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ORIGINAL ARTICLE

Spectroscopic investigation of Rose Bengal/cyclodextrin interactions in aqueous solution: the case of the hydroxypropyl-cyclodextrins

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Abstract The interaction of Rose Bengal (RB) with hydroxypropyl-α-cyclodextrin (HP-α-CD), hydroxypropyl-β-cyclodextrin (HP-β-CD) and hydroxypropyl-γ-cyclodextrin (HP-γ-CD) has been studied in water and in acetate buffer at pH 4.5 by UV–Vis absorption, fluorescence spectroscopy and Induced Circular Dichroism at 298 K. Evidence of the complex formation between the RB and all HP-CDs have been obtained both in water and in buffer. Binding constants and stoichiometry of RB/HP-CD complexes in water have been determined by applying the modified Benesi-Hildebrand equation to the fluorescence measurements.

Keywords Rose Bengal · Aqueous solutions · Hydroxypropyl-Cyclodextrins · Inclusion complexes · pH

Introduction

Rose Bengal (RB) is one of the most popular sensitizer in water and it is used in many applicative and investigative fields. This alogenated xantene is characterised by peculiar spectroscopic and photochemical properties such as a high absorption coefficient in the visible region of the spectrum, a high intersystem crossing efficiency from the first excited singlet to the triplet state and a tendency to transfer electrons in the excited state producing long lived radicals [1–3]. These features make it particularly suitable as a photosensitizer in many different areas such as conversion and storage of solar energy, photocatalysis in synthetic processes, wastewater treatment [4–6] and in medicine [7]. It is known that the photochemical and photophysical properties of dyes in solution strongly depend on the chemical–physical characteristics of the solvent (polarity, viscosity, hydrogen donating ability, etc.) [8, 9] and on its aggregation state. It is preferable to have the dye in the monomeric form because its aggregation impairs the photochemical response [5]. Recently the effect of complexation by natural cyclodextrins (CDs) on the photoreactivity of alogenated xanthenes have been the subject of studies [3, 10]. Data reported on the inclusion of RB in α, β and γ natural CDs in aqueous solutions have shown that RB binds only to γ-CD mainly in a 1:1 stoichiometry ratio with $K = 100\ dm^3\ mol^{-1}$ [3]. We extended the study to the case of some modified CDs with the aim of testing the ability of these CDs to prevent the dye's self aggregation [11–13] in water and in salt solution, where the tendency to aggregate is higher. The results of these studies, performed by calorimetric, spectrophotometric and electrochemical measurements, showed that some β and γ CDs form complexes with RB with a stoichiometry 1:1 and shift the equilibrium monomer-aggregates towards the formation of the monomer both in water and in salt solution. In this paper, we report the results of a spectrophotometric study on the interaction of RB with three hydroxypropyl-CDs, the α, β and γ in water. In order to understand the role played by the

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formation of hydrogen bonds in the complexation, we have also studied the interaction RB/HP-CD at pH 4.5.

Experimental section

Hydroxypropyl-α-cyclodextrin (HP-α-CD) DS = 3.0, hydroxypropyl-β-cyclodextrin (HP-β-CD) DS = 5.6, and hydroxypropyl-γ-cyclodextrin (HP-γ-CD) DS = 4.8 were purchased from ALDRICH. RPE ACS D(+)-glucose was purchased from Carlo Erba whereas Rose Bengal (RB) was purchased from Fluka. The molecular structures of RB is reported in Fig. 1. All chemicals were used as received.

Aqueous solutions were prepared with doubly distilled water. Two stock solutions of RB were prepared, one in water and the other in acetate buffer 0.1 M at pH 4.5. Solutions were prepared dissolving weighted amounts of CD or glucose in a 5 ml of a RB stock solution pipetted into 10 ml calibrated flasks and diluted to volume with water or with the buffer solution. This procedure ensures a constant concentration of RB both in the absence and in the presence of the various CD or glucose concentrations.

UV–Visible absorption spectra were recorded using a Varian CARY/3 spectrophotometer. Fluorescence measurements were carried out using a Varian Cary Eclipse fluorescence spectrophotometer exciting at 550 nm. Fluorescence spectra of RB/HP-γ-CD solution in water were recorded using an excitation power lower than that used for the other solutions. Circular dichroism spectra were recorded using a JASCO J810 spectropolarimeter and cuvettes having different optical length so that all samples had the same O.D.

Result and discussion

The absorption spectrum of RB in water is characterized by a maximum at 548.10 nm and a shoulder at about 512 nm. The presence in solution of HP-CDs produces a red shift of the absorbance maximum of RB

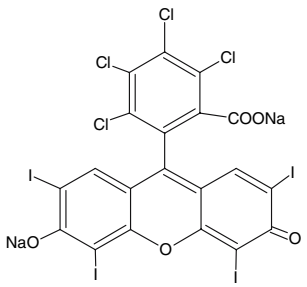

Fig. 1 Molecular structures of RB

(7×10^{-6} M). Figure 2 shows the dependence of this shift $\Delta\lambda$ on the CD concentration. The shift $\Delta\lambda$ has been calculated using the following expression $\Delta\lambda = \lambda_{max} - \lambda_o$, where λ_{max} and λ_o indicate the wavelengths of the maximum absorbance of RB in water and in CD solution respectively. The $\Delta\lambda$ risen by the addition of increasing amounts of D-(+)-glucose are also reported in Fig. 2. In agreement with that already observed at higher RB concentrations [11], the $\Delta\lambda$ obtained in presence of glucose are negligible. It results that the observed batochromic shift of RB spectrum upon the addition of CDs, similar to that obtained in solvent less polar than water [14], can be ascribed to the formation of complexes between RB and CDs and not to changes in the solvent properties. It is noteworthy that also HP-α-CD produces a shift of RB spectrum though only at concentrations higher than 0.04 g/ml.

This interaction is also confirmed by the RB fluorescence enhancement observed in presence of HP-CDs shown in Fig. 3. It is possible to observe that the increase of natural α-CD concentration does not produce any increase of the fluorescence intensity so confirming the non-inclusion of RB in the natural α-CD. Otherwise, an increase of fluorescence intensity is obtained at increasing of CD concentration, as general behaviour, for HP-α, HP-β and HP-γ-CDs.

The binding constants for the inclusion complexes have been calculated by applying the modified Benesi-Hildebrand treatment to the fluorescence measurements in the following form [15]:

$$\frac{F^0}{F - F^0} = \frac{1}{A} + \frac{1}{AK[\mathrm{CD}]^n}$$

where K is the binding constant, F^0 is the initial fluorescence intensity of free RB in water, F is the maximum fluorescence intensity of the RB in the

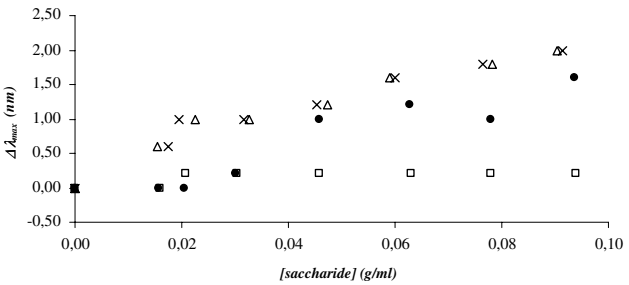

Fig. 2 Experimental shift of the wavelengths of the absorption maximum of RB 7×10^{-6} M, $\Delta\lambda = \lambda_{max} - \lambda_o$, as a function of the concentration of (□) glucose, (●) HP-α-CD, (Δ) HP-β-CD, (X) HP-γ-CD in water. λ_{max} and λ_o indicate the wavelengths of the maximum absorbance of RB in solution with and without CDs respectively. The saccharide concentrations are expressed as g/ml

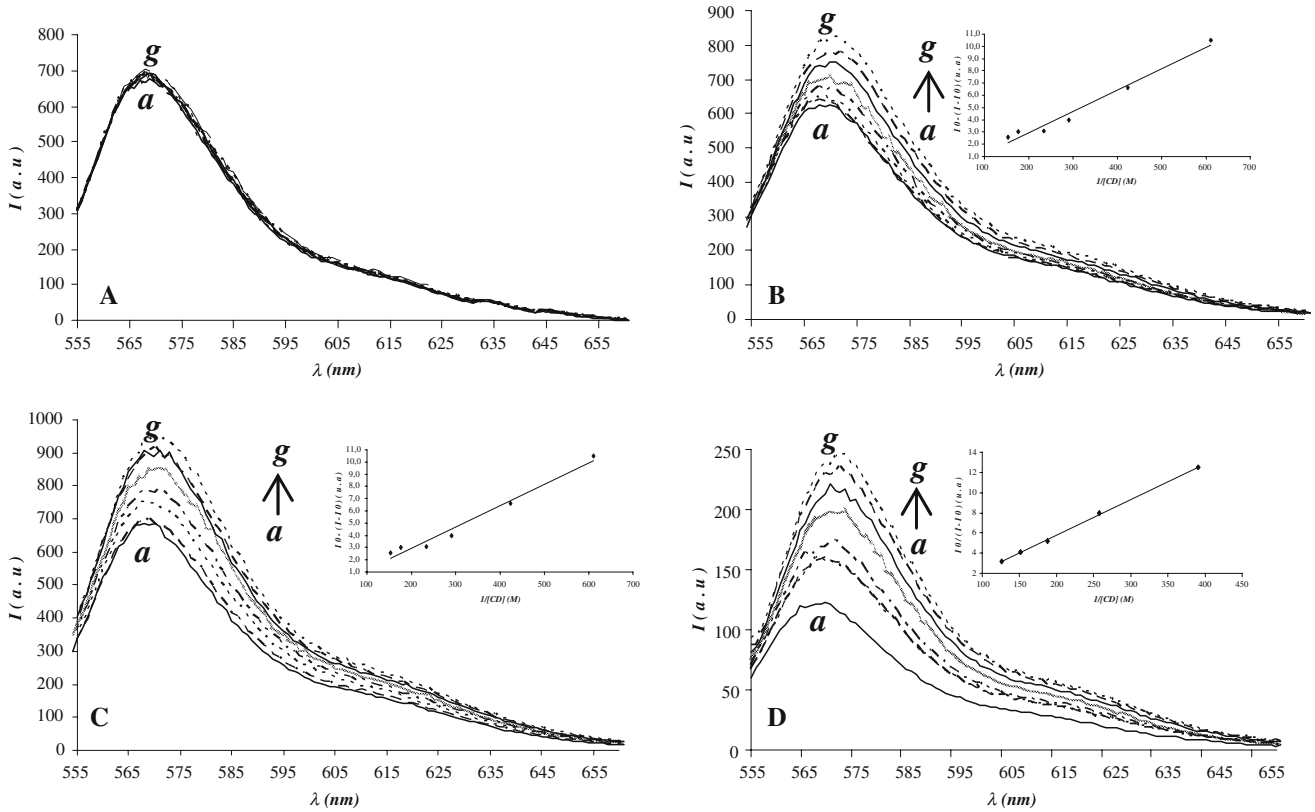

Fig. 3 Fluorescence spectra of RB, 7×10^{-6} M in aqueous solutions at different concentrations of α-CD (**A**), (a) 1.32×10^{-3} M, (b) 1.92×10^{-3} M, (c) 2.71×10^{-3} M, (d) 4.02×10^{-3} M, (e) 5.00×10^{-3} M, (f) 6.64×10^{-3} M, (g) 7.66×10^{-3} M; HP-α-CD (**B**), (a) 1.33×10^{-3} M, (b) 1.75×10^{-3} M, (c) 2.58×10^{-3} M, (d) 3.91×10^{-3} M, (e) 5.34×10^{-3} M, (f) 6.60×10^{-3} M, (g) 7.96×10^{-3} M; HP-β-CD (**C**), (a) 1.32×10^{-3} M, (b) 1.92×10^{-3} M, (c) 2.71×10^{-3} M, (d) 4.02×10^{-3} M, (e) 5.00×10^{-3} M, (f) 6.64×10^{-3} M, (g) 7.66×10^{-3} M; HP-γ-CD (**D**), (a) 1.47×10^{-3} M, (b) 1.65×10^{-3} M, (c) 2.69×10^{-3} M, (d) 3.84×10^{-3} M, (e) 5.08×10^{-3} M, (f) 6.48×10^{-3} M, (g) 7.75×10^{-3} M. Inset: Benesi-Hildebrand plots of $F^0/(F–F^0)$ vs. 1/[HP-CD]

cyclodextrine solution at the [CD] concentration, A is a constant, and n is the number of binding sites. In the insets of Fig. 3 the plots of $F^0/(F–F^0)$ vs. 1/[HP-CD] in solution are reported. The linearity of the plots $F^0/(F–F^0)$ vs. 1/[CD] obtained in the case of all HP-CDs reflects the formation of complexes 1:1 between the dye and the CDs as already observed in water and in salt solutions [11–13]. The binding constants evaluated by fluorescence measurements, 40 M^{-1} for HP-α-CD, 74 M^{-1} for HP-β-CD and 137 M^{-1} for HP-γ-CD, indicate that the complex stability increases at increasing of the cavity size.

The binding constants evaluated by fluorescence measurements are lower than those previously evaluated by absorption measurements [11]. A likely explanation of the discrepancy between these two sets of values can be ascribed to the different RB concentration used to study the binding by the two spectroscopic techniques. At the higher RB concentration used to analyze the modifications of the absorbance spectrum, the dye is partially aggregated as shown by the decrease of RB relative intensity of the shoulder to the peak at increasing of CD concentration already reported. [11]. It results that the spectral modifications observed at increasing CD concentration are due not only to the association of RB with CD but also to the dissociation of the dye's aggregate. The impossibility to discriminate the two effects can lead to an overvaluation of the binding constants. Differently at the RB concentration used in the fluorescence study no change in the relative intensity of the shoulder to the peak has been observed.

Further evidence of the formation of complexes between RB and HP-CDs is also given by the Induced Circular Dichroism (ICD) spectra reported in Fig. 4. The presence of an ICD signal proves the interaction between a chiral environment provided by CDs and the achiral molecule of RB. Moreover the ICD sign provides information on the orientation of the electric transition moment of RB with respect to the axis of the CD cavity [16–19]. In particular, a negative ICD signal is obtained in the case of the two smaller HP-CDs,

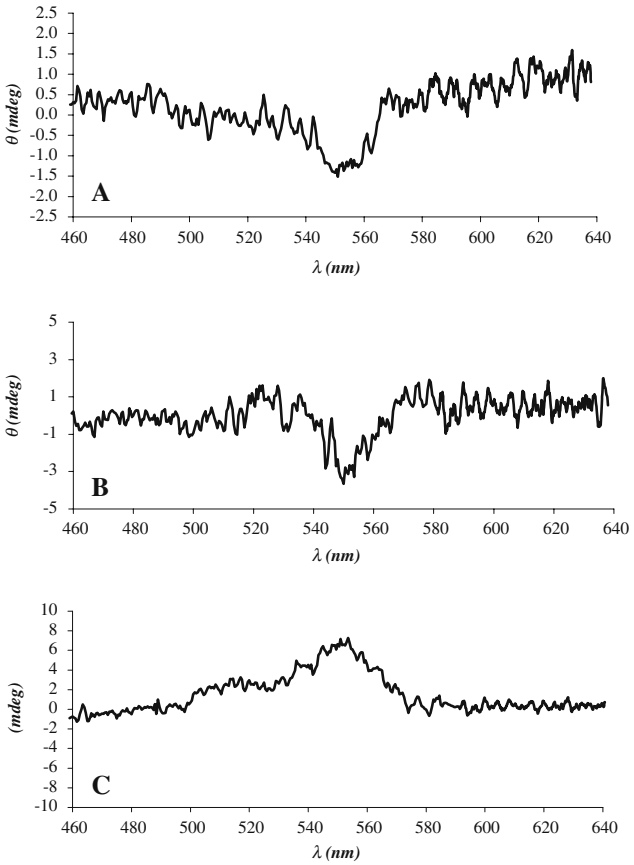

Fig. 4 Induced Circular Dichroism spectra of RB (7×10^{-6} M) in aqueous solution of HP-α-CD (7.96×10^{-2} M)(**A**),HP-β-CD (6.70×10^{-2} M) (**B**) and HP-γ-CD (6.20×10^{-2} M) (**C**)

α and β, whereas a positive one is obtained with the HP-γ-CD. This behaviour suggests a likeness in the geometry of RB/HP-α-CD and RB/HP-β-CD complexes. This is in agreement with one of Flamigni's studies which suggests the formation of two types of complexes: the type I complex, due to the inclusion of part of the xanthenic moiety, and the type II complex, formed by the inclusion of the pendant benzoic acid group [10]. The preferential formation of Type I or II complexes depends on the CD size cavity: CDs having a large cavity, like HP-γ-CD, preferentially form type I complexes whereas CDs having a small cavity, like HP-α-CD and HP-β-CD, form type II complexes. The intensities of the ICD signals produced by the different HP-CDs are in agreement with the stabilities of the various complexes: the ICD of HP-γ-CD is the most intense, followed by the progressively weaker signals of HP-β-CD and HP-α-CD.

In order to understand the role played by the formation of hydrogen bonds in the complexation we have studied the interaction RB/HP-CD at pH 4.5. Figure 5 shows the absorbance spectra of RB in water and in buffer solution at pH 4.5 in absence and in presence of HP-CDs. It is known that, depending on pH, RB can be present in solution in various forms differently ionized [20, 21], each of which showing a different spectrum. In particular at decreasing of the pH there is the presence of less coloured or colourless forms like the lactoid form obtained at very acid pH.

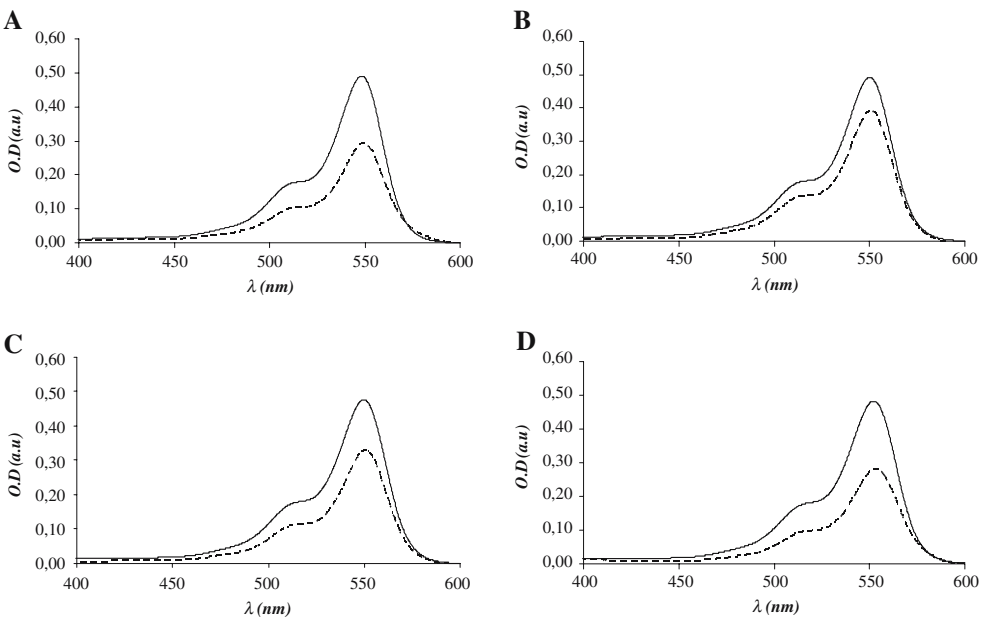

Fig. 5 Absorption spectra of RB (7×10^{-6} M) in water (—) and in acetate buffer 0.1 M at pH 4.5 (- - - -) alone (**A**) and with HP-α-CD 7.9×10^{-3} M (**B**), HP-β-CD 6.6×10^{-3} M (**C**) and HP-γ-CD 5.8×10^{-3} M (**D**)

The remarkable decrease in the absorbance of RB in the buffer solution at pH 4.5 compared to water can be associated to the shift of the complex series of pH dependent equilibrium among the different forms of RB towards species having lower absorbance coefficients.

Also in presence of HP-CDs the RB absorption spectra in buffer solution are less intense than in water. In particular the HP-γ-CD (Fig. 5D) produces the most marked effect, comparable to that obtained in absence of CD (Fig. 5A) whereas HP-β-CD (Fig. 5B) and HP-α-CD (Fig. 5C) cause a smaller effect. A possible explanation of this behaviour is that the type II complexes formed by the HP-α-CD and the HP-β-CD stabilize the bis anionic or monoanionic form of RB compared to the other forms probably due to the formation of a hydrogen bond between the CD and the carboxylic moiety [20].

In buffer solution at pH 4.5 also the fluorescence intensity of RB in absence and in presence of CDs is much lower than that observed in water as shown in Fig. 6. The presence of HP-CDs produces also in buffer solution an increase of the RB fluorescence at increasing of CD's cavity: the highest increase is observed in presence of HP-γ-CD, an intermediate increase in presence of HP-β-CD and the smallest increase in presence of HP-α-CD. These results are in agreements with that expected from the stability sequence of the various complexes in water. It is important to note that the highest enhancement in the fluorescence intensity is produced by the HP-γ-CD, the CD which induces a maximum decrease in the RB absorbance. This result further supports the hypothesis that there are differences between the complexes formed by RB and HP-γ-CD and those formed with the HP-α-CD and HP-β-CD. The observed interaction between RB and HP-α-CD or HP-β-CD is particularly interesting because it provides evidence that the ability of a molecule to associate with CD cannot be evaluated exclusively on the basis of a comparison between the diameter of the molecule and the diameter of the CD's cavity.

In literature [3], it is reported that natural α-CD and β-CD can associate with erythrosin B but not with RB. Since the difference between the two xanthenic dyes lies in the benzoic moiety which is completely substituted by chlorine in RB, Flamigni [3] suggested that the increase in the diameter of the benzoic moiety from 5 to 7.5 Å prevents RB from fitting into natural α and β-CD cavities. Instead, we observed that RB forms inclusion complexes with some modified β-CD and in particular the complexes with hydroxypropylated CD have an higher stability than those with methylated CD in water [11]. The difference in the behaviour which we have and that observed by Flamigni has been explained assuming that the substitution of OH groups with other chemical moieties can give rise to an increase of the dimension and opening of cavity making the complexation ability of modified CDs different from that of natural ones [22]. The higher stability of complexes with HP-CDs compared to those with methylated CDs has been ascribed to the occurrance of hydrogen bonds between the hydroxypropyl moiety of CD and one of the electron donor moiety of RB. It is likely that also in the case of HP-α-CD the formation of hydrogen bonds plays an important role in the association of RB with

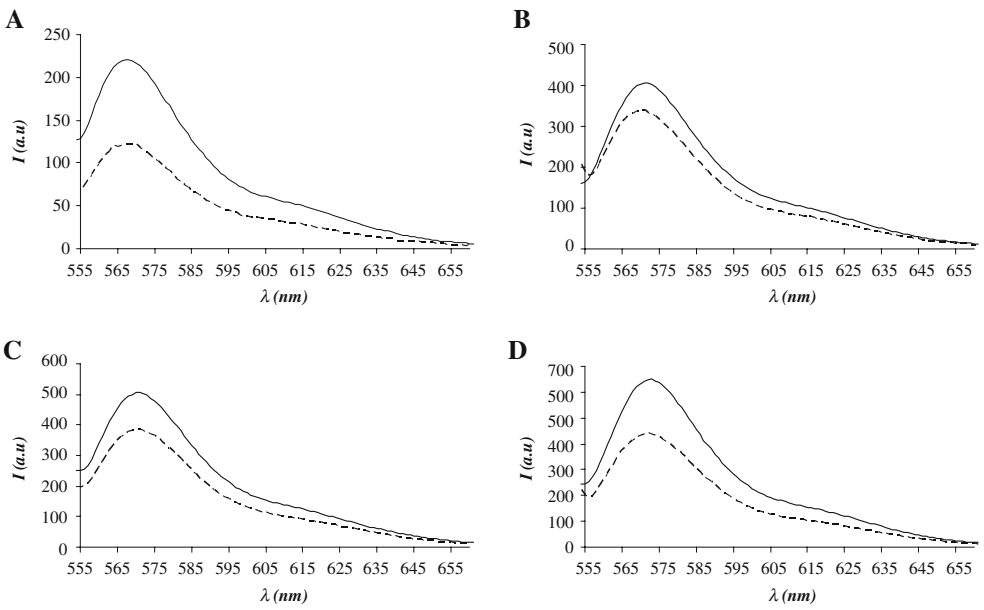

Fig. 6 Fluorescence spectra of RB (7×10^{-6} M) in water (—) and in acetate buffer 0.1 M at pH 4.5 (- - - -) alone (**A**) and with HP-α-CD 7.9×10^{-3} M (**B**), HP-β-CD 6.6×10^{-3} M (**C**) and HP-γ-CD 5.8×10^{-3} M (**D**)

this CD. In addition it is also necessary to take into account that the torouslike structure of cyclodextrins is not rigid [23]; the proximity of RB with the HP-α-CD, made easier by the formation of hydrogen bonds, can produce additional interactions which promote the deformation of the CD making the inclusion of part of RB possible.

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