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Comparative effect of cyclodextrin nanocavities versus organic solvents on the fluorescence of carbamate and indole compounds

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ABSTRACT

The effect of the addition of different amounts of organic solvents (**S**) on the fluorescence of aromatic compounds (**C**) and their inclusion complexes with β-cyclodextrin (**βCD**) and hydroxypropyl-β-cyclodextrin (**HPCD**) has been examined using steady-state measurements. Carbamate pesticides with different aromatic moiety, such as carbofuran (**CF**), promecarb (**PC**), carbaryl (**CY**) and bendiocarb (**BC**) were used, as well as indole derivatives with different polarity in their lateral chains, such as melatonin (**M**, neutral), 5-methoxytryptamine (**MT**, cation) and auxin (**IA**, anion). Their complexes in water show a fluorescence signal higher than that obtained for the free substrates in solvent:water mixtures (30%, v/v *n*-propanol or acetonitrile, and 50%, v/v methanol). The isofluorescent point (**IF**), the **%IF** and the $F_{85\%}$ are defined in order to evaluate the use of CD nanocavities as a non-polluting alternative for the analysis of the compounds analyzed.

Apparent formation constants (K_{AP}, M^{-1}) for the complexes of C:HPCD at different solvent percentages were determined for CF and PC with methanol (**MeOH**), *n*-propanol (**ProOH**) and acetonitrile (**ACN**), and for indole compounds with **ACN**. A decrease in the K_{AP} values for the **CF:HPCD** (120–30) and **PC:HPCD** (2000–400) complexes occurs in accordance with the solvent affinities for **CDs** (**MeOH** < **ACN** < **ProOH**). Nevertheless, in the indolic series, the polar characteristics of **MT**, **IA** and **M** determine their behaviour in the presence of **ACN**. For the neutral substrate **M**, K_{AP} decreases with the increasing percentage of **ACN** (100–10). In contrast, for **IA** and **MT** (ionic substrates) K_{AP} increases (10–100).

These results may be accounted for by two different mechanisms: the competition between **C** and **S** for the cavity of the receptor or the formation of ternary complexes **C:S:CD** with additional stabilization.

1. Introduction

Molecular fluorescence is a very useful technique for many analytical applications and it should be able to render lower limits of detection and greater selectivity than absorption spectroscopy. Although many aromatic compounds (**C**) show strong fluorescence in organic solvents, their intensity in water is generally weaker [1].

Organized systems, such as molecular aggregates (micelles, vesicles, monolayers, etc.) and polymeric species (polysilicates such as zeolites, polyethers such as crown ethers, or polysugars such as cyclodextrins) enhance the fluorescence emission intensity of several **C** [2]. For this reason, in the last decades there has been an increasing interest in the study of many fluorophores in micro-heterogeneous systems [3].

Cyclodextrins (**CDs**) are cyclic oligosaccharides consisting of six (**αCD**), seven (**βCD**) or eight (**γCD**) units of α-D-glucose linked by α-(1,4) bonds. The shape of cyclodextrin molecules is that of a hollow truncated cone (a toroid) of height 0.78 nm. Among the native **CD** derivatives, the hydroxypropyl-β-cyclodextrin (**HPCD**) has higher solubility and particular analytical advantages. These macrocycles can act as hosts to form inclusion complexes with guest molecules in solid state or in solution. The ability of **CDs** to incorporate guest molecules into their hydrophobic nanocavity (internal diameter/nm: 0.47–0.53; 0.60–0.65 and 0.75–0.83 for **αCD**, **βCD** and **γCD**, respectively) [4] leading to the formation of host–guest inclusion complexes [5] has allowed for further applications in different fields [6] because of their biocompatibility and the physical chemical changes produced in the substrate included.

Carbamate pesticides containing different fluorescent nuclei such as carbaryl (1-naphthyl methylcarbamate, **CY**) [7a], carbofuran (2-3-dihydro-2,2-dimethylbenzofuran-7-yl methylcarbamate, **CF**) [7b], bendiocarb (2,2-dimethyl-1,3-benzodioxol-4-yl methyl-

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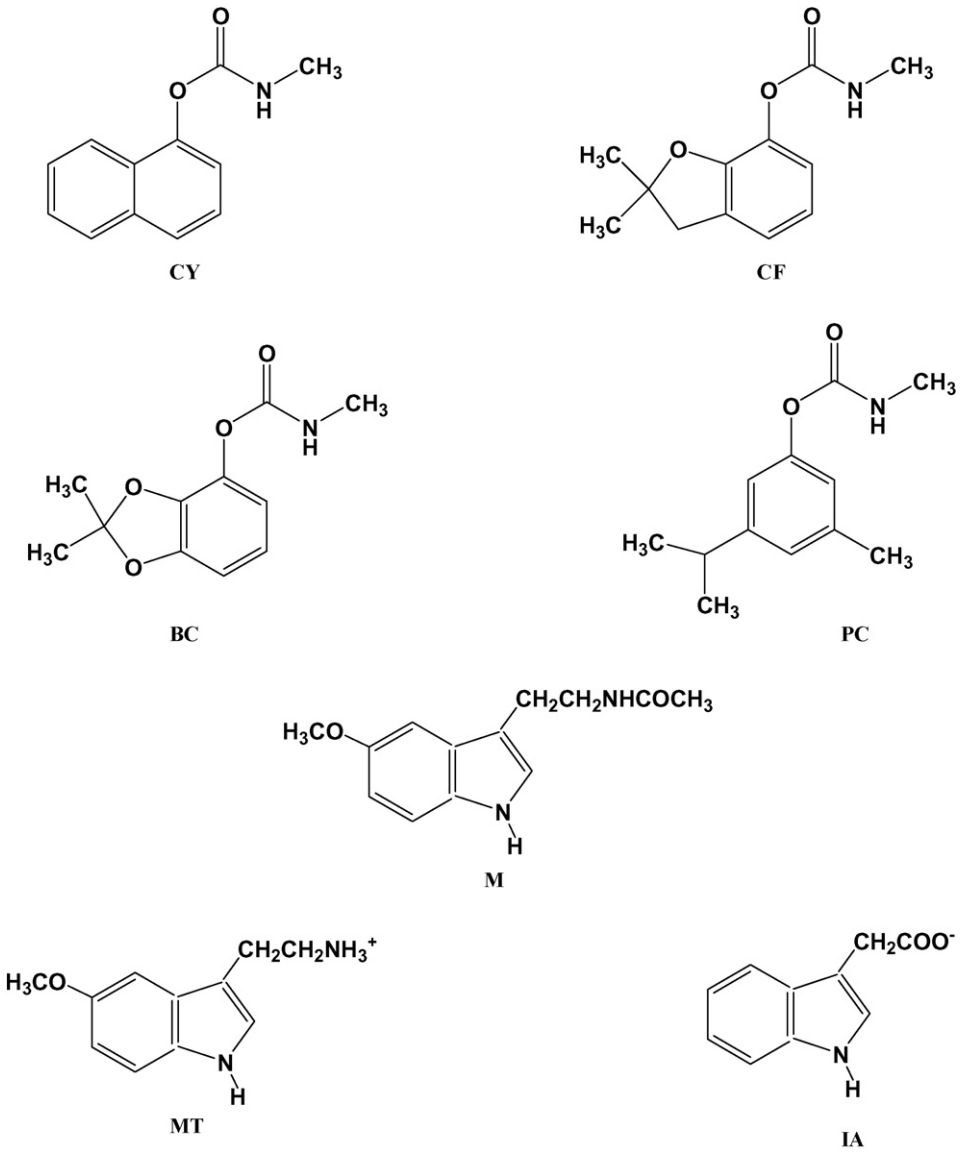


Scheme 1. Structures of carbamates and indoles studied at pH 7.00.

carbamate, **BC**) [7c] and promecarb (3-isopropyl-5-methylphenyl methylcarbamate, **PC**) [7d] are widely used as insecticides (Scheme 1) [8].

Indole derivatives comprise a wide range of **C** of biological, pharmaceutical and agrochemical interest. Melatonin (*N*-acetyl-5-methoxytryptamine, **M**) is a hormone produced in the pineal gland [7e]; it is involved in the physiology of circadian rhythms [9] and in mammalian reproduction [10] and it has possible genomic action [11]. Nowadays, it is extensively used as a sleep disorder controller. 5-methoxytryptamine ([5-methoxy-3-(2-aminoethyl)indole], **MT**) has been proposed as potential hypnotic and sedative compounds [7f]. Indole 3-acetic acid (auxin, **IA**) is a plant hormone that acts as growth regulator [7g] and a derivative of 5-hydroxy-3-indolilacetic acid, the principal metabolite of serotonin (5-hydroxy-tryptamine) (Scheme 1).

It has been previously reported [12–15] that these **C** form binary complexes with **βCD** and **HPCD**. Complexes of **CDs** with molecules of organic solvent (**S**) have also been reported for alcohols and other **S** [16–19].

Most studies about the stability of cyclodextrin complexes have been conducted in pure aqueous solutions. However, the addition of organic solvents might affect the host–guest interaction; therefore, studies of the formation of cyclodextrin complexes in aqueous:organic solvents media have been undertaken [19]. The nature of the solvent is of the utmost importance since it can determine the structure of the complex [20]. Moreover, cyclodextrin complexes in the presence of **S** have been found to be more stable in some cases and more unstable in others than in pure water [21].

Due to the increasing need for more sustainable strategies in chemistry, the use of water or biodegradable systems in water has aroused much interest [22].

The aim of this work is to show that **CDs** are a good alternative to organic **S** such as methanol (**MeOH**), *n*-propanol (**ProOH**) and acetonitrile (**ACN**) that are toxic, hazardous and environmentally unfriendly, and to compare the variation in the stability of the complexes with the addition of different percentages of **S**. It can prove an economic and less polluting method for the detection and the quantification of **C** to than the hazardous **S**.

2. Experimental

2.1. Apparatus

The UV–vis and the spectrofluorimetric determinations were carried out in a Shimadzu (Kyoto, Japan) UV-2101 **PC** and a Jasco (Tokyo, Japan) FP-777. Data analysis was performed with a Sigma Plot (Scientific Graph system) version 8.00 (Jandel Scientific).

2.2. Reagents

The water was obtained using a Millipore apparatus. Carbofuran, Promecarb (98% purity), Carbaryl, Bendiocarb (99% purity) (Chem Service, West Chester, PA, USA); indole-3-acetic acid and 5-methoxytryptamine (Sigma, St. Louis, MO, USA), melatonin (ICN, Costa Mesa, CA, USA), β-cyclodextrin (Roquete, Lestreme, France) and hydroxypropyl-β-cyclodextrin (degree of substitution 5.5 each **HPCD**) (Cerestar, Hammond, IN, USA) were used as received. All constituents of the buffers were commercial reagents of analytical grade and prepared according to literature procedures [23]. The reference buffer solution at pH 6.994 was obtained from monopotassium dihydrogenphosphate (0.02 M); disodium hydrogenphosphate (0.03 M) and sodium chloride (0.02 M) [23]. Methanol, *n*-propanol and acetonitrile were HPLC grade (Sintorgan, Villa Martelli, Bs. As., Argentina).

2.3. General procedure

Concentrated solutions of carbamates (10 mg/100 mL) or indoles (2 mg/10 mL) in water were maintained in the refrigerator for a few weeks and periodically checked before the adequate dilutions for fluorimetric determinations were prepared. Water solutions were prepared by adding the substrate solution to 95% by volume of the buffer solution prepared as detailed above and by diluting to the mark with water. In solutions containing a co-solvent, the buffer concentration was 68% of the above mentioned from a concentrated buffer solution at pH 6.994, in order to prevent salt precipitation and to maintain the pH. The solutions were prepared mixing the appropriate amount of the concentrated buffer solution and the required amounts of the organic solvent to obtain the desired co-solvent content and then diluting to the mark with water. Also a solution of each substrate at 85% of organic solvent without control of ionic strength was measured in order to extrapolate the solvent effect.

The photomultiplier gain was low for fluorescence emission spectra in the case of indoles and **CF**; very low for **CY**; and medium for **PC** and **BC**; with 10 nm emission and excitation bandwidths. The fluorescence emission spectra were taken with excitation wavelengths λ^{ex} = 280.0 nm for **IA**, **CY** and **BC**, 278.0 nm for **M**, 274.0 nm for **MT**, 260.0 nm for **PC**, and 273.0 and 273.6 with **CF** with **βCD** and **HPCD** respectively. 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 degassed. Solutions in water at pH 6.994 of 2.40, 18.0, 9.0, 50.0 and 60.0 μM of the indole compounds, **CF**, **CY**, **BC** and **PC**, respectively, were used as reference for the fluorimetric measurements. The ionic strength (μ) of all solutions was 0.124 M by adding NaCl.

For the determination of the overall association constant, two solutions of the same substrate concentration (one without receptor and the other with the maximum concentration of the receptor used) were mixed in adequate proportions for the variation of receptor concentration in order to minimize the changes in fluorescence by changes in substrate or co-solvent concentration [24]. For the spectrofluorimetric determination, the total area below the fluorescence spectrum (F) (Eq. (1)) was measured:

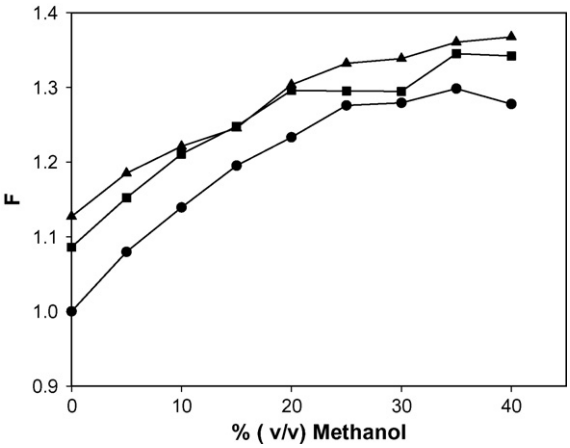


Fig. 1. Relative fluorescence of 2.40 μM **IA** in water at pH 7.00 as a function of **MeOH** concentrations in the presence of **CDs**. Curve: (●) buffer solution, (■) with 10.0 mM of **βCD** and (▲) with 10.0 mM of **HPCD**.

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

where B is a constant which depends on the instrumental set-up, ε_i is the molar absorptivity at the λ^{ex} and ϕ_i is the fluorescence quantum yield.

3. Results and discussion

3.1. Effect of the addition of cyclodextrins and organic solvents on the fluorescence of the substrates

Inclusion complexes (Eq. (2)) with stoichiometry 1:1 have been previously established between **CDs** (**βCD** and **HPCD**) and the **C** studied in water by fluorescence enhancement with association constant values (K_A) from 100 to 2000 M^{-1} [12–15]

$$\mathbf{C} + \mathbf{CD} \overset{K_A}{\rightleftharpoons} \mathbf{C-CD} \quad (2)$$

Here, the effect of variable percentages of **S**, such as **MeOH**, **ProOH** or **ACN**, on the fluorescence of the free and the complexed analyte was studied. Although each free or complexed substrate shows a particular behaviour with the addition of increasing percentages of organic co-solvent, some general tendencies can be analyzed.

For all the free compounds (pesticides and indoles), the addition of any **S** enhances the fluorescence with respect to water. Nevertheless, in the case of **C–CD**, the behaviour is mainly dependent on the **S** used and the polar characteristic of **C**. Figs. 1–3 show the general behaviour observed for a substrate with or without the presence of 10.0 mM of **βCD** and **HPCD** with **MeOH**, **ProOH** and **ACN**, respectively.

The addition of **MeOH** (increments of 5%, v/v from 0 to 50%, v/v) to buffer solutions of carbamates (**CY**, **CF**, **BC** or **PC**) or indoles (**M**, **MT** or **IA** (Fig. 1 is representative) in the presence of **βCD** or **HPCD** produces a fluorescence signal slightly higher than or practically the same as that without alcohol. In all cases the complexes with **HPCD** have the highest fluorescence.

On the other hand, in the presence of **ProOH**, the fluorescence for the complexed **CY**, **CF**, **BC**, **M**, **MT** or **IA** decreases up to 5–10% of **S** and then the fluorescence increases with similar or higher values than those for the **C** at the same **S** percentage. In the case of the complexed **PC** the fluorescence is practically the same as that without **S** (Fig. 2) as was observed for all the substrates with **MeOH**.

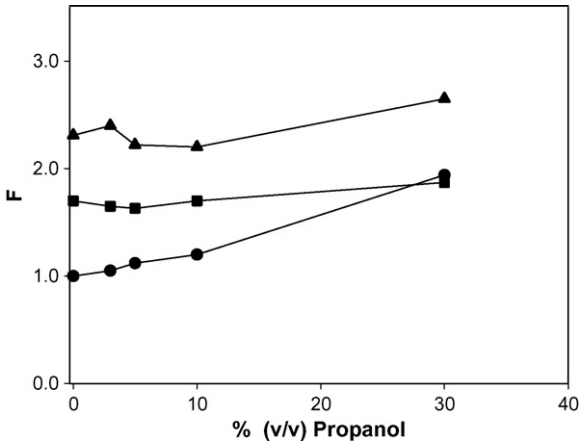


Fig. 2. Relative fluorescence of 60.0 μM **PC** in water at pH 6.994 as a function of **ProOH** concentrations in the presence of **CDs**. Curve: (●) buffer solution, (■) with 10.0 mM of **βCD** and (▲) with 10.0 mM of **HPCD**.

When **ACN** is added, the behaviour of the neutral guest studied (all of the carbamates and **M**) is similar to that observed with **ProOH** for most of the complexes (except **PC**). In these cases, the fluorescence of the complexes decreases up to 5–10% of **ACN** and then increases with the increasing percentage of **ACN** (up to 40%, v/v). Only a slight increase in fluorescence is found for **PC** at low percentages of **ACN** (≤1%). For the ionic species (**MT** and **IA**) the fluorescence increases (Fig. 3 is representative) in the presence of **ACN**. This indicates that in **ACN** the fluorescence of the complexes is dependent on the substrate polarity.

Accordingly, in order to meet our objectives, we have defined some parameters. The isofluorescence point (**IF**) has been explained as the coincident fluorescence value for the substrate in water with **CDs** (F_w^{CD}) and for the free substrate in the aqueous–organic solvent mixture ($F_{\%S}$) (indicated in Fig. 3 by the horizontal line from the first point of the curve in the presence of **βCD** (or **HPCD**) at 0% of organic solvent to intersect the curve without host in water–organic solvent mixture. Moreover, the percentage of **S** where that fact occurs has been named isofluorescence percentage (**%IF**) (indicated in Fig. 3 by the % of organic solvent reached by the vertical line from the **IF** in the curve without host, to the axis). The **%IF** indicates the amount of co-solvent that could be replaced by cyclodextrin in order to give ***F*** signals of equivalent value.

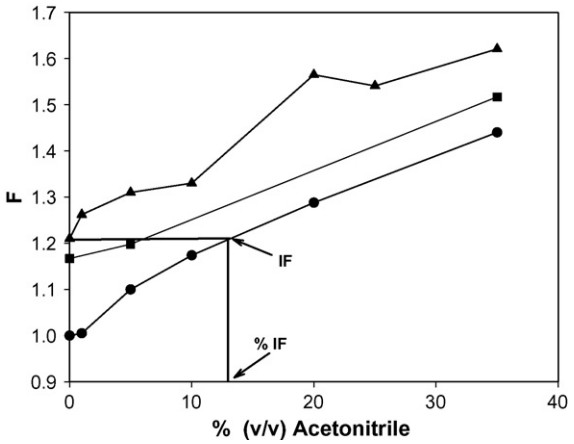


Fig. 3. Relative fluorescence at of 2.40 mM IA in water at pH 6.994 as a function of **ACN** concentrations in the presence of **CDs**. Curve: (●) buffer solution, (■) with 10.0 mM of **βCD** and (▲) with 10.0 mM of **HPCD**.

Table 1
Isofluorescence point (**IF**), isofluorescence percentages (%**IF**) and fluorescence at 85% of organic co-solvent ($F_{85\%}$)[a]

C	HPCD	MeOH		ProOH		CAN	
	IF	%IF	$F_{85\%}$	%IF	$F_{85\%}$	%IF	$F_{85\%}$
CY[b]	1.27	[c]	0.74	[c]	0.77	[c]	0.63
CF[d]	5.53	57	1.6	32	1.55	53	10.4
BC[e]	2.94	40	2.6	[c]	2.00	45	6.88
PC[f]	2.31	78	2.48	40	3.07	50	2.76
M[g]	2.06	60	2.48	20	2.57	35	2.45
MT[g]	1.28	30	1.69	23	1.68	17	1.55
IA[g]	1.13	10	1.28	10	1.47	20	1.49

[a] At pH 6.994, μ = 0.124 M and 25.0 °C, isofluorescence point (**IF**) in the presence of 10 mM of **HPCD**.
[b] 9.00 μM.
[c] (**%IF**) ≥ 85.
[d] 18.00 μM.
[e] 50 μM.
[f] 60 μM.
[g] 2.4 μM.

The **%IF** (for $\mathbf{IF} = F_w^{HPCD}$) determined for neutral substrates (Table 1) shows the variability with the substrate and with the solvent. The lower and higher values in each solvent are 40 (**BC**) and 78 (**PC**) in **MeOH**; 20 (**M**) and 40 (**PC**) in **ProOH** and 35 (**M**) to 50 (**CF**) in **ACN**, in the cases where this point is possible to be determined. The lowest **%IF** suggests that **HPCD** could be used instead of 20% of **ProOH**–water (**M**). In some cases there is not an isofluorescence point since the F_w^{HPCD} is higher than $F_{85\%}$ (defined as the fluorescence value without host at 85% of organic co-solvent). These are the cases for **BC** with **ProOH** and **CY** with **MeOH**, **ProOH** and **ACN**. The values of $F_{85\%}$ are also tabulated in Table 1.

In the cases of ionic substrates (**MT** and **IA**), the **%IF** observed are lower, with values from 10 to 30, which shows the higher affinity of the substrates for the polar media.

In all cases the same tendencies are observed with **βCD**, but the **%IF** are the same as or lower than those with **HPCD** (Table 1) since the fluorescence enhancements with **HPCD** are similar to or better than those with **βCD**.

The ratios ($\mathbf{IF}/F_{85\%}$) calculated from the values tabulated in Table 1 are around 0.8–1.0 in most of the cases. With **CY**, **CF** and **BC** these ratios are higher (between 1.1 and 3.6). Only in the case of **ACN** with **CF** and **BC**, the values are below 1 (0.5 and 0.4, respectively) but with **%IF** higher than 45. These results show the environmental benefit of using of **CD**, a less pollutant alternative to detecting and quantifying these compounds by fluorescence.

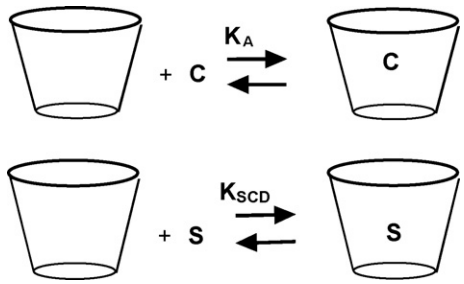
3.2. Stability of the complexes in aqueous:organic solvent media

In order to determine the stability constants in aqueous:organic solvent media, a spectrofluorimetric method was employed based on the determination of fluorescence at increasing concentrations of **HPCD** in the presence of a given co-solvent percentage for each **C**.

From non-linear regression analysis of the data according to Eq. (3) [24] where

$$\left(\frac{F^{CD}}{F_0}\right)_{\%S} = \left(\frac{1 + (\phi^{CCD}/\phi^{C})K_{AP}[CD]}{1 + K_{AP}[CD]}\right)_{\%S} \quad (3)$$

(F^{CD}/F_0) is the ratio between the fluorescence of **C** with and without **HPCD**, respectively; K_{AP} is the overall formation complex constant and (ϕ^{CCD}/ϕ^{C}) is the fluorescent quantum yield ratio between the complexed and the free substrate; all these terms at a given percentage of **S**. Eq. (3) is similar in the absence of **S** and K_{AP} was obtained from the same treatment as that previously employed [12–15].



Scheme 2. Equilibrium reactions in water.

In the derivation of Eq. (3), it was assumed that the concentration of free **C** is equal to its analytical concentration. This assumption is valid in the case of host concentration being much higher than one guest species concentration. It may not be true in the presence of a second guest species (Scheme 2) which can form weak complexes with **CD** but is present in solution at a much higher concentration than that of the first guest [17].

The K_{AP} (M^{-1}) in different percentages of **MeOH**, **ProOH** and **ACN** were determined for **CF:HPCD** and **PC:HPCD** as models of carbamate pesticides, since they have the lower (123) [13] and the higher (2050) [15] values of K_A. Furthermore, since the K_A for indoles (**M**, **MT** and **IA**) are similar (~100) [12,14], the K_{AP} in **ACN** for the indolic nucleus were determined in order to evaluate the effect of the polarity of the chain in position 3.

The results obtained by Eq. (3) with **MeOH** and **ProOH** as cosolvent are shown in Table 2, and the corresponding values with **ACN** are reported in Table 3. The K_{AP} values decrease with increasing **S** concentration for neutral compounds (**CF**, **PC** and **M**) indicating that the complexes are weaker or destabilized in the presence of **S**. For the ionic compounds, **IA** and **MT**, the behaviour is different. At low percentages of **ACN** (1%, v/v) K_{AP} for **IA:HPCD** is lower than K_A. After that, an increment of the K_{AP} value with the variation of the **S** percentage is observed up to 300 M^{-1} (20%, v/v), the same behaviour was found with **MT**, although K_{AP} is always lower than K_A. The increase in the K_{AP} values with the increase of the %**S** indicates an additional stabilization.

There are several hypotheses about the effect of **S** on the complexation of different compounds with **CDs**. One approach is that

Table 2
Overall formation constants (K_{AP}) of **C:HPCD** complexes in the presence of variable %**S**

C	S	% v/v	(K_{AP}, M^{-1})[a]	(K^T_{AP}, M^{-1})[b]	ϕ^{CCD}/ϕ^{C}
		0	(123 ± 7)	–	9.3 ± 0.4
	MeOH	10	(67 ± 5)	69	10.3 ± 0.4
CF[c]		20	(42 ± 12)[d]	48	26 ± 9[d]
		3	(46 ± 7)	50	11 ± 1
	ProOH	10	(29 ± 5)	21	9 ± 1
		0	(21 ± 2)10^2	–	2.4 ± 0.2
	MeOH	1	(17 ± 5)10^2	1905	2.1 ± 0.1
		10	(7 ± 2)10^2	1148	3.3 ± 0.1
PC[e]		0.5	(12 ± 1)10^2	1656	2.55 ± 0.03
	ProOH	1	(11 ± 2)10^2	1388	2.38 ± 0.03
		3	(7 ± 1)10^2	829	2.45 ± 0.04
		10	(3.8 ± 0.5)10^2	345	2.51 ± 0.07

[a] Buffer solutions at pH 6.994, 25.0 °C and ionic strength 0.124 M. The K_{AP} values were obtained measuring *F* with host concentrations between 0 and 14 mM. The errors are those calculated by the fitting program.
[b] Values obtained from Eq. (4).
[c] **CF** concentration 18.0 μM.
[d] Recalculated value taking into account the amount of **CD** consumed by the high S%.
[e] **PC** concentration 60.0 μM.

Table 3
Overall formation constants (K_{AP}) of **C:HPCD** complexes in the presence of variable %**ACN**

C	%**ACN** (v/v)	K_{AP} (M^{-1})[a]	K^T_{AP} (M^{-1})[b]	ϕ^{CCD}/ϕ^{C}
	0	(14 ± 3) 10	–	1.21 ± 0.02
	1	(12 ± 9)	120	2.3 ± 0.2
IA[c]	3	(37 ± 7)	92	1.4 ± 0.3
	5	(4 ± 1) 10	76	1.3 ± 0.3
	20	(33 ± 4) 10	32	1.1 ± 0.1
	0	(12 ± 4) 10	–	1.27 ± 0.06
	1	(19 ± 4)	102	2.2 ± 0.4
MT[c]	3	(10 ± 6)	79	2.1 ± 0.8
	5	(7 ± 1) 10	63	1.3 ± 0.2
	20	(8 ± 2) 10	27	1.1 ± 0.2
	0	(15 ± 7) 10	–	1.48 ± 0.03
	1	(106 ± 2)	128	1.47 ± 0.02
M[c]	5	(79 ± 3)	81	1.39 ± 0.05
	10	(33 ± 3)	55	1.5 ± 0.1
	20	(5 ± 2)[d]	34	1.7 ± 0.5[d]
	0	(21 ± 2) 10^2	–	2.40 ± 0.02
PC[e]	1	(28 ± 7) 10^2	1756	2.62 ± 0.05
	10	(7 ± 1) 10^2	759	3.14 ± 0.07
	0	(123 ± 7)	–	9.3 ± 0.4
CF[f]	1	(85 ± 7)	105	10.2 ± 0.5
	10	(40 ± 3)	45	12 ± 2

[a] Buffer solutions at pH 6.994, 25.0 °C and ionic strength 0.124 M. The K_{AP} values were obtained measuring *F* with host concentrations between 0 and 14 mM. The errors are those calculated by the fitting program.
[b] Values obtained from Eq. (4).
[c] **IA**, **MT** and **M** concentration 2.40 μM.
[d] Recalculated value taking into account the amount of **CD** consumed by the high S%.
[e] **PC** concentration 60.0 μM.
[f] **CF** concentration 18.0 μM.

S acts as a competitive guest displacing the analyte from the **CD** nanocavity [25–27]. This situation can be evaluated through the determination of the affinity constants for the overall system considering the **S:CD** complex formation [27]. Another hypothesis suggests that a ternary complex is formed between **S**, **C** and **CD**. In this case, the stability of the binary complexes increases when the **S** is added to an aqueous system [21,28].

One explanation provided for the results shown in Tables 2 and 3 is through Eq. (4) which can be deduced from the coexisting equilibrium reactions in Scheme 2 and represents a correlation between a theoretical K_{AP} (namely K^T_{AP}) and the S concentration, K_A and K_{SCD} being the formation constant of the **C:HPCD** complex in water and the **S:HPCD** complex, respectively [27]. The values of K_{SCD} (M^{-1}) for **MeOH** (0.3) and **ProOH** (3.7) employed in the calculation of K^T_{AP} (Tables 2 and 3) correspond to literature values [16]. Since there is not much agreement between the reported values of K_{SCD} (M^{-1}) for **ACN** (6 [17], 0.54 [18] and 0.7 (and K_{S2CD} = 0.6 M^{-2}) [19]), a value of 0.9 was estimated from the relation between ($K_{S\alpha CD}/K_{S\beta CD}$~6) for different alcohols [16], and the $K_{S\alpha CD}$ (5.6) informed for **ACN** [25]. This value is in the same order as the lower values reported in literature [18,19].

$$K^{T}_{AP} = \frac{K_A}{(1 + K_{SCD}[S])} \quad (4)$$

The concordance between the K^T_{AP} (Eq. (4)) and K_{AP} (Eq. (3)) (Tables 2 and 3) indicates that the suggested model (Scheme 2) is operating. On the other hand, a value of K^T_{AP} higher than K_{AP} could indicate that the difference in hydrophobicity of water:**S** media and the **HPCD** cavity at the same **S** percentage is smaller than that in water. This difference ($K^T_{AP} > K_{AP}$) could also be attributed to the presence of complexes of higher stoichiometry between **S** and **CD** (not taken into account in Eq. (4)).

In Tables 2 and 3 we can observe the correlation between the K_{AP}^{T} and the K_{AP} values for the complexes of neutral **C** (**CF**, **PC** and **M**) in **MeOH**, **ProOH** and **ACN** indicating the coexistence of the equilibriums shown in Scheme 2.

For the ionic **C** (**MT** and **IA**), the complexes up to 3% of **ACN** (K_{AP}) are much weaker than those at high percentages of S (20%) and those predicted by K_{AP}^{T}. Nevertheless, the K_{AP} for **IA** and **MT** at 20% (v/v) **ACN** are higher (3–10 times) than the calculated K_{AP}^{T}. In the case of **IA**, the K_{AP} value also indicates a complex ~2 times more stabilized in relation to in water (Table 3). The disagreement between the K_{AP} and K_{AP}^{T} suggests an additional stabilization of these complexes, probably due to a ternary complex formation (Eq. (5)) in the presence of **ACN**.

$$C + S - CD \overset{K_{CCDS}}{\rightleftharpoons} C - CD - S \quad (5)$$

In order to evaluate this possibility, the equilibrium reaction which considers the formation of the ternary complex (Eq. (5)) is added to the equilibrium reactions shown in Scheme 2. The K_{AP}^{T} for this situation is represented by Eq. (6).

$$K_{AP}^{T} = \frac{(K_A + K_{CCDS}K_{SCD})}{(1 + K_{SCD}[S])} \quad (6)$$

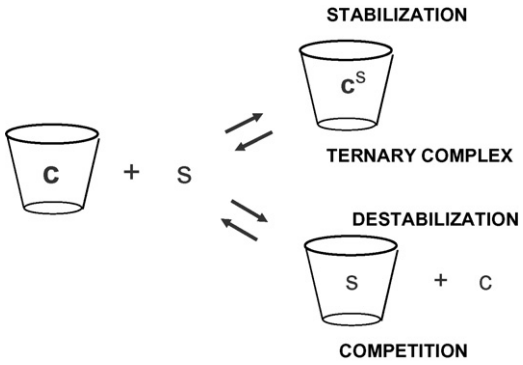
Considering the formation of a ternary complex, the K_{CCDS} (M^{-1}) were calculated from the experimental values of K_{AP} and K_{SCD}, which gave values of 386 for **IA** and 68 for **MT**.

The most important requirement for the formation of the complex is a good spatial fit between the interacting molecules [29]. In view of our results, the decrease in K_{AP} with the increase in the **S** concentration in the case of neutral (in alcohols and **ACN**) and ionic **C** (in alcohols) is attributed to the competition of the **S** for the **CD** cavity as reported in other cases [30]. The destabilization observed is only apparent and is ascribed to the decrease in the **CD** concentration available. The **S** concentration is always higher but with lower affinity for the cavity ($K_{SCD} < K_A$) than that of **C**. The correlation between K_{AP} and K_{AP}^{T} indicates that the affinity between **C** and **CD** (K_A) is not affected by changes in solvent polarity. This interpretation also applies to complexes of **βCD** with chloronitrobenzenes in different water:**S** mixtures [17]. The values of the (ϕ^{CCD}/ϕ^{C}) at different **S**% for neutral **C** (Tables 2 and 3) are also the same between the experimental error as those in water, showing that the fluorescent probe tests practically the same change in hydrophobicity for the cavity in all the water:**S** mixtures.

On the other hand, in the cases of charged **C** in water:**ACN** mixtures, there is stabilization for ternary complex formation, K_{CCDS} being smaller for **MT** and higher for **IA** than their respective K_A. A similar behaviour to **MT** was found with xantone–**CD** [21] and acridine–**CD** [26] complexes in the presence of alcohols. The complex pirene–**CD** is stabilized in the presence of t-butanol or cyclohexanol and the increase of the K_{AP} was attributed to a ternary complex formation [27] as interpreted for **IA**. Also, the space-filling role of the solvent has been put forth as an important factor for the formation of ternary complexes [21,26–28].

The different complexation observed in alcohol:water mixtures compared with **ACN**:water mixtures for **MT** and **IA** resembles that found with *o*-chloronitrobenzene [17]. In the latter case, the competition of the alcohol for the **CD** cavity, and a more hydrophobic cavity in **ACN** were held as responsible for the different behaviour observed.

There would be additional effects operating in **ACN** with respect to alcohols. For example, the higher polarity of the solvent has been mentioned in the flavour–**CD** complexes formation, in addition to the molecular size [31]. Also, for a group of guests with polar, anionic and cationic characteristics, the increase in the K_A has been attributed to a solvent with a weaker and less-structured hydration shell that facilitates the transfer of hydrated species to the hydrophobic environment of **CD** and it may also contribute to the optimization of the intracavity interactions to increase the complex stability [32].



Scheme 3.

In the present study, the (ϕ^{CCD}/ϕ^{C}) values at different **ACN**% for **IA** and **MT** (Table 3) show an effect opposite to that observed in K_{AP}. The lower (ϕ^{CCD}/ϕ^{C}) at higher **ACN**% may also suggest the co-inclusion of the solvent into the cavity and a decrease in the cavity hydrophobicity in relation to water.

All the results reported in this work can be explained from Scheme 3, in which the upper part shows the stabilization found for the charged compounds in **ACN**:water, and the lower part illustrates the destabilization observed for all the neutral compounds in all the solvent:water mixtures studied.

4. Conclusions

βCD and HPCD, instead of organic solvents, can be employed in the spectrofluorimetric determination of several compounds with different aromatic nuclei and different polarity. The % **IF** indicates that the use of **CD** replaces 15–70% (v/v) of methanol, 10–55% (v/v) of *n*-propanol and 17–53% (v/v) of acetonitrile. This will imply low cost, environmentally sustainable alternatives.

The apparent stability of the complexes **C:HPCD** for the neutral compounds decreases in the presence of any **S**, since **S** displaces the guest from the **CD** cavity according to the affinity of the **S** for the host (K_{SCD}). On the other hand, the increased stability of the complexes of ionic indoles in acetonitrile is not in agreement with the coexistence of two equilibrium reactions (Scheme 2), therefore it could be ascribed to the formation of a ternary complex **C:HPCD:S** (Scheme 3).

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