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A SIMPLE MODEL FOR THE BEHAVIOR OF MULTISITE RECEPTORS IN PHOTOCHEMICAL PROCESSES

by

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ABSTRACT

A previously established treatment for photochemical processes in the presence of one receptor has been extended for the case of solutions containing mixtures of two (or more) receptors. The treatment gives the variations of emission intensities and Stern-Volmer constants when the concentrations of receptors are changed. The quenching of 1-pyrene-carboxaldehyde by iodide in mixtures of β- and 2-hydroxypropyl- β-cyclodextrins has been used to check the new treatment. These mixtures are of three classes. Mixtures of class i) are a model of a receptor containing two kinds of binding sites with different affinities towards a ligand. The second class of mixtures, ii), are models for multisite receptors containing only one type of sites. These sites however can change their affinity for a given ligand as a consequence of external stimuli, such as a change in pH. Finally, mixtures of class iii) would be representative of polymers with two different binding sites in which the occupation of one of them produces, through a configurational change, the appearance of the previously hidden second class of sites.

KEYWORDS

Restricted geometry conditions, Photochemical Reaction, Pseudophase Model, Cyclodextrins and their Mixtures

INTRODUCTION

Biological receptors are generally complex molecules which are characterized by different types of binding modes. Thus, in the case of DNA, electrostatic, major groove, minor groove and intercalative binding have been described.[1-5] These different binding modes are in some cases specific for some ligands. In other cases, the same ligand can bind through different binding modes (different binding sites). Thus, the binding of surfactants to proteins starts when the surfactant monomers bind some high-affinity sites, placed on the protein structure, followed by the occupation of other sites of lower affinity.[6, 7] The complex character of the binding to biological receptors is increased by the fact that the union of a given ligand to a biological receptor can induce structural changes in the receptor. Thus, binding of inorganic ions to DNA in solution induces a change from random coil to helix conformation[8] and binding of the surfactant produces compactation of DNA to a globular state.[9] Of course, this modifies the characteristics of the biological molecule as receptor.[10, 11] Moreover, cooperativity effects are frequent in the case of biological systems.[12-15] These effects obviously change the characteristics of the binding sites, increasing or decreasing the affinity of the ligand by the receptor[16] (positive or negative cooperativity).

The above mentioned facts imply that the analysis of binding in the case of biological receptors is rather complex, in such a way that the different contributions to the binding of a given ligand are difficult to separate in a quantitative way. Consequently, it would be of interest to find simple models of biological receptors that permit us to know what are the consequences of the different factors (competition of different binding sites for the ligand, cooperativity and conformational changes of the receptors) on the binding. This, of course, implies the use of polytopic receptors (or, alternatively, models of these receptors) in which only one of the factors is present.

In this paper, as a first step in this direction, we propose a simple model of a receptor containing several binding sites, characterized by different affinities towards a given ligand. This model is constituted by a mixture of cyclodextrins that show different affinities to a ligand. We have used binary mixtures of cyclodextrins, that would correspond to a receptor with two different binding sites. As the cyclodextrin molecules in solution are separate entities the problem of configurational change of the receptor after binding is absent. Moreover we selected a ligand that forms a 1:1 complex with the two cyclodextrins employed in the study, in such a way that there are no cooperativity effects.

The mixtures of cyclodextrins were prepared using β-cyclodextrin (β-CD) and 2-hydroxypropyl- β-cyclodextrin (β-HCD). As ligand pyrene-1-carboxaldehyde (PyCHO) was used. Different mixtures of cyclodextrins were employed: i) mixtures in which the two receptors are at equimolecular concentrations, ii) mixtures in which [β-CD] + [β-HCD] is maintained as a constant and iii) mixtures containing a given concentration of β-CD and different β-HCD concentrations.

Mixtures of class i) are a model of a receptor containing two kinds of binding sites with different affinities towards a ligand. These sites, indeed, are separated enough so as to avoid interactions between the ligands bound to them, in such a way that cooperativity effects are absent. The second class of mixtures, ii), are models for multisite receptors containing only one type of sites. These sites however can change their affinity for a given ligand as a consequence of an external stimuli, such as a change in the pH. Thus, two populations of binding sites would appear as a consequence of the stimuli. In the case of a pH change, for example, protonated and unprotonated binding sites. Finally, mixtures of class iii) would be representative of

polymers with two different binding sites in which the occupation of one of them produces, through a configurational change, the appearance of the second class of sites, previously hidden.[7] Obviously, this work constitutes a first step in the analysis of complex systems.

Our studies have been developed through static fluorescence measurements and quenching processes of pyrene-1-carboxaldehyde, the ligand. As quencher iodide ion was employed.

An additional point of interest of our studies is the experimental verification of a treatment of quenching processes under restricted geometry conditions.[17, 18] This treatment has been extended here for mixtures of receptors (see Supporting Information).

RESULTS AND DISCUSSION

a) Solutions containing only one type of receptor

For these solutions the binding constant, K, of the fluorophore to the receptor present in the solutions were obtained from the emission intensities employing the equation:[19]

$$(I_{em})_0 = \frac{(I_{em})_{0f} + (I_{em})_{0i} K_i [CD_i]}{1 + K_i [CD_i]} \qquad (CD_i = \text{receptor present in the solution}) \quad (i = 1,2) \qquad (1)$$

In this equation $(I_{em})_0$ is the observed emission intensity in the presence of a given concentration of receptor, $[CD_i]$, and in the absence of the quencher. $(I_{em})_{0f}$ is the emission intensity of non-bounded fluorophore. $(I_{em})_{0i}$ (i=1,2) is the emission intensity at a concentration of receptor high enough to produce complete binding of the

fluorophore to the receptor. The binding constant corresponds to the equilibrium (in the ground state):

$$R_f + CD_i \underset{}{\overset{K_i}{\rightleftharpoons}} CD_i/R \qquad (CD_i/R = R_i) \quad (i=1, 2) \qquad (2)$$

Figure 1 gives a plot of the normalized emission intensities when the concentrations of CD_i change. As can be seen equation 1 fits the data well. From these fits the values of K_i were obtained. These values were K_1= 835±100 mol^{-1} dm^3 (for β-cyclodextrin) and K_2= 1550±120 mol^{-1} dm^3 (for hydroxypropyl-β-cyclodextrin). The values of $(I_{em})_{0f}$ and $(I_{em})_{0i}$ (i= 1,2) are given in Table 1.

On the other hand, Stern-Volmer constants were obtained from the equation:[20]

$$(K_{SV})_{obs} = \frac{\frac{(I_{em})_0 - (I_{em})_Q}{(I_{em})_Q}}{[Q]} \qquad (3)$$

$(I_{em})_Q$ being the emission intensity for a given quencher concentration, [Q].

The observed Stern-Volmer constants can be described through an equation similar to equation 1:[17,18]

$$(K_{SV})_{obs} = \frac{(K_{SV})_f + (K_{SV})_i (K_{app})_i [CD_i]}{1 + (K_{app})_i [CD_i]} \qquad (i = 1,2) \qquad (4)$$

In this equation $(K_{app})_i$ (i= 1,2) are different from the K_i appearing in equation 1, but are related to them:

$$(K_{app})_i = K_i \frac{a_i}{a_f} \qquad \text{(a)}$$

$$(i = 1,2) \qquad (5)$$

$$\frac{a_i}{a_f} = \frac{\varphi_i}{\varphi_f} \frac{\varepsilon_i}{\varepsilon_f} \qquad \text{(b)}$$

In equation 5b φ_i is the quantum yield of the fluorophore bound to receptor i, in the presence of the quencher and ε_i its molar extinction coefficient. φ_f and ε_f have the same meaning for the free fluorophore. It can easily be shown that[18]

$$\frac{a_i}{a_f} = \frac{(I_{em})_i}{(I_{em})_f} \qquad (i = 1,2) \qquad (6)$$

$(I_{em})_i$ and $(I_{em})_f$ being the emission intensities of the fluorophore bound to receptor i and free, respectively, *in the presence of the quencher*. Of course, $(I_{em})_f$ is an experimental datum and $(I_{em})_i$ can be obtained from the fit of emission data, in the presence of the quencher, to an equation similar to equation 1. The values of a_i/a_f for the receptors used here, as well as the values of $(K_{SV})_f$ and $(K_{SV})_i$ appear in Table 1. Using these values and equation 4 one can calculate $(K_{SV})_{obs}$. The results of this calculation are compared to the experimental values in Figure 2. This figure gives support to the treatment in references 17 and 18.

It is important to realize that K_{app} is neither the binding constant for the ground state of the fluorophore nor the binding constant of the excited state (see reference 17).

b) Solutions containing two receptors

In solutions containing the two cyclodextrins, in the absence of the quencher, the emission intensity is given by (see Supporting Information):

$$(I_{em})_0 = \frac{(I_{em})_{0f} + (I_{em})_{01} K_1[CD_1] + (I_{em})_{02} K_2[CD_2]}{1 + K_1[CD_1] + K_2[CD_2]} \qquad (7)$$

This equation, in fact, fits well the emission data for all the mixtures of the receptors employed in this work. The value of K_1 and K_2 obtained from the fit are,

respectively, 820±170 and 1230±230 mol^{-1} dm^3, in good agreement with the values obtained in solutions containing only one of the cyclodextrins.

On the other hand, it can be shown (see Supporting Information) that the observed Stern-Volmer constants (equation 3) are given by:

$$(K_{SV})_{obs} = \frac{(K_{SV})_f + (K_{SV})_1(K_{app})_1[CD_1] + (K_{SV})_2(K_{app})_2[CD_2]}{1 + (K_{app})_1[CD_1] + (K_{SV})_2[CD_2]} \quad (8)$$

where $(K_{app})_i$ (i=1,2) are given by equations 5.

The values of $(K_{SV})_{obs}$ calculated with this equation, employing the data in Table 1 are in a good agreement with the experimental values of this parameter, as can be seen in Figure 3. This figure, consequently, gives support to the treatment presented in the Supporting Information.

Now, the three types of the mixtures of receptors employed in this work will be considered in detail. These different mixtures were presented in the introduction of the paper and can be used as model of more complex receptors containing two (or more) types of binding sites.

Mixtures i) contain equimolar quantities of the two receptors, that is, $[CD_1]= [CD_2]= [CD]$. According to this, equation 7 can be put as:

$$(I_{em})_0 = \frac{(I_{em})_{0f} + ((I_{em})_{01}K_1 + (I_{em})_{02}K_2)[CD]}{1 + (K_1 + K_2)[CD]} \quad (9)$$

If one defines:

$$K = K_1 + K_2 \quad \text{(a)}$$

$$(I_{em})_{0b} = \frac{(I_{em})_{01}K_1 + (I_{em})_{02}K_2}{K} \quad \text{(b)} \quad (10)$$

equation 9 becomes

$$(I_{em})_0 = \frac{(I_{em})_{0f} + (I_{em})_{0b} K[CD]}{1 + K[CD]} \quad (11)$$

Figure 4 represents the fit of experimental data of mixtures of class i to equation 11. According to this result, a receptor containing two different types of binding sites cannot be distinguished, employing only data of emission intensity, from a receptor containing only one kind of binding site. This is so, because equation 11 is formally identical to equation 1 which corresponds on only one type of receptor. On the other hand, data in Table 2 corresponding to $(I_{em})_{0b}$ and K confirms the validity of equations 10a and 10b.

As to $(K_{SV})_{obs}$, it is easy to show that, in this kind of mixture, this parameter is given by:

$$(K_{SV})_{obs} = \frac{(K_{SV})_f + (K_{SV})_b K_{app}[CD]}{1 + K_{app}[CD]} \quad (12)$$

where

$$K_{app} = \left(\frac{a_{b1}}{a_{f1}}\right) K_1 + \left(\frac{a_{b2}}{a_{f2}}\right) K_2 \quad (13)$$

and

$$(K_{SV})_b = \frac{(K_{SV})_1 \left(\frac{a_{b1}}{a_{f1}}\right) K_1 + (K_{SV})_2 \left(\frac{a_{b2}}{a_{f2}}\right) K_2}{\left(\frac{a_{b1}}{a_{f1}}\right) K_1 + \left(\frac{a_{b2}}{a_{f2}}\right) K_2} \quad (14)$$

Equation 12 implies that quenching data ($(K_{SV})_{obs}$) does not permit us to know if the receptor contains more than one binding site because equations 12 and 4 are formally identical.

Figure 5 gives the fit of experimental data to equation 12 for mixtures of class i. On the other hand, in Table 2 a comparison of parameters ($(K_{SV})_b$ and K^*_{app}) in equation 12 appears. The *experimental* values of these parameters are obtained from the previously mentioned fit and the calculated values come from equations 13 and 14 and data in Table 1. These results confirm previous calculations.

Mixtures ii) contain CD_1 and CD_2 but, in this case $[CD_1]+[CD_2]$ is a constant, that is, $[CD_1]+[CD_2]=[CD]$. In this case, in the absence of the quencher:

$$(I_{em})_0 = \frac{(I_{em})_{0f} + (I_{em})_{01} K_1[CD_1] + (I_{em})_{02} K_2([CD] - [CD_1])}{1 + K_1[CD_1] + K_2([CD] - [CD_1])} \quad (15)$$

This equation can be written as:

$$(I_{em})_0 = \frac{(I'_{em})_{0f} + (I_{em})_{0b} K[CD_1]}{1 + K[CD_1]} \quad (16)$$

with

$$(I'_{em})_{0f} = \frac{(I_{em})_{0f} + (I_{em})_{02} K_2[CD]}{1 + K_2[CD]} \quad (a)$$

$$(I_{em})_{0b} = \frac{(I_{em})_{01} K_1 - (I_{em})_{02} K_2}{K_1 - K_2} \quad (b) \quad (17)$$

$$K = \frac{K_1 - K_2}{1 + K_2[CD]} \quad (c)$$

Figure 6 gives the fit of $(I_{em})_0$ to equation 16 for this kind of mixtures. The values of $(I'_{em})_{0f}$, $(I_{em})_{0b}$ and K obtained from this fit are given in Table 3. In this table also appear the calculated values of these parameters, from equations 17 and data in Table 1. As can be seen in the table, there is agreement between calculated and experimental parameters, giving support to the equations of the model.

As to $(K_{SV})_{obs}$ one can obtain:

$$(K_{SV})_{obs} = \frac{(K'_{SV})_f + (K_{SV})_b K_{app}[CD_1]}{1 + K_{app}[CD_1]} \tag{18}$$

where:

$$(K'_{SV})_f = \frac{(K_{SV})_f + (K_{SV})_2\left(\frac{a_{b2}}{a_{f2}}\right)K_2[CD]}{1+\left(\frac{a_{b2}}{a_{f2}}\right)K_2[CD]} \qquad \text{(a)}$$

$$(K_{SV})_b = \frac{(K_{SV})_1\left(\frac{a_{b1}}{a_{f1}}\right)K_1 - (K_{SV})_2\left(\frac{a_{b2}}{a_{f2}}\right)K_2}{\left(\frac{a_{b1}}{a_{f1}}\right)K_1 - \left(\frac{a_{b2}}{a_{f2}}\right)K_2} \qquad \text{(b)} \tag{19}$$

$$K_{app} = \frac{\left(\frac{a_{b1}}{a_{f1}}\right)K_1 - \left(\frac{a_{b2}}{a_{f2}}\right)K_2}{1+\left(\frac{a_{b2}}{a_{f2}}\right)K_2[CD]} \qquad \text{(c)}$$

Again in this case the experimental data, $(K_{SV})_{obs}$, are in agreement with the equations of the model, as can be seen in Figure 7. On the other hand, the values of experimental and calculated parameters are in reasonable agreement, as can be seen in Table 3. The difference of $(K_{app})_{exp}$ and $(K_{app})_{calc}$ comes from the fact that the latter is very dependent of the values of K_i employed in the calculation.

Finally, mixtures of class iii) will be considered. The mixtures as stated in the Introduction section contained a fixed concentration of one of the cyclodextrins (β-CD) and variable concentrations of the other (β-HCD). The results obtained for these mixtures ($(K_{SV})_{obs}$) are given in Figure 8. In this figure, the first part of the curve (up to the point marked with an arrow) corresponds to zero concentration of β-HCD and a variable concentration of β-CD. The portion of the curve after the arrow corresponds to a fixed concentration of β-CD, whereas the concentration of β-HCD is increased. Figure 8A represents the behavior that one would expect on intuitive grounds: the two cyclodextrins apparently produce additive effects, decreasing $(K_{SV})_{obs}$. However, this additive character disappears in the data given in Figures 8B and 8C: in the case of Figure 8B, the addition of β-HCD has no effect on $(K_{SV})_{obs}$, and in the case of Figure 8C the addition of β-HCD produces an increase $(K_{SV})_{obs}$ in spite of the fact that this cyclodextrin produces a decrease of K_{SV} when it is the only receptor in the medium.

The above mentioned results can be explained again making use of equation 8: first of all, it is interesting to note that the limiting values of $(K_{SV})_{obs}$ correspond in all cases to $(K_{SV})_2$, that is, the values of K_{SV} when all the fluorophore is bound to β-HCD. This result follows from equation 8 because this equation predicts $(K_{SV})_{obs}= (K_{SV})_2$ when the condition $(K_{app})_2[CD_2]>> (K_{app})_1[CD_1]$ holds. In other words: if the concentration of β-CD in the arrow has a value that produces $(K_{SV})_{obs}>(K_{SV})_2$ (Figure 8A), addition of β-HCD will decrease $(K_{SV})_{obs}$. If the concentration of β-CD produces $(K_{SV})_{obs}=(K_{SV})_2$ (Figure 8B) no effect of β-HCD will be observed. Finally when the concentration of β-CD has a value such that $(K_{SV})_{obs}<(K_{SV})_2$ (Figure 8C), addition of β-HCD will produce an increase of $(K_{SV})_{obs}$.

The results obtained with the mixtures of class iii show that the effect of the appearance of some binding sites in a receptor, when other sites are previously occupied, depends on the occupation fraction of the first sites when the occupancy of second sites starts: if the second sites appear to a level of occupation of the first sites that produces $(K_{SV})_{obs}>(K_{SV})_2$, a decrease of $(K_{SV})_{obs}$ will be observed. If the second sites appear when the fraction of the first sites occupied produces $(K_{SV})_{obs}=(K_{SV})_2$, no changes in $(K_{SV})_{obs}$ would be observed (and this could be, erroneously, interpreted as a saturation of the first class of binding sites). Finally, if the second sites appear when the level occupation of the first sites implies $(K_{SV})_{obs}<(K_{SV})_2$, $(K_{SV})_{obs}$ would increase, and this could be interpreted, erroneously, as the consequence of a release of the ligand to the medium.

EXPERIMENTAL

Materials

Pyrene-1-carboxaldehyde meeting USP specifications, β– and hydroxypropyl-β-cyclodextrin were from Aldrich. tert-Butanol and NaI were from Sigma-Aldrich.

Materials were used as received since preliminary experiments with purified materials showed that purification does not produce any changes in the experimental results.

Purified water used in the preparation of solutions was obtained from a Millipore Milli-Q water system. Its conductivity was less than 10^{-6} S m^{-1}.

Stock solutions of pyrene-1-carboxaldehyde were prepared by dissolving weighed amounts of the solid in tert-butanol. Working solutions were prepared by dilution with water to reach the desired concentration of the aldehyde ($5\cdot10^{-7}$ mol dm^{-3}).

The solutions always contained tert-butanol at 1% v/v. The quencher concentration (NaI) was maintained at 0.1 mol dm^{-3}.

Steady state fluorescence measurements

Fluorescence measurements were carried out in a PTI Fluorescence Master Systems spectrofluorimeter, equipped with LPS-220B Xe arc lamp light, interfaced to a PC for reading and handling of the spectra. These spectra were obtained in the range of 380 to 600 nm. The spectral slits for excitation and emission were 0.5 and 2 nm, respectively. The excitation wavelength was established as 356 nm. No magic angle polarizer conditions were employed.

All the measurements were carried out at 298.2 K.

CONCLUSIONS

We have developed a treatment that gives I_{em} and $(K_{SV})_{obs}$ in mixtures of receptors. This treatment has been checked for three different classes of mixtures. The first type would correspond to a complex receptor with two kind of independent binding sites each having a different affinity for the ligand and without cooperative effects. It has been shown that behavior of the complex receptor cannot be distinguished from behavior of a simple receptor using independent intensities of emission or quenching data. The second class of mixtures would correspond (for example) to a complex receptor with protonable sites in such a way that in changing the pH the proportion of the two sites (protonated and unprotonated) changes. Under these circumstances the behavior of the systems when the concentration of the sites changes can be described by equations 16 and 18, which are formally identical to the equations corresponding to classical two-state models, but with parameters having a different meaning than in these models (see equations 17).

The third class of mixtures shows that the effects of different receptors (or different binding sites on the same receptor) are not additive.

In this paper verification of equations 7 and 8 has been carried out using only two receptors. It is clear that generalization of these equations to three or more receptors is straightforward.

Finally, it is worth mentioning that the model presented is still valid for the case in which the concentration of free receptor was not close to the total concentration. In this case, however, the equations become formally more complex.

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Bibliographic references

1 A. Boggioni, Thesis, Pisa, 2008.

2 B. Pullman, *Adv. Drug Res.*. 1989, **18**, 1.

3 X. Gao, P. Mirau and D. J. Patel, *J. Mol. Biol.*. 1992, **223**, 259.

4 M. J. Hannon, *Chem. Soc. Rev.*, 2007, **36**, 280.

5 H. S. Schwartz, *Adv. Cancer Chemother.*, 1979, **1**, 1.

6 N. J. Turro, X.-G. Lei, K. P. Ananthapadmanabhan and M. Aronson, *Langmuir*, 1995, **11**, 2525.

7 E. L. Gelamo, R. Itri, A. Alonso, J. V. Da Silva and M. Tabak, *J. Colloid Interface Sci.*, 2004, **277**, 471.

8 Z.-J. Tan and S.-J. Chen, *Biophys. J.*, 2006, **90**, 1175.

9 H. Nakanishi, K. Tsuchiya, T. Okubo, H. Sakai and M. Abe, *Langmuir*, 2007, **23**, 345.

10 E. Grueso, F. Sanchez, V. I. Martin, E. García-Fernández and R. Prado-Gotor, *Chem. Phys.*, 2008, **352**, 306.

11 E. Grueso, E. Roldan and F. Sanchez, *J. Phys. Chem. B*, 2009, **113**, 8319.

12 S. M. Mel'nikov, V. G. Sergeyev and K. Yoshikawa, *J. Am. Chem. Soc.* 1995, **117**, 9951.

13 T. Lopes-Costa, F. Sanchez and P. Lopez-Cornejo, *J. Phys. Chem. B*, 2009, **113**, 9373.

14 M. Rahimian, Y. Miao and W. D. Wilson, *J. Phys. Chem. B*, 2008, **112**, 8770.

15 V. Gabelica, R. Maeda, T. Fujimoto, H. Yaku, T. Murashima, N. Sugimoto and D. Miyoshi, *Biochemistry.*, 2013, **52**, 5620.

16 J. D. Mc Ghee and P. H. von Hippel, *J. Mol. Biol.*, 1974, **86**, 469.

17 M. Marchena and F. Sanchez, *Prog. React. Kinet. Mech.*, 2010, **35**, 27.

18 E. Bernal, B. Sarrion, A. Barrios, P. Perez, A. Jimenez, F. Sanchez and M. Lopez-Lopez, *J. Photochem. Photobiol. A: Chem*, 2014, **278**, 25.

19 G. Nelson and I. M. Warner, *J. Phys. Chem.*, 1990, **94**, 576.

20 D. Meisel, M. S. Matheson and J. Rabani, *J. Am. Chem. Soc.*, 1978, **100**, 117.

FIGURES CAPTIONS

Figure 1.- Normalized emission intensities of 1-pyrene-carboxaldehyde in the presence of different concentrations of β-CD (●) and β-HCD (○). Symbols are the experimental data and the curves are the best fit to eq. 1.

Figure 2.- Plot of the values of $(K_{SV})_{obs}$ calculated using equation 4 and the parameters in Table 1 *vs.* the experimental $(K_{SV})_{obs}$ for the quenching of 1-pyrene-carboxaldehyde by the iodide ion at different concentrations of β-CD (●) and β-HCD (○).

Figure 3.- Plot of the values of $(K_{SV})_{obs}$ calculated using equation 8 and the parameters in Table 1 *vs.* the experimental $(K_{SV})_{obs}$ for the quenching of 1-pyrene-carboxaldehyde by the iodide ion at different mixtures of β-CD and β-HCD.

Figure 4.- Plot of normalized emission intensities of 1-pyrene-carboxaldehyde in the absence of a quencher in solutions containing mixtures of class i. Points correspond to experimental data and the curve is the best fit to eq. 11.

Figure 5.- Plot of $(K_{SV})_{obs}$ for the quenching of 1-pyrene-carboxaldehyde by the iodide ion ($[I^-]$= 0.1 mol dm^{-3}) *vs.* [CD] in several mixtures of class i. The curve is the best fit of experimental data to equation 12.

Figure 6.- Normalized emission intensities of 1-pyrene-carboxaldehyde in solutions containing mixtures of class ii *vs*. [β-CD]. The curve corresponds to the best fit to equation 16.

Figure 7.- Plot of $(K_{SV})_{obs}$ for the quenching of 1-pyrene-carboxaldehyde by the iodide ion ($[I^-]$= 0.1 mol dm^{-3}) *vs.* [β-CD] in mixtures of class ii. The curve corresponds to the best fit to equation 18.

Figure 8.- Plot of $(K_{SV})_{obs}$ for the quenching of 1-pyrene-carboxaldehyde by the iodide ion ($[I^-]$= 0.1 mol dm^{-3}) *vs.* $[CD]_T$ ($[CD]_T$= [β-CD]+[β-HCD]) in several mixtures of class iii. Points are experimental data and the curves are the best fit to equation 8. (The different types of mixtures of class iii (A, B and C) are described in the text). Insert corresponds to an enlargement of the lower part of the figure.

Table 1.- Values $(I_{em})_f$, $(I_{em})_i$ and K of 1-pyrene-carboxaldehyde in absence of quencher and of $(I_{em})_f$, $(I_{em})_i$, $(K_{SV})_f$, $(K_{SV})_i$ and a_i/a_f corresponding to the photochemical reaction between 1-pyrene-carboxaldehyde and iodide ion ($[I^-]$= 0.1 mol dm^{-3}) in the presence of β-CD and β-HCD in aqueous solution containing tBuOH 1%(v/v).

	No quencher			$[I^-]$= 0.1 mol dm^{-3}				
	$(I_{em})_{0f}$	$(I_{em})_{0i}$	K_i/mol^{-1} dm^3	$(I_{em})_f$	$(I_{em})_i$	$(K_{SV})_f$ /mol^{-1} dm^3	$(K_{SV})_i$ /mol^{-1} dm^3	a_i/a_f
β-CD	1.000	0.688	835	0.129	0.780	68	≈ 0	6
H-β-CD	1.000	0.677	1550	0.129	0.258	68	16	2

Table 2.- Calculated and experimental values of $(I_{em})_{0b}$ and K of 1-pyrene-carboxaldehyde in absence of quencher and K_{app} and $(K_{SV})_b$ corresponding to the photochemical reaction between 1-pyrene-carboxaldehyde and iodide ion ($[I^-]$= 0.1 mol dm^{-3}) in the presence of mixtures class i.

$(I_{em})_{0b\ calc}$[a]	$(I_{em})_{0b\ exp}$[b]	K_{calc}[c]/$mol^{-1}dm^3$	K_{exp}[b]/$mol^{-1}dm^3$	$(K_{app})^{d}_{calc}$ /$mol^{-1}dm^3$	$(K_{app})^{e}_{exp}$ /$mol^{-1}dm^3$	$(K_{SV})_{b\ calc}$[f] /$mol^{-1}dm^3$	$(K_{SV})_{b\ exp}$[e] /$mol^{-1}dm^3$
0.681	0.682	2400	2100	8100	7400	6.1	4.6

[a]Calculated from equation 10b and data in Table 1.[b]Obtained from the fit of data in Figure 4 to equation 11.[c]Calculated from equation 10a and data in Table 1.[d]Calculated from equation 13 and data in Table 1.[e]Obtained from the fit of data in Figure 5 to equation 12.[f]Calculated from equation 14 and data in Table 1.

Table 3.- Calculated and experimental values of $(I_{em}')_{0f}$, $(I_{em})_b$ and K of 1-pyrene-carboxaldehyde in absence of quencher and $(K_{SV}')_f$, $(K_{SV})_b$ and K_{app} corresponding to the photochemical reaction between 1-pyrene-carboxaldehyde and iodide ion ($[I^-]$= 0.1 mol dm^{-3}) in the presence of mixtures class ii.

$(I_{em}')_{0f\,calc}$ [a]	$(I_{em}')_{0f\,exp}$ [b]	$(I_{em})_{0bcalc}$ [c]	$(I_{em})_{0bexp}$ [b]	K_{calc} [d] /$mol^{-1}dm^3$	K_{exp} [b] /$mol^{-1}dm^3$	$(K_{SV}')_{f\,calc}$ [e] /$mol^{-1}dm^3$	$(K_{SV}')_{f\,exp}$ [f] /$mol^{-1}dm^3$	$(K_{SV})_{b\,calc}$ [g] /$mol^{-1}dm^3$	$(K_{SV})_{b\,exp}$ [f] /$mol^{-1}dm^3$	$(K_{app})_{calc}$ [h] /$mol^{-1}dm^3$	$(K_{app})_{exp}$ [f] /$mol^{-1}dm^3$
0.691	0.696	0.664	0.658	-31.5	-32.5	17.2	17.7	-25.5	-17.5	43.7*	73.4*

[a]Calculated from equation 17a and data in Table 1.[b]Obtained from the fit of data in Figure 6 to equation 16.[c]Calculated from equation 17b and data in Table 1.[d]Calculated from equation 17c and data in Table 1. [e]Calculated from equation 19a and data in Table 1.[f]Obtained from the fit of data in Figure 7 to equation 18.[g]Calculated from equation 19b and data in Table 1.[h]Calculated from equation 19c and data in Table 1.

*The difference obtained in the values of $(K_{app})_{calc}$ and $(K_{app})_{exp}$ is the consequence of this parameter being very sensitive to the values of K used in the calculation.