Spectroscopic, photophysical and thermodynamic studies of inclusion complexes of β-cyclodextrin and 2-hydroxypropyl-β-cyclodextrin with 10-methylbenzophenothiazine

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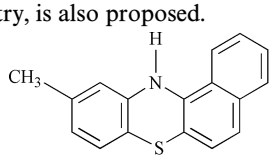
The inclusion complexes of 10-methyl-12*H*-benzo[*a*]phenothiazine (10-MeBPHT) with β-cyclodextrin (β-CD) and with 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) were investigated by electronic absorption and fluorescence spectroscopies in aqueous solutions. 10-MeBPHT fluorescence quantum yields were significantly larger in the presence of HP-β-CD ($\Phi_F = 0.097 \pm 0.02$) and β-CD ($\Phi_F = 0.025 \pm 0.004$) than in water ($\Phi_F = 0.004\,35 \pm 0.000\,07$). A 1 : 1 stoichiometry was found for these CD complexes and formation constants (K_f, evaluated by means of the Benesi–Hildebrand treatment) ranged from 14 ± 7 to 50 ± 11 M^{-1} (at 20 °C), depending on the nature of the CD. In the case of the 10-MeBPHT : HP-β-CD complex, the complexation reaction was endothermic ($\Delta H^0 = 12.4$ kJ mol^{-1}) and presented a positive entropy value ($\Delta S^0 = 75$ J mol^{-1} K^{-1}). AM1 semiempirical calculations of the 10-MeBPHT geometry indicate a partial inclusion of this molecule in HP-β-CD, in agreement with our experimental results.

Phenothiazines are planar heterocyclic compounds that have considerable biological and pharmacological interest because of their use as tranquillizing, antidepressant and psychotropic drugs.[1,2] Some phenothiazine derivatives have been studied for their photochemotherapeutic abilities against carcinoma[3] and other important biological and chemical effects.[4,5] Recently, the electronic and therapeutic properties of benzophenothiazine (BPHT) derivatives have received considerable attention.[6–10] Also, their photophysical and photochemical characteristics have been widely investigated.[11–14] However, because of their strong hydrophobicity, BPHTs are generally poorly soluble and unstable in aqueous media, which constitutes an important problem for physicochemical and biological studies. Moreover, from the analytical standpoint, BPHTs fluoresce weakly in aqueous solutions and, therefore, are barely detectable by fluorimetry.

In the last decade, organized media including cyclodextrins (CDs) and micellar solutions have been the subject of a number of studies. Indeed, these media are able to solubilize hydrophobic compounds in water and to modify significantly some physicochemical and biological properties of active molecules, owing to their capacity to form supramolecular systems.[15–18] In this respect, CDs constitute a good example of organized assemblies that can selectively include one or several organic molecules in their apolar and hydrophobic cavity, leading to the formation of inclusion complexes. The interactions with CDs may increase or decrease the absorbance and generally increase the fluorescence intensity of the included organic moiety.[15–17] The complexation of neutral organic species with CDs is mainly governed by geometric factors. Recently, our research group has demonstrated the existence of unsubstituted BPHT : 2-hydroxypropyl-β-CD (HP-β-CD) inclusion complexes with a 1 : 1 stoichiometry, yielding significant fluorescence enhancements.[13,14] In a preliminary communication,[19] we have also reported similar findings in the case of methylBPHT derivatives.

The goal of this paper is, first, to investigate by electronic absorption and fluorescence spectroscopies the hydrophobic interactions occurring between 10-methyl-12*H*-benzophenothiazine (10-MeBPHT) and two cyclodextrins (HP-β-CD and β-CD) in aqueous media. Secondly, stoichiometric and thermodynamic characteristics of the inclusion complexes, including a temperature effect on the complex formation constants, are determined. A molecular structure of the inclusion complexes, based on AM1 semiempirical calculations of the 10-MeBPHT geometry, is also proposed.



10-MeBPHT

Experimental

Reagents and instrumentation

High purity β-CD and HP-β-CD were purchased from Aldrich and were used as received. The synthesis of 10-methyl-12*H*-benzo[*a*]phenothiazine was performed as previously reported[8,9]. Ethanol (spectroscopy grade, Merck) and distilled water were utilized as solvents.

Absorption spectra were recorded on a Perkin–Elmer Lambda 2 spectrometer, using 1 cm quartz cuvettes at 20 ± 0.1 °C. Fluorescence spectra were obtained on a Perkin–

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Elmer LS-5 luminescence spectrometer equipped with a pulsed xenon lamp and interfaced to a Sony Trinitron CPD-1420E microcomputer. Fluorescence quantum yields (Φ_F) were measured on an SLM Aminco Bowman Series 2 Bioritech spectrofluorimeter. The excitation and emission bandwidths were 10 nm. All fluorescence measurements were carried out in 1 cm quartz cuvettes at different temperatures [5, 20, 35 and 45 $\pm$ 0.1 °C], using a cell holder with a thermostatically controlled Bioblock Model 18205 water bath.

Procedures

10^{-3} M 10-MeBPHT stock solutions were freshly prepared in ethanol and kept in the dark to avoid photooxidation and degradation. They were used for preparing 10^{-5} M 10-MeBPHT diluted solutions for spectroscopic studies. Stock solutions of cyclodextrins (10^{-1} M HP-β-CD and 2×10^{-2} M β-CD) were also prepared in distilled water. The general procedure was to add in the cuvette 30 μl of the 10-MeBPHT ethanolic stock solution to 3 ml of the CD initial solution or to 3 ml of pure ethanol or distilled water. Thus, whatever medium used, a 10^{-5} M concentration of 10-MeBPHT and concentrations of CD practically equal to those of the stock solutions were obtained in all cases. For all working aqueous solutions, the solvent ratio was 99 : 1 (water–ethanol, v/v).

The 10-MeBPHT fluorescence quantum yields were determined by using a 10^{-5} M quinine sulfate dihydrate aqueous solution in 0.05 M H_2SO_4 as a standard ($\phi_f = 0.55$)[20] and exciting at 340 nm the 10^{-5} M 10-MeBPHT solutions. Φ_F values were determined using the following simplified equation:

$$\Phi_{F,x} = \Phi_{F,r} \frac{A_r D_x}{A_x D_r} \quad (1)$$

where $\Phi_{F,x}$ and $\Phi_{F,r}$ are the fluorescence quantum yields of the analyte and the standard, respectively, A_x and A_r are the absorbances of the analyte and the standard solutions, respectively, and D_x and D_r are the integrated area of the emission fluorescence spectra, corrected for the solvent blank, for the analyte and the standard solutions, respectively.

Quantum mechanical calculations

Quantum mechanical calculations have been performed using Hyperchem® software (Hypercube, Inc., Waterloo, Ontario, Canada). Optimized geometry and dipole moment of 10-MeBPHT were calculated by choosing the semi-empirical AM1 method and the Polak–Ribiere optimization algorithm.

Results and discussion

Spectral properties

Electronic absorption spectra. UV-Visible absorption spectra of 10-MeBPHT were recorded in aqueous solutions of 10^{-1} M HP-β-CD, 10^{-2} M β-CD, ethanol and water (Table 1). As can be seen, 10-MeBPHT exhibits three main bands in the 221–223, 274–282 and 321–324 nm regions. The molar absorption coefficients (ε_{max}) values are larger than 10^4 M^{-1} cm^{-1} for the two short wavelength bands, which indicates that the corresponding electronic transitions are of the π–π* type. In the case of the high-wavelength band, ε_{max} values are higher than 10^3 M^{-1} cm^{-1}, suggesting a partial recuperation of the *n*–π* transitions by π–π* ones. The ε_{max} values do not vary significantly in ethanol and the CD media, and the shapes of the absorption spectra are alike in these media. This behavior indicates that the 10-MeBPHT electronic transitions are similar. However, the 10-MeBPHT spectra in cyclodextrins show a blue shift of the π–π* absorption band, upon going from water (282 nm) to cyclodextrins (274–275 nm), and a better resolution in CDs of the 324 nm *n*–π* band, which appears only as a shoulder in water (Fig. 1). The observed blue shift of the π–π* absorption band may be due to the decrease in polarity of the CD cavity relative to water. It is also interesting to stress that the ε_{max} values of the 221 and 275 nm bands increase in ethanol, β-CD and HP-β-CD relative to water. The ε_{max} increases are more significant in HP-β-CD than in β-CD.

Fluorescence spectra. Fluorescence excitation and emission spectra of 10-MeBPHT obtained in ethanol, and in water in the absence and the presence of HP-β-CD or β-CD are shown in Fig. 2. The excitation spectra are characterized by a strong band located at 276–279 nm and another one at about 340 nm; these spectral features are similar in the various media under study and resemble the long-wavelength region of the

Table 1 Electronic absorption and fluorescence spectral characteristics of 10-MeBPHT[a] in different media under study

Media	Absorption λ/nm ($\varepsilon_{max}/10^4$ M^{-1} cm^{-1})	Fluorescence λ_{ex}/nm	Fluorescence λ_{em}/nm	Stokes shift[b] /cm^{-1}	ϕ_F[c]
Water	222 (2.9) 255sh 282 (2.2) 321sh (1.2)	278sh 340sh	502 527sh	10435	0.004 35 $\pm$ 0.000 07
EtOH	221 (5.6) 258 (2.7) 276 (3.6) 323 (0.7)	276 341	500 519sh	9640	0.091 $\pm$ 0.002
β-CD (10^{-2} M)[d]	222 (4.0) 258sh 274 (2.4) 323 (0.7)	279 340	502sh 528	10799	0.025 $\pm$ 0.004
HP-β-CD (10^{-1} M)[d]	223 (4.7) 259sh 275 (3.0) 324 (0.5)	278 340	501sh 524	9839	0.097 $\pm$ 0.002

[a] 10-MeBPHT concentration is 10^{-5} M. [b] Stokes shift: $\bar{\nu}_{em} - \bar{\nu}_{ex}$. [c] Quantum yields measured at $\lambda_{ex} = 340$ nm relative to a 0.05 M H_2SO_4 quinine sulfate dihydrate aqueous solution ($\phi_f = 0.55$).[19] [d] Solvent: water–ethanol (99 : 1 v/v).

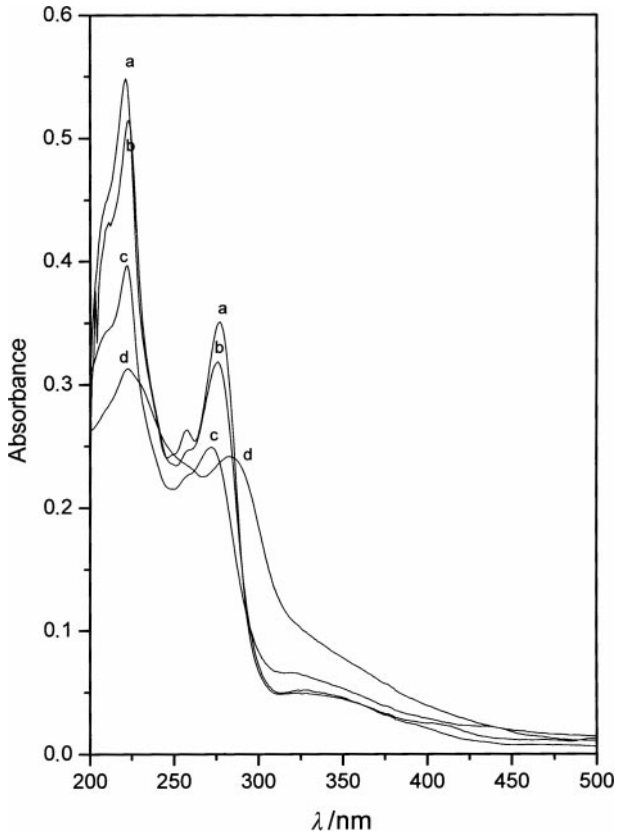

Fig. 1 10^{-5} M 10-MeBPHT electronic absorption spectra in (a) ethanol, (b) 10^{-1} M HP-β-CD water–ethanol [99 : 1 v/v (WE)] mixture, (c) 10^{-2} M β-CD WE mixture, (d) WE mixture.

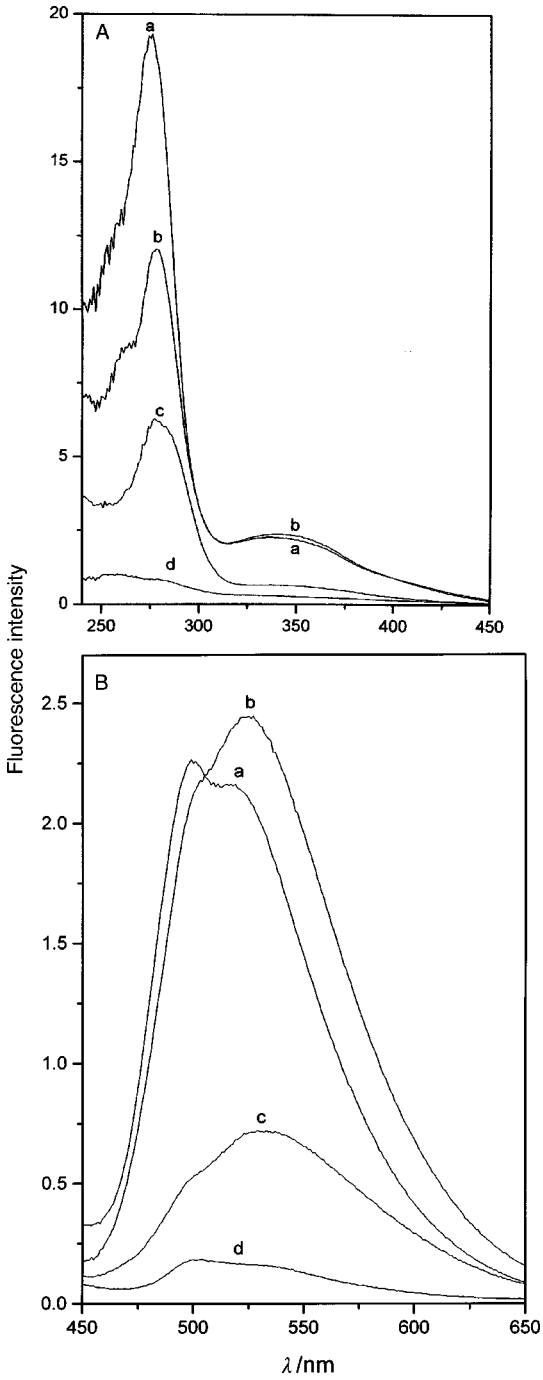

Fig. 2 Fluorescence excitation (A) and emission (B) spectra of 10^{-5} M 10-MeBPHT in (a) ethanol, (b) 10^{-1} M HP-β-CD WE mixture, (c) 10^{-2} M β-CD WE mixture, (d) WE mixture.

absorption spectra, which indicates that the excited molecular species are identical to the absorbing ones. The emission spectra exhibit a broad, structureless band, located at 502 nm in water, 500 nm in ethanol, 528 nm in β-CD and 524 nm in HP-β-CD media. Pronounced shoulders also appear between 501 and 527 nm in the various media. The shapes of the excitation and emission spectra are essentially similar in ethanol, water and CD media. However, as can be seen, red shifts ($\Delta\lambda \approx 22$–26 nm) of the emission maximum are observed in β-CD and HP-β-CD relative to water (Table 1). Comparable weak excitation or emission spectral shifts from water to CDs have also been reported in the case of unsubstituted phenothiazine,[13] Azure A[17] and several types of aromatic molecules.[21,22] These observations are in agreement with the formation of an inclusion complex between 10-MeBPHT and the CD, which would diminish the 10-MeBPHT singlet excited state energy.

The fluorescence quantum yields of 10-MeBPHT in HP-β-CD and in β-CD are, respectively, about 22 times and 6 times larger than in water, and a 4-fold increase is observed in HP-β-CD relative to β-CD (Table 1). This variation relative to water confirms the lower polarity of the cyclodextrin cavity as compared to water.[23]

Effect of the cyclodextrin concentration

We investigated the effect of increasing the HP-β-CD and β-CD concentrations on 10-MeBPHT UV-visible absorption and fluorescence spectra in the range 0–10^{-1} M for HP-β-CD and 0–10^{-2} M for β-CD. The absorbances and fluorescence intensities of 10-MeBPHT were enhanced progressively upon adding cyclodextrins. No significant spectral change was noted when increasing the CD concentration, except for a progressive displacement of the emission maximum from 508 to 520 nm for CD concentrations larger than 5×10^{-3} M (Fig. 3). This behavior could indicate formation of more than one host : guest complex. The absence of an isosbestic point in the UV absorption titration experiments further supports this interpretation.

When plotting 10-MeBPHT absorbance or fluorescence (F) intensity (at $\lambda_{em} = 524$ nm), *vs.* CD concentration, levelling off curves were obtained. In the case of *F vs.* [HP-β-CD] curves, a plateau region was reached for HP-β-CD concentrations higher than 10^{-1} M, indicating that a great majority of 10-MeBPHT molecules are complexed in the ground state (Fig. 4). In contrast, for *F* (at $\lambda_{em} = 528$ nm) *vs.* [β-CD] curves, no plateau region was observed because of the lower β-CD solubility limit, which occurs at about 1.5×10^{-2} M in water. All

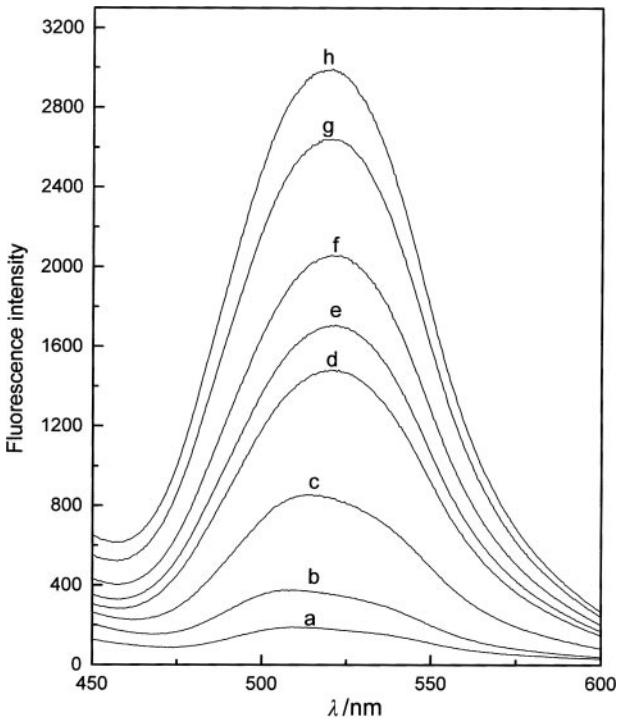

Fig. 3 Variation of the 10^{-5} M 10-MeBPHT fluorescence emission spectra with increasing [HP-β-CD]: (a) 0, (b) 1, (c) 5, (d) 10, (e) 20, (f) 30, (g) 70, (h) 100×10^{-3} M.

these observations suggest the formation of inclusion complexes of 10-MeBPHT with cyclodextrins.

Stoichiometry and association constants of the inclusion complexes

The stoichiometry and association constants of the inclusion complexes were calculated by means of the methods of Scatchard and Benesi–Hildebrand.[24,25] Assuming a 1 : 1 stoichiometry ratio, the equilibrium is given by:

$$[\mathrm{CD}] + [\mathrm{MeBPHT}] \rightleftharpoons [\mathrm{CD{:}\,MeBPHT}] \qquad (2)$$

The formation constant of the complex (K_f) is expressed by:

$$K_f = \frac{[\mathrm{CD{:}\,MeBPHT}]}{[\mathrm{CD}] \cdot [\mathrm{MeBPHT}]} \qquad (3)$$

where [CD], [MeBPHT] and [CD: MeBPHT] are the corresponding equilibrium concentrations of these species, respectively.

The relation between the increase of the 10-MeBPHT fluorescence intensity and the CD concentration can be represented by the following equation in the case of a 1 : 1 stoichiometry:[25]

$$\frac{1}{F - F_o} = \frac{1}{F_\infty - F_o} + \frac{1}{(F_\infty - F_o) \cdot K_f \cdot [\mathrm{CD}]_o} \qquad (4)$$

If the stoichiometry of the complex is assumed to be 2 : 1, eqn. (5) becomes applicable:

$$\frac{1}{F - F_o} = \frac{1}{F_\infty - F_o} + \frac{1}{(F_\infty - F_o) \cdot K'_f \cdot [\mathrm{CD}]_o^2} \qquad (5)$$

In eqn. (4) and (5), F_o and F_∞ denote, respectively, the fluorescence intensity of 10-MeBPHT, in the absence of CD and when all fluorophore molecules are essentially complexed with CD. F is the measured fluorescence at each CD concentration tested. $[\mathrm{CD}]_o$ is the initial CD concentration, K_f and K'_f are, respectively, the association constants of the complex with 1 : 1 and 2 : 1 stoichiometries. If the stoichiometry is 1 : 1, a linear plot of $1/(F - F_o)$ *vs.* $1/[\mathrm{CD}]_o$ should be obtained. In the case that a 2 : 1 stoichiometry is predominant, a plot of $1/(F - F_o)$ *vs.* $1/[\mathrm{CD}]_o^2$ should give a straight line. In both cases, the linear plot can be used to obtain K_f or K'_f by simply dividing the intercept by the slope.

However, although the representation of $1/F - F_o$ *vs.* $1/[\mathrm{CD}]_o$ or *vs.* $1/[\mathrm{CD}]_o^2$, known as a double-reciprocal plot,[24,25] allows one to establish the stoichiometry of the inclusion complex, the use of Benesi–Hildebrand plots to determine K_f or K'_f gives only estimated values of the association constants. Indeed, in this approach, more emphasis is placed on the lower CD concentration values than on the higher ones, and the data are not weighted properly.[23,26,27] A better estimation of K_f can be made by using nonlinear regression (NLR) analysis.[17,23] The rearrangement of data leads to a direct relationship between the measured fluorescence intensity (F) and $[\mathrm{CD}]_o$:

$$F = F_o + \frac{(F_\infty - F_o) \cdot (K_f) \cdot [\mathrm{CD}]_o}{1 + K_f \cdot [\mathrm{CD}]_o} \qquad (6)$$

Typical linear double-reciprocal plots were obtained from absorption and fluorescence data when eqn. (4) was applied ($r = 0.99$), indicating a 1 : 1 stoichiometry for the inclusion complexes of 10-MeBPHT with β-CD and HP-β-CD (Fig. 5, curve A). In contrast, a downward curve was observed, when these data were fitted to a 2 : 1 complex, using eqn. (5) (Fig. 5, curve B). This curved plot means that simultaneous formation of a 2 : 1 host: guest complex is not taking place. However, the sequential formation of 1 : 1 plus 2 : 1 complexes, with both complexes occurring at the equilibrium, could also give a nonlinear plot.

The K_f values were determined by an iterative solution of the NLR analysis [eqn. (6)]. The initial parameters were estimated from the linear plot. We have summarized, in Table 2, the K_f values, determined by using the absorption and fluorescence data. At 20 °C, they range from 14 to 50 M^{-1}, depend-

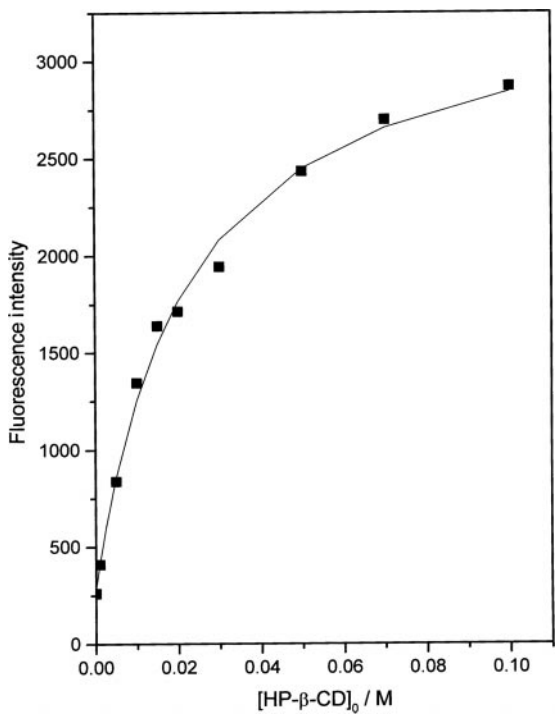

Fig. 4 Influence of HP-β-CD concentration on the fluorescence intensity (at $\lambda_{em} = 524$ nm) of a 10^{-5} M 10-MeBPHT aqueous solution. The solid line was calculated by applying eqn. (6), assuming a 1 : 1 stoichiometry and using F_∞ and K_f values obtained from a nonlinear regression analysis.

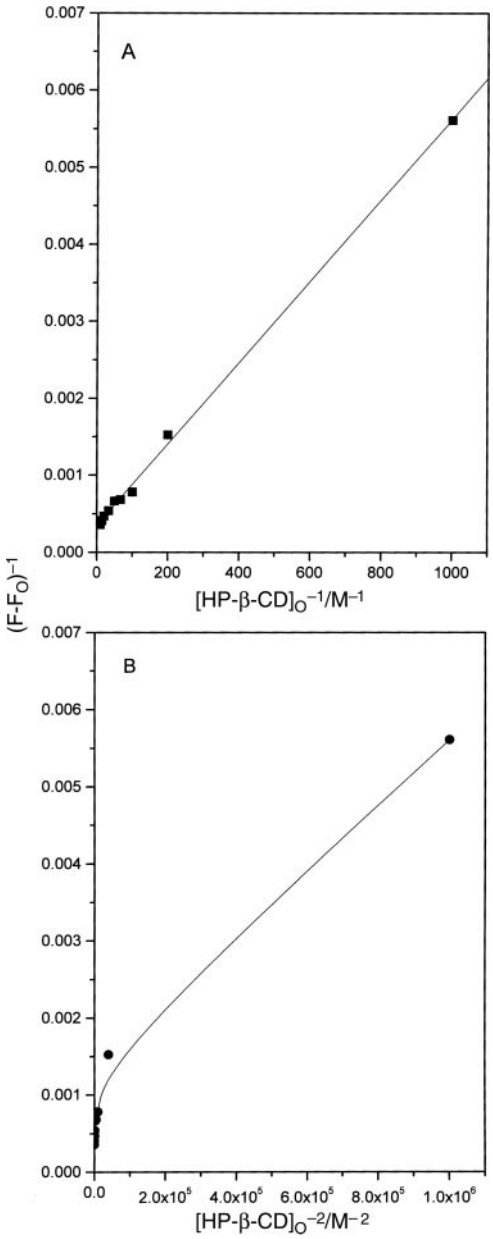

Fig. 5 Double-reciprocal plots of the fluorescence, measured at $\lambda_{em} = 524$ nm, of the inclusion complex of 10-MeBPHT (10^{-5} M) with varying [HP-β-CD]. (A) Data plotted using eqn. (4) and assuming a 1 : 1 10-MeBPHT : HP-β-CD stoichiometry. (B) Data plotted using eqn. (5) and assuming a 1 : 2 10-MeBPHT : HP-β-CD stoichiometry.

Table 2 Association constants for the complexation reaction of 10-MeBPHT[a] in cyclodextrin media determined by UV-visible and fluorescence methods at 20 °C

Complex	Method	K_f/M^{-1}
10-MeBPHT : β-CD[b]	Fluorescence	14 ± 7
10-MeBPHT : HP-β-CD[c]	Fluorescence	50 ± 11
10-MeBPHT : HP-β-CD[c]	Abs UV-vis	43 ± 17

[a] [10-MeBPHT] = 5×10^{-5} M for absorbance measurements and 10^{-5} M for fluorescence measurements. [b] In H_2O–EtOH (99 : 1 v/v) in the presence of β-CD concentrations varying between 0–1.5×10^{-2} M; $\lambda_{ex} = 340$ nm and $\lambda_{em} = 528$ nm. Absorbance of the solution was 0.057 at $\lambda_{ex} = 340$ nm. [c] In H_2O–EtOH (99 : 1 v/v) in the presence of HP-β-CD concentrations varying between 0–10^{-1} M; $\lambda_{ex} = 340$ nm and $\lambda_{em} = 524$ nm. Absorbance of the solution was 0.0525 at $\lambda_{ex} = 340$ nm.

ing on the nature of the CD; both sets of spectral data give similar K_f values, which validates our results. In the case of HP-β-CD, these K_f values are about two times smaller than those found for unsubstituted benzophenothiazine (100 ± 20 M^{-1}).[13] In agreement with other literature results[16,28] on the complexing ability of CDs with a variety of organic compounds, the 10-MeBPHT inclusion complexes are more difficult to form with β-CD ($K_1 = 14 \pm 7\,M^{-1}$) than with HP-β-CD ($K_1 = 50 \pm 11\ M^{-1}$).

Temperature effects

We studied the temperature effects on the 10-MeBPHT : HP-β-CD inclusion complex fluorescence spectra and the thermodynamic characteristics in the 5–45 °C range. No significant change was found in the structure and position of the emission fluorescence spectra upon varying the temperature, whereas a moderate increment of fluorescence intensity was observed upon increasing the temperature. This latter effect suggests that the formation of the inclusion complex is favored at higher temperatures. Indeed, the inclusion complex formation constant values increased from 40 to 76 M^{-1} between 5 and 45 °C (Table 3).

To evaluate the thermodynamic parameters of the complexes, we plotted ln K_f *vs.* $1/T$. A linear relationship was obtained (Fig. 6), indicating that the Vant' Hoff equation is obeyed. The value of the formation enthalpy (ΔH^0) obtained for the

Table 3 Temperature effect on K_f for the complexation reaction of 10-MeBPHT and 10^{-1} M HP-β-CD

T/°C	K_f/M^{-1} [a]
5	40 ± 7
20	50 ± 11
35	66 ± 9
45	76 ± 18

[a] K_f values obtained by fluorescence spectroscopy.

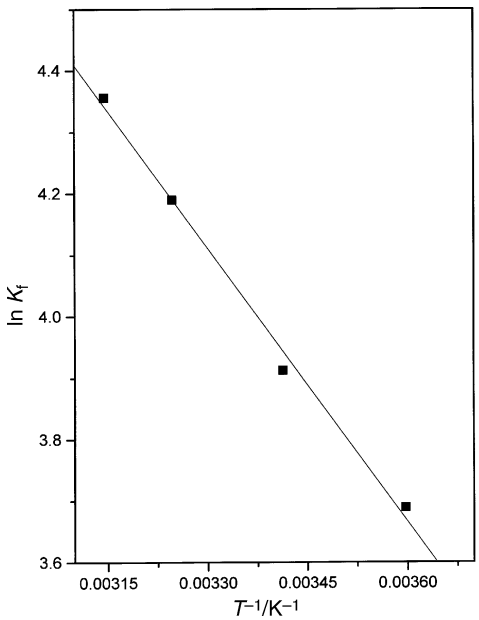

Fig. 6 Vant' Hoff plot of ln K_f *vs.* $1/T$ for the 10-MeBPHT : HP-β-CD inclusion complex.

complex of 10-MeBPHT with HP-β-CD was 12.40 ± 0.08 kJ mol^{-1}, which shows that the complexation process is endothermic. The large positive value of the formation entropy ($\Delta S^0 = 75.0 \pm 0.3$ J mol^{-1} K^{-1}) means that there is an important increase of the disorder during the complexation reaction, resulting from the contribution of water molecules to the process. Indeed, it can be expected that 10-MeBPHT hydrophobic molecules are surrounded by water molecules, forming a kind of structured solvent cage. When the 10-MeBPHT molecules become included in the relatively hydrophobic HP-β-CD cavity, these structured cages would be destroyed; as a consequence, the water molecules would have more freedom, leading to a positive value of ΔS^0. However, the Gibbs free energy value at 298 K is negative ($\Delta G^0 = -10.1$ kJ mol^{-1}), indicating that the formation of the inclusion complexes is energetically favorable.

In most cases, the formation of cyclodextrin inclusion complexes implies negative entropy and enthalpy changes, which can be attributed to stabilization of the complexes by hydrogen bonds and guest–cyclodextrin dipole–dipole interactions which are more important than solvent effects.[23,29,30] However, positive entropy values have also been reported for the inclusion complexes found between β-CD and some anilinonaphthalene sulfonates,[30] and between β-CDs and nabumetone, a naphthalenyl-butanone derivative.[31] In the latter cases, as well as in that of 10-MeBPHT, the "linear" shape of the molecules allows for a stronger, more ordered solvent cage, when the molecules are free in aqueous solutions. Upon complexation, this solvent shell is broken up, producing an entropy increase that offsets the entropy loss corresponding to the complexation itself.[30]

Proposed structure of the 10-MeBPHT: HP-β-CD inclusion complex

We propose a molecular structure for the 10-MeBPHT: HP-β-CD inclusion complex, based on AM1 semiempirical calculations and geometrical considerations relative to the size of both species and the way in which 10-MeBPHT is included in the cyclodextrin cavity. The HP-β-CD cavity dimensions are similar to those of β-CD (length 7.9 Å and internal diameter 7.8 Å).[15,32] The Hyperchem program was used to optimize the most stable form of the 10-MeBPHT molecule. In the AM1 method, the molecules are considered to be isolated and without solvent molecules. It allows the calculation of the values of the interatomic distances in 10-MeBPHT, and the deduction of the most probable modes of approach of an incoming 10-MeBPHT molecule to a HP-β-CD molecule.

The calculated molecular structure of 10-MeBPHT is shown in Fig. 7. The calculated dimensions of 10-MeBPHT (interatomic distances, bond lengths, bond and torsion angles) are given in Table 4. Because of its length [$r(H_{20}–H_{28}) = 11.4$ Å], 10-MeBPHT cannot be included equatorially in HP-β-CD. The only possible modes of approach are axial, which should be thermodynamically favored orientations. However, even in the axial inclusion, 10-MeBPHT would be only partially included in the HP-β-CD cavity, since its longitudinal dimension is larger than the cavity length. Similar remarks were made in the case of inclusion complexes formed between β-CDs and 1-substituted naphthalene,[30] phenothiazine derivatives[33] and unsubstituted benzophenothiazine.[13] In fact, from the calculated geometrical dimensions of 10-MeBPHT there are two possible, different moieties for inclusion in the HP-β-CD cavity: one being the naphthalene moiety [$r(H_{26}–H_{24}) = 5.6$ Å] and the another the benzenic ring linked to the heteroatoms N and S [$r(H_{21}–H_{22}) = 5.0$ Å] (Fig. 7). We must point out that 2 Å, corresponding to the radii of two hydrogen atoms, should be added to each interatomic distance reported in Table 4, in order to estimate more accurately the steric hindrance of the different parts of the 10-MeBHT molecule. The interatomic distances cited above would clearly be in favor of the inclusion in the cavity of the second moiety (heteroatom-linked benzo group).

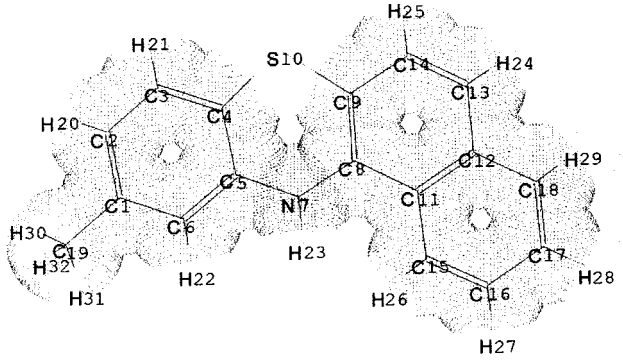

Fig. 7 Calculated molecular structure of 10-MeBPHT.

Two additional factors should also be taken in consideration, the effects of the Van der Waals radii of the 10-methyl group and the nonplanar shape of the 10-MeBPHT molecule. Indeed, owing to its lateral position, the 10-methyl group should provoke a steric inhibition to the inclusion process, while achieving maximum contact with the apolar HP-β-CD cavity. As a matter of fact, the 10-MeBPHT formation constant value is significantly lower ($K_f = 50 \pm 11$ M^{-1}) than that of unsubstituted BPHT ($K_f = 100 \pm 20$ M^{-1})[13] confirming that the presence of the methyl group hinders and slows down the complexation process. On the other hand, the "butterfly wing" shape of the 10-MeBPHT molecule, indicated by the $C_6–C_5–N_7–C_8$ torsion angle calculated value of 158.3°, should not impede inclusion on both sides, but the

Table 4 Geometrical dimensions of 10-methyl BPHT[a]

Interatomic distance[b]/Å		Bond length[b]/Å		Bond angle[b]/°		Torsion angle[b]/°	
		N7–C5	1.408	C4–S10–C9	102.8	H32–C19–C1–C6	84.7
H20···H28	11.408	N7–C8	1.406	C5–N7–C8	121.8	H31–C19–C1–C6	−34.9
H20···H22	4.317	S10–C4	1.695	C5–N7–H23	113.8	C4–C5–N7–C8	−24.7
H21···H22	4.990	S10–C9	1.696	C8–N7–H23	115.5	C5–N7–C8–C9	24.4
H26···H29	4.993	N7–H23	0.998			C9–S10–C4–C5	15.0
H26···H24	5.598	C8–C11	1.445			C1–C2–C3–C4	−0.9
H26···H25	6.019	C5–C6	1.410			C1–C6–C5–C4	1.3
H23···H22	2.345	C8–C9	1.396			C6–C5–N7–C8	158.3
H23···H26	1.923	C1–C6	1.397			C5–N7–C8–C11	158.7
		C1–C19	1.482			C3–C4–S10–C9	−165.9
		C19–H31	1.118			C3–C4–C5–N7	−176.6
		C17–H28	1.100			C6–C5–C4–S10	179.4

[a] Calculated by the AMI method (see Experimental for details). [b] Atom numbering corresponds to that given in Fig. 7.

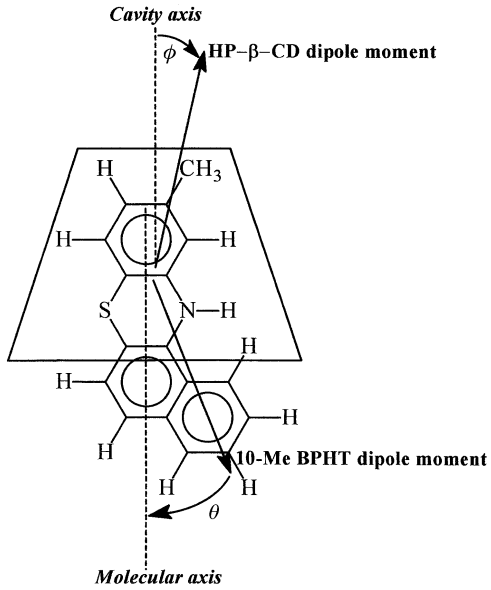

Fig. 8 Geometry of the 10-MeBPHT–HP-β-CD inclusion complex, showing the direction of the respective dipole moment vectors of HP-β-CD and the 10-MeBPHT guest molecule. The direction of the HP-β-CD dipole moment vector, running from the wide, secondary hydroxyl rim, towards the narrower primary hydroxyl rim, is defined by the tilting angle φ relative to the symmetry axis of HP-β-CD. The 10-MeBPHT dipole moment is defined by the tilting angle θ compared to its molecular symmetry axis.

"boomerang" type curvature due to the difference of the dihedral torsion angles at N and S (C_3–C_4–S_{10}–C_9 = −165.9° and C_6–C_5–N_7–C_8 = 158.3°, respectively), should also make inclusion more difficult on the naphthalene side.

Finally, we computed the ground state dipole moment (μg) of 10-MeBPHT and found a value of 2.05 D, in good agreement with a previously reported value [2.72 D, calculated by a Pariser–Parr–Pople (π-LCI-SCF-MO) method].[12] In the inclusion complex, the 10-MeBPHT dipole should take a nearly antiparallel orientation relative to the HP-β-CD dipole, in order to maximize the dipole–dipole attractions, as shown in computational studies of cyclodextrins.[34] Fig. 8 presents a simplified drawing for the molecular modelling of the expected inclusion complex structure. In particular, this dipole configuration in the modelled inclusion complex also sustains the aforementioned insertion of 10-MeBPHT by the heteroatom-linked benzo group side in the HP-β-CD cavity.

Conclusion

The study of the electronic absorption and fluorescence spectral features of 10-MeBPHT in CD aqueous solutions as well as the significant increase of the absorbance and fluorescence intensity values with CD concentration clearly demonstrate the formation of inclusion complexes with CDs. Typically, the fluorescence quantum yields of 10-MeBPHT are enhanced by 22 and 6 times in HP-β-CD and in β-CD, respectively, relative to water. Also, using the Scatchard and Benesi–Hildebrand treatments, we found a 1 : 1 stoichiometry for the inclusion complexes and association constants ranging from 14 to 50 M^{-1} at 20 °C, depending on the CD. These K_f values are significantly smaller than those previously reported for unsubstituted BPHT,[13] indicating that the presence of a methyl group hinders the complexation process. The AM1-based model proposed for the structure of the 10-MeBPHT: HP-β-CD complex supports the above conclusions. Moreover, our AM1 calculations allow us to conclude that 10-MeBPHT is included partially along the molecular axis. Geometrical considerations and dipole moment calculations indicate that the insertion of 10-MeBPHT in the HP-β-CD cavity takes place very probably on the heteroatom-linked benzo group side. As shown by the strong positive entropy value obtained for complexation, the inclusion process, which involves important dipole–dipole interactions, is also characterized by a disruption of the solvent cage surrounding the 10-MeBPHT molecules.

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