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Cyclodextrin enhanced fluorimetric method for the determination of tryptamine

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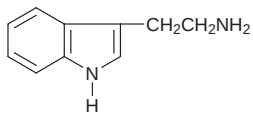
The effect of native cyclodextrins (α-, β- or γ- with six, seven or eight glucose units, respectively), hydroxypropyl-β-cyclodextrin, β-cyclodextrin solubilized in urea, soluble starch and glucose in water solution on the fluorescence behaviour of tryptamine [3-(2-aminoethyl)indole] (T) was determined. In addition, the effect of methanol and propanol with and without β-cyclodextrin or hydroxypropyl-β-cyclodextrin was ascertained. From the fluorescence changes with pH and with β-cyclodextrin or hydroxypropyl-β-cyclodextrin, the values of the pK_a of the ground and excited states and the association constants of T and TH [3-(2-ammoniumethyl)indole] with the two hosts were determined. The values are pK_a = 9.5 ± 0.2 and pK_a^* = 8.4 ± 0.2; K_{Assoc}^{TH} = (1.6 ± 0.3) × 10^2 mol^{-1} dm^3 and K_{Assoc}^{T} = (2.8 ± 0.3) × 10^2 mol^{-1} dm^3 with β-cyclodextrin, K_{Assoc}^{TH} = (1.8 ± 0.5) × 10^2 mol^{-1} dm^3 and K_{Assoc}^{T} = (4.9 ± 0.9) × 10^2 mol^{-1} dm^3 with hydroxypropyl-β-cyclodextrin. The ratio of the fluorescence quantum yields for the bound and free substrate (Φ^b/Φ^f) were in the range 1.25–1.33. The detection limit for the better conditions where the host–guest interactions produce fluorescence enhancement was 0.454 ± 0.002 ng ml^{-1} for the complex T–hydroxypropyl-β-cyclodextrin in water. The method is simpler than others reported previously.

Keywords: *Tryptamine determination; fluorescence; cyclodextrin complexes*

Supramolecular chemistry has seen intensive development in recent years with the use of receptors such as polysilicates (zeolites), polysugars (cyclodextrins), polyethers (crown ethers) and other derivatives. In this area we have studied several aspects of the chemistry of cyclodextrins,[1–4] which are doughnut-shaped molecules formed by six, seven or eight glucose units (α, β and γ, respectively) and are able to form inclusion complexes with a great variety of compounds.[5,6] Frequently, these supramolecular species enhance the luminescence properties of some compounds and this has been reported to have analytical implications.[7,8]

In this paper, we report on the influence of different hosts and reaction media in the absorption and fluorescence spectra of tryptamine [3-(2-aminoethyl)indole] (T), which was chosen as



T

the model for a series of compounds of biological interest and whose analytical determinations might be improved. Tryptamine is a precursor of serotonin [5-hydroxy-3-(2-aminoethyl)-indole], a hormone carried in the blood by platelets which functions as a neurotransmitter. Other compounds containing the indole nucleus are used in pharmacy and agriculture and sensitive analytical methods are necessary for their monitoring.

Native cyclodextrins (α-, β- and γ-CD), hydroxypropyl-β-cyclodextrin (HP-β-CD) and urea-solubilized-β-CD (U-β-CD) were used as receptors. For comparison, soluble starch and glucose were employed. The possibility of co-inclusion of methanol and propanol in the presence of β-CD and HP-β-CD was also investigated, taking into account that enhanced fluorescence detection has been reported in several cases in the presence of this host system[9] and also their analytical application.[10]

Experimental

Apparatus

UV/VIS and spectrofluorimetric determinations were carried out with Shimadzu UV 260 and Jasco FP-777 instruments, respectively. pH was measured with an Orion Model 720 A pH meter at 25.0 ± 0.1 °C using a Ross combination pH electrode. The pH meter was calibrated using standard buffers prepared according to the literature.[11] Data analysis was performed with Sigma Plot (Scientific Graph system) Jandel Scientific, version 1.00.

Reagents

High-purity water was obtained from a Milli-Q apparatus (Millipore, Bedford, MA, USA). Tryptamine (Sigma, St. Louis, MO, USA), native cyclodextrins and hydroxypropyl-β-cyclodextrin (Roquette, Lestrem, France), urea (Timper, Cap. Fed., Bs. As., Argentina), α-D-glucose (dextrose, Anedra, San Fernando, Bs. As., Argentina) and soluble starch (Reidel-de Haën, Seelze, Germany) were used as received. All constituents of the buffers were commercial reagents of analytical-reagent grade. The buffers used were hydrochloric acid (pH 2.70–1.00), acetic acid–sodium acetate (pH 4.70),[12] monopotassium dihydrogenphosphate–disodium hydrogenphosphate (pH 7.00),[11] borax (pH 8.30–10.68),[12] disodium hydrogenphosphate–trisodium phosphate (pH 10.70 and 12.00)[12] and sodium hydroxide (pH 11.00–13.00). Methanol and propanol were of HPLC grade (Sintorgan, Villa Martelli, Bs. As., Argentina).

Procedure

A concentrated solution of tryptamine (2 mg in 10 ml) in water was kept in a refrigerator for about 2 weeks and checked periodically before preparing the adequate dilutions for the fluorimetric determination. Solutions were prepared by adding the T solution to 95% v/v of the appropriate buffer solution prepared as indicated above and diluting to volume with water. The photomultiplier gain was low for emission fluorescence spectra and very low for excitation with 10 nm emission and excitation bandwidths. The fluorescence emission spectra were

taken with excitation wavelength λ^{ex} = 279.0 nm. All the determinations were made at 25.0 ± 0.1 °C and the temperature of the cell compartment was controlled with a Haake circulator. The solutions were not de-gassed. A solution of T ≈ 4.6 μmol dm^{-3} at pH 7.00 was used as reference for the fluorimetric measurements. The ionic strength (μ) of all solutions was 0.124 mol dm^{-3}, adjusted by adding NaCl. For the determination of association constants, two solutions of the same substrate concentration (one without receptor and the other with the maximum concentration of receptor used) were mixed in suitable proportions for the variation of receptor concentration in order to minimize the changes in fluorescence by changes in substrate concentration.[13] For spectrofluorimetric determinations, the total area below the fluorescence spectrum (*F*) [eqn. (1)] and the fluorescence intensity at a fixed emission wavelength (F_λ) [eqn. (2)] were measured:

$$F = B\sum\varepsilon_i\Phi_i[i] \quad (1)$$

$$F_\lambda = B\sum\varepsilon_i\Phi_i\gamma_i[i] \quad (2)$$

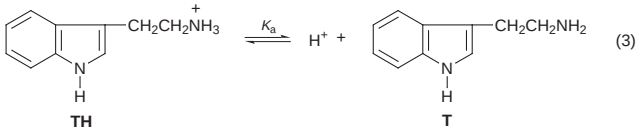
where *B* is a constant which depends on the instrumental set-up, ε_i is the molar absorptivity, Φ_i is the fluorescence quantum yield, γ_i is the fraction of the total emission intensity at a given wavelength and [*i*] indicates the concentration of each fluorescent species *i*. In all cases the absorbance of the solution was < 0.050 where eqns. (1) and (2) are valid.

Results and discussion

Effect of pH *and buffer concentration*

The UV/VIS spectra of the substrate (not shown) presented a maximum at 278.6 nm (pH 2.00) and 280.5 nm (pH 12.00) and it did not change significantly in the presence of the receptors studied. The molar absorptivity of the reference solution (pH 7.00) was (55 ± 1) × 10^2 dm^3 mol^{-1} cm^{-1} at 279.0 nm.

The fluorescence emission spectra (Fig. 1) show a red shift in the maximum emission wavelength (λ_{max}^{em}) and an increase of about 20% in fluorescence intensity when the pH changes from 7.00 to 10.70. These differences are attributed to the acid–base equilibrium shown in eqn. (3). The values of *F* and F_λ were



(3)

measured as a function of pH† and a value of pK_a = 9.5 ± 0.2 was calculated. This value is slightly higher than that corresponding to tryptophan (pK_a = 9.39)[14] and is consistent with the electron-withdrawing effect of the carboxylate group. Similar differences in pK_a have been reported for phenylalanine (pK_a = 9.13) and phenylethylamine (pK_a = 9.78).[14]

Spectral shifts between an acid–base pair can be attributed to the excited state deprotonation. The difference in pK_a between ground and excited species can be calculated using the Förster cycle,[15,16] giving a value for the excited state (pK_a^*) of 8.4 ± 0.2. This value indicates that excitation increases the acidity of the emitting species. Similar results were obtained with other indole derivatives.[17]

There is no change in fluorescence between pH 7.00 and 3.00. Quenching of fluorescence is observed (compare the spectra in Fig. 1 at pH 2.00 and 12.00) below pH 3.00 and above pH 11.00, in agreement with the decrease in fluorescence quantum yields reported for indole derivatives.[18–22] In most cases neither the identity nor concentration of the buffer produces any change in the emission spectrum. At pH 12.00 with Na_2HPO_4–Na_3PO_4, some quenching is observed when the total buffer concentration changes from 0.010 to 0.074 mol dm^{-3}. This effect is attributed to the increase in phosphate trianion, since no effect is observed at pH 7.00 with KH_2PO_4–NaH_2PO_4 buffer.

Fluorescence in cyclodextrins and related media

The effect of the addition of native CD (α-, β- or γ-) on the fluorescence of TH (pH 7.00) and T (pH 10.70) was investigated (see Table 1). The λ_{max}^{em} of a solution of TH (pH 7.00) shifts from 355 to 353 nm when β-CD (10 mmol dm^{-3}) is added, along with an increase in the total emission intensity (*F*). On the other hand, there is no change when α- or γ-CD is added. In the case of T, native CD (α-, β- or γ-) produces a blue shift in the λ_{max}^{em} and an increase in fluorescence intensity, but a stronger effect is observed with β-CD.

Since some β-CD derivatives are more water soluble and have been reported to be more effective in fluorescence enhancement,[9] the effect of HP-β-CD (0.85 average molar substitution) was investigated. At 10 mmol dm^{-3} concentration it produces a shift in λ_{max}^{em} of 4 nm for TH and of 13 nm for T. For both substrate species, T and TH, the changes in λ_{max}^{em} and *F* induced by HP-β-CD are greater than those produced by β-CD. Based on literature data for compounds of similar structure,[17,23] we suggest that an inclusion complex of 1 : 1

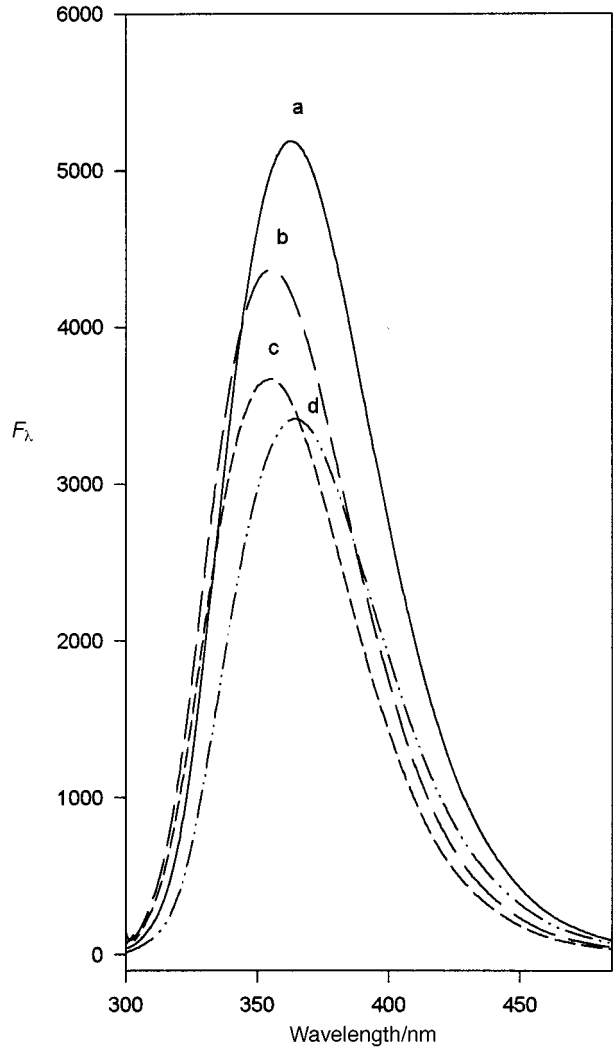


Fig. 1 Fluorescence emission spectra of 4.6 μmol dm^{-3} tryptamine in water at pH (a) 10.70, (b) 7.00, (c) 2.00 and (d) 12.00. Excitation wavelength, 279 nm.

† Available as supplementary material (SUP 57386; 3 pp.) deposited with the British Library. Details are available from the editorial office.

stoichiometry is formed. The association constants (K_{Assoc}, Scheme 1) for the 1 : 1 complex were determined from the values of the fluorescence at different receptor (host) concentrations from non-linear regression analysis of the data (Table 2).[17,24] The same values of K_{Assoc} were obtained using F or F_λ. Further, the data plotted according to a linearized equation[24,25] (Fig. 2, inset) also gave the same values of K_{Assoc} within experimental error. The results confirm that the stoichiometric ratio of the complex is 1 : 1.[26]

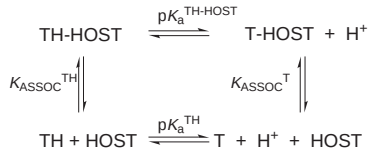
The values of K_{Assoc} determined in this study are similar to others reported for indole derivatives.[9,17] The higher K_{Assoc} for the neutral substrate (T) indicate a better interaction with the hosts, probably owing to its greater hydrophobicity. (A referee suggested that another explanation for the greater binding of T than TH might be that the free primary amino nitrogen in T can hydrogen bond better to the hydrogen atom of the hydroxyl group on the rim of the CD cavity. We think that this effect should be at least partially compensated for by the better hydrogen bond from the ammonium group to the oxygen at the rim of the CD cavity.) The ratios Φ^b/Φ^f, determined as reported in the literature,[17,24] were > 1 in all cases, indicating the more protected environment of β-CD or HP-β-CD for the corresponding excited state of the bound substrate (TH or T).

The pK_a values for the complexed substrate with β-CD or HP-β-CD (Table 2) were determined from the thermodynamic cycle shown in Scheme 1. Since the $K_{Assoc}{}^{T} > K_{Assoc}{}^{TH}$, an increase in acidity for the complexed substrate is expected. In both cases the acidity of the bound substrate shows this trend.

Table 1 Fluorescence parameters of tryptamine in different aqueous media*

	TH (pH 7.00)†		T (pH 10.70)‡	
Medium	$\lambda_{em}{}^{max}$/nm§	F¶	$\lambda_{em}{}^{max}$/nm§	F¶
—‖	355	1.00	364	1.27
αCD**	355	1.00**	359	1.27
γCD**	355	1.00**	359	1.27
β-CD**	353	1.18	355	1.49
HP-β-CD**	351	1.19	351	1.61

* Tryptamine concentration ≈ 4.6 μmol dm^{-3} at 25 °C and ionic strength 0.124 mol dm^{-3}. † Buffer prepared as in ref. 11. ‡ Buffer prepared as in ref. 12. § λ_{ex} = 279 nm. ¶ Relative fluorescence calculated from the integrated areas of the uncorrected emission spectra divided by the corresponding value for TH at pH 7.00. ‖ Aqueous buffer. **In the presence of 10 mmol dm^{-3} of the corresponding compounds.



Scheme 1

The use of more concentrated solutions of HP-β-CD (0.12 or 0.24 mol dm^{-3}) results in higher blue shifts of the $\lambda_{max}{}^{em}$ (18 or 21 nm, respectively) of T without the fluorescence enhancement expected for the greater concentration of bound substrate (98 or 99%) with respect to the more dilute solutions (83% with 10 mmol dm^{-3} HP-β-CD). These effects could be produced by some absorption of the HP-β-CD used in this study at the λ^{ex}, which is as high as 0.100 at 0.24 mol dm^{-3}.

It is known that the aqueous solubility of β-CD (15 mmol dm^{-3}) increases in aqueous urea (U), reaching concentrations of 100 mmol dm^{-3} (β-CD) in 4.0 mol dm^{-3} U and of 200 mmol dm^{-3} (β-CD) in 8.5 mol dm^{-3} U. The binding constants of some analytes are higher and others are lower in U–β-CD than in β-CD.[9] Also, enhanced fluorescence was observed in U–β-CD compared with β-CD.[9] This system is also used in analytical applications as a more convenient concentrated stock of β-CD solutions.[26] The systems 10 mmol dm^{-3} β-CD in 0.8 mol dm^{-3} U or 100 mmol dm^{-3} β-CD in 4.5 mol dm^{-3} U produce the same (for T) or smaller changes (for TH) in fluorescence intensity as β-CD alone. The smaller changes in fluorescence intensity for TH may be due to a decrease in the binding constant of this species in the presence of urea. Quenching by urea is discarded, since the addition of 0.8 or 4.5 mol dm^{-3} U has no effect on fluorescence intensity or $\lambda_{em}{}^{max}$ of T or TH.

The effect on the fluorescence of T and TH of glucose and starch in an equivalent mass of 10 mmol dm^{-3} β-CD was studied for comparison with that of β-CD. Glucose produces a small blue shift of about 1 nm in the $\lambda_{max}{}^{em}$ of T or TH without changes in fluorescence intensity. Soluble starch produces a

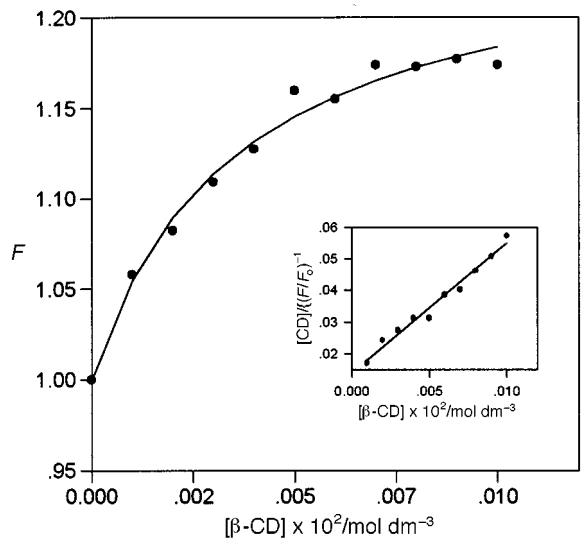


Fig. 2 Relative fluorescence intensities of 4.6 μmol dm^{-3} tryptamine at pH 10.70 as a function of β-cyclodextrin concentration. Inset: plot according to a linearized equation as in refs. 22 and 23.

Table 2 Calculated relative fluorescence quantum yields and equilibrium constants for TH and T*

	TH		T		
Host	K_{Assoc}/ 10^2 mol^{-1} dm^3†	Φ^b/Φ^f‡	K_{Assoc}/ 10^2 mol^{-1} dm^3†	Φ^b/Φ^f‡	p$K_a{}^{THCD}$§
β-CD	1.6 ± 0.3	1.29 ± 0.03	2.8 ± 0.3	1.25 ± 0.01	9.3 ± 0.2
HP-β-CD	1.8 ± 0.5	1.30 ± 0.04	4.9 ± 0.9	1.33 ± 0.02	9.1 ± 0.2

* Tryptamine concentration ≈ 4.6 μmol dm^{-3} at 25 °C and ionic strength 0.124 mol dm^{-3}; the substrate is indicated as TH at pH 7.00 and as T at pH 10.70. † The values of K_{Assoc} are the averages of the values obtained using F_λ and F as in refs. 17 and 24, with standard deviations no higher than 6%. The errors are the averages of those calculated by the fitting program. ‡ Values calculated as in refs. 17 and 24; the errors are given by the fitting program. § Values calculated from Scheme 1.

blue shift of about 7 nm in the λ_{max}^{em} of T but acts as quencher of fluorescence of both species of the substrate (27% for TH and 43% for T). Both results contrast with those found with β-CD and HP-β-CD, indicating that there is some specific interaction with the last two receptors.

Influence of the presence of alcohol in the medium

The addition of increasing concentrations of methanol to water or water–β-CD solutions of T (Fig. 3) enhances not only the fluorescence intensity of T, but also that of the complexes T–β-CD and T–HP-β-CD. The fluorescence of T and T–β-CD reaches the same value at 25% v/v methanol and the fluorescence of T–HP-β-CD is slightly higher; in all three cases no important changes were observed at higher percentages of methanol.

Solutions containing more than 55% v/v methanol could not be used owing to the low buffer solubility. The effect of propanol is clearly different (Fig. 4), since at 5% v/v propanol an increase in the fluorescence of T but decreases in the fluorescence of T–β-CD and T–HPβ-CD are observed. At this propanol concentration, all solutions have the same fluorescence intensity. The dissimilar behaviour of methanol and propanol may be interpreted as a competition of propanol with the substrate for the cavity of the host,[23] which produces the displacement of T, whereas methanol stabilizes the binary complexes[27] probably because it is co-included, forming a ternary complex.[28] These results are in agreement with the higher affinity of β-CD for propanol (K_{Assoc} = 3.7 mol^{-1} dm^3)[29] than for methanol (K_{Assoc} = 0.32 mol^{-1} dm^3).[29] The formation of a ternary complex with methanol is suggested because the calculated fluorescence of the solution taking into account the amount of free and complexed substrate is always lower than the measured value.

Analytical parameters

Taking into account the experimental results described above, pH 10.70 was selected for the determination of T. The solvent chosen was water or methanol–water (20% v/v) in the presence of β-CD or HP-β-CD at 25 °C.

Determinations carried out at 15 °C showed no improvement in the detection limit, which became worse at 40 °C.

The calibration graph obtained by plotting relative fluorescence intensity *versus* T concentration was linear in the range studied (0.04–6.00 μmol dm^{-3}) with good correlations (at least 0.998). Therefore, the data can be represented by the equation

$$F = F_B + m[T] \quad (4)$$

The slope of the line is the analytical sensitivity m and F_B is the blank measure. The detection limits, expressed as a concentration c_L, were determined as defined by IUPAC.[30,31] The values of c_L given in Table 3, were calculated with the equation

$$c_L = \frac{ks_B}{m} \quad (5)$$

where s_B is the standard deviation of the blank calculated as the square root of eqn. (6) for a number of observations n ($n \geqslant 20$)

$$s_B^2 = \frac{\sum_{j=1}^{j=n} \left(F_B^j - \overline{F}_B\right)^2}{n-1} \quad (6)$$

and k = 3 for a confidence level of 99.86%. The dynamic linear range is between $c_L \times 10/3$ and 6.00 μmol dm^{-3}.

The value of m (Table 3) increases in aqueous solutions with β-CD and HP-β-CD as receptors, being higher with the latter. The best c_L determined is 0.454 ± 0.002 ng ml^{-1} for T complexed with HP-β-CD in water, followed by T complexed with β-CD in water (0.509 ± 0.002 ng ml^{-1}) or complexed with HP-β-CD in methanol–water (20% v/v) (0.51 ± 0.01 ng ml^{-1}). Although the values of m obtained in methanol–water (20% v/v) are higher than those corresponding to the aqueous solution, the values of s_B are also higher so the values of c_L are similar in both solvents.

The precision of the method was determined by analysing 10 replicate samples containing 0.5 μg ml^{-1} of analyte and the relative error was not greater than 3%.

Other fluorescence determinations for T have been published but they are indirect methods, since several reaction paths are necessary,[32] or require drastic experimental conditions, such as high temperature[32] or strongly acidic media,[33–35] to produce the adequate fluorophore. The detection c_L limits are similar or higher, *i.e.*, 0.5,[32] 1.0[33,34] or 4.0 ng ml^{-1}.[35] The method described here is direct and simpler, requiring only a solution of

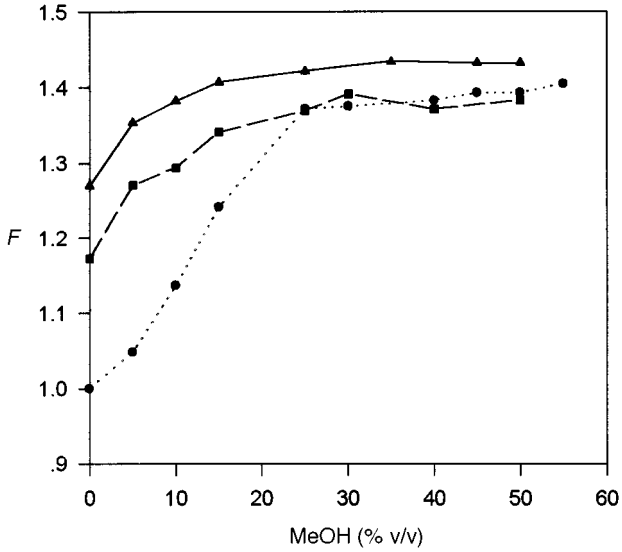


Fig. 3 Relative fluorescence of tryptamine at pH 10.70 as a function of methanol–water composition: (●) without host; (◆) in the presence of 10 mmol dm^{-3} of β-CD; and (▲) in the presence of 10 mmol dm^{-3} of HP-β-CD.

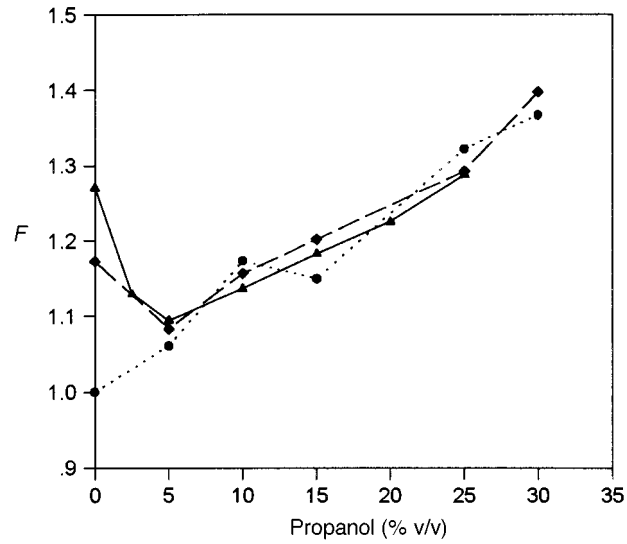


Fig. 4 Relative fluorescence of tryptamine at pH 10.70 as a function of propanol–water composition: (●) without host; (◆) in the presence of 10 mmol dm^{-3} of β-CD; and (▲) in the presence of 10 mmol dm^{-3} of HP-β-CD.

Table 3 Analytical parameters*

Solvent	Species	$s_B/10^{-4}$†	$m/10^5$ mol^{-1} dm^3‡	c_L/ng ml^{-1}§
Water¶	T	3.4	2.86 ± 0.01	0.570 ± 0.002
	T–β-CD	3.2	3.02 ± 0.02	0.509 ± 0.003
	T–HP-βCD	3.4	3.60 ± 0.01	0.454 ± 0.002
20% Methanol–water¶	T	4.3	3.65 ± 0.02	0.572 ± 0.003
	T–β-CD	5.0	3.68 ± 0.02	0.653 ± 0.004
	T–HP-β-CD	4.3	4.04 ± 0.08	0.51 ± 0.01

* At 25 °C and ionic strength 0.124 mol dm^{-3}, tryptamine concentration from 0 to approximately 4.6 μmol dm^{-3}, pH 10.70. † Calculated as the square root of eqn. (6), see text. ‡ Calculated as the slope of a plot according to eqn. (4). The errors are those calculated by the fitting program. § Calculated from eqn. (5). The error is calculated by error propagation. ¶ The solutions in water and in methanol have 95% and 65%, respectively, of the buffer solution prepared as in ref. 12.

receptor in a suitable buffer and addition of an aliquot of substrate to be determined. The receptor can be prepared from a more concentrated aqueous solution in the case of HP-β-CD.

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