

Adduct Formation Between γ -Cyclodextrin and the Bifluorophore 1,3-Bis(1-pyrenyl)propane

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The complexation of the bifluorophore 1,3-bis(pyrenyl)propane with γ -cyclodextrin in water has been studied by means of steady state and time-resolved fluorescence spectroscopy. It was found that in the association with γ -cyclodextrin the propane chain of 1,3-bis(pyrenyl)propane folds and the two pyrene units enter the same cyclodextrin cavity where they form weakly bound ground state dimers, which upon excitation emit excimer fluorescence. In addition to this 1:1 excimer emitting complex, two more complexes were detected, which emit monomer pyrene fluorescence. One has 1:1 stoichiometry, i.e. *isomeric* to the previous complex, and the other, with 2:1 stoichiometry, is comprised of two γ -cyclodextrin units and one 1,3-bis(pyrenyl)propane.

Keywords: Cyclodextrin; 1,3-Bis(1-pyrenyl)propane; Fluorescence; Excimers; Complexation

INTRODUCTION

Adduct formation with a variety of organic molecules in aqueous media is one of the most remarkable properties of cyclodextrins (CD). Whenever guest molecules can fit inside the cyclodextrin cavity, even partially, weak complexes can be formed, which usually demonstrate simple guest:CD stoichiometries, primarily 1:1, 1:2 and 2:2 [1,2]. Occasionally however supramolecular assemblies such as catenates, rotaxanes, polyrotaxanes [3,4], threaded cyclodextrins [5], nanotubes [6–11] etc., are produced which involve several cyclodextrin and guest molecules. All these complexes, simple and supramolecular, which are held together by non-covalent chemical bonds, are usually formed in solution and therefore they can, in principle, be

characterized by high resolution spectroscopic techniques. Fluorescence spectroscopy, both steady state and time-resolved, is among the most appropriate methods for such studies [12]. One of the guest molecules that has been studied at length as far as its complexation with cyclodextrins is concerned, is pyrene which is known to form complexes with β - and γ -cyclodextrin in water [13–19]. In its use as a probe, pyrene has the great advantage that, under appropriate conditions, it can form excimers which facilitate the study of structural and other properties of systems in which this molecule is dispersed.

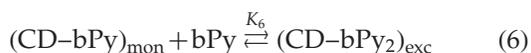
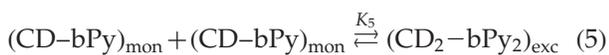
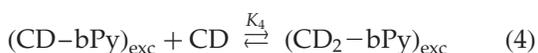
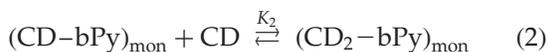
Another pyrene derivative which also exhibits excimer fluorescence is the bifluorophore 1,3-bis(1-pyrenyl)propane [20] (abbreviated as bPy). However, this molecule has a more complicated behavior, as far as its association with cyclodextrins in aqueous media is concerned, than its precursor molecule pyrene. Specifically, bPy is practically insoluble in pure water [21], rendering impossible, the determination by absorption-fluorescence methods of the free amount of this molecule in aqueous solutions, only γ -CD bound bPy can be detected spectroscopically. Also, the presence in the bPy molecule of two pyrene units which can emit either monomeric or excimeric fluorescence, has as consequence in the formation of a number of *isomeric* bPy/ γ -CD inclusion compounds with different emitting properties. In view of the above and the existing data on the complexation of pyrene with γ -CD [17], it is interesting to examine what sort of arrangements bPy will adopt in its complexation with γ -CD in water. It is therefore the objective of the present study to investigate, by means of fluorescence spectroscopy, the details of

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the adduct formation between bPy and γ -CD in aqueous environment.

RESULTS AND DISCUSSION

Eqs. (1)–(6) and Scheme 1 depict all the conceivable interactions between bPy and γ -CD, along with the resulting complexes (for simplicity in the Eqs. and in Scheme 1, γ -CD is

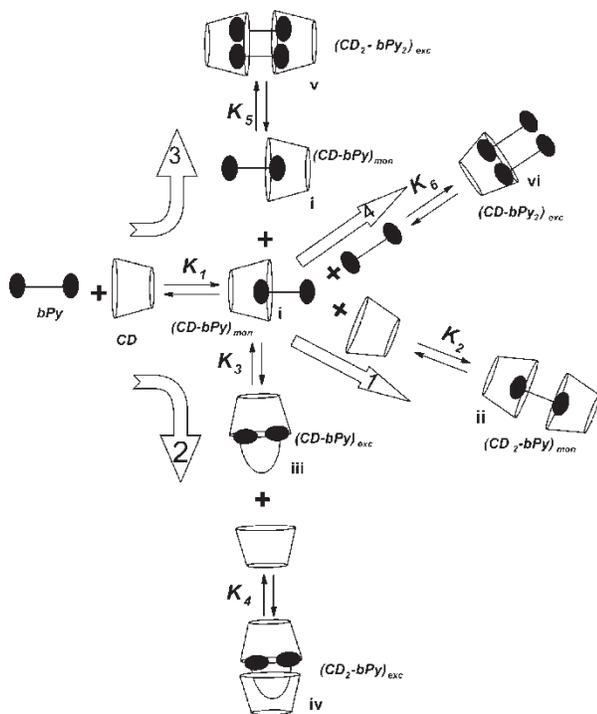


shown as CD). Note that the presence of two fluorophore units in the bPy molecule gives rise to a number of bPy/ γ -CD *isomers* with different photo-physical properties. More specifically, although complexes **i** and **iii** (see Scheme 1) have the same stoichiometry, 1:1, the former is expected to produce monomeric fluorescence spectra and the latter

excimeric; for this reason they have been labelled $(\text{CD-bPy})_{\text{mon}}$ and $(\text{CD-bPy})_{\text{exc}}$ respectively. Similarly, **ii** and **iv** both have 2:1 stoichiometry, but **ii** will give monomer and **iv** excimer fluorescence, hence the notation $(\text{CD-bPy-CD})_{\text{mon}}$ for **ii** and $(\text{CD-bPy-CD})_{\text{exc}}$ for **iv**. Moreover, from structural considerations it is clear that complexes **i** and **ii** are expected to emit monomer fluorescence, while **iii**, **iv**, **v** and **vi** will give excimer emission. The question to be answered here is which of the reactions (1)–(6) really occur and which of the species **i**–**vi** are present in the solution.

Analyses of the fluorescence decays of aqueous solutions containing $[\text{bPy}] = 10^{-6} \text{ M}$ and $[\gamma\text{-CD}] = 10^{-2} \text{ M}$, have shown that there are present two different monomeric fluorescent species (decay at 370 nm), but only one excimer emitting species (decay at 480 nm). The lifetimes of the two monomers are $\tau_1 = 42 \text{ ns}$ and $\tau_2 = 163 \text{ ns}$, while that of the excimer is $\tau_{\text{exc}} = 152 \text{ ns}$. Since, according to Scheme 1, only species **i** and **ii** are expected to emit monomer fluorescence, we conclude that these two are the ones which correspond to the two different monomer fluorescence lifetimes. On the contrary, there are four complexes capable of emitting excimer fluorescence, while there is only one experimentally confirmed excimer lifetime, therefore only one of them is present in the solution. The possibility that two or more of these four species have the same lifetime is rather unlikely because of their very different structural features.

In view of the structure of complex **vi**, it is clear that the single decay of the excimer fluorescence observed, is not compatible with this complex. Indeed, species **vi** should give rise to two different excimeric fluorescence spectra; one coming from the two pyrene groups restricted inside the CD cavity, and the other from those lying outside the cavity, where they can move around with considerable freedom. These two excimers should have approximately equal intensities, but different lifetimes, the former longer and the latter shorter, because the excimer formed inside the CD cavity is better protected from quenchers, such as oxygen, than the other excimer formed outside the cavity. More importantly, however, is the fact that the decay profile of the excimer formed outside the cavity should exhibit a discernible rise time, since it will take a couple of nanoseconds for the two pyrene groups outside the cavity to approach each other and form the excimer. Since we did not observe either two lifetimes or any rise time, we conclude that species **vi** does not form in the solution and consequently route 4 in Scheme 1 should be ignored. A different argument against the presence of species **vi** is based on the fact that its formation requires bPy molecules dissolved in water (see Eq. 6 and route 4 in Scheme 1), but this molecule is highly insoluble in water ($< 10^{-8} \text{ M}$).

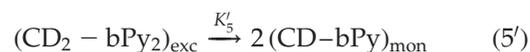


SCHEME 1

The Effect of pH

Based on the structure of the complexes which can possibly form between bPy and γ -CD (shown in Scheme 1) and on the known fact that at $\text{pH} > \text{ca. } 12.3$ the secondary hydroxy groups of γ -CD lose their hydrogens [22], one can expect that the effect of pH on the photophysics of the remaining species of Scheme 1, may help to further reduce the number of the complexes which exist in the solution. Thus, it is well established that when simple pyrene molecules interact with γ -CD at $\text{pH} = 7$, a barrel-type 2:2 complex is formed which accounts for the strong excimer fluorescence of Py: γ -CD in aqueous solutions [17]. It is also known that when the pH of this solution increases above ca. 12.3, the 2:2 complex breaks down to two Py: γ -CD, 1:1 complexes, shown by the replacement of the excimer with the monomer fluorescence of pyrene [17]. This transformation of the 2:2 to the 1:1 complex has been rationalized in terms of the acid-base ionization constant ($\text{p}K_a$) for which a value of ca. 12.3 has been reported for γ -cyclodextrin [23,24]. In a similar experiment we steadily increased the pH of an aqueous solution of bPy: γ -CD, from $\text{pH} = 7$ up to $\text{pH} = 12.5$, recording at the same time the absorption and emission spectra

of the sample, as shown in Fig. 1a. Note that all the fluorescence spectra were obtained by excitation at the isosbestic point, 336 nm, (see Fig. 1b) in order to keep the number of absorbed photons constant. Due to the aforementioned $\text{p}K_a$ value of the cyclodextrins, we expected that when the pH became more than ca. 12.3, species **ii**, **iv** and **v** would dissociate to their constituents, according to Eqs. (2'), (4') and (5'), *i.e.* the reverse of Eqs. (2), (4) and (5). However,



reaction (2') transforms species **ii**, which emits monomeric fluorescence, to species **i**, which also emits monomeric fluorescence, likewise, reaction (4') transforms species **iv**, which forms excimers to species **iii** which also forms excimers. Therefore these two reactions will not affect drastically the intensity of the fluorescence, apart from possible differences in the quantum yields of the complexes involved. On the other hand, reaction (5') transforms

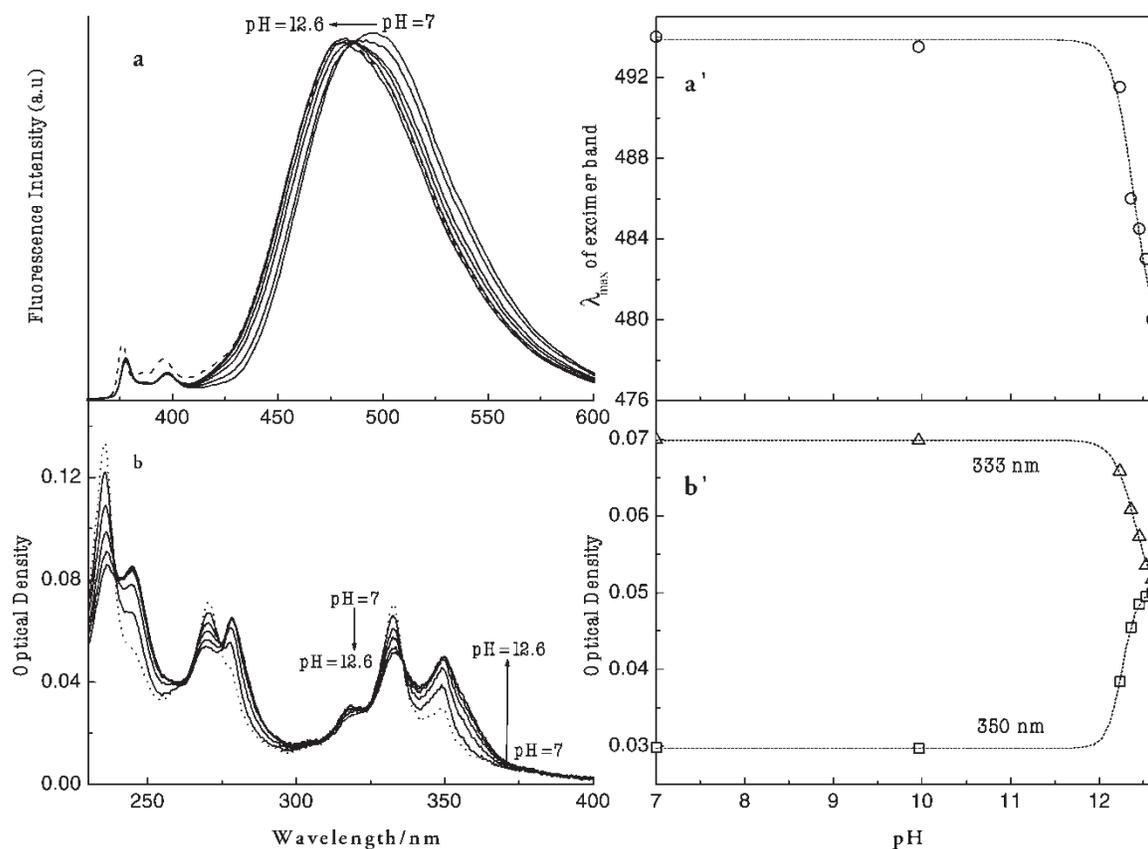


FIGURE 1 Effect of pH on the spectra of an aqueous solution, containing 10^{-6} M bPy and 10^{-2} M γ -CD: (a) fluorescence spectra of bPy/ γ -CD on changing pH from 7 to 12.6, dotted line fluorescence of bPy/hexane. All spectra were normalized at the same intensity; (a') plot of λ_{max} of the excimer fluorescence of bPy/ γ -CD vs. pH; (b) absorption spectra of bPy/ γ -CD on changing pH from 7 to 12.6; (b') plot of the optical densities, at $\lambda = 333$ nm (∇) and at $\lambda = 350$ nm (\square), vs. pH.

species **v**, which is expected to emit excimeric fluorescence, to species **i** which emits monomeric fluorescence, therefore, if species **v** exists in the solution, the overall excimer fluorescence should decrease at $\text{pH} > 12.3$ (due to the dissociation of **v**), while at the same time the monomer fluorescence should increase (due to the formation of **i**). Contrary to this prediction, our experiments show (Fig. 1a) that the monomer and excimer fluorescence intensity is independent of pH , except for a blue shift of the excimer fluorescence when the pH increased from 7 to 12.6, which will be discussed at the end of this section. We take this independence as strong evidence that species **v** does not form in the complexation of bPy with γ -CD.

Fluorescence Quenching

After the elimination of species **v** and **vi** from the complexation scheme, we performed quenching experiments of the excimer fluorescence, in order to distinguish between the two remaining potential excimer emitters, viz. species **iii** and **iv**. Thus, when the small ion Ag^+ was used as quencher, the Stern–Volmer plot of the quenching of the excimer fluorescence, turned out to be a straight line ($R^2 = 0.9999$) with a slope corresponding to a quenching constant $k_q = 7.4 \times 10^7 \text{ M s}^{-1}$. The linearity of the Stern–Volmer plot shows that there is only one excimer emitting species in the solution, which is quenched by Ag^+ . Recall that a similar conclusion was obtained from the fluorescence decay analysis, which produced only one excimer fluorescence lifetime. For comparison, we repeated the same quenching experiment with pyrene: γ -CD instead of bPy: γ -CD, and we also found a single straight line for the Stern–Volmer plot ($k_q = 6.9 \times 10^7 \text{ M}$), reflecting the known fact that between pyrene and γ -CD only one inclusion compound is formed which emits excimer fluorescence, namely the barrel-type 2:2 complex [17]. Evidently, since Ag^+ , because of its small size, can reach the pyrene molecules in the $(\text{pyrene})_2:(\gamma\text{-CD})_2$ complex and quench the excimer emission, it will also be able to reach the fluorophores, not only in species **iii**, but in species **iv** as well. Therefore this quenching experiment cannot tell with certainty which one of the two complexes, **iii** or **iv**, is the one quenched by Ag^+ . However, when we used a larger quencher, such as triethanolamine (TEA), we observed excimer fluorescence quenching in the case of bPy: γ -CD ($k_q = 9.7 \times 10^6 \text{ M s}^{-1}$) but not in the case of pyrene: γ -CD ($k_q = 0$). This shows that the emitting species in bPy: γ -CD is the complex **iii**, which because of its open structure allows TEA to approach the excited pyrene units and quench their fluorescence. On the contrary, in species **iv** such an approach would be impeded by the barrel-type

structure of this complex, similar to what we saw happening in pyrene: γ -CD. In conclusion, fluorescence quenching experiments confirm that only one excimer emitting complex is formed between bPy and γ -CD, namely species **iii**.

Time-resolved Fluorescence

Next we analyzed the decay profiles of the monomer and the excimer fluorescence obtained from solutions containing 10^{-6} M bPy and increasing amounts of γ -CD, from 10^{-2} to $7.9 \times 10^{-2} \text{ M}$. As we mentioned earlier, we found only one decay time for the excimer fluorescence (emitted from species **iii**), equal to 152 ns, without any evidence of rise time. On the other hand, solutions of bPy in *n*-alkanes have been reported to demonstrate two excimer decay times, $\tau_1 = 35.6 \text{ ns}$ and $\tau_2 = 140.3 \text{ ns}$, along with a short rise time equal to 2.5 ns. These two different decay times have been attributed to two different configurations of bPy in homogeneous solutions [23], while the rise time corresponds to the time needed for the excited pyrene group to approach the unexcited one, of the same bPy molecule, and form the excimer. In species **iii**, however, where the two pyrene units of bPy are packed inside the γ -CD cavity, the fluorophores are restricted in their movements and evidently they can assume only one configuration, therefore only one excimer is formed and only one decay is observed as shown in Fig. 2. The fact that the two pyrene units of bPy in species **iii** are tightly packed inside the γ -CD cavity is also confirmed by the lack of a rising branch in the excitation-decay profile of bPy: γ -CD (see Fig. 2).

Contrary to the excimer fluorescence, which originates from only one emitting species (**iii**), monomeric emission comprises of two different decay times, $\tau_1 = 41 \text{ ns}$ and $\tau_2 = 163 \text{ ns}$, the percentages of which vary as the concentration of γ -CD varies. Thus, at $[\gamma\text{-CD}] = 10^{-2} \text{ M}$, the percentage of τ_1 is 44% and that of τ_2 56%, whereas at $[\gamma\text{-CD}] = 7.9 \times 10^{-2} \text{ M}$, τ_1 corresponds to 10% and τ_2 to 90%. Therefore as the concentration of γ -CD increases the percentage of the monomer with decay time τ_2 increases at the expense of the monomer with decay time τ_1 . This behavior, in connection with the reaction Scheme 1, suggests that the species with $\tau_1 = 41 \text{ ns}$ is the 1:1 complex $(\text{bPy-CD})_{\text{mon}}$ (species **i**), whereas the one with $\tau_2 = 163 \text{ ns}$ is the 2:1 complex $(\text{CD-bPy-CD})_{\text{mon}}$ (species **ii**). This assignment is also supported by the quenching of the monomer fluorescence at high γ -CD concentration. Thus, the species with $\tau_2 = 163 \text{ ns}$, apart from being predominant (90%) at $[\gamma\text{-CD}] = 7.9 \times 10^{-2} \text{ M}$, it should also be more susceptible to quenching due to its long lifetime, 163 ns, compared to $\tau_1 = 41 \text{ ns}$ for the less abundant (10%) species. Thus, if the species with $\tau_2 = 163 \text{ ns}$ were the 1:1 complex (**i**), quenching by the bulky

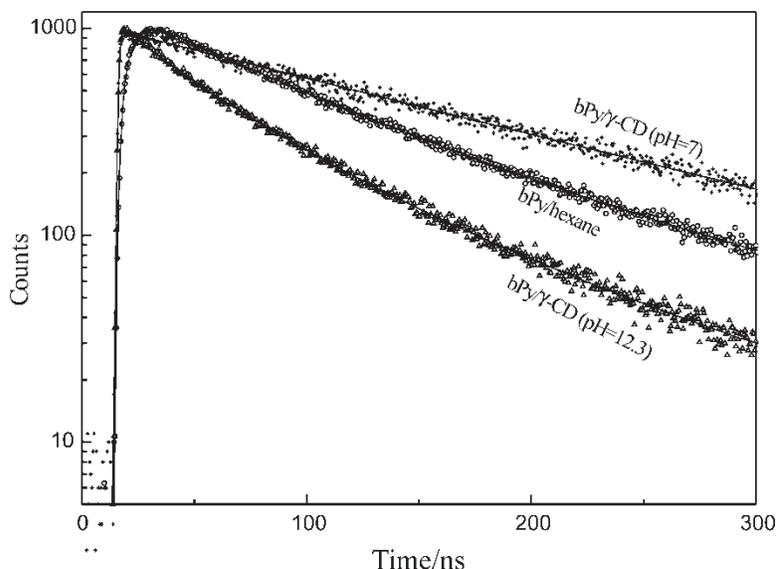


FIGURE 2 Excimer decay profiles (at 480 nm): (o) 10^{-6} M bPy in hexane; (+) bPy/ γ -CD in water at pH = 7; and (∇) bPy/ γ -CD in water at pH = 12.3. Solid lines represent fitting curves.

quencher TEA should be quite effective, since in this case one of the pyrene groups would be exposed to the quencher. In the opposite case, viz. if the predominant species with $\tau_2 = 163$ ns were the 2:1 complex (ii), TEA should not be able to quench the monomer fluorescence effectively, because pyrene would be protected by the barrel structure of the two γ -CD units. In an experiment in which we added 5×10^{-2} M TEA to an aqueous solution of bPy in γ -CD (7.9×10^{-2} M) we did not observe quenching of the monomer fluorescence. Therefore, from the two monomer emitting complexes we have concluded that, species i has life time $\tau_1 = 41$ ns and species ii has $\tau_2 = 163$ ns, and as the concentration of γ -CD increases species i is transformed into species ii.

Interpretation of the Effect of pH

Now, that we have concluded that only three complexes, viz. i, ii and iii, are formed between bPy and γ -CD in aqueous solutions, we are in a position to rationalize the results of the pH experiment shown in Fig. 1. Thus, in our earlier discussion we examined the effect of pH only on the species containing two CD units, and we based our arguments on the fact that at pH > 12.3 these CD units should separate due to electrostatic repulsive forces between the oxygen ions formed on the rim of the secondary OH groups of γ -CD. However, at this high pH value, the same electrostatic forces will also affect species i and iii, which contain only one CD unit, since the mutual repulsions between the aforementioned secondary oxygen ions—this time on the same dextrin unit—will widen the opening on this side of the CD cavity. Widening this opening will result in increasing the space available for movement of pyrene units hosted in

the γ -CD cavity. Admittedly, this will not have a great effect on species i, since only one pyrene unit is associated with the γ -CD, but it will relax the packing of the two pyrene units (of the same bPy molecule) inside the CD cavity in species iii. This means that, at pH > 12.3, the two pyrene groups of iii will be able to move more—than they do at pH = 7—and assume mutual orientations closer to the ones allowed in homogeneous solutions. Therefore the ground state dimerization of the two pyrene units of bPy inside the CD cavity, will be weakened at pH > 12.3, leading to a situation similar to that prevailing in homogeneous solutions of bPy [25].

The above argument is consistent with the experimental findings of Fig. 1. Thus, the position of the excimer fluorescence spectrum of an aqueous solution containing 10^{-6} M bPy and 10^{-2} M γ -CD (evidently the fluorescence spectrum of iii) at pH > 12.3, is shifted to the blue by approximately 600 cm^{-1} , compared to its position at pH = 7. Interestingly, this blue-shifted spectrum is identical with the spectrum of a homogeneous solution of bPy in *n*-hexane (see dotted line in Fig. 1a). Moreover, although at pH = 7 the time-resolved excimer fluorescence of bPy/ γ -CD consists of only one decay with $\tau_{\text{exc}} = 120$ ns, at pH > 12.3 a second decay appeared with $\tau = 47$ ns (see Fig. 2). As we have already mentioned, similar behavior, viz. two different decays ($\tau_1 = 63$, $\tau_2 = 142$ ns), has also been observed in homogeneous solutions of bPy in hexane. However, there is a crucial difference between the fluorescence spectra of bPy/ γ -CD and bPy/hexane, namely that the decay profile of bPy/hexane exhibits a rise time, which is not present in the corresponding spectra of bPy/ γ -CD (see Fig. 2). This rise time, as we mentioned earlier,

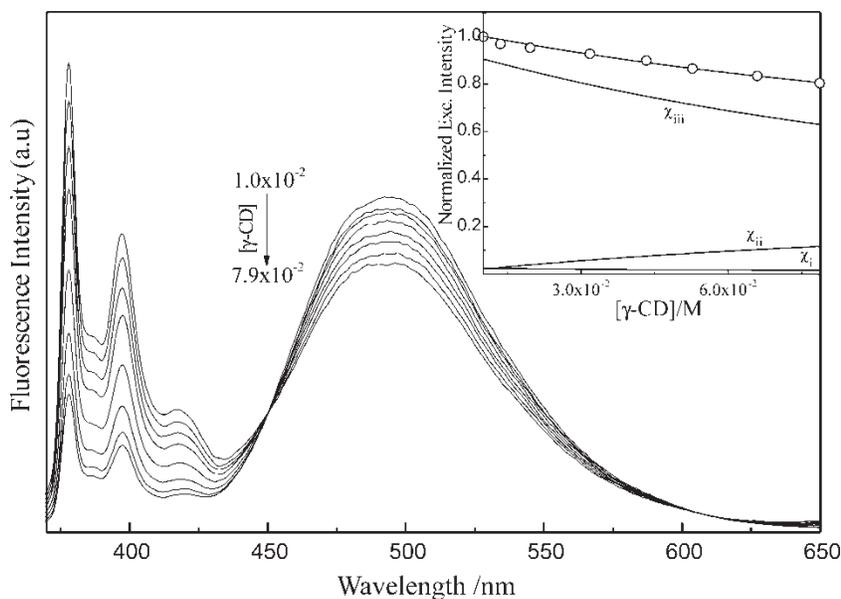


FIGURE 3 Fluorescence spectra of $[bPy] = 10^{-6} M$ at varying $[\gamma\text{-CD}]$. Inset: excimer fluorescence intensities plotted *vs.* $[\gamma\text{-CD}]$; the molar fractions (χ) of the species involved are also shown, for $K_2 = 170 M^{-1}$ and $K_3 = 45 M^{-1}$.

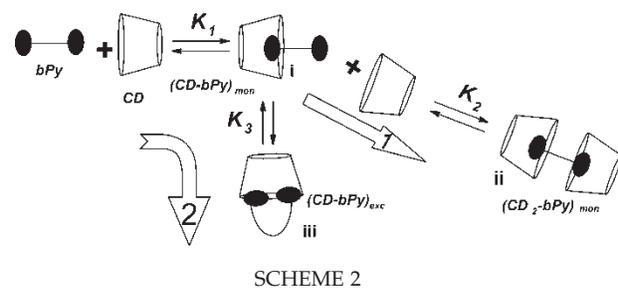
has been attributed to the movement of the two pyrene groups of a single bPy molecule towards excimer formation [25]. Since such a rise time was not observed in the decay profile of bPy/ γ -CD either at pH = 7 or at pH > 12.3 (see Fig. 2), we have concluded that the movement of the two pyrene groups in species **iii** takes place, in a time scale shorter than 500 ps (the limit of our instrument). In other words, the dimerization of the two pyrene groups of bPy in species **iii**, is not broken at pH > 12.3, although it is considerably weaker than at pH = 7.

In conclusion, at pH = 7, due to the tighter structure of the γ -CD cavity, the two pyrene groups of **iii** are close enough to form a dimer ground state, which upon excitation produces excimer fluorescence with intensity maximum at 495 nm (see Fig. 1a). The absorption spectra of bPy/ γ -CD confirm the formation of such a ground state dimer, by the enhancement of the absorption band at 333 nm (see Fig. 1b). Recall that the formation of ground state dimers in the case of pyrene is followed by increase of the absorption at ca. 333 nm and decrease at ca. 350 nm [26]. At pH > 12.3 however, due to the looser structure of the cavity (mutual oxygen ion repulsions), the two pyrene groups of **iii** are able to move somewhat (always remaining within the cavity) thus weakening their ground state dimerization. The enhancement of the absorption band of bPy/ γ -CD at 350 nm (see Fig. 1b) reflects this weakening of the ground state dimer. Upon excitation the weak dimers produce excimer fluorescence with intensity maximum at 481 nm (see Fig. 1a). Finally, Figs. 1a' and b'

show the changes of the position of the fluorescence maximum and the changes of the absorption intensities at 333 nm and 350 nm, as the pH of the bPy/ γ -CD solution was increased from 7 to 12.6. The sudden changes of these three parameters at ca. pH = 12.3 is evident and reflects the loss of the hydrogens of the secondary hydroxy groups of γ -CD [22].

Fluorescence Intensities

Fig. 3 shows the total fluorescence (monomer and excimer) of $10^{-6} M$ bPy as a function of $[\gamma\text{-CD}]$ in water, while in the inset the experimental intensity of the excimer fluorescence alone is shown. From the possible routes of Scheme 1 we are left with the simple Scheme 2 which describes the complexation processes compatible with our experimental findings. However, since K_1 is impossible to determine, because of the extremely low solubility of bPy in water, we have further reduced our scheme to Eqs. (2) and (3), by assuming complex **i** to be the starting reactant. Evidently, as the reaction



proceeds, species **i** either transforms to species **iii** by the unimolecular reaction (3), or produces species **ii** by interaction with one γ -CD unit. Due to the fact that there are two different monomer emitters, viz. species **i** and **ii**, with different quantum yields each, whereas there is only one excimer emitting complex, it is much simpler to try to fit the excimer fluorescence intensity data, I_{exc} , shown in the inset of Fig. 3. From such fittings we found that the fitting Eq. (7) can be compatible with our data, whenever the ratio

$$I_{\text{exc}} = \frac{1 + K_3 + K_2 10^{-2}}{1 + K_3 + K_2 [\text{CD}]_{\text{tot}}} \quad (7)$$

$K_2:K_3$ is equal to ca. 3.7–3.8, but the exact values of these two *equilibrium* constants cannot be determined, because the plot of the experimental points—excimer fluorescence intensity vs. $[\gamma\text{-CD}]$, inset Fig. 3—does not exhibit any characteristic features. When though we assigned to the equilibrium constant K_2 a value similar to the corresponding parameter in the complexation of pyrene with γ -CD, viz. 170–200 M^{-1} , the constant K_3 turned out to be equal to 45–50 M^{-1} . In the inset of Fig. 3 along with the experimental data and the fitting curve, the molar fractions (χ) for species **i**, **ii** and **iii**, are also shown, as obtained from the fitting. The simplicity of the complexation is further confirmed by the presence of the clear-cut isostilbic point in Fig. 3 at ca. 450 nm, which suggests that the underlying scheme does not involve equilibria among several monomer and excimer emitting species. For instance, if Eq. (4) were part of the complexation scheme, the addition of γ -CD would have produced species **iv**, along with species **ii**, thus destroying the simple relation between monomer and excimer fluorescence, which evidently is responsible for the occurrence of the isostilbic point.

CONCLUSIONS

The complexation of the bifluorophore 1,3-bis(1-pyrenyl)propane with γ -cyclodextrin in water involves the formation of three main inclusion compounds, of which two have stoichiometry (bPy: γ -CD) 1:1 and the third 1:2. The 1:2 complex and one of the two 1:1 *isomers* produce typical monomer pyrene fluorescence, differentiated only by their lifetimes. The other 1:1 complex, in which the bPy has folded in such a way that its two pyrene units enter the same γ -CD cavity, produces strong excimer emission which originates from a weakly-bound ground state dimer. The 2:2 barrel-type complex, which characterizes the excimer fluorescence of pyrene: γ -CD, does not form in

bPy: γ -CD. In spite of the simple interaction scheme which applies to the complexation of bPy with γ -CD in water, the magnitudes of the equilibrium constants involved cannot be directly determined.

MATERIALS AND METHODS

1,3-Bis(pyrenyl)propane, purchased from Molecular Probes, was further purified by recrystallization from an ethanol–chloroform mixture. γ -CD, obtained from Cyclolab, was of the highest purity and therefore it was used without any further purification. A severe experimental difficulty with bPy for this sort of study, is that this molecule is practically totally insoluble in water, solubility $< 10^{-8}$ M. Indeed, when we formed a thin film of bPy on the walls of an Erlenmeyer flask—by evaporation of an appropriate solution in chloroform—then added water and left it overnight under mild stirring, we were unable to detect any fluorescence signal, even at the highest instrumental sensitivity. Only when the stirring in water took place in the presence of γ -CD, were we able to obtain absorption and fluorescence spectra characteristic of bPy. From the optical densities of these absorption spectra, which were determined using the molar absorption coefficient of bPy in hexane, ($\log \epsilon = 4.67$ at 343.6 nm), we estimated the approximate amount of bPy solubilized in γ -cyclodextrin.

The details of the instruments used for this work and the methods of data analysis have been reported at length in previous publications [9,10].

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