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Spectral and photophysical properties of 2-dibenzofuranol and its inclusion complexes with cyclodextrins

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Abstract

The absorption and fluorescence spectral features of a dioxin-like compound, 2-dibenzofuranol (DBFOH), have been studied in aqueous and non-aqueous media and in the presence of cyclodextrins (CDs). The fluorescence of DBFOH is structured in cyclohexane and shifts towards red when the polarity of the solvent is increased. The p*K*s in the ground and first electronic excited states are pK = 9.95 and pK^* = 1.5, as calculated by the Förster cycle from the absorptometric and fluorimetric data. DBFOH forms inclusion complexes with β and γ-CDs but not with α-CD, the spectral behavior being dependent on the type of macrocycle. β-CD enhances the emission of DBFOH by forming a 1:1 complex, whereas the γ-CD forms 1:1 and 2:1 stoichiometries, $(DBFOH)_2$:γ-CD, as deduced from absorption, fluorescence, and ^{1}H NMR experiments. No excimer emission is perceived, unlike the case of dibenzofuran (DBF) and 2-dibenzofurancarboxylic acid with γ-CD. The complexation does not modify the excited-state reactivity of DBFOH, in contrast to other condensed aromatic alcohols. The binding constants have been obtained from the emission spectra by multivariable non-linear regression analysis at several wavelengths and, from the dependence on the temperature, the thermodynamic parameters of the binding, enthalpy and entropy, have been deduced. The effect of the hydroxyl group on the stability and on the proton transfer in the excited state of DBFOH is compared to the non-substituted DBF and the corresponding carboxylate derivative (DBFC). The similarities between the enthalpies indicate that the complex formation is governed by the inclusion of the aromatic part in the cavity, the functional group having a negligible effect on the binding.

Keywords: Cyclodextrins; 2-Dibenzofuranol; Excimer; Excited-state proton transfer; Steady-state fluorescence; NMR; Absorption spectroscopy; Binding constants

1. Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides built up from α-D-glucopyranose residues linked by α-1,4 glycosidic bonds, the most common being those formed by six, seven or eight glucose units (α, β, and γ-CD, respectively). This family of macrocycles display a torus-like shape, with a hydrophobic cavity and two hydrophilic rims in which the primary and secondary OH groups are inserted. The main feature that makes CDs of interest is their capacity to form inclusion complexes with a variety of guest molecules in solution or in solid phase [1], a property that yields many interesting applications that have been extensively described in the literature [2].

In the case of fluorescent guests, the inclusion can affect the ground and/or excited states of the fluorophore, producing remarkable modifications in the spectral properties of the substrate upon inclusion. Fluorescence enhancement is the most common situation, ascribed to factors such as the protection against collisional quenching, changes in the polarity of the microenvironment or an increase in the rigidity of the guest, amongst others [3]. The opposite effect (quenching of fluorescence), although less common, has also been observed for inclusion complexes, for example in the case of certain heterocycles, such as acridine [4] and phenazine [5], or some naphtalene derivatives, such as 2-acetylnaphtalene [6,7], and pyrene [8,9].

In addition to the changes in the spectral properties of the guest upon the inclusion, its photochemistry can also be modified [10]. Thus, the CD behaves as a protective casing for the fluorophore against photobleaching or side re-

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actions or, on the contrary, it can act as a catalyst, inducing excimer formation [11,12]. The reactivity in the excited state, e.g. proton transfer, can also be altered in the presence of CD due to interactions of a protonable group with the edges of the cavity as in the case of 2-naphtol, for example [13–15]. The CDs have also proven to restrict the twisting of functional groups in molecules that display twisted intramolecular charge transfer (TICT), for example 4-*N*,*N*-dimethylaminocinnamic acid [16] or 2-(4′-*N*,*N*-dimethylaminophenyl) benzimidazole [17].

In previous works we have investigated the spectral properties and photophysics of some dioxin-like compounds, dibenzofuran (DBF) and 2-dibenzofuran carboxylic acid (DBFCA) and its inclusion within natural CDs [18,19]. In DBF, some important differences with respect to nitrogen containing heterocycles in the stoichiometries and binding constants were found, especially with α-CD, and γ-CD. The latter CD was also found to induce excimer emission by complexes of 2:2 stoichiometry at high γ-CD/DBF ratios. In DBFCA, the emission is very sensitive to the pH and to the type of CD used, yielding also stoichiometries different from 1:1 in the case of the γ-CD. In addition, 2D ROESY experiments combined with molecular dynamics calculations proved that the carboxylate group of the ionized DBFCA (DBFC) is located at the narrower edge of the β-CD [20].

In this framework, we have carried out an investigation with 2-hydroxydibenzofuran (DBFOH), a heterocycle capable of giving proton transfer in the excited state. The electron donor group −OH produces a different behavior with respect to the −COOH group in DBFCA. Our research has been focused in the study of the spectral features and photophysics of this molecule in water and other solvents, and in the presence of natural CDs. The effect that the complexation may have in the excited-state proton transfer has also been investigated. The thermodynamic parameters of the binding, deduced from the temperature dependence of the formation constants, and the effect of the −OH group on the stability have been analyzed by multivariable non-linear squares fitting and compared with those of the parent DBF, and with DBFC.

2. Experimental

β-CD was manufactured by Roquette, α-CD and γ-CD by Wacker. The water contents determined by thermal analysis were 10.6, 8.29, and 7.91%, respectively. 2-Hydroxydibenzofuran (DBFOH) with 98% purity was obtained from Aldrich, purified by precipitation in water from an ethanol solution. The rest of reagents were used as received. Cyclohexane (Panreac), ethanol (Merck), and acetonitrile (Scharlau), were of spectrophotometric grade. Water solutions were prepared with type I-grade water.

UV spectra were acquired with a HP 8452A diode array spectrophotometer (1s integration time). Steady-state fluorescence measurements were performed using a Perkin-Elmer LS-50B spectrofluorimeter. The excitation wavelength in each case corresponded to the λ_{max} in the absorption spectrum at its longest wavelength. The scan rate was 300 nm/min, and the excitation and emission slits fixed at a spectral bandpass of 6.0 and 2.5 nm, respectively. 1.000 cm-pathlength or 0.400 cm-pathlength quartz cells were employed both in absorption and fluorescence measurements, keeping constant the temperature with an external heated circulating bath ($25.0 \pm 0.1\,^{\circ}C$ for the absorption spectra and 15, 25, 35 and $45^{\circ} \pm 0.1\,^{\circ}C$ for the fluorescence measurements).

In the experiments with CDs, the DBFOH concentration was fixed in 3.63×10^{-5} M for UV measurements. For fluorescence, it was 5.4×10^{-6} M for α- and β-CD, and 2.71×10^{-5} M for γ-CD. The concentration of DBFOH was always kept within the interval of linearity in all the cases. The ratio CD/DBFOH was varied by direct titration in the cell with a stock solution containing DBFOH + CD.

^{1}H NMR spectra were recorded at 300 K in a Bruker Avance 400 Ultrashield spectrometer (9.36 T) by averaging 600 scans. The assignation of the resonances of the DBFOH has been done with the aid of the 1D and COSY spectra and the simulation of spin subsystems with MestRe-C software [21]. The solutions were prepared in D_2O (Aldrich, 99.9% minimum in D) by adding solutions of α-, β- and γ-CD to vials containing weighed amounts of DBFOH. The Job method has been used in order to ascertain the stoichiometry of the complex in the case of the γ-CD, by mixing stock solutions of DBFOH and γ-CD [22].

3. Results

3.1. Spectral properties of DBFOH

The absorption spectrum of DBFOH in water, at pH 7.4, displays a broad long wavelength band (LW) at ca. 320 nm overlapped to a more intense one at 288 nm, and a sharp peak at 252 nm (Fig. 1). The bands are red shifted with re-

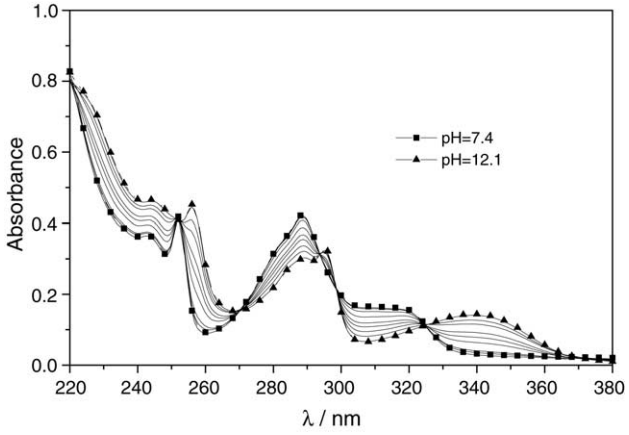

Fig. 1. Absorption spectra of DBFOH as a function of pH.

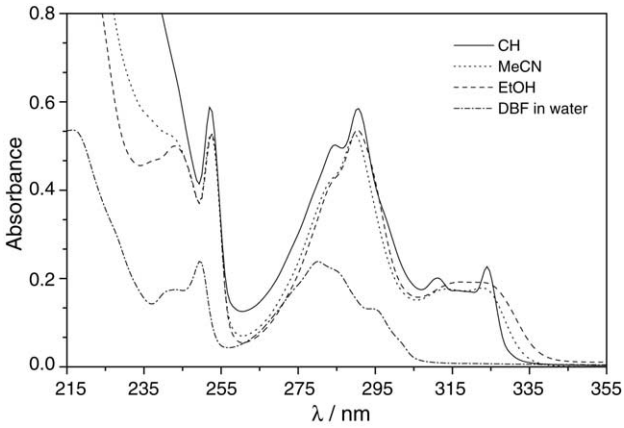

Fig. 2. Absorption spectra of DBFOH in several solvents (CH: cyclohexane; MeCN: acetonitrile; EtOH: ethanol), and DBF in water.

spect to the non-substituted DBF, due to the auxochromic effect of the electron donor –OH group (Fig. 2). The shape of the spectrum does not change substantially in organic media, such as cyclohexane, ethanol and acetonitrile, the cyclohexane being the solvent that yields the best resolution of the vibronic structure (Fig. 2). The spectrum is much less structured in water and the bands are slightly blue shifted. At pHs at which ionization of the hydroxyl occurs (above ≈ pH 10), the appearance of the absorption spectrum changes dramatically, with the LW band centered at 340 nm and two other more intense bands at 296 and 256 nm. The $-O^-$ group has a higher tendency than –OH to donate electrons to the aromatic ring, which increases the conjugation of the π system with the subsequent reduction in the energy of the 0–0 transition.

The emission is strongly pH dependent (Fig. 3a) and dual fluorescence can be observed, with bands at 352 and 443 nm, and two isoemissive points at 415 and 433 nm, for pH intervals 0.1–7.8, and 8.7–12.5, respectively. The emission at basic pH, coming from the excitation of the anion, is much less intense than the observed in acidic conditions. Organic solvents, such as cyclohexane, acetonitrile and ethanol can not accept a proton from excited DBFOH, so the fluorescence comes from neutral DBFOH. The emission at 352 nm is attributed to molecular DBFOH, whereas that at 443 nm corresponds to the ionic form. The band is more structured in cyclohexane, showing two maxima (Fig. 4). When the dielectric constant of the solvent increases, the emission loses its structure and the Stokes shift increases according to the general solvent effect [23] in the sequence cyclohexane < acetonitrile < ethanol < water (acid) ≈ water (neutral). The ethanol, despite having a lower dielectric constant than acetonitrile, possesses hydrogen-bonding ability and produces a higher relative Stokes shift. The quantum yields decrease in the order ethanol > cyclohexane > acetonitrile > water (acid) > water (neutral) > water (basic). All these results are collected in Table 1.

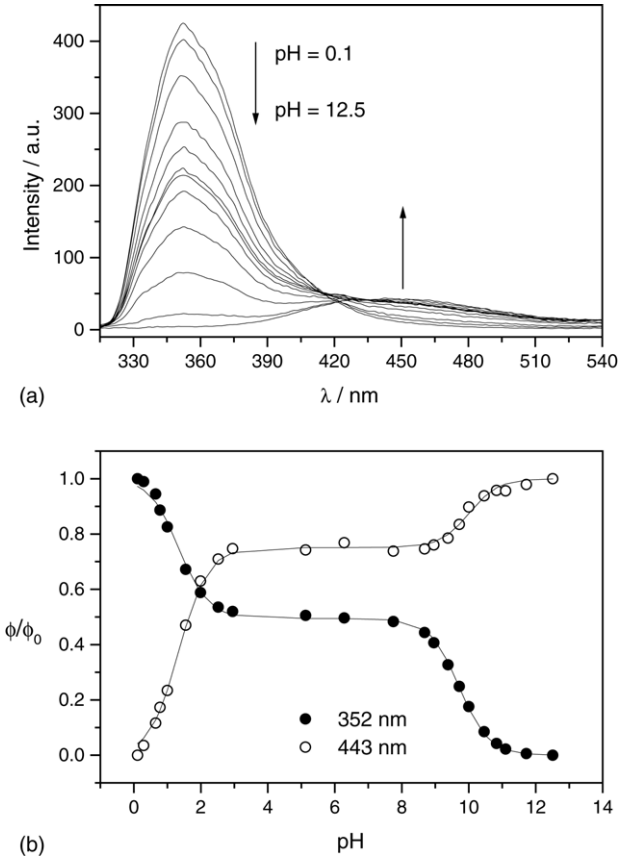

Fig. 3. (a) Fluorescence emission of DBFOH as a function of pH (λ_{ex} = 299 nm, [DBFOH] = 5.4 × 10^{-6} M); (b) normalized fluorimetric titration of DBFOH at 352 and 443 nm. The solid lines are the fits to Eq. (3).

Table 1
Spectral characteristics of DBFOH in several solvents at 298 K

Solvent	λ_{max}	log(ε)	λ_F (nm)	Quantum yield, ϕ	Stokes shift (cm^{-1})
Cyclohexane	252	4.26	326	0.354	189.4
	290	4.25	340		
	324	3.84			
Acetonitrile	252	4.27	348	0.289	2320
	290	4.26			
	322	3.86			
Ethanol	252	4.20	351	0.408	2957
	290	4.22			
	318	3.77			
Water (pH 0.1)	252	4.09	352	0.190	3849
	288	4.10			
	310	3.63			
Water (neutral)	252	4.09	352	0.079	3849
	288	4.10			
	310	3.63			
Water (pH 13)	256	4.13	443	0.035	6838
	296	3.99			
	340	3.64			

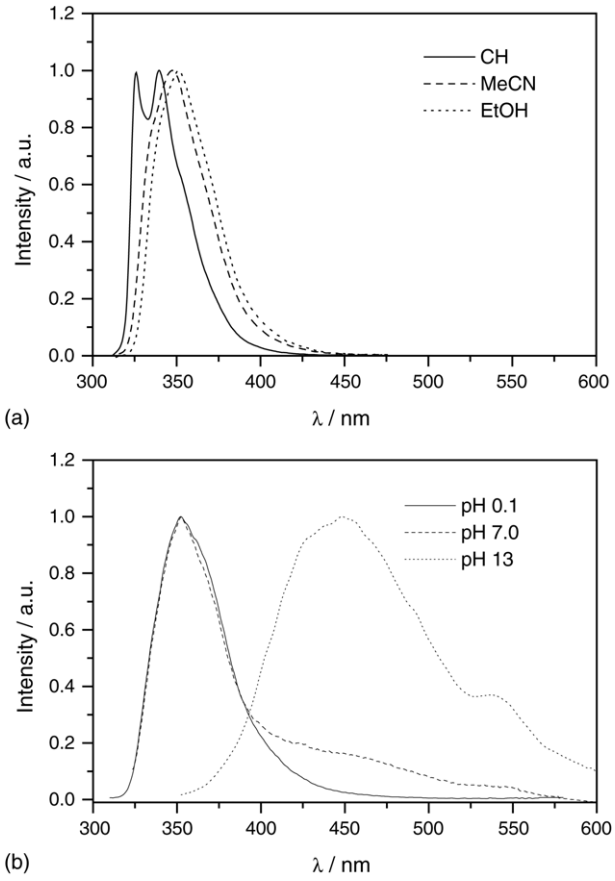

Fig. 4. (a) Normalized fluorescence emission of DBFOH in some organic solvents (CH: cyclohexane; MeCN: acetonitrile; EtOH: ethanol); (b) in water, at different pHs.

3.2. Ground and excited-state pK's of DBFOH

The pK in the ground state can be calculated from the changes in the absorbance by a non-linear least-squares fit to the Henderson–Hasselbach equation. By choosing $\lambda_{max} = 288$ nm the pK was 9.95 ± 0.02, a value close to that of other aromatic alcohols, such as 2-naphthol (9.5) [24], 4-hydroxydiphenyl ether (9.6) [25], and 1-naphthol-2-sulfonate (9.6) [26].

It is possible to calculate the acidity constant in the excited state, pK^*, by means of the Förster cycle [27] with absorption and emission data:

$$\mathrm{p}K - \mathrm{p}K^* = \frac{E_{HX} - E_X}{2.303RT} \quad (1)$$

where E_{HX} and E_X are the energies of the electronic 0–0 transition for the acid and base pair, respectively, T the temperature and R the universal gas constant. The determination of such energies in acid–base equilibria has been discussed by Grabowski and Grabowska [28]. They can be obtained by averaging the energies corresponding to the maxima in the absorption and fluorescence spectra (expressed in wavenumbers) of both the acid and base forms, i.e.,

$$E_{HX} = \frac{N_A hc}{2}(\bar{\nu}^a_{HX} + \bar{\nu}^f_{HX}); \quad E_X = \frac{N_A hc}{2}(\bar{\nu}^a_X + \bar{\nu}^f_X) \quad (2)$$

where a and f refer to absorption or fluorescence, respectively. This method is appropriate provided the spectra have a well-resolved vibrational structure. In our case, the poorly defined maximum of the LW band in alkaline conditions can produce a considerable error in the estimation of $\bar{\nu}^a_{HX}$, as well as the widening of the basic emission does in $\bar{\nu}^f_X$. This approach produces p$K^* = 0.85$. As an alternative, the 0–0 transition can be obtained by calculating the wavenumber corresponding to the intersection point of the normalized absorption and emission spectra. These values are 30,379 cm^{-1} for the neutral DBFOH and 26,690 cm^{-1} for the ionised form, yielding p$K^* = 2.22$. In any case DBFOH becomes a stronger acid in its excited state. $\Delta\mathrm{p}K = \mathrm{p}K^* - \mathrm{p}K = -8.38$, calculated using 1.54 as the mean value, falls within the same range than the reported for 2-naphthol (−6.7) [24], 4-hydroxydiphenyl ether (−9.4) [25] and 1-naphthol-2-sulfonate (−8.0) [26].

Fluorescence titrations can give information about the time scale in which fluorescence decay and excited-state proton exchange occur. When exciting at the isosbestic point $\lambda = 299$ nm the normalized fluorescence at 352 and 443 nm versus the pH, ϕ/ϕ_0, gives a double sigmoid curve, with a wide plateau region between 3 and 8 pH units, and two inflection points at 1.2 and 9.8 (Fig. 3b). In these plots, the emission at 443 nm has been corrected for the contribution from the protonated form at this wavelength, the correction factor given by F_{443}/F_{352} at the lowest pH. The two-step shape shows a reversible two-state model that reflects the equilibria corresponding to the acid–base reactions of the excited and ground states. The curves for the ionic or neutral forms of DBFOH intersect at pH 2, $\phi/\phi_0 = 0.6$, and the plateau extends along six pH units. This is the characteristic case in which both the rate constant of dissociation of DBFOH* and the rate of pseudo first order protonation of the excited anion are comparable to the rate of the fluorescence. This can be proved by fitting the curves to the equations [29]:

$$\frac{\phi}{\phi_0} = \frac{|\mathrm{H}^+|/(|\mathrm{H}^+| + K_a) + k_4/k_2|\mathrm{H}^+|}{1 + k_3/k_1 + k_4/k_2|\mathrm{H}^+|},$$
$$\frac{\phi'}{\phi'_0} = \frac{K_a/(|\mathrm{H}^+| + K_a) + k_3/k_1}{1 + k_3/k_1 + k_4/k_2|\mathrm{H}^+|} \quad (3)$$

k_1 and k_2 being the rate constants of fluorescence of the neutral and ionic form, and k_3, k_4, the rate constants for the deprotonation of DBFOH*/protonation of (DBFO$^-$)*. The resulting fits are the solid curves in Fig. 3b. Ideally, the sum $\phi/\phi_0 + \phi'/\phi'_0$ in the flat regions should be 1, but this is not the case (ca. 1.26). This may be due to overlapping of the fluorescence between both forms of the molecule, and consequently, the calculated values will be different [25]. In any case, the fits yield $k_4/k_2 = 63$, and $k_3/k_1 = 2.1$, taken as the mean values from the neutral or ionic form, i.e., the fluorescence of excited DBFOH is contemporaneous with the proton trans-

Table 2
Spectral characteristics of DBFOH with natural CDs at 298 K

	Absorption	Emission	λ_F (nm)	Stokes shift[a] (cm^{-1})
α-CD	No effect	No effect		
β-CD	Defined isosbestic points	Enhancement	352	3236
γ-CD	Three defined isosbestic points; two isosbestic points that shift with [γ-CD]	Low [γ-CD]: quenching; high [γ-CD]: enhancement	352	3236

[a] λ_{ex} = 316 nm.

fer in the lowest excited state, so thus DBFOH* is partially dissociated before the emission takes place. In such case, the acid–base reaction in S_1 cannot reach the equilibrium and the pK^* obtained from the inflection point in the titration curves would be only an estimation. Yet, the value obtained from the inflection point (1.2) is not far from the 1.54 calculated by the Förster cycle. Time-resolved fluorescence experiments should be used in order to complete these results.

3.3. Studies of DBFOH + CD in water

3.3.1. Absorption spectroscopy

The effects produced by the addition of α-, β-, and γ-CD on the UV spectrum of DBFOH in water have been collected in Table 2. β-CD produces defined isosbestic points at 254, 260, 292, 302, and 316 nm, together with redshifts of 2 and 4 nm for the bands at 288 and 310, respectively. With the wider macrocycle, γ-CD, isosbestic points also appear at 254, 270, 294, 306 and 320 nm, the last two points shifting slightly with the CD concentration. There is also a redshift of 2 nm for the band at 288 nm (Fig. 5). The α-CD does not modify the DBFOH spectrum.

The absorption data suggest some sort of interaction in the ground state between DBFOH and β- or γ-CD. In the case of β-CD, the isosbestic points remained constant for all the concentration range. In contrast, for γ-CD, the points at 306 and 320 nm vary, which suggests the possibility of different stoichiometries [30]. Finally, the red shifts detected both with β- and γ-CD, resembling the spectrum in ethanol (Fig. 2), indicate a less polar environment for the molecule, similar to that inside the CD cavity, what suggests the inclusional nature of the complex [31].

3.3.2. ^{1}H NMR spectroscopy

Fig. 6 shows the proton spectrum of DBFOH and its assignation. The addition of β- or γ-CD produces important shifts in the DBFOH protons, as well as changes in those of the CDs, specially the inner H5 and H3 ones. These evidences undoubtedly indicate that DBFOH is lodged within the cavity of the CD, in agreement with the UV and fluorescence experiments. With α-CD, no changes in any of the resonances either of the host or the guest are perceived.

In the case of β-CD, all the protons move upfield. Those of the cavity, H3 and H5, shift in −0.10 and −0.17 ppm, respectively, whereas the outer protons shift in a lesser extent (H1: −0.05, H2: −0.08, H4: −0.03, and H6: −0.08 ppm). According to the dimensions of both molecules and to steric considerations just a single guest can be lodged completely inside the β-CD cavity, along its longitudinal axis.

The protons of the γ-CD shift upfield in the presence of DBFOH. As with the β-CD, the changes in the inner protons are the most remarkable (H3: −0.14, and H5: −0.20 ppm). The signals of the H6, at the primary rim of the cavity, move considerably, in −0.13 ppm which may suggest the participation of the narrower edge of this CD in the complex. The external protons H1, H2, and H4, change −0.08, −0.05, and −0.07 ppm, respectively. In the case of this wider CD, two DBFOH molecules can be lodged in the cavity, yielding a complex other than 1:1. In order to elucidate the actual stoichiometry, we performed a NMR titration according to the Job method [22], as shown in Fig. 7. The abscissa is the mole fraction of host or guest, obtained by mixing solutions

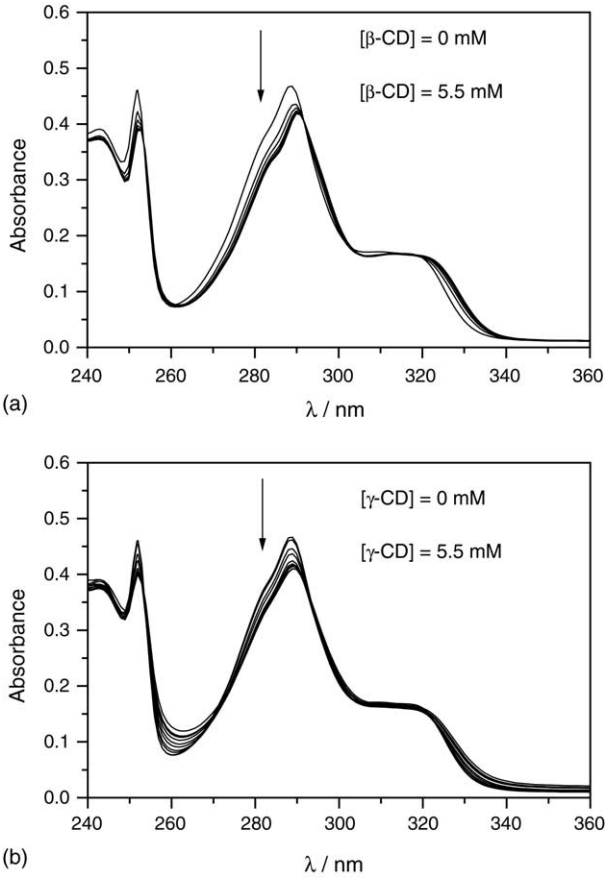

Fig. 5. (a) UV spectra of DBFOH 3.6×10^{-5} M in the presence of variable amounts of β-CD (arrows indicate the changes in intensity with increasing [β-CD]); (b) in the presence of γ-CD.

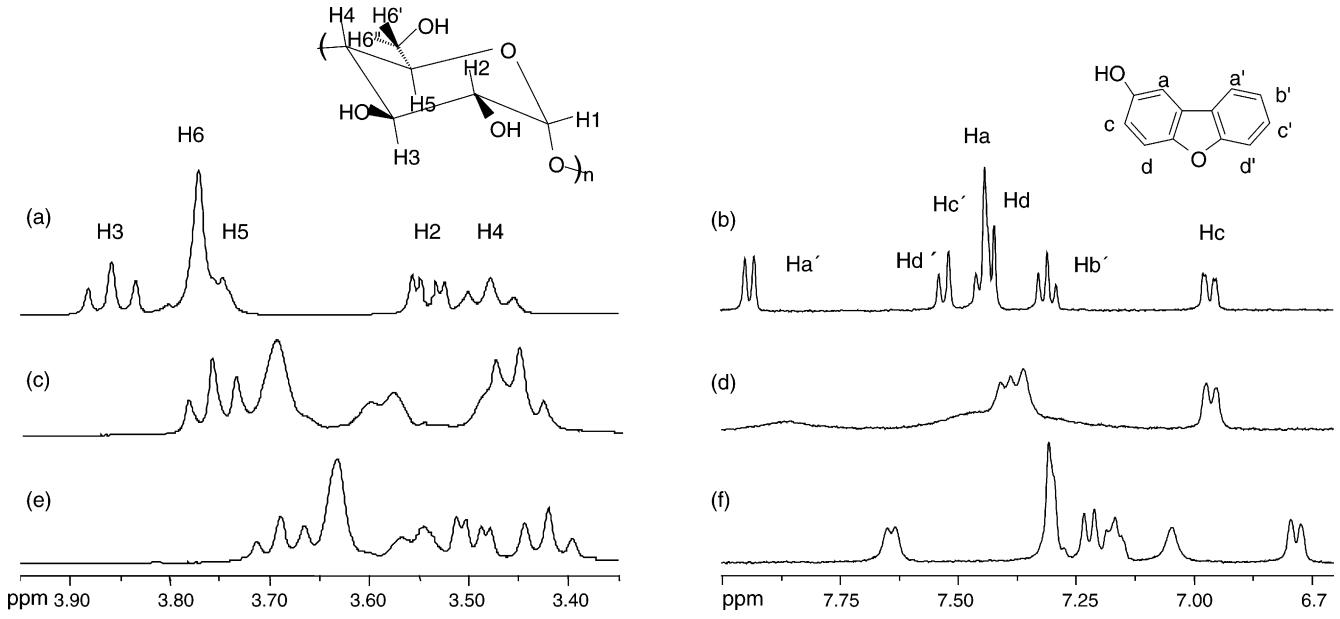

Fig. 6. Selected zones of the ^{1}H NMR spectra of (a) β-CD 3×10^{-4} M; (b) DBFOH 4×10^{-4} M; (c and d) 9×10^{-4} M DBFOH + 3×10^{-4} M β-CD; (e and f) 1×10^{-3} M DBFOH + 3×10^{-4} M γ-CD.

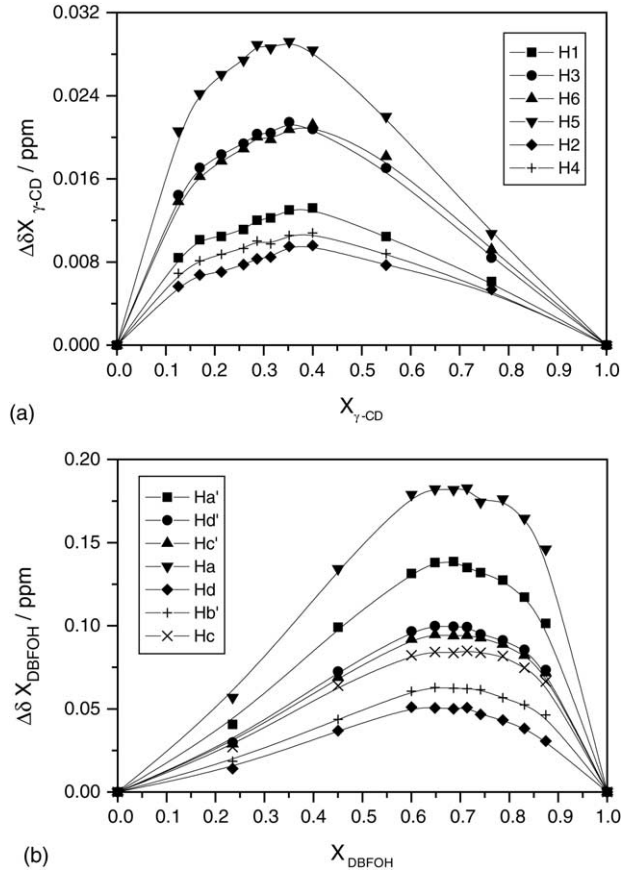

Fig. 7. Job plot of protons of (a) γ-CD and (b) DBFOH.

of fixed concentration. The maximum is reached at a molar ratio $X_{\gamma\text{-CD}} = 0.35$ and $X_{\text{DBFOH}} = 0.65$, proving that the complex is a $(\text{DBFOH})_2$:γ-CD type. The remarkable changes in the chemical shifts are in accordance to tight packing of two DBFOH molecules within the γ-CD.

3.3.3. *Steady-state fluorescence*

The experiments with CDs have been carried out at neutral pH. The samples were excited at the highest wavelength isosbestic point in the absorption spectrum (316 nm). This guarantees that both the DBFOH and the complexes are excited according to their relative concentrations, yielding an emission proportional to their quantum yields.

(a) *α-Cyclodextrin*. As it was expected, there are no changes in the fluorescence spectra. The narrower α-CD either does not form complex with DBFOH or the binding is weak. 2-Dibenzofuran carboxylate [19] does not form complexes either, although DBF and 2-dibenzofuran carboxylic acid can form a relatively weak complex with this CD [18,19].

(b) *β-Cyclodextrin*. Fig. 8 shows the fluorescence spectra of DBFOH upon the addition of β-CD. The intensity of the whole spectrum enhances and neither isoemissive points nor band shifting are perceived. This suggests that the complexation with β-CD does not affect the acid–base equilibrium in the excited state, and the –OH group must feel the same microenvironment than in the absence of CD, i.e., hydratation outside the cavity of the β-CD. This same effect was found for 2-dibenzofuran carboxylate with β-CD [20], in which the $-COO^-$ remains outside the CD, with a preferred orientation towards the narrower edge of the cavity. This behavior is in contrast with that

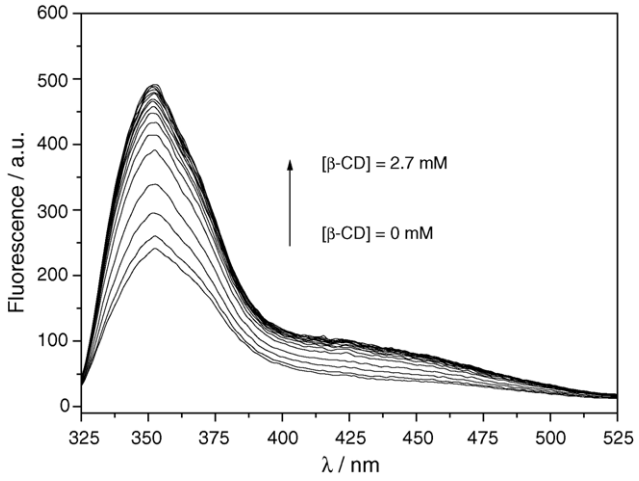

Fig. 8. Fluorescence spectra of DBFOH 5.4×10^{-6} M in the presence of variable amounts of β-CD at 25 °C (arrows indicate the changes in intensity with increasing [β-CD]).

of 2-naphthol [13] or 1,6-naphthalenediol [14] in which β-CD favors the emission of the molecular form with respect to the ionic one, by diminishing the rate constant of ionization in the excited state. The DBFOH is axially longer than 2-naphthol so that the –OH group can be exposed to the solvent.

(c) *γ-Cyclodextrin*. With γ-CD, DBFOH initially undergoes fluorescence quenching at low ratios [CD]/[DBFOH], then, at medium and high ratios the intensity increases up to reaching a plateau (Fig. 9). The emission band does neither shift nor has an isoemissive point within the entire range of γ-CD concentration. Unlike the DBF and DBFCA, the DBFOH does not give excimer-type emission, although this behavior of the DBFOH is similar to that of 2-dibenzofuran carboxylate.

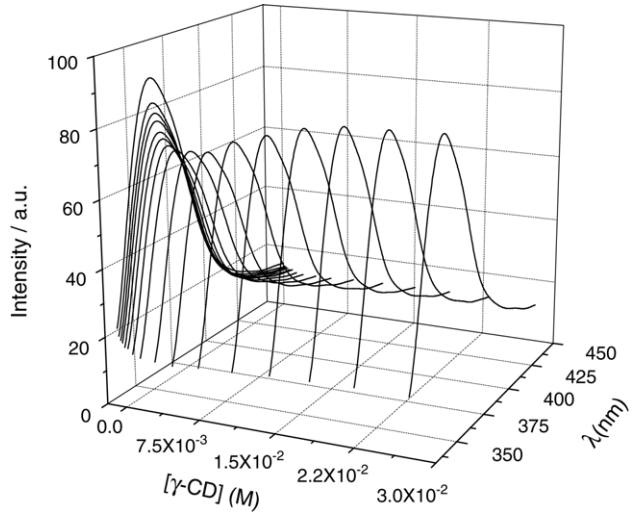

Fig. 9. Fluorescence spectra of DBFOH 2.71×10^{-5} M in the presence of variable amounts of γ-CD at 15 °C.

Taking into account that the stoichiometry is 2:1, the initial quenching detected must be due to the formation of a complex of such stoichiometry, due to the higher proportion of fluorophore. This complex coexists with the 1:1, which has a quantum yield of fluorescence similar to that of DBFOH. When [γ-CD] increases, the equilibrium is shifted to the 1:1 complex. The ratio between the emissions at 352 and 443 nm as a function of [γ-CD] is constant, what indicates that the acid–base equilibrium remains the same for both stoichiometries, suggesting again that the –OH group must be outside the cavity, irrespectively of the stoichiometry of the complex.

3.3.4. Estimation of the binding constants

For a 1:1 complex the estimation of the association constant by fluorescence reduces to the recording of the emission intensity at a certain wavelength (currently at λ_{max}) versus the CD concentration. Afterwards, the data are linearised by the Benesi–Hildebrand approach or fitted directly by a non-linear least squares fitting procedure. In the general case of having several emissive species, each one of concentration c_i, and provided we are working within the range of linearity, the fluorescence at a certain CD concentration is

$$F_j = \sum_{i=0} k_i c_i \tag{4}$$

where k_i is a proportionality constant related to the quantum yield of each one of the fluorophores. In the absence of CD, the fluorescence is due solely to the guest, $F_0 = k_0 c_0$, so thus

$$\frac{F_j}{F_0} = \sum_{i=1} q_i \chi_i \tag{5}$$

where $q_i = k_i/k_0$ and χ_i the mole fraction of the fluorophore. The corresponding mass balance and mass action law connect the concentrations of all of the components in solution, with a constant for each binding step K_i. From the numerical point of view, the problem reduces to finding a vector $(K_1 \ldots K_i \ldots q_1 \ldots q_i)$ that minimizes the sum of the squares of the residuals at each concentration. However, in the case of stoichiometries different than 1:1, the increasing number of fitting parameters produces broader confidence limits, sometimes making the results unreliable. This problem may be overcome if a wider set of experimental data is used, for example, by taking into account the emission measured at other wavelengths. In this way, a multivariable analysis of the whole set of wavelengths under study can be performed, by imposing the condition that the binding constants be the same for each wavelength. In this case, Eq. (5) must be rewritten as:

$$\left(\frac{F_j}{F_0}\right)_\lambda = \sum_{i=1} q_{i,\lambda} \chi_i \tag{6}$$

Table 3
Binding constants, $K \times 10^{-3}$ (L mol^{-1}), q_i, and thermodynamic parameters for DBFOH complexes with CDs

		15 °C	25 °C	35 °C	45 °C	ΔH (kJ mol^{-1})	ΔS (J mol^{-1} K^{-1})
β-CD	K_1	3.63 ± 0.05	2.95 ± 0.04	2.17 ± 0.03	1.67 ± 0.02	−20 ± 1	0 ± 4
	q_1	2.083 ± 0.004	2.161 ± 0.004	2.333 ± 0.005	2.505 ± 0.005		
γ-CD[a]	K_1	0.332 ± 0.009	0.39 ± 0.02	0.31 ± 0.02	0.240 ± 0.006		
	K_2	28.3 ± 0.5	13.2 ± 0.3	7.4 ± 0.2	11.2 ± 0.1		
	q_1	1.053 ± 0.006	1.070 ± 0.005	1.156 ± 0.005	1.413 ± 0.004		

[a] The parameter q_2 has been set to zero in the fit.

and now, the error function to be minimized becomes

$$E = \sum_{\lambda} \sum_{j} \left((F_j/F_{0,\lambda})^{\text{cal}} - (F_j/F_{0,\lambda}) \right)^2 \quad (7)$$

where the λ subindex stands for the wavelength. For accomplishing this calculation, we have written a routine in MATLAB® based on the Newton–Raphson algorithm, which switches to the Levenberg–Marquardt in the case of poor convergence. The input parameter is a vector that contains the initial guess for the binding constants and q_i, and the output are the estimation of the parameters with their error bounds, defined as the confidence intervals corresponding to a significance level, $\alpha = 0.16$. A weight factor, w_λ, is introduced at each wavelength in Eq. (7), taken as the absolute value of the difference between $F_{0,\lambda}$ and the maximum value reached in the binding. In this way, the wavelengths at which the changes in intensity are more important contribute with a higher statistical weight.

The fitted parameters for β- and γ-CD at each temperature are collected in Table 3. The results of the multivariable calculation show that $q_1 = 2.16$ for the β-CD and $q_1 = 1.07$ for the γ-CD, at 25 °C, i.e., the fluorescence of the 1:1 γ-CD complex is practically the same than that of DBFOH. This explains the fall in intensity at the beginning of the plot in Fig. 9 (since $q_2 = 0$). If the concentration of γ-CD were high enough, and consequently, all the DBFOH were in the form of 1:1 complex, the intensity should reach a value close to that of free DBFOH, at 15 and 25 °C, and higher at 35 and 45 °C.

The binding constants for β-CD are close to those of DBF and DBFC (1.8×10^3 M^{-1}), indicating that the driving force in the inclusion must be similar in the three complexes. This makes sense since the DBFC:β-CD complex has the carboxylate outside the cavity [20] and the driving force is the inclusion of the aromatic part of the molecule. Something similar is expected to happen with DBFOH, in which the results prove that the acid–base equilibrium in the excited state is not affected by the presence of the β-CD. Regarding the γ-CD, the fact that K_2 is more than two orders of magnitude higher than K_1 reveals that the inclusion of a second molecule of DBFOH is more favorable than the inclusion of the first one. The confidence interval of the fitted parameters widens in this case with respect to the 1:1 complex, due to the increased number of variables. On the other hand, the relatively low value for K_1 in the γ-CD compared to that of β-CD agrees with according to the wider cavity of γ-CD, in which the first DBFOH molecule that enters must fit loosely. The same trend in the association constants was observed in the complexes between γ-CD with DBFC.

The thermodynamic parameters of the binding, enthalpy and entropy can be obtained from the dependence of K on the temperature through the van't Hoff equation, provided these magnitudes remain constant within the working temperature range. The entropy and enthalpy have been calculated by a weighed least-squares method, the weights given by the standard deviations of K at each temperature (Table 3). In the case of β-CD complex, ΔH is negative, as usual in CD complexes, and the entropy is negligible within the experimental uncertainty. The binding enthalpy is practically the same than that for DBF and DBFC with this CD (-22 ± 3 and -19.1 ± 0.4 kJ mol^{-1}, respectively), confirming what has been said about the main driving force in the binding. The functional group in the 2 position has either no effect on the formation of the complex or it is negligible.

For the γ-CD complexes, although the errors in the parameters are higher than those with β-CD, we could estimate the enthalpy in each binding step by fitting K_1 and K_2 to the van't Hoff equation separately. The enthalpy for the 1:1 step is -9 kJ mol^{-1}, lower than -26 kJ mol^{-1} for the second one. The result is in accordance to the loose fit of DBFOH in the cavity of the γ-CD (1:1 complex), which becomes tighter once the first guest molecule is lodged.

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References

[1] (a) K.A. Connors, Chem. Rev. 97 (1997) 1325;
(b) M.V. Rekharsky, Y. Inoue, Chem. Rev. 98 (1998) 1875.
[2] (a) J. Szejtli, Cyclodextrins and Their Inclusion Complexes, Kluwer Academic Publishers, Dordrecht, 1988;

(b) D. Duchene, Cyclodextrins and Their Industrial Uses, Editions de Santé, Paris, 1987;
(c) D. Duchene, New Trends in Cyclodextrins and Derivatives, Editions de Santé, Paris, 1991;
(d) A.R. Hedges, Chem. Rev. 98 (1998) 2035.
[3] W.L. Hinze, F. Dai, R.P. Frankewich, K.N. Thimmaiah, J. Szejtli, Cyclodextrins as reagents in analytical chemistry and diagnosis, in: J. Szejtli, T. Osa (eds.), Comprehensive Molecular Chemistry, vol. 3: Cyclodextrins, Pergamon Press, Tarritown, NY, 1996, p. 588.
[4] J.M. Schuette, N. Thilivali, A. Muñoz de la Peña, K.L. Greene, C.K. Williamson, I.M. Warner, J. Phys. Chem. 95 (1991) 4897.
[5] J.M. Schuette, N. Thilivali, I.M. Warner, J. Phys. Chem. 96 (1992) 5309.
[6] E.K. Fraiji, T.R. Cregan, T.C. Werner, Appl. Spectrosc. 48 (1994) 79.
[7] J.H. Larose, T.C. Werner, Appl. Spectrosc. 54 (2000) 284.
[8] G. Patonay, A. Shapira, P. Diamond, I.M. Warner, J. Phys. Chem. 90 (1986) 1963.
[9] A. Muñoz de la Peña, T. Ndou, J.B. Zung, I.M. Warner, J. Phys. Chem. 95 (1991) 3330.
[10] K. Takahashi, Chem. Rev. 98 (1998) 2013.
[11] C. Retna Raj, R. Ramaraj, Chem. Phys. Lett. 273 (1997) 285.
[12] G. Pistolis, Chem. Phys. Lett. 304 (1999) 371.
[13] H.-R. Park, B. Mayer, P. Wolschann, G. Köhler, J. Phys. Chem. 98 (1994) 6158.
[14] R.A. Agbaria, B. Uzan, D. Gill, J. Phys. Chem. 93 (1989) 3855.
[15] T. Yorozu, M. Hoshino, M. Imamura, H. Shizuka, J. Phys. Chem. 86 (1982) 4422.
[16] S. Panja, S. Chakravorti, Chem. Phys. Lett. 336 (2001) 57.
[17] G. Krishnamoorthy, S.K. Dogra, J. Photochem. Photobiol. A: Chem. 123 (1999) 109.
[18] P. Rodríguez, M. Sánchez, J.R. Isasi, G. González-Gaitano, Appl. Spectrosc. 56 (2002) 1490.
[19] P.R. Sainz-Rozas, J.R. Isasi, M. Sánchez, G. Tardajos, G. González-Gaitano, J. Phys. Chem. A 108 (2004) 392.
[20] G. González-Gaitano, P.R. Sainz-Rozas, J.R. Isasi, G. Tardajos, A. Guerrero-Martínez, J. Phys. Chem. B 108 (2004) 14154.
[21] MestRe-C, Version 3.7.9.0, Departamento de Química Orgánica, Universidad de Santiago de Compostela, 2004.
[22] K.A. Connors, Binding Constants: The Measurement of Molecular Stability, Wiley, New York, 1987, Chapter 2.
[23] E. Von Lippert, Z. Electrochem. 61 (1957) 962.
[24] J.F. Ireland, P.A.H. Wyatt, Adv. Phys. Org. Chem. 12 (1976) 131.
[25] N. Rajendiran, M. Swaminathan, Int. J. Chem. Kinet. 29 (1997) 861.
[26] R. Krishnan, T.G. Fillingim, J. Lee, G.W. Robinson, J. Am. Chem. Soc. 112 (1990) 1353.
[27] T. Förster, Z. Elektrochem. 54 (1950) 42.
[28] Z.R. Grabowski, A. Grabowska, Z. Phys. Chem., Neue Folge 101 (1976) 197.
[29] A. Sharma, S.G. Schulman, Introduction to Fluorescence Spectroscopy, Wiley, New York, 1999, Chapter 2.
[30] K.A. Connors, Binding Constants: The Measurement of Molecular Stability, Wiley, New York, 1987, Chapter 4.
[31] L. Liu, Q.X. Guo, J. Incl. Phenom. Macro. 42 (2002) 1.