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# Spectrofluorimetric Study of an Inclusion Complex of 2-Hydroxypropyl-β-Cyclodextrin with the Antitumour 11-Methyl Benzophenothiazine. Analytical Application to Urine

MAME DIABOU GAYE SEYE[1,2], C. PARRAIN[2], J.J. AARON[2,*] and N. MOTOHASHI[3]
[1]*Département de Chimie, Faculté des Sciences et Techniques, Université Cheikh Anta Diop, Dakar, Sénégal;* [2]*Interfaces, Traitement Organisation et Dynamique des Systèmes (CNRS UMR 7086), Université Paris-7-Denis Diderot, 1, rue Guy de la Brosse, 75005, Paris, France;* [3]*Department of Medicinal Chemistry, Meiji College of Pharmacy, 1-22-1 Yato-Gho, Tanashi-Shi, 188, Tokyo, Japan*

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**Abstract**

The electronic absorption and fluorescence spectral properties of 11-methyl-12H-benzo[a]phenothiazine (11-MeBPHT) were investigated in various media (water, ethanol, β-cyclodextrin (β-CD) and 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) aqueous solutions). Fluorescence quantum yields were respectively about 20 and 2 times larger in HP-β-CD and β-CD than in water. The formation of a 1:1 stoichiometry inclusion complex between 11-MeBPHT and HP-β-CD (association constant $K_f = 118 \pm 3\ M^{-1}$ at 20 °C) was studied in aqueous medium by fluorescence spectroscopy. Analytical figures of merit were satisfactory for 11-MeBPHT with linear dynamic ranges over at least two orders of magnitude and limits of detection (LODs) between 0.2 and 1 ng/ml according to the medium. An analytical application to the determination of 11-MeBPHT in human urine samples by the standard addition procedure led to satisfactory recovery percentages (91–108%).

## Introduction

Phenothiazines are widely used as antidepressant and psychotropic tranquilizing drugs [1, 2] for pharmaceutical preparations. Some phenothiazine derivatives have been investigated for their photochemotherapeutic abilities against carcinoma [3] and others have been used as dyes or indicators [4–6]. A new family of benzo[a]phenothiazines (BPHTs) has been synthetized and has received considerable attention, due to their therapeutic, physicochemical and photophysical properties [7–13]. They have been utilized as pigments for synthetic polymers [14], and some BPHTs are potential antihelmintics [7a] and possess an antiviral activity, inhibiting the multiplication of encephalomyocarditis viruses in tissue cultures [7b]. Also, Motohashi et al. [15–17] have established relationships between the anti-tumour effect of BPHTs and their π-electron density, using quantum mechanical calculations.

BPHTs have a very rigid, unsaturated planar structure, but they are weakly soluble and unstable in aqueous media. Obviously, the latter properties constitute a drawback for the biomedical as well as for the analytical applications. A way to improve the BPHT solubility and stability consists to add cyclodextrins (CDs) as complexing agents to the medium of interest [13, 18–21]. Indeed, CDs constitute a good example of organized assemblies which can include selectively in their apolar and hydrophobic cavity, one or several organic molecules, leading to the formation of inclusion complexes. This type of complexation generally improves the solubility of the included organic compounds and also produces an increase in the fluorescence signal of the included moiety [13, 18–20].

Because of the BPHT potential anti-tumour effects, it is important to establish a convenient analytical methodology for their determination. Several analytical methods, including TLC, HPLC, flow injection analysis, photochemically induced fluorescence and spectrofluorimetry, have been reported for the determination of simple phenothiazines [22–24]. However, until now, practically no analytical study has concerned BPHTs, to the exception of our preliminary results [12].

In this work, we investigated the interactions occurring between 11-methyl-12H-benzo[a]phenothiazine (11-MeBPHT) and β-CDs in aqueous media by electronic absorption and fluorescence spectroscopies. We determined the stoichiometry and formation

* Author for correspondence. E-mail: aaron@paris7.jussieu.fr

constant ($K_f$) of the HP-$\beta$-CD:11-MeBPHT complex. Also, we developed a new CD-enhanced spectrofluorimetric method for the determination of 11-MeBPHT in aqueous medium in the presence of HP-$\beta$-CD. Finally, a standard addition procedure was applied by means of this method for the assay of 11-MeBPHT in fortified urine samples.

## Experimental

### *Reagents*

11-Methyl-12H-benzo[a]phenothiazine was synthesised in our laboratories, as previously described [17, 25]. High purity $\beta$-CD (Acros) and HP-$\beta$-CD (Aldrich) were used as received. Distilled water and ethanol (spectroscopic grade, Merck) were used for the preparation of solutions.

### *Apparatus*

Absorption spectra were recorded on a Perkin-Elmer Lambda 2 Spectrometer, using 1-cm quartz cuvettes at 20 ± 0.1 °C. Fluorescence excitation and emission spectra and fluorescence intensities ($F$) were measured in 1-cm quartz cuvettes at 20 ± 0.1 °C on a Perkin-Elmer LS-5 luminescence spectrometer (equipped with a pulsed xenon lamp and interfaced to a Sony Trinitron CPD-1420 E microcomputer), and on a SLM Aminco Bowman Series 2 Biorritech spectrofluorimeter equipped with a 150 W continuous xenon lamp, interfaced to a GPIB card, and driven by a PC 386 microcomputer. In the case of the Perkin-Elmer LS-5 instrument, the excitation and emission bandwidths were fixed at 10 nm. For the Aminco Bowman spectrofluorimeter, a monochromator scan rate of 8 nm/s was used; data acquisition and analysis were performed using an AB 2 software, version 1.40, running under OS/2 2.0. Fluorescence quantum yields ($\Phi_F$) were determined on the Aminco Bowman spectrofluorimeter.

### *Methods and sample preparation*

Stock solutions of $10^{-3}$ M 11-MeBPHT were prepared in ethanol, and kept in the dark to avoid photodegradation. Working aqueous solutions of $10^{-5}$ M 11-MeBPHT were obtained by serial dilution. Stock solutions of CDs ($10^{-1}$ M HP-$\beta$-CD and $10^{-2}$ M $\beta$-CD) were also prepared in distilled water. The general dilution procedure consisted to directly add in the cuvette 30 $\mu$l of the 11-MeBPHT ethanolic stock solutions to 3 ml of the CD initial solution, pure ethanol or distilled water. Thus, in all cases and whatever the medium used, a $10^{-5}$ M 11-MeBPHT concentration was obtained, and the CD concentrations were practically equal to those of the stock solutions. For all working aqueous solutions, the solvent ratio was 99:1 (water–ethanol, v/v). It was assumed that, for this very low amount of ethanol, no significant competition of ethanol molecules occurred with the 11-MeBPHT inclusion process in the CD cavities [26].

For the HP-$\beta$-CD (or $\beta$-CD) concentration effect studies, several CD solutions of various concentrations were prepared (0–$10^{-2}$ M for $\beta$-CD and 0–$10^{-1}$ M for HP-$\beta$-CD) with a $10^{-5}$ M 11-MeBPHT constant concentration.

The 11-MeBPHT fluorescence quantum yields were determined by using a $10^{-5}$ M quinine sulphate dihydrate aqueous solution in 0.05 M $H_2SO_4$ as a standard ($\Phi_F = 0.55$) [27], and exciting at 340 nm the $10^{-5}$ M 11-MeBPHT solutions. $\Phi_F$ values were determined by means of the following, simplified equation:

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

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

### *Standard addition procedure*

For the determination of 11-MeBPHT in urine, the standard addition procedure was applied. 1 ml of a $10^{-3}$ M 11-MeBPHT ethanol solution and 1 ml of filtrated human urine sample were introduced in a 10-ml flask and completed to the mark with a $10^{-1}$ M HP-$\beta$-CD aqueous solution. Then 30 $\mu$l volumes of the resulting mixture were put in a quartz cuvette and completed to 3 ml with 11-MeBPHT aqueous standard solutions of increasing concentrations (from $5 \times 10^{-8}$ M to $10^{-5}$ M).

## Results and discussion

### *Electronic absorption spectral properties*

The UV–visible absorption spectral properties of $10^{-5}$ M 11-MeBPHT in aqueous solutions of $10^{-1}$ M HP-$\beta$-CD and $10^{-2}$ M $\beta$-CD, ethanol and water are presented in Table 1. It appears two main bands located in the 220–223 nm and 273–281 nm regions, and shoulders between 330 and 347 nm (Figure 1). There is a great similarity between the absorption spectra in ethanol and HP-$\beta$-CD media. Except a 7-nm red shift of the second band in water and in $\beta$-CD relative to the other media, spectral features do not vary significantly with the medium.

The molar absorption coefficient ($\varepsilon_{max}$) values are larger than $10^4$ $M^{-1}$ $cm^{-1}$ for the two main bands located at shorter wavelengths, which indicates that these bands correspond to $\pi$–$\pi^*$ electronic transitions.

*Table 1.* Electronic absorption and fluorescence spectral characteristics of 11-MeBPHT in the various media under study[a]

| Media | Absorption $\lambda$/nm ($\epsilon_{max}/10^4$ $M^{-1}$ $cm^{-1}$) | Fluorescence | | $\Delta\nu$[b]/$cm^{-1}$ | $\Phi_F$[c] |
|---|---|---|---|---|---|
| | | $\lambda_{ex}$/nm | $\lambda_{em}$/nm | | |
| Water | 209sh[d] | 327 | 501 | 15,627 | 0.0024 ± 0.0003 |
| | 220 (2.7) | 380sh[d] | 530sh[d] | | |
| | 265sh[d] | | | | |
| | 281 (2.1) | | | | |
| | 347sh[d] | | | | |
| Ethanol | 207sh[d] | 321 | 497 | 16,509 | 0.025 ± 0.001 |
| | 222 (5.4) | 380sh[d] | 514sh[d] | | |
| | 273 (3.2) | | | | |
| | 347sh[d] | | | | |
| $\beta$-CD $10^{-2}$M[e] | 209sh[d] | 327 | 501sh[d] | 16,790 | 0.0043 ± 0.0002 |
| | 222 (2.9) | 380sh[d] | 532 | | |
| | 265sh[d] | | | | |
| | 281 (2.1) | | | | |
| | 347sh[d] | | | | |
| HP-$\beta$-CD $10^{-1}$M[e] | 211sh[d] | 330 | 504sh[d] | 17,436 | 0.049 ± 0.002 |
| | 223 (4.5) | 380sh[d] | 521 | | |
| | 258sh[d] | | | | |
| | 273 (2.6) | | | | |
| | 347sh[d] | | | | |

[a]11-MeBPHT initial concentration = $10^{-5}$M.
[b]$\Delta\nu$ = Stokes shift = $\nu_{abs}-\nu_{em}$.
[c]$\Phi_F$ = fluorescence quantum yields measured in triplicate at $\lambda_{ex}$ = 340 nm relative to a 0.05 M $H_2SO_4$ quinine sulphate aqueous solution ($\Phi_F$ = 0.55) [27].
[d]sh = shoulder.
[e]In water–ethanol (99:1 v/v).

The shoulders appearing at about 347 nm with much smaller $\epsilon_{max}$ values in all media under study could be attributed to an overlapped band resulting from $n$–$\pi$* electronic transitions.

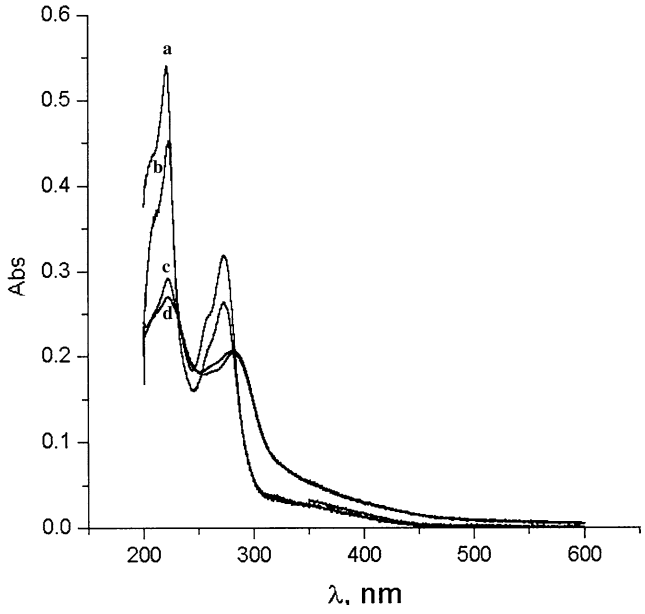


*Figure 1.* Electronic absorption spectra of $10^{-5}$ M 11-MeBPHT in (a) ethanol, (b) $10^{-1}$ M HP-$\beta$-CD water–ethanol (WE) [99:1 v/v] mixture, (c) $10^{-2}$ M $\beta$-CD WE mixture and (d) water.

*Fluorescence spectral properties*

Fluorescence excitation spectra are characterized by two broad bands in the 321–330 nm and 380 nm regions, with similar features in the various media under study. Fluorescence emission spectra display only one broad band located at 497 nm for ethanol, 501 nm for $H_2O$, 532 nm for $\beta$-CD and 522 nm for HP-$\beta$-CD (Figure 2). It appears also shoulders between 501 and 530 nm. The fluorescence maxima are red shifted ($\Delta\lambda$ = 20–31 nm) in HP-$\beta$-CD and in $\beta$-CD relative to water (Table 1). Similar behaviours, concerning the CD effects on the fluorescence spectra, were observed in the case of 10-MeBPHT [13], other phenothiazine derivatives [11b, 20], and aromatic compounds [28].

The Stokes shift ($\nu_{abs}-\nu_{em}$) values obtained for 11-MeBPHT were found to be slightly larger in HP-$\beta$-CD and $\beta$-CD media than in ethanol (Table 1). Using a previously-established Bakhshiev solvatochromic relationship for 11-MeBPHT [10] in which the Stokes shifts were plotted against a solvent polarity function, we substituted in this relationship our Stokes shift values obtained for 11-MeBPHT in 0.01 M $\beta$-CD and 0.1 M HP-$\beta$-CD solutions. We obtained solvent polarity function values for $\beta$-CD and HP-$\beta$-CD, relatively close to that for ethanol, which seems to indicate that the inclusion complexes expected to be formed between 11-MeBPHT and $\beta$-CD and HP-$\beta$-CD present a rather

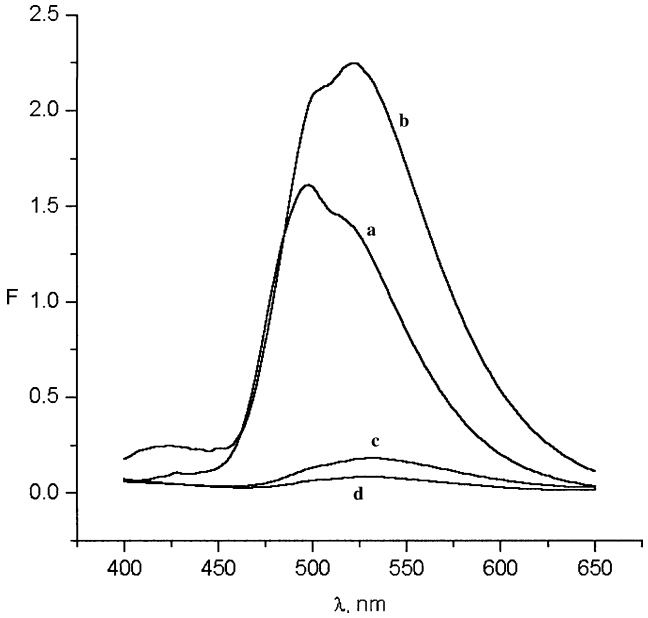


*Figure 2.* Fluorescence emission spectra of 11-MeBPHT in various media: (a) ethanol, (b) $10^{-1}$ M HP-$\beta$-CD WE mixture, (c) $10^{-2}$ M $\beta$-CD WE mixture and (d) water.

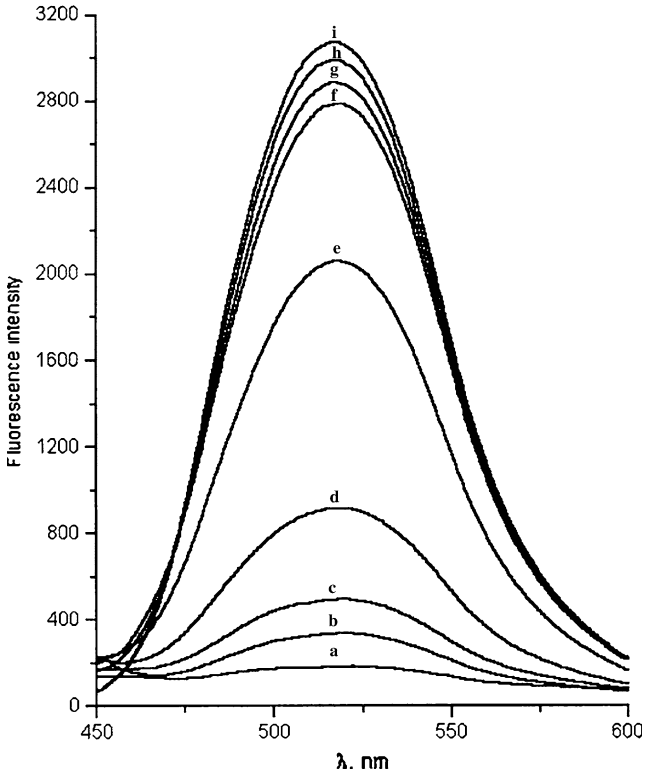


*Figure 3.* Concentration effect of HP-$\beta$-CD on $10^{-5}$ M 11-MeBPHT fluorescence emission spectra: (a) 0 M, (b) $5 \times 10^{-4}$ M, (c) $10^{-3}$ M, (d) $2.5 \times 10^{-3}$ M, (e) $7.5 \times 10^{-3}$ M, (f) $2.5 \times 10^{-2}$ M, (g) $5 \times 10^{-2}$ M, (h) $8 \times 10^{-2}$ M and (i) $10^{-1}$M.

polar character. Our results agree well with some previous literature data suggesting that the $\beta$-CD environment polarity is similar to ethanol and other alcohols, although there are very large discrepancies in the literature on the polar character of the CD cavity, as pointed out by Nigam and Durocher [26].

The fluorescence quantum yield ($\Phi_F$) values of 11-MeBPHT were very weak, ranging between 0.0024 and 0.025 according to the medium (Table 1). Nevertheless, a significant increase was noted upon going from water to CD aqueous solutions and to ethanol. $\Phi_F$ values were respectively about 21 and 2 times larger in HP-$\beta$-CD and $\beta$-CD than in water, in agreement with the lower polarity of the CD cavity as compared to water [26]. These results underline the usefulness of using CD aqueous solutions for the analytical fluorescence studies of 11-MeBPHT in biological media. Moreover, the variation of the order of the 11-MeBPHT $\Phi_F$ values with the nature of CD was similar to that found for 10-MeBPHT in a previous study [13]. Therefore, due to the larger $\Phi_F$ value of 11-MeBPHT in HP-$\beta$-CD than in $\beta$-CD, we selected the former medium for a detailed study of the 11-MeBPHT interactions with CD and of the resulting inclusion complex characteristics.

### *Effect of HP-β-CD concentration*

The effect of HP-$\beta$-CD concentration on the fluorescence emission spectra of a $10^{-5}$ M 11-MeBPHT solution is shown in Figure 3. As can been seen, the fluorescence intensity increased progressively with the HP-$\beta$-CD concentration, but no significant spectral change was noted.

An exponential curve was obtained in the 0–0.1 M HP-$\beta$-CD concentration range, when plotting the 11-MeBPHT fluorescence intensity (recorded at $\lambda_{em} = 521$ nm) vs. HP-$\beta$-CD concentration. The fluorescence intensity increased more steeply at the beginning of the curve and then slowly levelled off at concentration values higher than $2.5 \times 10^{-2}$ M (Figure 4). This fluorescence enhancement behaviour suggests that, in the ground state, a great majority of the 11-MeBPHT molecules have been included inside the HP-$\beta$-CD cavity, forming an inclusion complex.

### *Characteristics of the HP-β-CD inclusion complex*

The stoichiometry of the inclusion complex was calculated by the methods of Scatchard and Benesi-Hildebrand [29, 30]. Assuming a 1:1 stoichiometry ratio, according to the following equilibrium:

$$[\text{HP-}\beta\text{-CD}] + [\text{MeBPHT}] \leftrightarrow [\text{HP-}\beta\text{-CD:MeBPHT}] \quad (2)$$

the formation constant of the complex ($K_f$) is given by Equation (3):

$$K_f = \frac{[\text{HP-}\beta\text{-CD:MeBPHT}]}{[\text{HP-}\beta\text{-CD}][\text{MeBPHT}]} \quad (3)$$

where [HP-$\beta$-CD], [MeBPHT], and [HP-$\beta$-CD:MeBPHT] are, respectively, the corresponding equilibrium concentrations of these species. Taking into account the fact that, because of the very small 11-MeBPHT concentration, the complex concentration can be considered

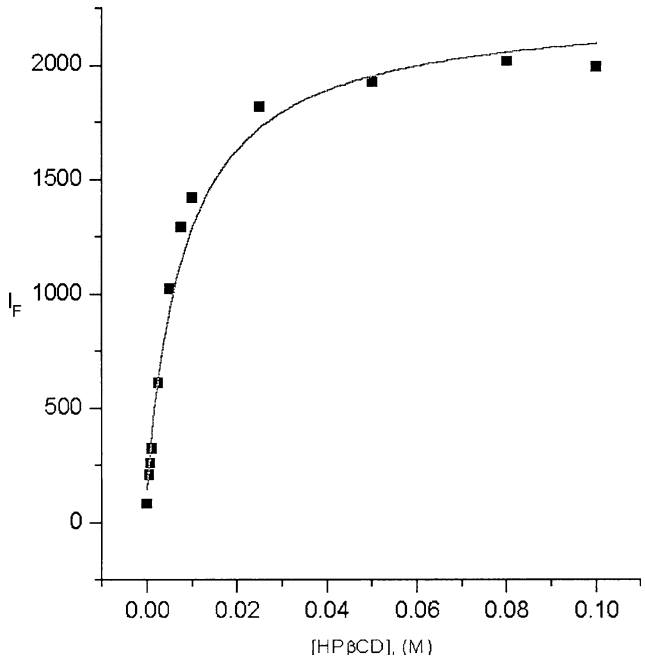


*Figure 4*. Influence of HP-$\beta$-CD concentration on the fluorescence intensity (at $\lambda_{em} = 520$ nm) of a $10^{-5}$ M 11-MeBPHT aqueous solution. The solid line was calculated through the use of Equation (7), assuming a 1:1 stoichiometry and using $F_\infty$ and $K_f$ values obtained from a non-linear regression analysis (Correlation coefficient $r^2 = 0.987$).

as practically negligible compared to the initial HP-$\beta$-CD concentration ([HP-$\beta$-CD]$_0$), the equilibrium HP-$\beta$-CD concentration can be approximated as:

$$[\text{HP-}\beta\text{-CD}] = [\text{HP-}\beta\text{-CD}]_0 - [\text{HP-}\beta\text{-CD:MeBPHT}] \sim [\text{HP-}\beta\text{-CD}]_0 \quad (4)$$

and from the mass balance, it can be written:

$$[\text{MeBPHT}]_0 \sim [\text{MeBPHT}] + [\text{HP-}\beta\text{-CD}][\text{MeBPHT}]$$

where [MeBPHT]$_0$ is the 11-MeBPHT initial concentration. In these conditions, the relation between the increase of 11-MeBPHT fluorescence intensity and the CD concentration can be represented by Equation (5) in the case of a 1:1 stoichiometry.

$$\frac{1}{F - F_0} = \frac{1}{F_\infty - F_0} + \frac{1}{(F_\infty - F_0) \cdot K_f \cdot [\text{HP-}\beta\text{-CD}]_0} \quad (5)$$

where $F_0$ and $F_\infty$ denote, respectively, the 11-MeBPHT fluorescence intensity, in the absence of HP-$\beta$-CD and when all 11-MeBPHT molecules are essentially complexed with HP-$\beta$-CD, $F$ is the measured fluorescence at each HP-$\beta$-CD concentration tested, and the remaining symbols are the same than in Equations (3) and (4).

Assuming that the stoichiometry is 1:1, a linear plot of $1/(F-F_0)$ vs. $1/[\text{HP-}\beta\text{-CD}]_0$ should be obtained. In the case that 2:1 stoichiometry is predominant, the application of equation (6) should give a linear plot.

$$\frac{1}{F - F_0} = \frac{1}{F_\infty - F_0} + \frac{1}{(F_\infty - F_0) \cdot K_f' \cdot [\text{HP-}\beta\text{-CD}]_0^2} \quad (6)$$

where $K_f'$ is the formation constant of the complex with 2:1 stoichiometry, the other symbols remaining the same than in Equation (5).

The representation of $1/F-F_0$ vs. $1/[\text{HP-}\beta\text{-CD}]_0$ or vs. $1/[\text{HP-}\beta\text{-CD}]_0^2$, is known as a double reciprocal plot [29, 30], and allows one to establish the stoichiometry of the inclusion complex. However, the use of the Benesi–Hildebrand plots to determine $K_f$ and $K_f'$ gives only estimated values of the formation constants, because it tends to place more emphasis on the lower HP-$\beta$-CD concentration values than on higher ones, and the data are not weighted properly [26, 31, 32]. Then, the slope of the line is more sensitive to the ordinate value of the point having the smallest concentration. Therefore nonlinear regression analysis (NLR) constitutes an alternative approach to provide a better estimation of $K_f$ [26, 29].

The rearrangement of the data, leads to a direct relationship between the measured fluorescence intensity ($F$) and [HP-$\beta$-CD]$_0$:

$$F = F_0 + \frac{(F_\infty - F_0) \cdot K_f \cdot [\text{HP-}\beta\text{-CD}]_0}{1 + K_f \cdot [\text{HP-}\beta\text{-CD}]_0} \quad (7)$$

A typical linear double reciprocal plot was obtained from fluorescence data when Equation (5) was applied ($r = 0.999$), indicating a 1:1 stoichiometry for the inclusion complex of 11-MeBPHT with HP-$\beta$-CD (Figure 5). In contrast, when the data were fitted to a 2:1 complex, using Equation (6), a downward curve was observed, which confirms that the stoichiometry is not 2:1 (Figure not shown).

NLR analysis provided an evaluation of $K_f$ by fitting the fluorescence data through iteration (Equation 7).

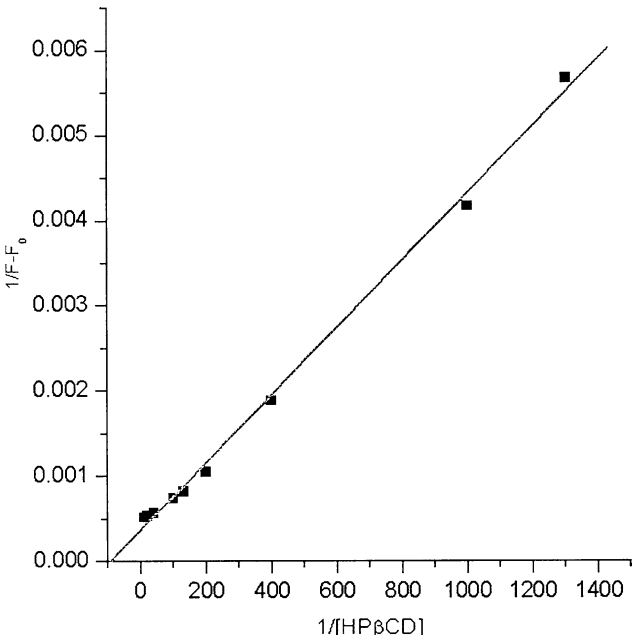


*Figure 5*. Double-reciprocal plot for HP-$\beta$-CD:11-MeBPHT complex. A linear relationship was obtained when the data are plotted, assuming a 1:1 stoichiometry (see Equation 5 in the text).

The initial parameters were estimated from the linear plot. We found a $K_f$ value of 118 ± 3 $M^{-1}$ which is very close to the value of 141 ± 11 $M^{-1}$ obtained in preliminary study, using a more limited range of HP-$\beta$-CD concentrations [12]. This 11-MeBPHT:CD complex $K_f$ value is rather larger than those measured for unsubstituted BPHT with HP-$\beta$-CD (100 ± 20 $M^{-1}$) [11b], and for 10-MeBPHT with HP-$\beta$-CD (50 ± 11 $M^{-1}$) and with $\beta$-CD (14 ± 7 $M^{-1}$) [13]. This comparison shows that 11-MeBPHT seems to interact more easily with HP-$\beta$-CD and to form a more stable inclusion complex relative to the other BPHT derivatives. Also, we evaluated the molar fraction of complexed 11-MeBPHT molecules ($f$), using Equation (8), developed by Flamigny [33].

$$f = 1 - \frac{1}{1 + K_f \cdot [\text{HP-}\beta\text{-CD}]} = \frac{F - F_0}{F_\infty - F_0} \qquad (8)$$

We found $f$ values of 0.74 and 0.63, respectively for $[\text{HP-}\beta\text{-CD}]_0 = 10^{-1}$ M and $2.5 \times 10^{-2}$ M, which indicates that very large proportions of 11-MeBPHT molecules were effectively complexed in the ground state.

*Analytical figures of merit*

As already mentioned, the complexation reaction of 11-MeBPHT with HP-$\beta$-CD is also interesting from an analytical standpoint. To demonstrate this usefulness, the analytical figures of merit for the fluorimetric determination of 11-MeBPHT were compared under various medium conditions (Table 2).

Linear log–log calibration graphs were established by plotting on a logarithmic scale the fluorescence intensity vs. 11-MeBPHT concentrations in all media under study. For each concentration the fluorescence measurements were performed in triplicate. Linear dynamic ranges (LDRs) of at least two orders of magnitude were found with satisfactory correlation coefficient ($r$) values ranging between 0.990 and 0.998. However, close examination of the slope values of log–log calibration plots revealed two different behaviours: in ethanol and HP-$\beta$-CD solution, these values were close to unity, whereas, in water and $\beta$-CD solutions, they were found to be much lower than unity (Table 2). This indicates that the classical, direct calibration curves obtained in the former media would be linear, while the ones drawn in the later media would be non-linear. In these conditions, meaningful limit of detection (LOD) and limit of quantification (LOQ) values could not be calculated for water and $\beta$-CD solution. The LODs calculated for 11-MeBPHT in ethanol and HP-$\beta$-CD solution were very low, ranging between 0.2 and 1.0 ng/ml, as well as the LOQ ones (0.8–5.0 ng/ml). The fact that the LOD value was lower in ethanol than in HP-$\beta$-CD solution can be related to the larger blank fluorescence signal obtained for HP-$\beta$-CD than for ethanol. Nevertheless, taking into account the relatively closeness of these LODs, and in order to avoid the use of an organic solvent, we decided to choose the HP-$\beta$-CD medium rather than ethanol for the spectrofluorimetric determination of 11-MeBPHT.

Our LOD value obtained in HP-$\beta$-CD solution is smaller than those obtained by the same spectrofluorimetric method in HP-$\beta$-CD for 9-MeBPHT (LOD = 7 ng/ml) [11a] and for 10-MeBPHT (LOD = 9 ng/ml) [12a]. Clearly, the CD-enhanced spectrofluorimetric method provides a good sensitivity and a satisfactory detectability for the quantitative analysis of 11-MeBPHT, as well as for the other MeBPHT derivatives.

*Application to urine analysis*

By taking advantage of the HP-$\beta$-CD fluorescence enhancement and of the improvement of solubility of 11-MeBPHT in CD media, we have applied this fluorimetric method to the determination of 11-MeBPHT in spiked human urine samples, using the standard addition procedure in HP-$\beta$-CD solution.

A good linearity and a good precision were obtained for the standard addition plots. Slopes were very close to those measured for the calibration curves, which indicates the absence of significant interference from matrix effects in the urine samples. Satisfactory recoveries, ranging from 97 to 108%, were found at various

*Table 2.* Analytical figures of merit for the fluorimetric determination of 11-MeBPHT in various media

| Medium | LDR[a] (ng/ml) | $r$[b] | Slope[c] | LOD[d] (ng/ml) | LOQ[e] (ng/ml) | RSD[f] (%) |
|---|---|---|---|---|---|---|
| Ethanol | 10–8000 | 0.998 | 0.97 | 0.2 | 0.79 | 6.2 |
| Water[g] | 100–13,000 | 0.990 | 0.52 | –[j] | –[j] | 6.8 |
| HP-$\beta$-CD[h] | 10–3000 | 0.996 | 0.90 | 1.0 | 5 | 9.0 |
| $\beta$-CD[i] | 1–13,000 | 0.993 | 0.49 | –[j] | –[j] | 5.2 |

[a]LDR = Linear dynamic range.
[b]$r$ = Correlation coefficient.
[c]Slope of the log–log calibration curves.
[d]LOD = Limit of detection defined as the concentration (in ng/ml) of compound giving a signal-to-noise ratio of 3.
[e]LOQ = Limit of quantification defined as the concentration (in ng/ml) of compound giving a signal-to-noise of 10.
[f]RSD = Relative standard deviation.
[g]Water–ethanol (99:1 v/v) mixture.
[h]$[\text{HP-}\beta\text{-CD}] = 10^{-1}$ M.
[i]$[\beta\text{-CD}] = 10^{-2}$ M.
[j]The LOD and LOQ values could not be calculated in these media (see text for details).

*Table 3*. Spectrofluorimetric determination of 11-MeBPHT in human urine sample

| 11-MeBPHT[a] ($\mu$g/ml) | | Recovery (%)[c] |
|---|---|---|
| Added | Found[b] | |
| 0.41 | 0.39 | 96.8 |
| 0.42 | 0.38 | 91.3 |
| 0.52 | 0.53 | 102.0 |
| 0.65 | 0.68 | 103.2 |
| 1.69 | 1.83 | 108.1 |
| 2.99 | 3.06 | 102.3 |

[a]Spiked with a constant concentration of 0.26 $\mu$g/ml of 11-MeBPHT.
[b]Number of replicate measurements = 3 for each 11-MeBPHT concentration.
[c]Mean relative standard deviation value = 5%.

11-MeBPHT concentrations in the urine samples under study (Table 3).

## Conclusion

In this work, we have shown that the 11-MeBPHT fluorescence quantum yields were significantly increased in the presence of $\beta$-CD and HP-$\beta$-CD relative to water, because of the formation of inclusion complexes with the CDs. Using this fluorescence enhancement, we have been able to develop a sensitive CD-enhanced spectrofluorimetric method for the determination of 11-MeBPHT in an HP-$\beta$-CD medium. Also, we have satisfactorily applied this analytical method to the assay of 11-MeBPHT in urine samples.

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