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Inclusion complexes of Rose Bengal and cyclodextrins

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Abstract

The interaction of Rose Bengal (RB) in aqueous solution with four different cyclodextrins, namely hydroxypropyl-β-cyclodextrin (HP-β-CD), hydroxypropyl-γ-cyclodextrin (HP-γ-CD), heptakis(2,6-di-*O*-methyl)-β-cyclodextrin (DIMEB), heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin (TRIMEB), was studied by spectrophotometric and calorimetric measurements. An original extension of the Benesi–Hildebrand treatment was proposed to evaluate the binding constants analysing spectrophotometric data. The results suggest that, at the concentration taken in exam, RB includes in all modified CDs considered forming complexes 1:1 with different stability depending on cavity size and kind of derivative.

Keywords: Inclusion complexes; Rose Bengal; Cyclodextrins; ITC; Spectroscopy

1. Introduction

Rose Bengal (RB) is an alogenated xanthene (Fig. 1) characterised by an high absorption coefficient in the visible region of the spectra, 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]. As a result of its photochemical and photophysical properties RB is widely used as ideal reagent in processes where the production of singlet oxygen or reactive radicals are required. For example, it has been proved that RB is able to inactivate microorganisms and cause photodamage in vitro on different cell models [4]. It is known that the photochemical and photophysical properties of dyes in solution strongly depend on chemical–physical characteristics of the solvent (polarity, viscosity, hydrogen donating ability, etc.) [5,6].

Recently the effect of complexation by cyclodextrins on the photoreactivity of alogenated xanthenes have been the subject of consideration [3,7]. Cyclodextrins are cyclic oligosaccharides which have the ability to include molecules of organic compounds into their cavity [8]. The complexation generally produces modifications in the physicochemical properties of guest molecules [9,10], particularly in their photoreactivity [11,12]. The dye chromophore included in a cyclodextrin cavity, finds a relatively hydrophobic environment and often has the possibility to form hydrogen bonds with the cyclodextrin [13]. In addition the presence of cyclodextrins in a solution can be helpful in preventing the dye's self-aggregation and its photooxidation and therefore in avoiding a decrease of dye efficiency.

The study of these complexes can also provide interesting information on the photodamaging mechanism in biological systems; in fact, cyclodextrins can be considered as a simple model to mimic enzymes which bind chemical species by means of hydrophobic interactions [14].

Data reported in literature on the inclusion of RB in α, β and γ natural cyclodextrins in aqueous solutions have shown that RB binds only to γ-cyclodextrins mainly in a 1:1 stochiometric ratio with $K_b = 100\,dm^3\,mol^{-1}$ [3].

We have extended the study on the formation of inclusion complexes between RB and cyclodextrins to the case of some modified cyclodextrins. The reason for such interest is that these cyclodextrins are generally more soluble than natural ones and therefore make possible to enlarge the field of applicability of such systems. Moreover, the modified cyclodextrins differ from the natural ones in the cavity access and internal size [15,16]; consequently the results

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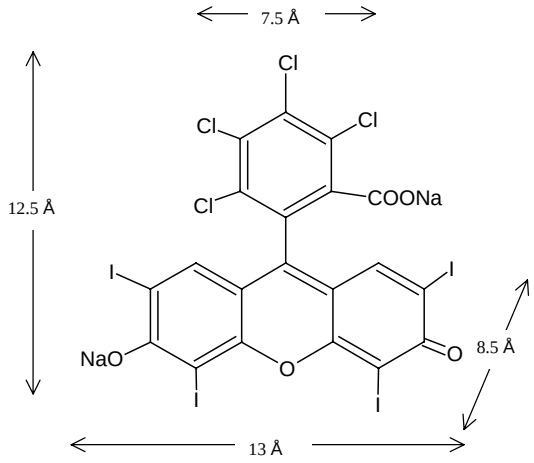

Fig. 1. Molecular structure and dimension [3] of RB.

obtained on natural cyclodextrins cannot be extended to functionalised ones.

2. Experimental

Hydroxypropyl-β-cyclodextrin (HP-β-CD) DS = 5.6, Hydroxypropyl-γ-cyclodextrin (HP-γ-CD) DS = 4.8, heptakis(2,6-di-*O*-methyl)-β-cyclodextrin (DIMEB), heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin (TRIMEB) were purchased from Aldrich. RPE ACS D-(+)-glucose and RB were purchased from Carlo Erba and Fluka, respectively. CDs, glucose and RB were used as received. All aqueous solutions were prepared with doubly distilled water. Solutions for spectrophotometric measurements were prepared dissolving weighted amounts of CD or glucose in a 5 ml of a RB aqueous stock solution pipetted into 10 ml calibrated flasks and diluted to volume with water. This procedure ensured a constant concentration of RB both in the absence and in the presence of the various CD or glucose concentrations.

Visible absorption and circular dichroism were recorded using a Varian CARY/3 spectrophotometer and a JASCO J810 spectropolarimeter, respectively.

Calorimetric measurements were performed using an LKB 2277 (TAM) microcalorimeter equipped with a thermometric 2250 titration unit. The isothermal titration calorimeter (ITC) was calibrated electrically and its performance was tested as previously described [17,18]. Each calorimetric titration consisted in three experiments. In the first one the thermal effects (Q_{tot}) associated to the injection of 10 μl aliquots of a CD aqueous solution (50 mM) into the sample cell containing 1 ml of a RB solution (1 mM) is measured. These heats are the result of three contributes associated to the interaction of RB with CDs (Q), to the dilution of the RB aqueous solution (Q'_{dil}) and to the dilution of the CD aqueous solutions (Q_{dil}):

$$Q_{tot} = Q + Q'_{dil} + Q_{dil} \quad (1)$$

In order to isolate from the total effect that associated to the interaction (Q), other two experiments were carried out replicating the corresponding binding experiment but filling the sample cell or the syringe with water to obtain Q_{dil} and Q'_{dil}, respectively. The heats associated to the dilution of the RB aqueous solutions (Q'_{dil}) resulted negligible.

In addition to these measurements some inverse titrations, in which the syringe was filled with a RB solution and the sample cell with a CD solution, were also carried out. In these experiments the solution concentrations were kept lower than those used in the direct titration in order to limit the self-aggregation of RB in solution. Consequently lower thermal effects were obtained which were used only to evaluate the enthalpy associated to the saturation of the binding sites. All measurements were performed at 298 K.

3. Results and discussion

3.1. Formation of complexes between RB and cyclodextrins

The absorption spectra of RB in water, ethanol, DMF and in an aqueous solution of HP-β-CD 0.049 M are shown in Fig. 2. In water the absorption spectra is characterised by a maximum at 548.10 nm. The relative intensity of the shoulder to the peak, 0.37, indicates that RB in aqueous solution 2.5×10^{-5} M is mainly present as monomer [2]. In solvent less polar than water, like EtOH and DMF, the λ_{max} of RB shifts to longer wavelength. An analogous batochromic effect is produced by the presence in solutions of CDs (Fig. 2).

The wavelengths corresponding to the maximum absorbance of RB in aqueous solutions of different CDs as a function of CD concentration are reported in Fig. 3. It is interesting to note that increasing CD concentration λ_{max} of RB increases up to two constant values, 555 nm in the case of the HP-CDs and 557 nm in the case of the methylated CDs. The trend of experimental data suggests the formation

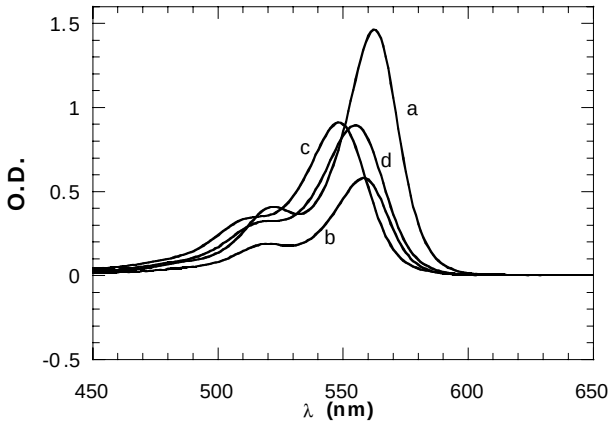

Fig. 2. Absorption spectra of RB (2.5×10^{-5} M, cell = 5 mm) in various solvents: (a) DMF; (b) EtOH; (c) water; (d) aqueous solution of HP-β-CD 0.049 M.

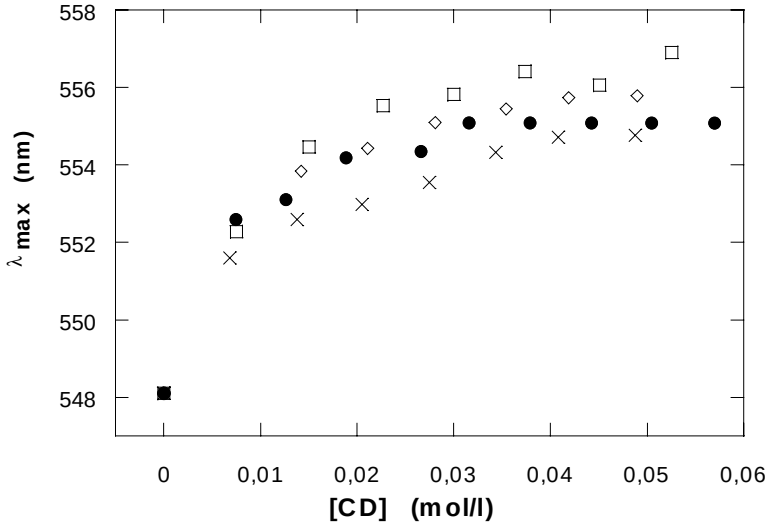

Fig. 3. Experimental wavelengths of the absorption maximum of RB 2.5×10^{-5} M, in the spectral range 500–600 nm, as a function of the concentration of (□) DIMEB, (◇) TRIMEB, (×) HP-β-CD and (●) HP-γ-CD.

of inclusion complexes between RB and CDs; the observed batochromic shift, in fact, can be ascribed to the changes in the polarity of the environment of RB chromophore, when it moves from the polar aqueous media to the apolar cyclodextrin cavity [19].

In order to confirm the formation of complexes and to distinguish between the spectral modifications produced by the inclusion of RB in the CDs cavity from other effects, like the presence of nonspecific interactions between RB and CD or changes in the solvent properties produced by the adding of CDs, the same set of experiments were also carried out in the presence of D-(+)-glucose. Amounts of D-(+)-glucose comparable to the used amounts of CD produces only a slight red shift whose maximum value is nearly 1 nm. This is a good indication that the spectral changes observed in the presence of CDs were mainly due to specific interactions between CD and RB [9,20].

Clues on the formation of complexes are also provided by the decrease of the relative intensity of the shoulder to the peak observed at increasing of CD concentration which is slight in water and more evident in salt solution where the amount of RB aggregated is higher than in water [21]. The formation of complexes promoted by an increase of the CD concentration, in fact produces a shift of the equilibrium monomer/aggregate of RB towards the monomeric form.

The analysis of the spectra of RB obtained at different concentration of cyclodextrins shows also the presence of a single isosbestic point for each CD which indicates the equilibrium between two species, the RB free and complexed, and therefore the preferential formation of a complex having stoichiometry 1:1 [20].

Further evidence of the formation of complexes between RB and β- and γ-CDs is also given by the presence of signals of induced circular dichroism which indicate that there is an interaction between the chiral environment provided by CDs and the achiral molecule of RB. In Fig. 4, the ICD spectra of RB in aqueous solution of HP-γ-CD 0.045 M is reported as an example.

3.2. Association constants of the complexes

The association constants for the inclusion complexes were calculated applying the Benesi–Hildebrand treatment to the absorption measurements [11,19,20,22].

Generally, this treatment takes into consideration the difference between the absorbance of solutions of complexed and free dye. In the case of RB the presence of CD in solutions essentially produces a shift of the RB spectrum to longer wavelength without changes in the absorbance intensity as shown in Fig. 2. Consequently the Benesi–Hildebrand

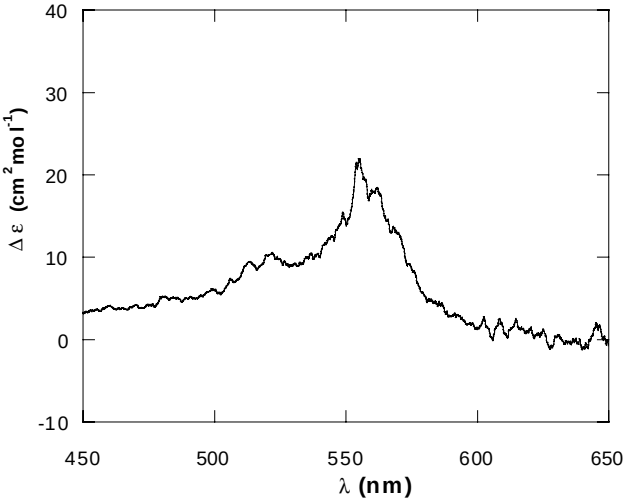

Fig. 4. Induced circular dichroism spectra of RB (1.9×10^{-5} M) in aqueous solution of HP-γ-CD 0.045 M.

treatment was rewritten in terms of the shift of the maximum wavelength.

Assuming that CDs taken into consideration form 1:1 inclusion complexes with RB:

$$\mathrm{RB} + \mathrm{CD} \leftrightarrows \mathrm{RB{-}CD} \tag{2}$$

The equilibrium constant of the complex is given by

$$K = \frac{[\mathrm{RB{-}CD}]}{[\mathrm{RB}][\mathrm{CD}]} \tag{3}$$

where [RB–CD], [RB] and [CD] are the concentrations of the complex RB–CD, RB and CD at the equilibrium. The relative amount of RB complexed, $[\mathrm{RB{-}CD}]/[\mathrm{RB}]_0$, is related to the observed λ_{max} by the following expression:

$$\frac{[\mathrm{RB{-}CD}]}{[\mathrm{RB}]_0} = \frac{\Delta\lambda_{exp}}{\Delta\lambda_0} \tag{4}$$

where $[\mathrm{RB}]_0$ is the total concentration of RB present in solution, complexed and free, $\Delta\lambda_{exp}$ the difference between λ_{max} of RB in an aqueous solution of a given CD concentration and that of RB free in aqueous solution and $\Delta\lambda_0$ is the difference between λ_{max} of RB included in CD, the plateau value of λ_{max} obtained at high CD concentration, and that of RB free in aqueous solution.

Using Eq. (4) to express equilibrium concentrations of RB free and complexed it is obtained that

$$[\mathrm{RB}] = [\mathrm{RB}]_0(1 - \Delta\lambda_{exp}/\Delta\lambda_0) \tag{5}$$

for

$$[\mathrm{RB}] = [\mathrm{RB}]_0(1 - \Delta\lambda_{exp})/\Delta\lambda_0 \tag{6}$$

Introducing Eqs. (5) and (6) in Eq. (3) and considering that the total concentration of CD, $[\mathrm{CD}]_0$, is at least 1000 times higher than $[\mathrm{RB}]_0$ and therefore [CD] can be considered almost equal to $[\mathrm{CD}]_0$, the equilibrium constant becomes

$$K = \frac{[\mathrm{RB}]_0(\Delta\lambda_{exp}/\Delta\lambda_0)}{[\mathrm{RB}]_0(1 - (\Delta\lambda_{exp}/\Delta\lambda_0))[\mathrm{CD}]_0} \tag{7}$$

which can be easily transformed into a new form of the double reciprocal linear equation known as Benesi–Hildebrand equation:

$$\frac{\Delta\lambda_0}{\Delta\lambda_{exp}} = \frac{1}{K[\mathrm{CD}]} + 1 \tag{8}$$

According to this approach, the stoichiometry of the complex is considered 1:1 if a linear double-reciprocal plot is obtained; when it is nonlinear other stoichiometries are taken in consideration and different equations are developed [12].

For the systems under examination the double reciprocal plots (Fig. 5) of the experimental data show a satisfactory linear correlation with $R > 0.97$; in addition all intercepts values are very close to 1 in agreement with Eq. (4). These findings indicate that 1:1 complexes are mainly formed in the studied systems. This result is corroborated by the presence of an isosbestic point for each CD as already reported in the previous section, and by the results of the analysis of some preliminary fluorescence measurements (data not shown). In particular we observed that the fluorescence intensity (I) increases at increasing of the CD concentration and that there is a linear correlation of $1/I$ versus 1/[CD] as expected in the case of the formation of complexes having a stoichiometry 1:1 [23].

The values of K calculated by the slopes of linear double reciprocal plot are reported in Table 1.

In order to have a more detailed knowledge on the thermodynamics of formation of these complexes they were studied also by calorimetric measurements. Calorimetric data were analysed using the treatment proposed by Eftink and Biltonen [24].

According to this treatment and assuming the formation of a complex 1:1, Q_i, the sum of the heats Q associated to i injections of a solution of CD in a solution of RB, is proportional to n_b, the moles of RB complexed:

$$n_b = \frac{Q_i}{\Delta H^\circ} \tag{9}$$

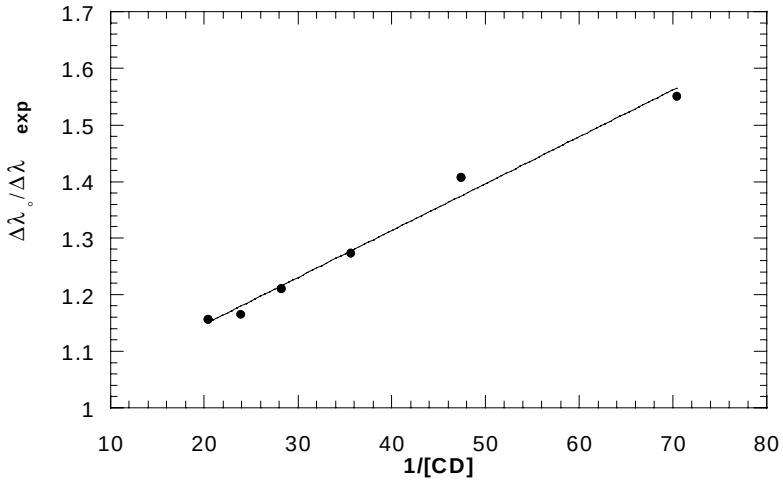

Fig. 5. Benesi–Hildebrand plot of $\Delta\lambda_0/\Delta\lambda_{exp}$ vs. 1/[CD] of RB in presence of TRIMEB.

Table 1
Thermodynamic parameters for the association between RB and CDs at 298 K[a]

CD	K[b] (M^{-1})	K[c] (M^{-1})	ΔH° (kJ/mol)	ΔG° (kJ/mol)	$T\Delta S^\circ$ (kJ/mol)
DIMEB	63 ± 2	68 + 15	−17.5 ± 10	−10.4 ± 0.5	7.1 ± 1.5
TRIMEB	120 ± 7	90 ± 10	+9.1 ± 0.5	−11.1 ± 1.0	20.2 ± 1.5
HP-β-CD	140 ± 30	130 ± 20	−13.4 ± 1.3	−12.0 ± 0.3	1.4 ± 1.6
HP-γ-CD	190 ± 30	210 ± 20	−9.7 ± 0.3	−13.2 ± 0.2	3.5 ± 0.5

[a] The errors reported in table are the standard deviations.
[b] Values obtained from the spectrophotometric study.
[c] Values obtained from the calorimetric study.

where ΔH° is the molar standard enthalpy of complexation. The moles, n_f, of RB free in solution are given by the following equation:

$$n_f = [RB]_0 V_0 - \frac{Q_i}{\Delta H^\circ} \quad (10)$$

where $[RB]_0$ is the initial concentration of RB in the sample cell, before the addition of the CDs, and V_0 is its volume.

Using Eqs. (9) and (10) to express the concentration of RB free and complexed in Eq. (3), the association constant becomes

$$K = \frac{Q_i/\Delta H^\circ}{[CD]([RB]_0 V_0 - Q_i/\Delta H^\circ)} \quad (11)$$

Eq. (11) can be rewritten in the following form which correlates experimental data $Q' = Q_i/[RB]_0 V_0$ with the concentration of CD free in solution [CD]:

$$Q' = \frac{\Delta H^\circ K[CD]}{1 + K[CD]} \quad (12)$$

The [CD] is calculated by the following equation:

$$[CD] = [CD]_T - [RB]_0 Q'/\Delta H^\circ \quad (13)$$

where $[CD]_T$ is the total CD molarity.

The values of ΔH° and K (Table 1) were obtained from Eqs. (12) and (13) by an iterative least squares method. The first values of [CD] were evaluated assuming that $\Delta H^\circ = Q'_{sat}$ that is the value of $Q_i/[RB]_0 V_0$ at saturation at the end of the titration when CD is present in large excess. The iteration were stopped when two successive values of ΔH° differed by less than 2% as reported in literature [25,26]. In order to verify if the assumed stoichiometry 1:1 is correct the formation of complexes was also studied performing inverse titrations [25]. The agreement, within the experimental uncertainties, between the values of ΔH° evaluated from the direct and the inverse titrations indicate mainly the formation of complexes having stoichiometry 1:1. In fact if the stoichiometry of the complexes was 1:2 or 2:1 the inverse titration should lead to values of ΔH° about half or twice those calculated through the direct experiments.

From K and ΔH° values obtained by calorimetric measurements the standard free energy and entropy of association were calculated (Table 1). The values of K obtained by the calorimetric and spectrophotometric study are in a satisfactory agreement within the experimental uncertainty.

These results indicate that RB includes in all modified CDs considered forming complexes whose stability depends on cavity size and kind of derivatives. As shown by Flamigni's study [3] and our unreported results, natural α- and β-CDs are not able to include RB. In the case of α-CDs because of molecular dimensions, RB does not fit into the CD cavity. In the case of β-CDs the drawback to the inclusion can be ascribed more than to cavity dimension to other structural differences which are often considered responsible of the changes in the complexation ability of modified β-CDs respect to the natural CDs [27]. The substitution of OH groups with other chemical moieties can increase the cavity dimension providing additional surface for interaction; in addition an increase or decrease of the cavity opening is realised according to the substituents are canted outwards or have a high steric hidrance, respectively [15,16]. In agreement with these findings our experimental results indicate that, conversely to natural β-CD, the DIMEB is able to include RB in its cavity. The TRIMEB forms with RB inclusion complexes more stable than DIMEB because the complete methylation of the hydroxyl groups of CDs leads to an increase of both cavity opening and internal diameter and provides an additional surface for interaction [16]. In the case of HP-CDs the difference between the values of K obtained with β- and γ-CD can be ascribed to the different cavity size. The higher stability of complexes with HP-CDs compared to those with methylated CDs suggests the occurring of hydrogen bonds between the hydroxypropyl moiety of CD and one of the electron donor moieties of RB besides the hydrophobic interactions, which are characteristic of inclusion complexes.

The data reported in Table 1 indicate that the formation of inclusion complexes produces negative changes in enthalpy for DIMEB, HP-β-CD and HP-γ-CD, as shown from most of the studies reported in literature on the formation of inclusion complexes [13], whereas it is positive for TRIMEB. At the best of our knowledge there are no other calorimetric on the formation of inclusion complexes between CDs and guest molecules having structure similar to RB; therefore it not possible to compare our thermodynamic data with other data reported in literature.

The changes of the complexation thermodynamics of CDs are generally the results of several and contrasting effects mainly originated from the penetration of the hydrophobic part of the guest molecule into the CDs cavity, the

dehydration of the guest, the formation of hydrogen bonds, the release of the water molecules originally included in the CD cavity to bulk water and the conformational changes of CD upon the inclusion of the guest molecule [8,13].

In the case of TRIMEB the permethylation of hydroxyl groups makes impossible the formation of hydrogen bonds between the CD and RB. It results that the exothermic contribution associated to this effect disappears. Moreover, it needs to be considered that the permethylation leads to structural modifications of CD both in size and shape [16]. These modifications can give rise to some differences in the interaction of TRIMEB and DIMEB with RB which can make endothermic processes, like the release of water molecules included in the cavity and the changes of the hydration shell of the guest molecule, more important than exothermic ones. A further indication that the release of ordered water plays an important role in the complexation of RB with TRIMEB is also given by the high positive value of ΔS° [13,25,26].

4. Conclusions

In aqueous solution RB includes in all modified CDs considered forming complexes with a stoichiometry 1:1. The inclusion produces modifications in the absorption spectra of RB which were used to evaluate the association constants. The values of the association constants determined by spectrophotometric and calorimetric measurements indicate that the stability of the complexes depends on cavity size and kind of CD derivatives. The formation of these complexes produces an increase of entropy for all CD derivatives considered; whereas for the enthalpy it was obtained a decrease or increase depending on the kind of CD.

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References

[1] P. Murasecco-Suardi, E. Gassmann, A.M. Braun, E. Oliveros, Helv. Chim. Acta 70 (1987) 1760.
[2] S.D.M. Islam, O. Ito, J. Photochem. Photobiol. A: Chem. 123 (1999) 53.
[3] L. Flamigni, J. Phys. Chem. 97 (1993) 9566.
[4] G. Bottiroli, A.C. Croce, P. Balzarini, D. Locatelli, P. Baglioni, P. Lo Nostro, M. Monici, R. Pratesi, Photochem. Photobiol. 66 (3) (1997) 373.
[5] R.S. Becker, Theory and Interpretation of Fluorescence and Phosphorescence, Wiley/Interscience, New York, 1969 (Chapters 4 and 10).
[6] E. Kohen, R. Santus, J.G. Hirschberg, Photobiology, Academic Press, London, 1995 (Chapters 4 and 5).
[7] L. Flamigni, J. Chem. Soc. Faraday Trans. 90 (16) (1994) 2311.
[8] J. Szejtli, Chem. Rev. 98 (1998) 1743.
[9] M. Ilanchelian, C. Retna Raj, R. Ramaraj, J. Inclusion Phenom. Macrocycl. Chem. 36 (2000) 9.
[10] T. Yorozu, M. Hoshino, M. Imamura, J. Phys. Chem. 86 (1982) 4426.
[11] M. Nowakowska, M. Smouloch, D. Sendor, J. Inclusion Phenom. Macrocycl. Chem. 40 (2001) 213.
[12] R.S. Murphy, C. Bohne, Photochem. Photobiol 71 (1) (2000) 35.
[13] M.V. Rekharsky, Y. Inoue, Chem. Rev. 98 (1998) 1875.
[14] P.K. Zarzycky, H. Lamparczyk, J. Pharm. Biomed. Anal. 18 (1998) 165.
[15] V. Zia, R.A. Rajewski, V.J. Stella, Pharm. Res. 17 (2000) 936.
[16] R. Reinhardt, M. Richter, P. Mager, Carbohydr. Res. 291 (1996) 1.
[17] P. Fini, M. Castagnolo, J. Therm. Anal. Cal. 66 (2001) 91.
[18] L.E. Briggner, I. Wadso, J. Biochem. Biophys. Meth. 22 (1991) 101.
[19] Y. Dotsikas, E. Kontopanou, C. Allagiannis, Y.L. Loukas, J. Pharm. Biomed. Anal. 23 (2000) 997.
[20] V.K. Indirapriyadharshini, P. Karunanithi, P. Ramamurthy, Langmuir 17 (2001) 4056.
[21] P. Fini, F. Longobardi, L. Catucci, P. Cosma, A. Agostiano, Bioelectrochemistry, in press.
[22] Y.L. Loukas, J. Pharm. Biomed. Anal. 16 (1997) 275.
[23] S. Nigam, G. Durocher, J. Phys. Chem. 100 (1996) 7135.
[24] M. Eftink, R. Biltonen, in: A.E. Beezer (Ed.), Biological Microcalorimetry, Academic Press, London, 1980, pp. 343–412.
[25] G. Castronuovo, V. Elia, F. Velleca, G. Viscardi, Thermochim. Acta 292 (1997) 31.
[26] G. Castronuovo, V. Elia, D. Fessas, A. Giordano, F. Velleca, Carbohydr. Res. 272 (1995) 31.
[27] R.A. Rajewski, V.J. Stella, J. Pharm. Sci. 85 (1996) 1142.