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Influence of the Cyclodextrin Size Cavity in the Complexation of Tetrahydroharmane

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Abstract. The interactions between cyclodextrins and tetrahydroharmane, 1-methyl-1,2,3,4-tetrahydro-9H-pyrido/3,4-b/indole, have been investigated in pH 10.1 aqueous media. Absorption and fluorescence studies, together with lifetime measurements show that tetrahydroharmane forms ground state inclusion complexes with α-CD and β-CD but not with γ-CD. Steady state experiments detect only one kind of tetrahydroharmane-β-CD complex, whereas time resolved measurements reveal the existence of two different types of 1 : 1 association complexes. The results in α-CD are very similar to those in β-CD, but the interactions are much weaker.

Key words: cyclodextrin, tetrahydroharmane, tetrahydrobetacarboline, inclusion complexes, steady-state and time-resolved fluorescence

1. Introduction

The study of inclusion complexes formed by selective binding of model substrates with natural cyclodextrins as molecular receptors is currently a significant topic in chemistry and biochemistry [1–4]. The encapsulation of the guest into the hydrophobic CD cavities leads to changes in the intermediate environment of the guest molecule. Besides, geometric restrictions are imposed on the guest molecules due to space limitations in the host cavity. The reduced polarity and the restricted geometry of the CD cavity are found to modify the energetics and dynamics of the photophysical and photochemical processes of the guest molecule [5].

Host complexation is primarily determined by the tightness of the fit, i.e. by the size and shape matching between the guest and the CD cavity. Imperfect fit of the guest in the CD cavity diminishes the host-guest interactions and leads to a decrease of the association constants. On the other hand, depending on the sizes of the host CD cavity and the guest molecule, different guest to host stoichiometries are possible.

In a recent study from this laboratory [6], it was found that tetrahydrobetacarboline (1,2,3,4-tetrahydro-9H-pyrido/3,4-b/indole), (**1**), forms two different 1 : 1

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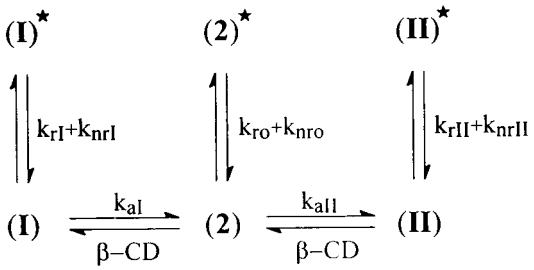
inclusion complexes with β-CD. On the basis of geometrical and spectroscopic considerations, it was assumed that each complex corresponds to the two different topologies for the entry of the substrate into the β-CD cavity, i.e. by its benzene or by its piperidinic moiety. The association constant for the former complex is greater than for the latter.

On the other hand, the complex involving the encapsulation of the benzene moiety enhances the intensity, quantum yield and lifetime of the tetrahydrobetacarboline fluorescence. Conversely, the encapsulation of the piperidinic moiety of the tetrahydrobetacarboline produces only minor changes in its fluorescent properties.

In the present communication we wish to extend this study to the analysis of the influence of the CD cavity size on the complexation process with tetrahydrobetacarbolines. For this purpose we have studied the interactions of a methylated derivative, tetrahydroharmane (**2**) [7, 8], with α-, β- and γ-cyclodextrins. The photophysical properties of (**1**) and (**2**) are entirely similar [9, 10]. Thus, the choice of (**2**) will allow us to inspect also the influence of its methyl group on the complexation process.

(**1**) (**2**)

Scheme 1.



Scheme 2.

2. Experimental

Tetrahydroharmane, (**2**), 1-methyl-1,2,3,4-tetrahydro-9H-pyrido/3,4-b/indole, was prepared by the Pictet-Splenger reaction [11] of pyruvic acid and tryptamine. Cyclodextrins, CD, were commercial products (Fluka) of the best available quality (>98%)and were used without further purification.

Aqueous stock solutions for spectroscopic studies contained a fixed concentration of (**2**) ($\sim 2 \times 10^{-5}$ M) and varying concentrations (always in excess) of CD. To guarantee the exclusive presence of neutral (**2**) species (pK_a = 8.68) [10], the pHs of the solutions were adjusted to 10.1. At this pH value, the cyclodextrins are practically unionized ($pK_a \sim 12$) [2]. The pH of the media was maintained by the addition of diluted sodium hydroxide solutions and no buffers were used. All the solutions were freshly prepared in water just before the experiments and kept in the dark to avoid photodecompositions. Since quenching by oxygen was unimportant, the fluorescence measurements were made with non-degassed solutions. For fluorescence measurements, the absorbances of the samples at the excitation wavelengths were smaller than 0.1 to minimize inner filter effects and reabsorption phenomena. All the spectroscopic measurements were done at 298 ± 0.1 K.

The UV-visible absorption spectra were recorded on a Perkin Elmer Lambda-5 spectrophotometer. Uncorrected fluorescence emission spectra were obtained in a Perkin-Elmer 650-40 spectrofluorometer interfaced to a Multitech Acer 500 PC for the handling of the spectra. Time-resolved fluorescence measurements were carried out with a FL-900 CD Edinburgh Analytical Instrument employing the time-correlated single photon counting technique. The excitation source was a nanosecond flash lamp filled with H_2 (0.4 bar) operating at 6.8 kV, with a repetition rate of 40.0 kHz. Fluorescence decays were acquired to 2×10^4 counts in the peak. Fluorescence decay data were fitted by reference deconvolution to a sum of exponentials:

$$I(t) = \sum \alpha_i \exp(-t/\tau_i) \tag{1}$$

with amplitudes, α_i, and lifetimes, τ_i. Decay curves were individually and globally analysed by using single, double and triple exponentials. Goodness of the individual fits was judged by the magnitude of the reduced χ_r^2 and the shape of the autocorrelation function of the weighted residuals. To analyse the lifetime data at different CD concentrations, a global analysis [12] program based on the Marquardt algorithm was used. The results were judged by the statistical fitting parameter χ_g^2 for the global analysis.

3. Results

The addition of CDs produces only minor changes in the absorption spectrum of (**2**). These changes, as shown typically in Figure 1, are barely outside the experimental errors. The changes induced by the CDs on the fluorescence emission spectrum of (**2**) markedly depend on the size of the CD cavity. In general, the addition of CD induces small blue shifts and broadening of the fluorescence band of (**2**), together with an enhancement of its intensity. However, as Figures 2–4 show, these changes are hardly apparent in γ-CD solutions, moderate with α-CD and very important in β-CD solutions.

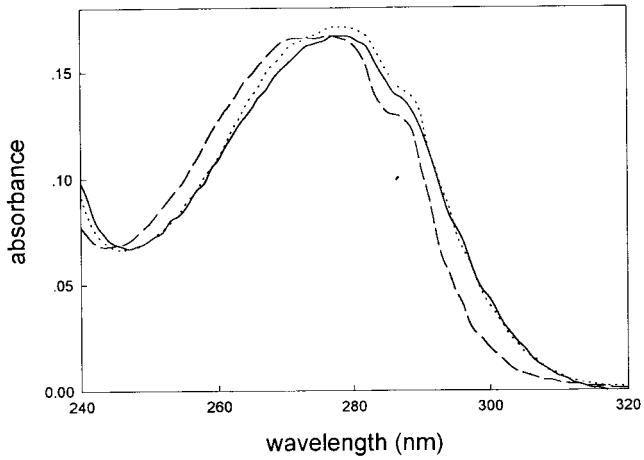


Figure 1. Absorption uv-vis spectra of (**2**) in β-CD solutions of different concentrations.

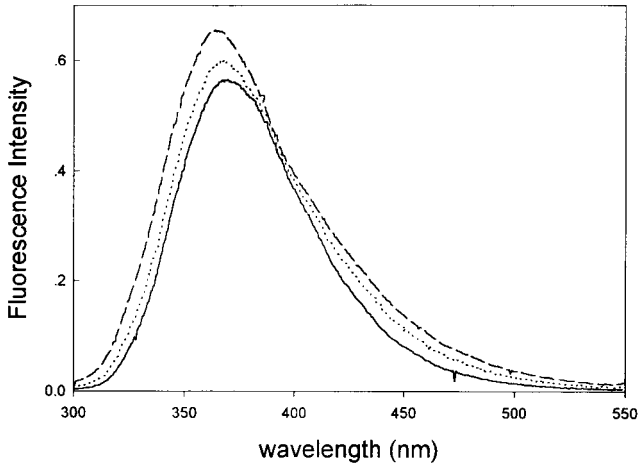


Figure 2. Fluorescence emission spectra of (**2**) in 0 M (full line), 4×10^{-3} M and 1.7×10^{-2} M solutions of α-CD.

The plot of the ratio of the integrated fluorescence intensities of (**2**) in β-CD solutions and in water, I/I_0, against β-CD concentrations, Figure 5, tends to saturation as the β-CD concentration increases. A Benesi Hildebrand plot of $1/(I - I_0)$ against $1/[\beta\text{-CD}]_0$, inset of Figure 5, shows an excellent linear regression (r^2 = 0.996). These results point to the formation of 1 : 1 (**2**)-β-CD complexes. Unfortunately, the smaller changes of the fluorescence spectrum of (**2**) in the presence of α- or γ-CD preclude the perfomance of a similar analysis for these systems.

To provide additional evidence of the formation of complexes, the fluorescence decays of (**2**) in solutions of CDs were measured. While in the absence of CD, the

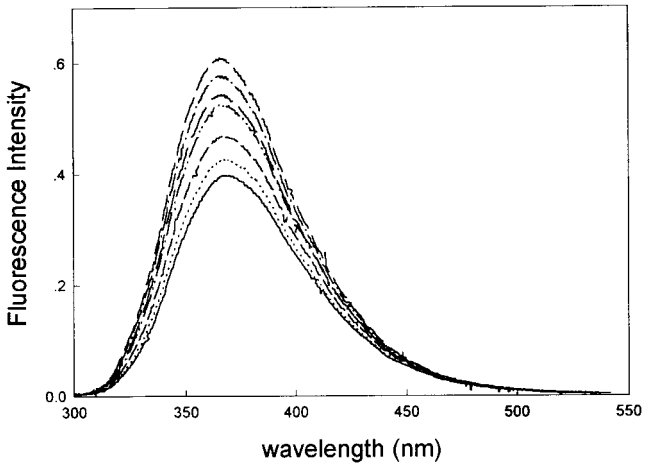


Figure 3. Fluorescence emission spectra (**2**) in 0 M (full line), 1.4×10^{-3} M, 2.8×10^{-3} M, 4.2×10^{-3} M, 5.6×10^{-3} M, 8.4×10^{-3} M and 1.12×10^{-2} M solutions of β-CD.

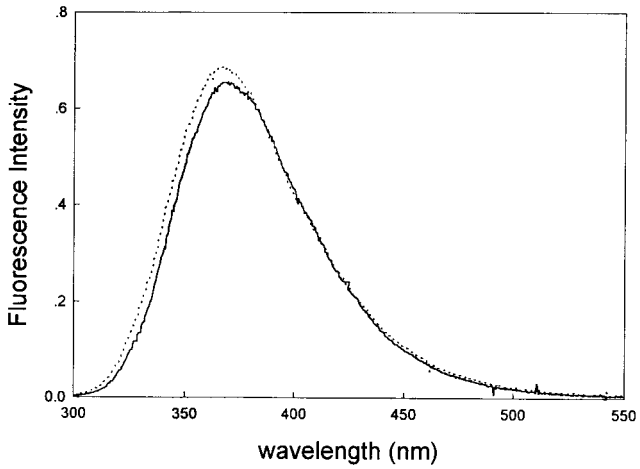


Figure 4. Fluorescence emission spectra of (**2**) in 0 M (full line) and 1.25×10^{-2} M solutions of γ-CD.

fluorescence decays monoexponentially, in α- and β-CD solutions they could be fitted by a linear combination of two exponentials:

$$I(t) = \alpha_1 \exp(-t/\tau_1) + \alpha_2 \exp(-t/\tau_2) \tag{2}$$

where α_1 and α_2 stand for the contribution at zero time of the two components with lifetimes τ_1 and τ_2. Triple exponential analyses were also attempted, but neither χ_r^2 nor χ_g^2 improved. Conversely, the emission decays of (**2**) in the presence of γ-CD could still be analysed as single exponentials with lifetimes close to that in water (4.64 ns at 298 K). This result conclusively shows that (**2**) does not form inclusion complexes with γ-CD.

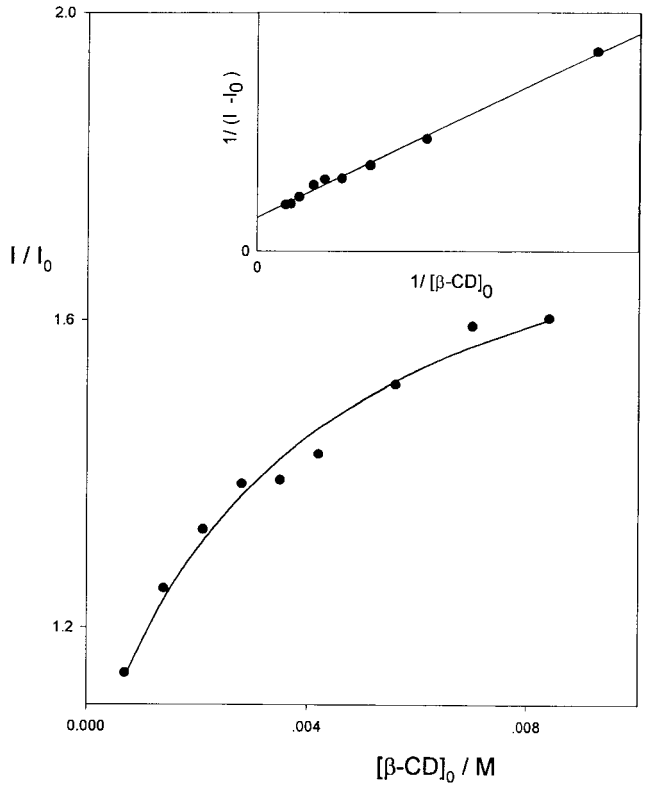


Figure 5. Plot of I/I_0 versus $[\beta\text{-CD}]_0$ (points). Non linear regression analysis (NLR) of the steady state integrated fluorescence intensity data according to Equation (3) (full line). In the inset, Benesi Hildebrand plot of $1/(I - I_0)$ *versus* $1/[\beta\text{-CD}]_0$.

Tables I and II report the results of the biexponential global analysis of the fluorescence decays carried out over a concentration range of α- and β-CD. It can be observed that the shorter lifetime of the decays, τ_1, is very close to that of (**2**) in water. Hence it might be assigned to the non complexed molecules of (**2**), whereas the longer lifetime, τ_2, should be assigned to those molecules complexed with CD. On the other hand, while the contribution of the shorter component to the total fluorescence, α_1, decreases with the increase of CD concentration, that of the larger component, α_2, increases. The plots of α_2/α_1 versus $[CD]_0$ are downward curved, Figures 6 and 7.

The changes induced in the absorption and fluorescence spectra of (**2**) by the addition of β-CD are entirely similar to those observed in the spectra of (**1**) upon complexation with the same CD. Similarly, the downward curvature in the α_2/α_1 *vs* $[CD]_0$ plot is reminiscent of that observed in the (**1**)-β-CD system [6]. Therefore, the behaviour of the (**2**)-β-CD system can also be rationalized assuming the competing formation of two different 1 : 1 inclusion complexes between (**2**) and β-CD, Scheme 2. These complexes represent the two different topologies for the entry of

Table I. Global analysis of the fluorescence decays of (**2**) in α-CD solutions at 298 K.

[α-CD]/M	τ_1 (ns)	τ_2 (ns)	α_2/α_1
0.014	4.32	6.08	0.34
0.024	4.27	5.98	0.57
0.043	4.46	6.28	0.86
0.058	4.39	6.41	1.03
0.072	4.20	6.30	1.26
0.086	4.50	6.58	1.42
0.100	4.78	6.86	1.54
0.120	4.81	6.94	1.70
			$\chi_g^2 = 1.127$

Table II. Global analysis of the fluorescence decays of (**2**) in β-CD solutions at 298 K.

10^3[β-CD]/M	τ_1 (ns)	τ_2 (ns)	α_2/α_1
0.7	4.48	8.38	0.17
1.4	4.36	8.50	0.35
2.1	4.46	8.54	0.48
2.8	4.50	8.61	0.61
4.2	4.54	8.64	0.89
5.6	4.60	8.58	1.15
8.4	4.62	8.89	1.43
11.2	4.86	8.95	1.81
			$\chi_g^2 = 1.187$

the guest into the β-CD cavity, i.e. by the benzene (type I) or by the piperidinic (type II) rings, Figure 8.

Owing to the different structures of the proposed type I and type II complexes, they are expected to affect differently the singlet excited state behaviour of (**2**). Thus, since in the type I complexes the indole chromophore of (**2**) is almost completely isolated from water and it is provided with a constrained environment more hydrophobic in nature, its formation would induce important enhancements of the quantum yield and fluorescence lifetime of the substrate. Conversely, due to the poorest penetration of the indole ring into the CD cavity, the formation of type II complexes would have a very litle effect on these parameters, i.e. they should behave as the free chromophore. On this basis, and due to the known difficulty to resolve components with lifetimes within 1 ns or less, the triexponential de-

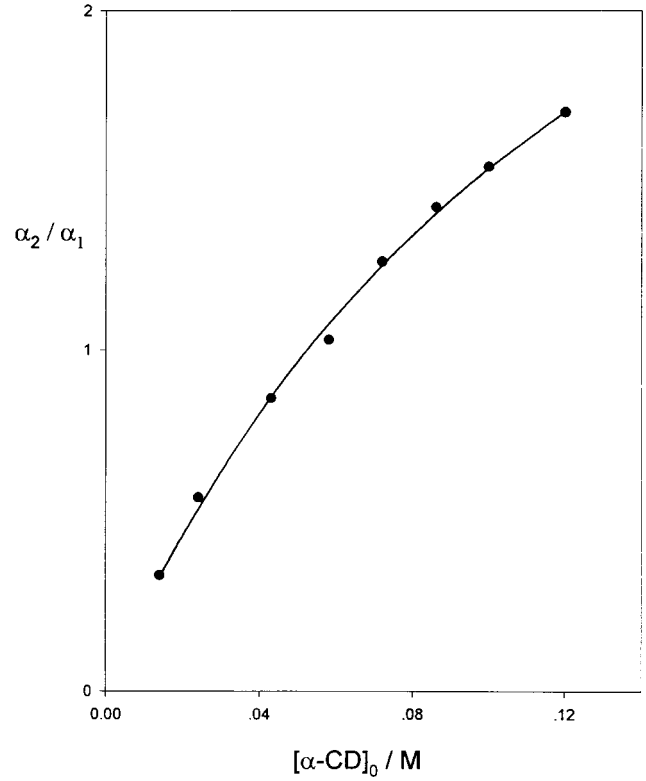


Figure 6. Plot of the amplitude ratios α_2/α_1 against α-CD concentrations. Points: experimental data. Full line: non linear regression according to Equation (4).

cay predicted by Scheme 2 could apparently give rise to a biexponential one, as experimentally observed. Furthermore, this could explain the smooth increase of the lifetimes extracted from the biexponential analysis with the increase of the CD concentration.

Thus, if we assume that the free molecule and the type II complex have entirely similar fluorescence characteristics, the variations of the steady state integrated fluorescence intensities, I/I_0, and of the amplitude ratios of the apparent biexponential decays, α_2/α_1, can be expressed [6] by Equations (3) and (4)

$$\frac{I}{I_0} = \frac{1 + [K_{\mathrm{aI}}(\phi_{\mathrm{I}}/\phi_0) + K_{\mathrm{aII}}][\mathrm{CD}]_0}{1 + (K_{\mathrm{aI}} + K_{\mathrm{aII}})[\mathrm{CD}]_0} \tag{3}$$

$$\frac{\alpha_2}{\alpha_1} = \frac{k_{\mathrm{rI}}}{k_{\mathrm{r0}}} \frac{K_{\mathrm{aI}}[\mathrm{CD}]_0}{(1 + K_{\mathrm{aII}}[\mathrm{CD}]_0)} \tag{4}$$

where Φ_0 and Φ_{I} are the quantum yields of the unbounded molecule and the type I complex and k_{r0} and k_{rI} their respective radiative rate constants. In agreement with experimental results, Equations (3) and (4) predict downward curvatures as the concentration of CD increases.

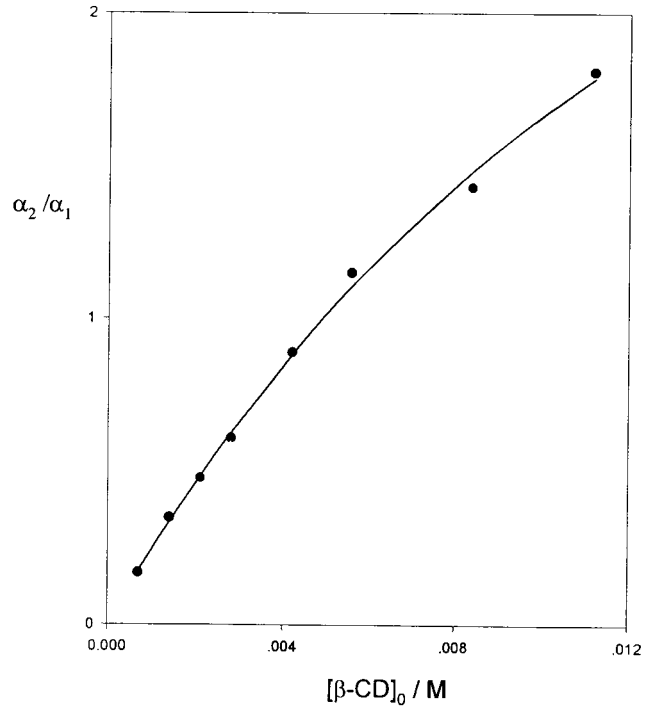


Figure 7. Plot of the amplitude ratios α_2/α_1 against β-CD concentrations. Points: experimental data. Full line: non linear regression according to Equation (4).

In Table III we report the values of the association constants, K_{aI} and K_{aII}, the relative quantum yield, $\Phi_I\Phi_0$, and the ratio of the radiative rate constants, k_{rI}/k_{r0}, for the (**2**)-β-CD complex. These values have been calculated from the non linear regression fits of the steady state and time resolved experimental data to Equations

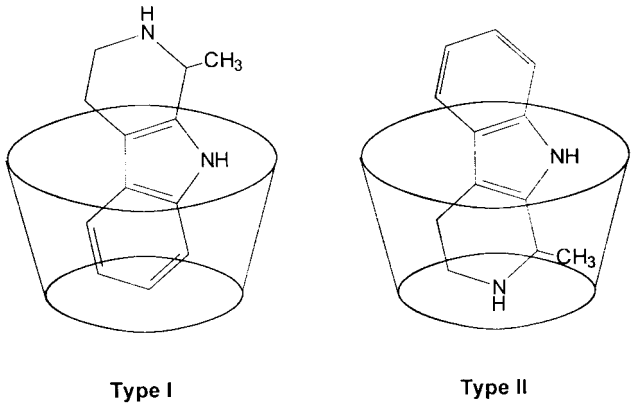


Figure 8. Pressumed structures of the type I and type II (**2**)-CD complexes.

Table III. Thermodynamic and photophysical data of the tetrahydrobetacarboline-CD type I and II complexes at 298 K.

	K_{aI}/M^{-1}	K_{aII}/M^{-1}	ϕ_I/ϕ_0	k_{rI}/k_{r0}
(**2**)-[α-CD]	26 ± 1	7 ± 0.7	–	–
(**2**)-[β-CD]	213 ± 44	56 ± 7	2.10	1.18
(**1**)-[β-CD]	245 ± 25	95 ± 3	2.41	1.22

(3) and (4). Unfortunately, because of the small changes in the fluorescence spectra, a similar analysis cannot be achieved with the (**2**)-α-CD system. However, if we assume that, as for β-CD complex, the rate constant for the radiative decay of (**2**) does not appreciably change upon complexation with α-CD, using Equation (4) we can estimate the association constants K_{aI} and K_{aII} reported in Table III. Also, for comparison purpose, the corresponding data for the (**1**)-β-CD complexes [6] have been included in Table III.

4. Discussion

A perusal of the data in Table III shows that, as expected, the presence of the methyl group in (**2**) sterically hinders the encapsulation of the piperidinic moiety, but it has a minor effect on the inclusion of the indole moiety. Also, as for the (**1**)-β-CD system, the formation of (**2**)-type I complexes increases the quantum yield and fluorescence lifetime of (**2**). Conversely, in agreement with the small variations of the absorption spectra, this complexation process leaves the rate constant for the radiative decay of (**2**), k_r, practically unchanged. These results indicate that the increase of the fluorescence quantum yield and lifetime of (**2**) upon complexation with β-CD is mainly due to a decrease in the rate constant for the nonradiative decay, k_{nr}. This behaviour is typical for the encapsulation of indole derivatives into the β-CD cavity [13, 14].

As data in Table III show, α-CD behaves similarly to β-CD but its ability to associate with (**2**) is considerably smaller. However, the capability of α-CD to form complexes with indole and with (**2**) seems to be different. Thus, although Ricci [15] has reported that indole forms 1 : 2 stoichiometric complexes in α-CD solutions, our experimental data do not provide any evidence on the formation of 1 : 2 (**2**)-α-CD complexes. As we previously demonstrated [6], such complexes would give upward curvatures in the plots of I/I_0 and α_2/α_1 versus $[CD]_0$.

One of the most interesting results of the present study is the lack of complexation of (**2**) with γ-CD. The small enhancement of the fluorescence of (**2**) in concentrated γ-CD solutions is similar to that observed in the spectra of indole derivatives upon the addition of the non cyclic saccharide glucose [6, 14]. There-

fore, these changes would be better related with a solvent effect due to the high concentration of CD. This fact emphasises the importance of the size-fit relation between the host and the guest in the complexation process.

The different ability of CDs to associate with (**2**) seems to be related with the matching degree of the substrate into the CD cavity. Thus, since molecule (**2**) is approximately 6 Å in width [16], it should be tightly packed into the β-CD cavity ($\approx$7.8 Å), but loosely in the γ-CD cavity ($\approx$9.5 Å). Similarly, only a part of the molecule (**2**) can be accommodated in the α-CD cavity ($\approx$5.7 Å), leaving the rest of the molecule protruding into water. The shorter lifetime of the (**2**)-α-CD type I complex as compared with that of the β-CD complex agrees well with a minor encapsulation degree of the indole chromophore in the (**2**)-α-CD complex.

Extensive studies of molecular recognition by CDs have shown that the stabilities of the CD inclusion complexes are governed by the simultaneous contribution of several weak forces such as hydrogen bonding, hydrophobic, dispersive and dipolar van der Waals interactions [1–4]. All these forces are mainly determined by size fit of the guest molecule into the CD cavity.

Solvent can also contribute to the different stabilization of the (**2**)-CD complexes. Örstand and Ross [13] claimed that solvent surface tension is the major solvent factor affecting the stability of the indole-β-CD complex. Thus, when indole enters the β-CD cavity, the decrease in the surface area exposed to the solvent greatly favours the stabilization of the complex. Hence, this factor would additionally contribute to make the (**2**)-β-CD complex more stable than the (**2**)-α-CD complex.

Solvent extrusion from the CD cavity is another factor which can also contribute to the stabilization of the (**2**)-CD complexes. The removal of the highly ordered water molecules from the CD cavity is usually considered to be one of the main contributions to the driving force for the formation of the CD complexes. This effect must negatively affect the inclusion of (**2**) into the γ-CD cavity. Thus, since the γ-CD cavity is wider than those of the α- and β-CD oligomers, the water molecules inside the γ-CD cavity are expected to be less ordered and their displacement might result in a smaller energetic gain.

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