proton transfer equilibria of norharmane different sets of experiments were developed. Thus successive aliquots of HCl 1.0 M were added to the different solutions of the inclusion complexes at the different pH values studied. The results were compared with those obtained with the addition of HCl to the solutions without CDs. After each addition the fluorescence emission spectra were recorded. The same procedure was used for the additions of NaOH, in these cases successive aliquots of 1.0 and 10.0 M NaOH were added.

3. Results and discussion

CDs are widely used in chromatography because of the enhancement of the efficiency and resolutions of separations [28], but they have perhaps been most used as intensity enhancement agents in analytical fluorimetric measurements. Fluorimetric detection is frequently used in the analysis of β-carbolines by HPLC [29] and therefore the existence of proton transfer equilibria affects the sensitivity of the detection when CDs are added to the mobile phases.

3.1. Evidence for the formation of norharmane-CDs inclusion complexes

The high electronic density and low polarity afforded by CD cavities modify the spectral characteristics of the included molecules and therefore shifts in the excitation and emission wavelengths and increases in the fluorescence intensity should be expected as a consequence of the inclusion process. Fig. 1 shows the superimposed spectra of norharmane in different environments. In the case of ethanolic solution an emission band with two maxima at 366 and 382 nm corresponding to the neutral species is present. In buffered aqueous solution the behaviour depends on the pH of the media. As can be observed in Fig. 1, at pH 7.8 (close to the pK_a value of norharmane, 6.85 [15] and 7.8 [12] for the equilibrium cationic–neutral species) the emission bands for the neutral and cationic species are observed in the case of β-CD and HPβ-CD, but for the DMβ-CD and TMβ-CD only the emission band corresponding to the neutral species is detected. The spectral shape corresponding to the neutral form is not resolved as in ethanolic solution. No significant shifts in the emission wavelength for the cationic form (442 nm for β-CD and HPβ-CD) were detected with regard to those observed in buffered aqueous solution without CDs (450 nm). Besides changes in the fluorescence intensity were detected. Considering that in buffered aqueous solution without CD at this pH value it is only possible to observe the emission due to the cationic form and that in CD solution the emission corresponds to cationic and neutral species, it can be deduced that norharmane has been included into the CD cavities. The appearance of the neutral emission band confirms that norharmane remains in an environment of lower polarity than the bulk aqueous solution in concordance with the results described by Mallick and Chattopadhyay for norharmane in micellar solutions [30] and for the interaction of norhamane with bovine serum albumine [31]. The protonation of the neutral species in the buffered aqueous solution is a consequence of the rapid protonation of the excited species of norharmane. This protonation takes place in protic solvents like water. In

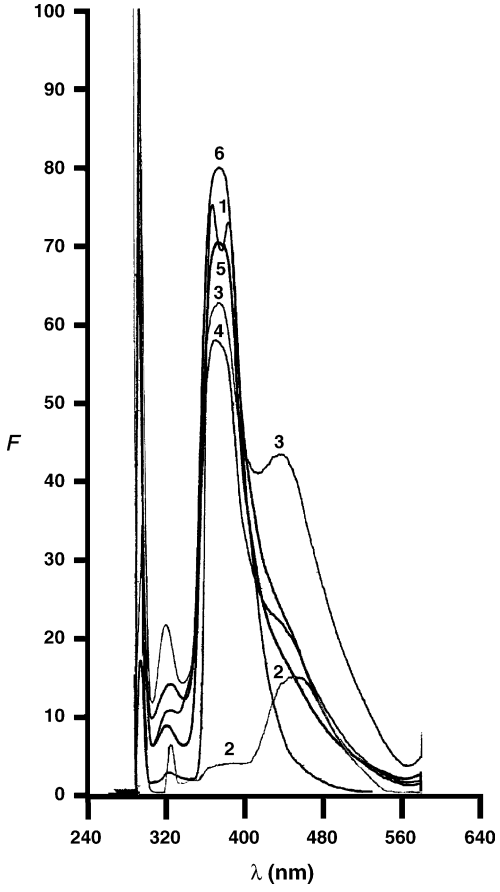


Fig. 1. Fluorescence emission spectra of norharmane (1 μM) in ethanol (1) and in buffered aqueous solution at pH 7.8: without CD (2), with β-CD (3), HPβ-CD (4), DMβ-CD (5) and TMβ-CD (6). Fluorescence intensity for solution 2 is 27 times higher than for the rest of solutions. F: fluorescence intensity in arbitrary units, λ: emission wavelength in nm.

organic solvents the protonation is not possible except by the addition of acids [18].

Similar spectral changes were observed at pH 10.0. Thus, in the presence of β-CD and HPβ-CD the emission of the neutral species was higher than that of the cationic species, while in buffered aqueous solution without CDs the emission of the cationic species predominates with regard to the neutral form. At pH 13 in buffered aqueous solutions without CDs, the emission band corresponding to neutral form and a shoulder around to 500 nm corresponding to the zwitterionic form were observed. In the presence of CDs the emission of the neutral form appears together with the well-defined band of the zwitterionic species. A notable increase in the fluorescence intensity with regard to the aqueous solution was observed for both species (see Table 1).

When the experiments were carried out at pH 4.0 only the emission corresponding to the cationic form was observed. The maximum was not shifted and slight changes in the fluorescence emission intensity were detected. A possible explanation could be concluded that the cationic species remains in the bulk aqueous solution and it is not included. However, considering that the fluorescence quantum yield [15] of the cationic species is five times higher than the fluorescence quantum yield of the neutral species, the decrease in the fluorescence intensity of the cationic norharmane-CDs solutions with regard to the buffered aqueous solutions allows to establish that neutral norharmane is included into CD cavities. On the other hand the behaviour of the inclusion complexes with the additions of HCl and NaOH at this pH value demonstrates differences among free norharmane and the inclusion complexes as will be described below. The emission maxima and the fluorescence intensities obtained for the complexes are summarized in Table 1.

3.2. Effects of complexation on the phototransfer acid–base equilibria

Fig. 2 shows the effect of the additions of HCl on the solutions of the inclusion complexes of norharmane/β-CD and norharmane/HPβ-CD at pH 7.8. As can be observed, the presence of increasing amounts of HCl shifts the acid–base equilibrium from the neutral to the cationic form. The presence of an isoemissive point confirms the existence of only two species in the equilibrium in the excited state. The same is observed for the complexes with DMβ-CD and TMβ-CD. This behaviour clearly differs from the one observed in buffered aqueous solution without CDs where only a cationic band is present and the additions of HCl increase the fluorescence emission intensity of norharmane due to the fact that, in the excited state and inside the cyclodextrin environment, norharmane is a stronger base than OH^- or phosphate. The behaviour of the norharmane complexes at pH 10.0 with regard to the additions of HCl is close to the above described for pH 7.8. In the case of the solutions at pH 13.0 the cationic band appears after addition of 50–60 μL of HCl for the aqueous solution without CDs while for the inclusion complexes additions of 300–450 μL are required, depending on the nature of the CD. Therefore the environment afforded by the lower polarity cavities of CDs affects the protonation of norharmane and hampers seriously the proton transfer photoreaction of norharmane. Slight differences in this photoreaction were observed among the modified CDs, although for β-CD lower amounts of HCl (300 μL) than for HPβ-CD, DMβ-CD and TMβ-CD were necessary. This behaviour can be explained by assuming that at pH 13.0 hydroxyl groups on β-CD can be ionized and therefore the local concentration of H^+ should be high and, the norharmane included in β-CD requires lower proton concentration from HCl to achieve its protonation. For the experiments carried out at pH 4.0 the presence of increasing amounts of HCl causes an increase in the fluorescence intensity of the cationic band for all CDs and aqueous solutions studied.

Fig. 3 shows the effect of the additions of 10.0 M NaOH to the solutions of the complexes of norharmane at pH 4.0. As can be appreciated a decrease in the fluorescence intensity of the emission corresponding to the cationic species was produced as a consequence of the increasing amounts of OH^- in the media. After addition of 80 μL of NaOH the

Table 1
Excitation and emission wavelengths and fluorescence intensities of the inclusion complexes of norharmane/CDs in different buffered aqueous solutions. Norharmane concentration was 1.0×10^{-6} M, CD concentration was 1.0×10^{-2} M. The fluorescence intensities are normalized at the same scale for all the experiments

Solution	λ (nm)	pH 4.0		pH 7.8		pH 10.0		pH 13.0	
		λ	FI[a] $\pm \sigma_{n-1}$[b]	λ	FI[a] $\pm \sigma_{n-1}$[b]	λ	FI[a] $\pm \sigma_{n-1}$[b]	λ	FI[a] $\pm \sigma_{n-1}$[b]
Water	λ_{ex}[c]	302		290		290		288	
	λ_{em}[d]	450	274.95 ± 43.55	384	8.30 ± 0.78	392	10.50 ± 0.20	390	13.33 ± 0.40
	λ_{em}[d]	–		446	16.40 ± 1.23	450	20.02 ± 0.59	–	
β-CD	λ_{ex}[c]	302		290		288		290	
	λ_{em}[d]	450	225.01 ± 5.81	382	40.18 ± 6.84	384	50.96 ± 2.30	384	18.81 ± 2.48
	λ_{em}[d]	–		442	26.83 ± 5.11	446	28.70 ± 2.21	506	6.77 ± 0.86
HPβ-CD	λ_{ex}[c]	302		290		290		288	
	λ_{em}[d]	450	212.93 ± 12.02	374	96.43 ± 13.78	374	100.09 ± 3.73	380	24.36 ± 1.44
	λ_{em}[d]	–		450		–		508	7.75 ± 0.58
DMβ-CD	λ_{ex}[c]	302		290				288	
	λ_{em}[d]	450	284.28 ± 24.44	376	116.03 ± 7.81			380	64.94 ± 2.78
	λ_{em}[d]	–		–				496	3.92 ± 0.44
TMβ-CD	λ_{ex}[c]	302		290				290	
	λ_{em}[d]	450	249.89 ± 11.06	378	103.10 ± 2.47			378	88.85 ± 3.53
	λ_{em}[d]	–		–				498	6.01 ± 1.28

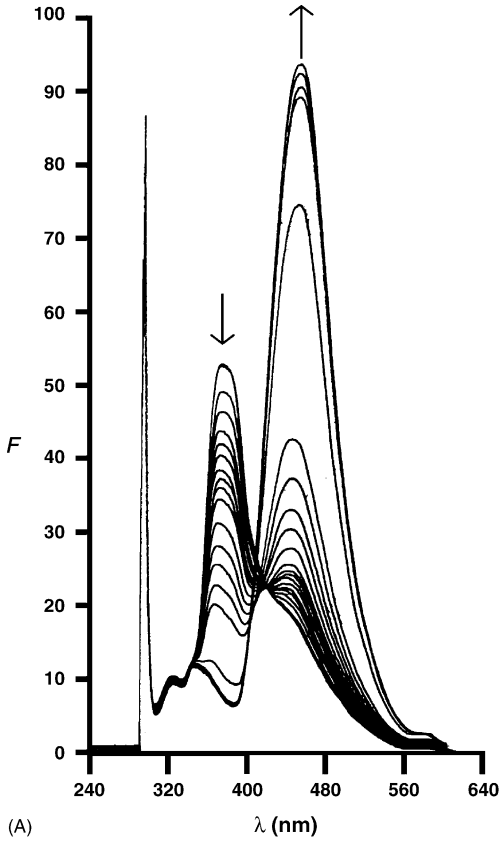
[a] FI: normalized fluorescence intensity values.
[b] σ_{n-1}: standard deviation.
[c] λ_{ex}: excitation wavelength in nm.
[d] λ_{em}: emission wavelength in nm.

weak emission corresponding to the neutral form can be detected showing an isoemissive point in the case of DMβ-CD, TMβ-CD and HPβ-CD complexes. The increase in the pH value produces the deprotonation of pyridinic nitrogen and the appearance of the emission band corresponding to the neutral form. This behaviour is possible due to the low polarity environment afforded by CDs and also considering the compartimentalization effect of the analytes in organized media [32,33]. For the norharmane/β-CD complexes, 10.0 M NaOH additions shifts the acid–base equilibria to the neutral and zwitterionic forms requiring 150 μL of base to observe this effect. However the addition of OH^- to the aqueous solution at pH 4.0 produces a decrease in the fluorescence intensity of the cationic band and the appearance and increase of the emission corresponding to the neutral and zwitterionic forms, 180–200 μL of 10.0 M NaOH being necessary to shift the acid–base equilibrium. The differences observed in the results for the native β-CD and modified β-CDs could be related with the effective protection in the case of modified β-CDs because chemical modification results in more favourable interactions between guest and CDs molecules [34].

Fig. 4 shows the effects of the additions of 10.0 M NaOH to the complexes at pH 10.0. In this case, considering the similar behaviour detected for pH 7.8 and 10.0 only two CDs were assayed, β-CD and HPβ-CD as a modified CD. For both CDs at this pH value the emission of the neutral form is higher than the cationic species while in buffered aqueous solution the cationic band predominates with regard to the neutral emission band. When sodium hydroxide was added to the aqueous solution the emission due to the cationic band as well as the emission corresponding to the neutral band slowly decreased, however the weak emission of the zwitterionic form was increased.

The absence of an isoemissive point is due to the existence of several species in the equilibria in the excited state. The norharmane/β-CD complexes exhibit the neutral and zwitterionic forms after additions of NaOH. However the emission intensity of the zwitterionic band is similar or higher than the intensity of the neutral form. An equivalent behaviour was observed in the case of the complexes with HPβ-CD.

When the study was carried out at pH 13.0 (Fig. 5) important changes were observed with regard to pH 10.0 or 7.8. Thus in buffered aqueous solutions without CDs the neutral and the zwitterionic species coexist and the emission intensity corresponding to the neutral band being higher than that of the zwitterionic band. No maxima in the region due to the cationic band was observed, but the shape of the spectra corresponds to a broad band suggesting that an appreciable amount of the cationic species is present as a consequence of the protonation of neutral norharmane in the excited state at this pH value. The addition of NaOH decreases the emission intensity of all spectra (neutral and zwitterionic species). In the presence of CDs at this pH value the emission of the neutral and zwitterionic species is observed. Addition of 10.0 M NaOH decreases the emission intensity of neutral band and induces a weak increase in the emission of the zwitterionic band. The neutral band was more intense than the zwitterionic one. The spectral shapes were well defined with regard to those observed in aqueous solution without CDs and therefore



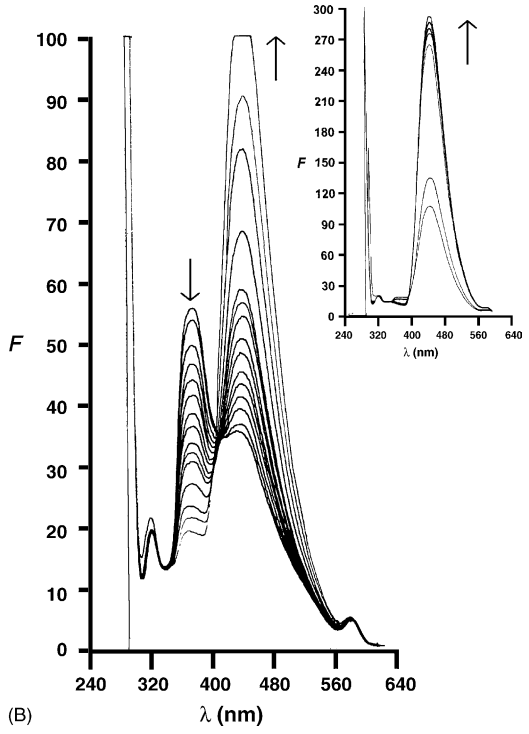
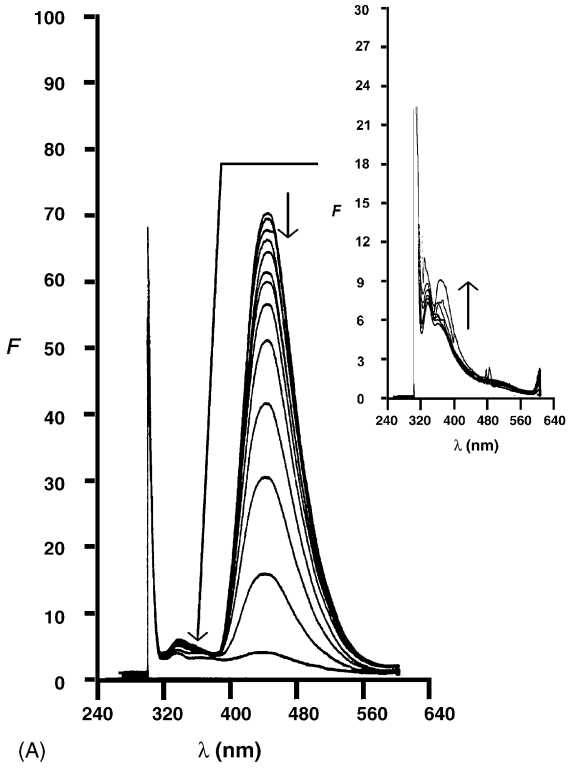


Fig. 2. Effect of the addition of HCl 1.0 M on the acid–base equilibrium of norharmane in the presence of buffered aqueous solution at pH 7.8 with HPβ-CD (A) and with β-CD (B). F: fluorescence intensity in arbitrary units, λ: emission wavelength in nm.



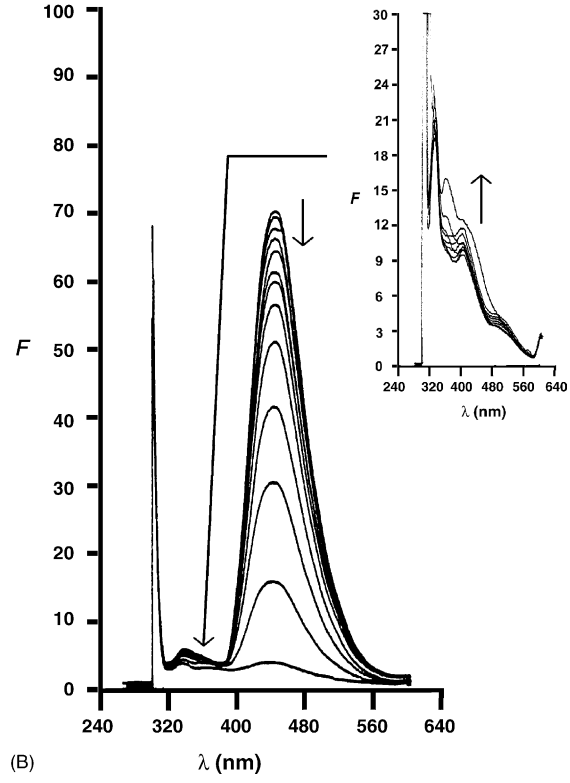


Fig. 3. Effect of the addition of NaOH 10.0 M on the acid–base equilibrium of norharmane in buffered aqueous solution at pH 4.0 with DMβ-CD (A) and with HPβ-CD (B). F: fluorescence intensity in arbitrary units, λ: emission wavelength in nm.

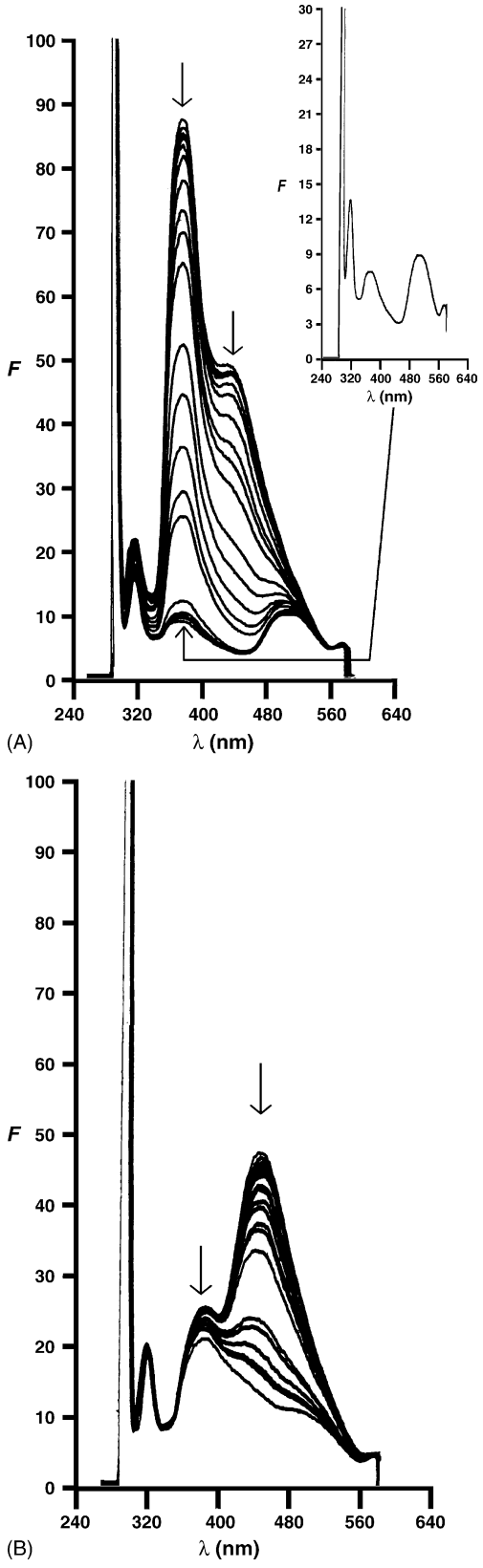


Fig. 4. Effect of the addition of NaOH 10.0 M on the acid–base equilibrium of norharmane in buffered aqueous solution at pH 10.0 with β-CD (A) and without CD (B). F: fluorescence intensity in arbitrary units, λ: emission wavelength in nm.

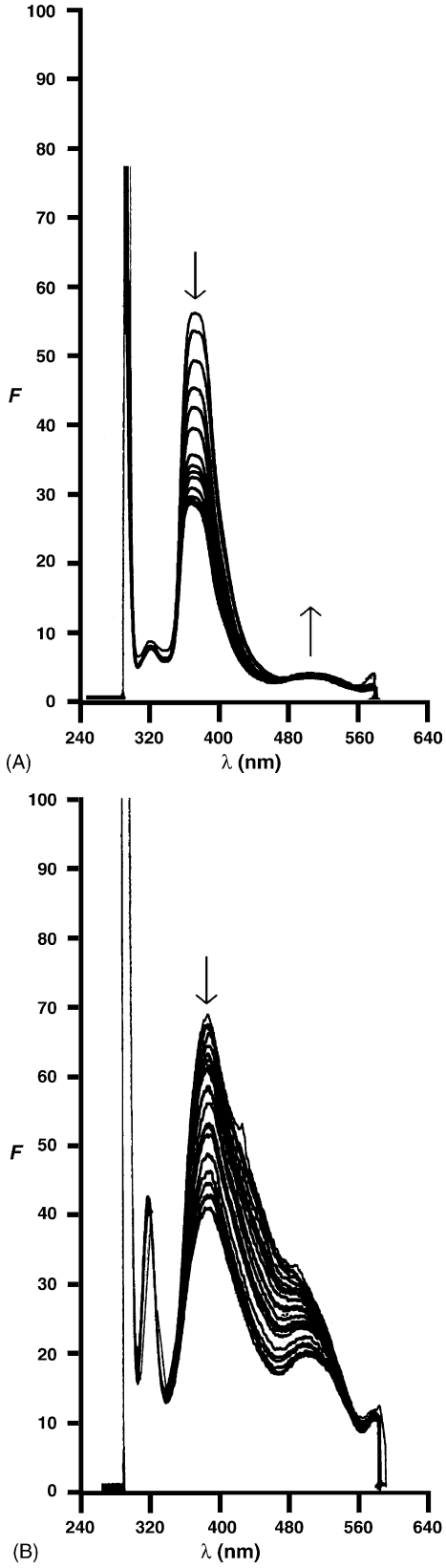


Fig. 5. Effect of the addition of NaOH 10.0 M on the acid–base equilibrium of norharmane in the presence of buffered aqueous solution at pH 13.0 with TMβ-CD (A) and without CD (B). F: fluorescence intensity in arbitrary units, λ: emission wavelength in nm.

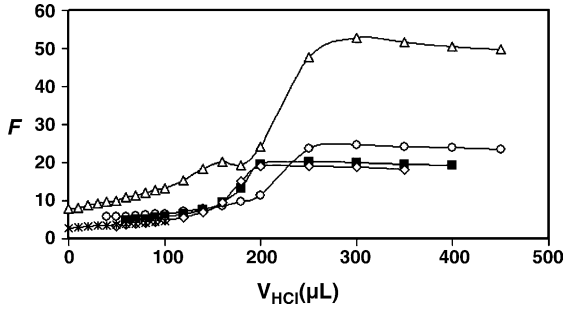


Fig. 6. Acid–base titration curves of norharmane in the presence of buffered aqueous solution at pH 7.8 without CD (✱), with β-CD (△), with HPβ-CD (◯), with DMβ-CD (■) and with TMβ-CD (◊) (titrating agent: HCl 1.0 M). F: Fluorescence intensity in arbitrary units, V: volume of HCl added in μL.

the emission due to the neutral form was a narrow band, although in the case of DMβ-CD and TMβ-CD the band was narrower than for HPβ-CD and β-CD. Thus the environment afforded by DMβ-CD and TMβ-CD is of lower polarity than in the case of HPβ-CD and β-CD. Another possible explanation is that the DMβ-CD and TMβ-CD cavities provide a more efficient fit for norharmane.

The differences in the acid–base behaviour with regard to the environment of the CDs and water are represented by the changes in the titration curves obtained for the different inclusion complexes of norharmane at pH 7.8 (Fig. 6). It is well known that norharmane is a weak base in the ground state (pK_a (S_0) 7.2 [13] or 7.9 [12]) and its basicity is greatly increased in the excited state (pK_a (S_1) 13.0 [13] or 14.7 [12]). Addition of 1.0 M HCl to the buffered aqueous solutions without CDs progressively increases the fluorescence intensity of the cationic band due to the fact that norharmane in buffered solution at this pH value (7.8) and in excited state behaves as a strong base and is easily protonated. In the presence of CDs, access of the protons to the pyridinic nitrogen is hampered because the environment inside CD cavities resembles a medium of lower polarity than water, and the basicity of organic bases such as anilines is known to decrease with solvent polarity [35]. This effect is observed in the case of CD solutions, where, contrary to aqueous solutions, it was possible to obtain a titration curve. The observed acid–base behaviour was dependant on the structure of the CD, each one requiring different volumes of HCl to reach the equivalence point, which led to the conclusion that the pK_a value of norharmane varies according to the type of CD employed. Thus, in the case of DMβ-CD and TMβ-CD, only 170–180 μL of HCl were necessary to reach the equivalence point, while this value rose to 210 μL for HPβ-CD and 220 μL for β-CD. It can be deduced from Fig. 6 that the apparent pK_a of norharmane in β-CD was lower than in HPβ-CD, DMβ-CD and TMβ-CD, and therefore norharmane is less basic in β-CD than in the modified CDs.

These changes can be related with the formation of water clusters in weak acid–base systems in the presence of CDs according to the effect described by Takahashi [22]. This behaviour can have important analytical consequences not only on the acid–base equilibria but also considering the different distribution of neutral and charged species of norharmane in partitioning equilibria in chromatography.

4. Conclusions

CDs exert a significant influence on the proton transfer photoreaction of norharmane. The protonation process is hampered by CDs due to the low polarity environment inside their cavities and the lower diffusion rate of protons into these cavities. The differences observed in the spectral shape and the fluorescence intensities obtained for titration of the inclusion complexes with HCl and NaOH allow to conclude that in the cyclodextrin cavities the acid–base equilibrium is not reached during the lifetime of the excited state.

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