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Binding of dibenzofuran and its derivatives to water-soluble β-cyclodextrin polymers

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Abstract

The binding capacity of β-cyclodextrin polymers (β-CDP), cross-linked with epichlorohydrin, has been compared with that of unimeric β-cyclodextrin for the inclusion of dibenzofuran (DBF) and two of its derivatives: 2-hydroxydibenzofuran (DBFOH) and dibenzofuran 2-carboxylate (DBFC). Gel permeation chromatography, ^{1}H and ^{13}C NMR, and dynamic light scattering, together with membrane ultrafiltration, were used to characterize and fractionate the polymeric samples. Their interactions with the dibenzofuran derivatives have been analyzed by absorption and fluorescence spectroscopies. Fluorescence anisotropy titrations were used to obtain the apparent binding constants. The affinity of the neutral dibenzofurans for the polymers is significantly higher than those of the unimeric β-CD, irrespective of the molecular weight or the microstructure of the polymer, whereas the charge in the anionic dibenzofuran derivative hinders the formation of the complex. The most hydrophobic DBF shows a higher affinity for the polymer with a higher content in β-CD, whereas the opposite behavior is displayed for DBFOH. The results are explained in terms of the synergic effect produced by the glyceryl cross-linking bridges, the relative hydrophobicity of the guest–host system and the density of the polymer network.

Keywords: β-Cyclodextrin; Cyclodextrin polymers; Dibenzofurans; Fluorescence anisotropy; Binding constants

1. Introduction

The main feature that makes cyclodextrins (CDs) of interest is their ability to form inclusion complexes with a wide diversity of molecules, either in solution or in solid phase, a property that offers many attractive applications described extensively in the literature [1]. In the case of fluorescent guests, the inclusion can affect the ground and/or excited electronic states of the fluorophore, consequently changing the spectral properties of the substrate. Fluorescence enhancement is the most common situation, which has found a number of interesting analytical applications, and can be attributed 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 [2]. The quenching of fluorescence, although less common, has also been observed [3–5]. In addition to this, the photochemistry of the substrate may also be changed [6]. The CD behaves as a protective casing against photobleaching or side reactions or, on the contrary, it can act as a catalyst, inducing excimer formation [7,8]. 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 [9,10]. The CDs have also been proven to inhibit the twisting of functional groups in molecules that display twisted intramolecular charge transfer (TICT) [11,12].

The field of applications expands when the CDs are chemically modified by selective conversion of the hydroxyl groups to other functionalities (methylation, for example, in order to increase the solubility) [13]. In the last few years, the interest has been focused in the synthesis of polymeric cyclodextrins (CDPs) [14]. These are usually obtained by reaction of the native CDs with a cross-linking agent, such as epichlorohydrin (EP). The properties of such polymers can be tailored according to the type of cyclodextrin, the spacer, or the molar

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ratio between both [15,16]. In this way, the resulting product provides other possible binding sites to the host molecules different from the cavity of the CD, thus increasing the versatility and potential applications of the material. Depending on the polymerization conditions, insoluble polymers can be produced, which have found applications in the removal of water pollutants [17], in chromatography [18] or in pharmaceutical [19] and food industries [20].

In continuation with our previous works dealing with the spectral features of CDs and dibenzofurans [21–24], a family of dioxin-like compounds, and their interactions with insoluble β-CD polymers [25], we have extended the study to water-soluble polymers. The advantage of these polymers is their high solubility in water compared to the native β-CD (ca. 18 g L^{-1}). The effect in the binding of the molecular weight and the microstructure of the polymer and the type of substrate has been investigated by spectroscopic methods.

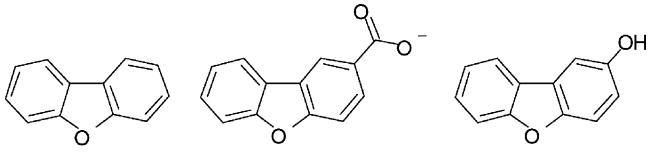
2. Experimental

2.1. Chemicals

Dibenzofuran (DBF) and 2-hydroxydibenzofuran (DBFOH) were obtained from Aldrich with purities of 99 and 98%, respectively. 2-Dibenzofuran carboxylic acid (DBFCA) was also obtained from Aldrich (Rare Chemicals Library). DBFOH was purified from an ethanol solution by precipitation in water, whereas the rest of the reactants were used as received. All the solutions were prepared with deionized water using a Wasserlab, Type I-Reagent Grade-Water equipment. The chemical structures are shown in Scheme 1.

Two β-CD polymers were used in this work: one was synthesized in this laboratory (β-CDP1), and the other one (β-CDP2) was obtained from Cyclolab (batch no. CYL-265). The commercial polymer has a β-CD content of 50–55% in mass, according to the manufacturer specifications. Polyethylenglycol (PEG) was from Panreac (average molecular weight of 3500–4500), and polyvinylalcohol (PVA) from Aldrich (average molecular weight of 85,000–146,000, with 4% acetylation degree).

The β-CD used for the synthesis of β-CDP1 was kindly donated by Roquette Laisa España S.A. (purity 99%). The epichlorohydrin (EP) (99%) and sodium hydroxide (97%) were obtained from Aldrich.



DBF DBFC DBFOH

Scheme 1. Structures of dibenzofuran and derivatives.

2.1.1. Preparation of the β-CD polymers (β-CDP)

The β-CDP1 has been synthesized by suspension polymerization of epichlorohydrin and β-CD under alkaline conditions, according to the procedure described by Zhu and Brizard [26]. The degree of polymerization is known to depend critically on the NaOH concentration, reactant ratios, reaction time and temperature [16]. In order to obtain a soluble polymer of adequate molecular weight, the molar ratio β-CD:EP was 1:16, the NaOH concentration was 40% in weight, and the temperature was 35 °C. After 50 h, the resulting polymer was neutralized with HCl 6 M, washed with deionized water and ethanol and purified by Soxhlet extraction, using hexane and ethanol as solvents. The actual content of β-CD in the polymer was determined by ^{1}H and ^{13}C NMR, as described in the literature [27].

2.1.2. Ultrafiltration of the β-CD polymers

In order to isolate the different fractions, diluted solutions of the polymers of β-CD were ultrafiltrated under nitrogen through membranes with molecular weight cut-off (MWCO) of 5, 50 or 100 kDa (polyethersulfone, Amicon Bioseparation, Millipore). We isolated a fraction from β-CDP1 retained by the 100 kDa MWCO membrane (β-CDPH). In the case of the β-CDP2 we selected the intermediate fraction collected by filtering with the membranes of 5 and 50 kDa (β-CDPL).

2.2. Techniques

2.2.1. Gel permeation chromatography (GPC)

The characterization of the polymers was performed using a GPC system which consisted of a Waters 600 pump and 717 autosampler, coupled to a PDA detector (Waters 996) and RI detector (Waters 2414). The columns were either an Aquagel OH-30 or an Aquagel OH-40 (Polymer Laboratories). For the estimation of the molecular weights the columns were calibrated with standards of polyethyleneglycol and polyethyleneoxide (Polymer Laboratories). Sample volumes of 100 μL with concentration less than 0.5% (w/w) were injected. The eluent was 0.2 μm filtered water and the flow 1 mL/min.

2.2.2. Dynamic light scattering (DLS)

DLS measurements were performed at a scattering angle of 90° using a DynaPro-MS/X photon correlation spectrometer equipped with a 248-channel multi-tau correlator and a Peltier effect temperature unit. The wavelength of the laser was 825.2 nm. The size distribution was obtained from the intensity autocorrelation function by regularization analysis, implemented in the Dynamics™ software package, and the hydrodynamic radii were calculated from the diffusion coefficients by means of the Stokes–Einstein equation. Temperature was 25.0 ± 0.1 °C unless otherwise stated. All the samples were filtered through 0.45 μm pore size syringe filters before the measurements.

2.2.3. Spectroscopic techniques

UV spectra were acquired with a HP 8452A diode array spectrophotometer (10 spectra per second, 4 s 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 the longest wavelength. The scan rate was 300 nm/min, and the excitation and emission slits were both fixed at 6.0 nm. Quartz cuvettes of 0.400 cm-pathlength were employed both in absorption and fluorescence measurements, keeping the temperature constant with an external heated circulating bath at 25.0 °C. Fluorescence anisotropy was measured with the same spectrofluorimeter in the L-format method, by exciting at the longest wavelength maximum at its red edge, in order to avoid absorption by the polarizer. The wavelengths of excitation and emission were 300 and 340 nm for DBF, 300 and 374 nm for DBFC, and 316 and 352 nm for DBFOH. In the case of the studies with DBFC, the pH was above 8. For the calculation of the binding constants, the concentration of the DBF derivatives was always kept within the interval of linearity, and the ratio polymer:DBF-X varied by direct titration in the cell with a stock solution containing the polymer and the aromatic derivative. The non-linear regression analysis of the anisotropy plots was performed with a program written in MATLAB® [28].

For the ^{13}C and ^{1}H NMR measurements 91.8 and 28.6 mg of the β-CDPH and β-CDPL polymers, respectively, were dissolved in 0.5 mL D_2O (Aldrich, 99.9% minimum in D), and transferred to NMR tubes. The spectra were recorded at 300 K in a Bruker Avance 400 Ultrashield spectrometer (9.36 T) by averaging either 25,000 scans for the ^{13}C spectra or 200 for the ^{1}H NMR.

3. Results and discussion

3.1. Characterization of the polymers: GPC, DLS, and NMR

The GPC of β-CDP1 reveals a heterogeneous mixture of a large component, detected at a low elution time and a polydisperse region at elution times between 6 and 11 min (Fig. 1). The large component represents 11% of the mass and its quick elution, above the exclusion limit of the Aquagel OH-40 column, indicates a molecular weight according to the PEO standards higher than 1000 kDa. Renard et al. have found that the formation of hydrosoluble polymeric CD of high molecular weight by polycondensation usually occurs when the reaction time is long enough (close to the gel point), although small molecular weight components are always present in the final product [27]. This also seems to occur in our case, in contrast to the results found by other authors who claim that the limit for the gel point is at ca. 10 kDa [29–32]. By filtration through a membrane of 100 kDa MWCO we could isolate this fraction of high molecular weight (β-CDPH).

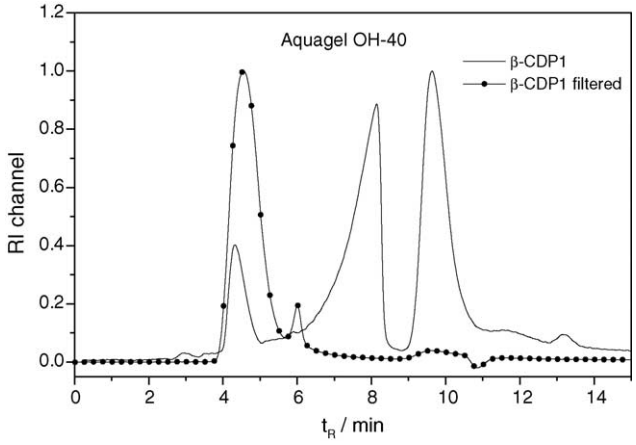


Fig. 1. Normalized chromatogram (Aquagel OH-40 column) of β-CDP1 and the high molecular weight fraction after ultrafiltration with a 100 kDa MWCO membrane.

The size distribution obtained by DLS according to the regularization method is shown in Fig. 2a. The scattered intensity is portioned between two overlapped components, having mean hydrodynamic radii of 19 ± 6 and 129 ± 67 nm. The slow relaxation mode, although represents an important fraction of the scattered light, has a scarce contribution in mass to the overall scattered intensity (ca. 4% in mass), the first peak being more representative of the polymer. To discard that slow mode is due to some kind of aggregation process in solution, as occurs with the native β-CD [33], we added NaOH to the samples up to pH 14. This produces the ionization of the non-reacted hydroxyl groups of the CD, a fact that seems to break the aggregation, in the same sense than the addition of chaotropic agents such as urea or salts. No changes in the intensity or size distribution were detected, not even when temperature was varied from 5 to 60 °C. The equivalent molecular mass, according to a general hydrodynamic equation for branched polysaccharides, based upon the Mark–Houwink–Sakurada equation [34], is 1.5×10^6 Da; a result that agrees with the fast elution time peak observed in the chromatogram and with the results obtained by Renard et al. [27].

In the case of the commercial sample (β-CDP2), its GPC analysis with the Aquagel OH-30 column shows two peaks (Fig. 3a). The first one corresponds to the polymer, whereas the second one is attributed to unimeric β-CD that has partially reacted with the EP. The ultrafiltration of the mixture with a 5 kDa MWCO membrane permits to recover the polymer (Fig. 3a). Apparently, the chromatogram is the same than that obtained by other authors with this same polymeric sample [35]. However, when analyzing this fraction with a column that covers a wider range of molecular weights, we obtained two elution peaks (Fig. 3b). That at 4.6 min resembles the large component obtained in our polymerization, isolated as β-CDPH. This suggests that ultrafiltration with a 5 kDa MWCO membrane is not enough for obtaining a single distribution. In order to achieve a less heterogeneous sample

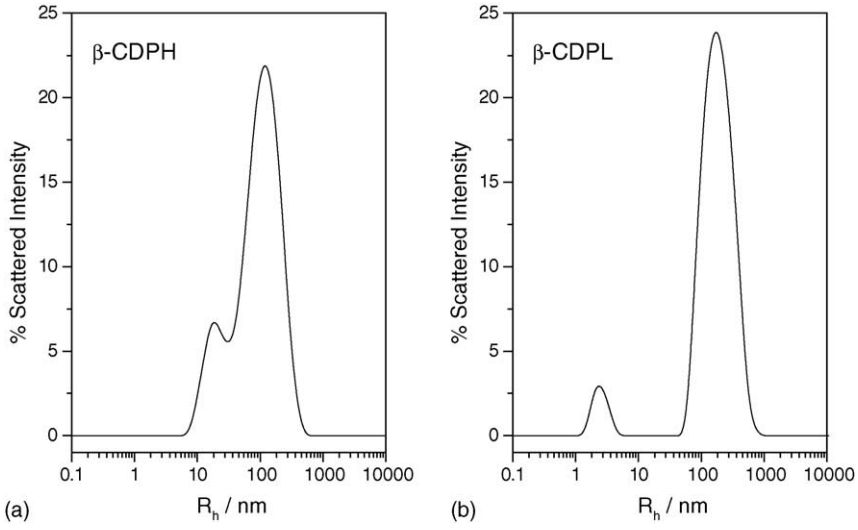


Fig. 2. DLS size distribution for (a) β-CDPH and (b) β-CDPL (25 °C, 5 mg/mL of sample).

for the binding studies we isolated the fraction of intermediate molecular weight at 9.5 min as shown in Fig. 3b (β-CDPL hereafter), by using a membrane of 50 kDa cut-off. The DLS reveals, as in the former case, two peaks with hydrodynamic radii of 2.5 ± 0.5 and 200 ± 100 nm (Fig. 2b). The first one is the polymer, having a calculated molecular weight of 15 kDa. The second peak must be ascribed to a component of high molecular weight, which represents a negligible contribution to the total mass. Neither using alkaline conditions nor heating up to 60 °C eliminates this slow diffusive mode, which precludes a possible effect of aggregation.

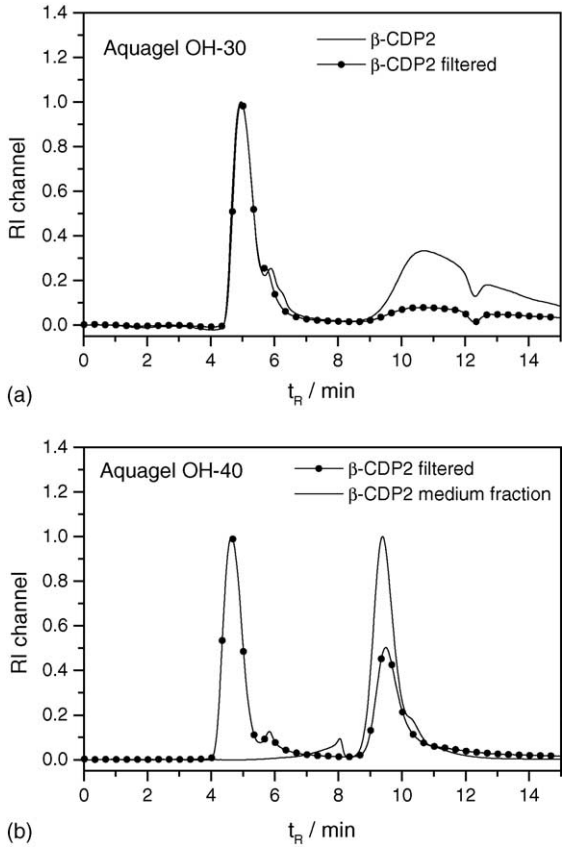


Fig. 3. (a) Normalized chromatogram of β-CDP2 and the fraction after ultrafiltration by 5 kDa MWCO membrane (Aquagel OH-30 column); (b) normalized chromatogram of the 5 kDa MWCO filtered β-CDP2 and its intermediate fraction between 5 and 50 kDa MWCO (by using the column Aquagel OH-40).

The β-CD content in each polymer can be deduced from ^{1}H NMR. The proton spectra in both cases are broad (Fig. 4c and d), since most of the β-CD protons appear in the same region than those of the hydroxypropyl ether linkers (between 3.2 and 4.3 ppm). However, the doublet of the non-interchangeable H1 of β-CD, integrating for seven protons, appears at 5.04 ppm, well apart from the rest of the signals, and it can be used to estimate the amount of β-CD in the polymer.

These polymers are inevitably heterogeneous in their microstructure in what refers to: (1) the number of linkers directly bonded to each single cyclodextrin; (2) the length of the polyhydroxypropyl ether chains that link two CDs; and (3) the place of substitution in the β-CD (primary or secondary border). ^{13}C NMR can provide some information on these points. Each β-CD possesses 21 hydroxyl-reacting groups that can attach a spacer. According to the assignation by Renard et al. [27] (see scheme in Fig. 4), the integration of the signal at 61 ppm in the ^{13}C spectrum gives the amount of non-reacted C6 of the β-CD, and by difference, the substitution at the primary rim can be obtained. The signal due to the $-CH_2-OH$ of the spacer is also distinguishable at 62.5 ppm (C9 signal), and provides the number of free terminal polymerized EPs. Although less reliable, the substitution at the C2 and C3 of the β-CD, appearing at 79 and 77 ppm, respectively, can also be calculated by integrating jointly both resonances and with the aid of signal deconvolution and fitting (Fig. 4a and b). By combining the data of molar ratio of EP and β-CD in the polymer, more precisely known from the proton spectra, and the amount of terminal EP, from the ^{13}C data,

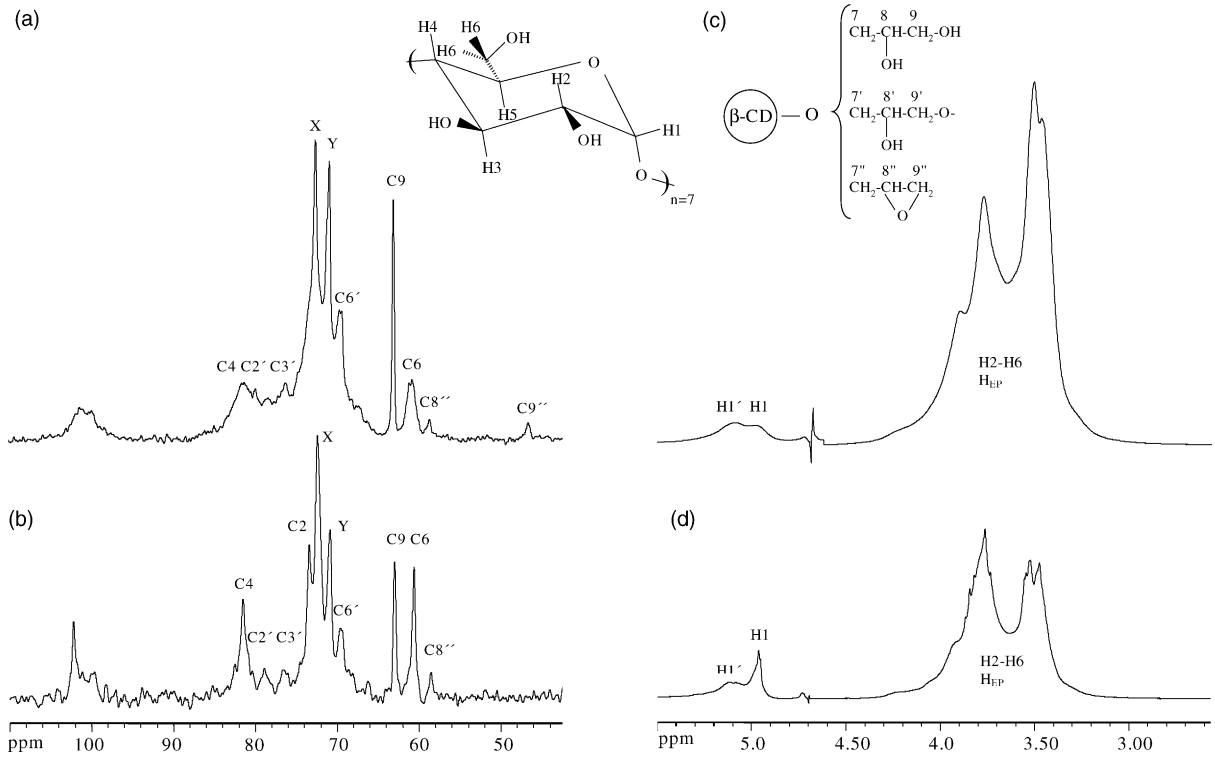


Fig. 4. ^{13}C NMR spectra of β-CDPH (a), and β-CDPL (b); ^{1}H NMR spectra of β-CDPH (c), and β-CDPL (d). X = C3, C5, Y = C7, C8, C7′, C8′, C9′.

the average number of hydroxypropyl ether units per β-CD, n_{EP}, can be deduced. The main features of the polymers have been compiled in Table 1.

3.2. Binding studies

3.2.1. Spectral features of the complexes

The absorption spectra of all the dibenzofurans display changes upon addition of the β-CD polymers, indicating the association of the guest in its ground electronic state. The changes produced are qualitatively similar irrespective of the polymer used (Figs. 5–7). A common feature of all the guests studied is the bathochromic shift of the bands that appear at longer wavelengths in each case. For example, in DBF, the addition of β-CDPH produces a redshift of all the bands, being the most manifest at 280 nm (around 2 nm). This band is also resolved into two defined components, as in the spectrum of DBF in ethanol [24], which indicates a less polar environment than water (Fig. 5a). With DBFOH the red shifting is ca. 4 nm for the bands at 288 and 310 nm, and isosbestic points (poorly resolved) arise at 292 and 302 nm, as it occurs with β-CD (Fig. 6a). In the DBFC, the band at 286 nm is red shifted to 288 nm, and there is also an overall increase of the absorption and the absence of isosbestic points. This contrasts with the results for β-CD, which produces isosbestic points at 296 and 302 nm, and the decrease in absorbance for the whole wavelength range (Fig. 7a).

As far as the fluorescence is concerned, the spectral features in the presence of the polymers are similar for both and depend on the DBF derivative. Thus, the addition of the polymer produces quenching of fluorescence and the arising of one isoemissive point at 356 nm for DBF and 396 nm for DBFC, respectively (λ_{ex} = 280 and 286, in each case), which indicates the presence of species emitting in different chemical environments (Fig. 8). At equal concentration of β-CD, the quenching is stronger for the polymer, with respect to the unimeric CD. It is worthy to mention that DBFC does not yield an isoemissive point when forming the complex with β-CD. In the case of DBFOH, the effect of the polymer is just the opposite, i.e., emission enhancement, as it occurs with β-CD, although it is more intense at the same concentration of guest and CD.

Table 1
Features of the β-CD polymers

	$\bar{M}_w$ (kDa)	R_h (nm)	% mass β-CD[a]	C6 substitution[b]	C2 + C3 substitution[b]	Terminal EPs (per CD)	n_{EP}
β-CDPL	15	2.5 ± 0.5	64	1.5/7	3.1/14	1.5	4.1
β-CDPH	1.5×10^3	19 ± 6	42	1.8/7	4.2/14	2.1	14.5

Molecular weight from DLS (see text).
[a] Deduced from ^{1}H NMR.
[b] Deduced from ^{13}C NMR.

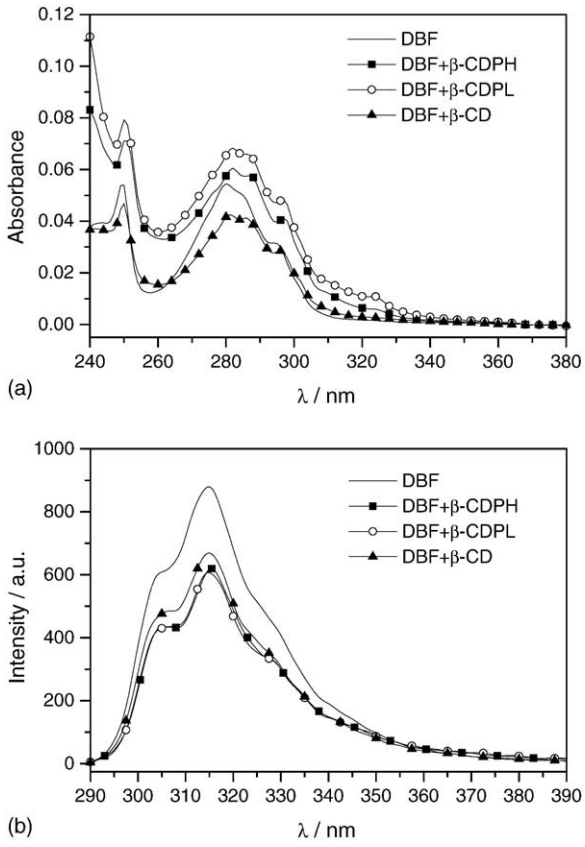


Fig. 5. Absorption (a), and fluorescence spectra (b) of DBF 1.17×10^{-5} M in the presence of β-CDPH, β-CDPL and β-CD. Equivalent [β-CD] = 1.74×10^{-3} M.

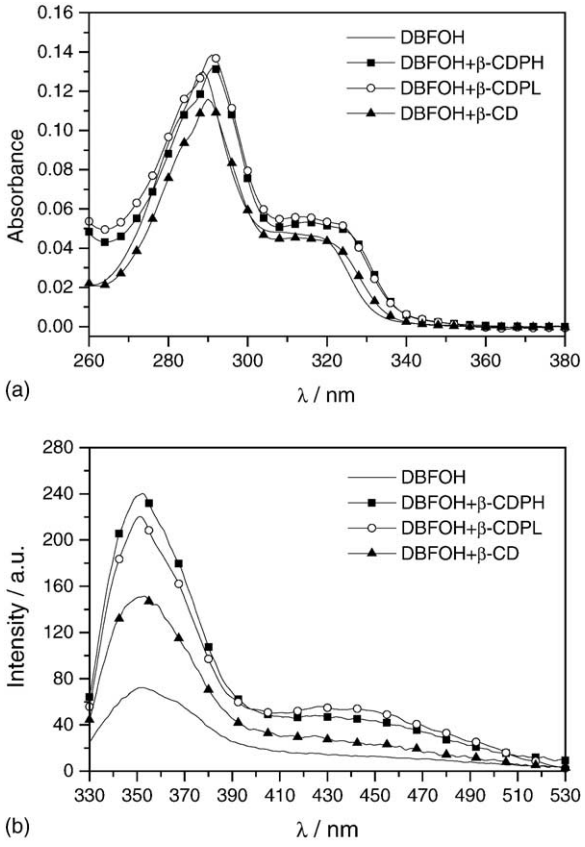


Fig. 6. Absorption (a), and fluorescence spectra (b) of DBFOH 2.76×10^{-5} M in the presence of β-CDPH, β-CDPL and β-CD. Equivalent [β-CD] = 1.75×10^{-3} M.

These evidences suggest that the interactions of the dibenzofurans with the polymers are qualitatively similar, although somewhat stronger to those occurring with β-CD. The type of interaction seems to be the same in all the cases, irrespective of the molecular weight or the composition of the polymer employed.

3.2.2. Anisotropy measurements

The anisotropy of the fluorescence emission is a very suitable magnitude for studying systems in which association occurs. The radiation emitted by the fluorophore in its free form is largely depolarized due to the quick rotation of the molecule during the time elapsed between the excitation and the emission. If this fluorophore binds to a large molecule, as β-CDP, its rotation is limited to that of the polymer and the emission will partially retain the state of polarization of the excitation. This effect can be quantitatively described by the measurement of the anisotropy, defined as:

$$r = \frac{I_{VV} - GI_{VH}}{I_{VV} + I_{VH}} \quad (1)$$

where I_{VV} is the intensity of the fluorescence measured with both polarizers (excitation and emission) in vertical position and I_{VH} that measured with the emission polarizer rotated at 90°. The G factor accounts for the depolarization due to instrumental sources, such as the wavelength dependence of the polarization due to the gratings or other optical components of the apparatus.

The anisotropy titration curves for the three derivatives with both polymers are shown in Fig. 9. The graphs have been plotted on a β-CD concentration basis, by considering the total mass of the polymer and its cyclodextrin content determined by ^{1}H NMR. The fraction of bound guest increases with the concentration of polymer as the equilibrium is shifted, which is reflected by the increase in anisotropy. In the limit of high concentration of polymer, r reaches a constant value that must be, necessarily, lower than the theoretical value of 0.4. With β-CDPH, the anisotropy is higher than with β-CDPL, as emerges from its largest size. Only the curve for DBFC does not reach a plateau, which suggests a poorer affinity for this polymer.

In quantitative terms, the anisotropy represents the sum of the fractional intensities due to the free and bound fluorophore according to the following equation [36]:

$$r = r_f^0 f_f + r_b^0 f_b \quad (2)$$

in which r_f^0 and r_b^0 are the anisotropies of the free and bound DBF-X, respectively, and f the fractional intensity for each species. If there is no change in the emission upon binding, then the fractional intensities equal the mole fractions, $f_i = \chi_i$,

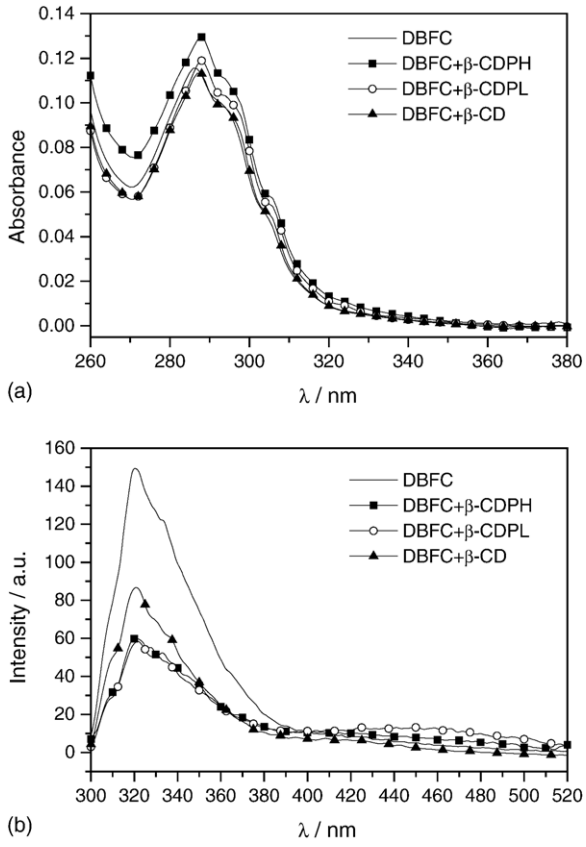


Fig. 7. Absorption (a), and fluorescence spectra (b) of DBFC 2.42×10^{-5} M in the presence of β-CDPH, β-CDPL and β-CD. Equivalent [β-CD] = 1.09×10^{-3} M.

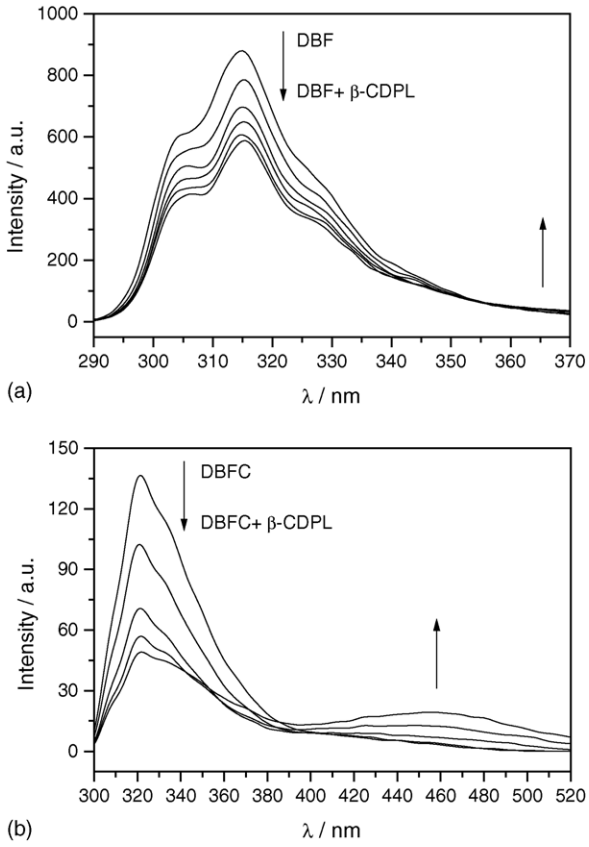


Fig. 8. Fluorescence spectra in the presence of β-CDPL of (a) DBF 1.17×10^{-5} M, and (b) DBFC 2.42×10^{-5} M.

and Eq. (2) can be written as a function of χ_i. Otherwise, the intensities of the fluorophore in each form must be taken into account. Thus,

$$r = \frac{\chi_f F_f^0 r_f^0 + \chi_b F_b^0 r_b^0}{\chi_f F_f^0 + \chi_b F_b^0} \quad (3)$$

F_i^0 being the intensity of the fluorophore in its free or bound form. The fraction of bound DBF-X can be deduced from this equation as:

$$\chi_b = \frac{r - r_f^0}{(r - r_f^0) + R(r_b^0 - r)} \quad (4)$$

where $R = F_b^0/F_f^0$ can be obtained as the ratio between the intensity measured at high β-CDP concentration and in the absence of substrate. One of the objectives of this work is to evaluate the affinity of the DBF-X for the polymer with respect to that of β-CD, which forms a 1:1 complex with any of the derivatives. Thus, it seems reasonable to assume a 1:1 model for the binding, by expressing the concentration in terms, not of the concentration of polymer, but of the concentration of β-CD. In this way, we will obtain an apparent association constant, K_{ap}, that can be compared with the thermodynamic one of the monomeric β-CD [37,38]. The binding constant may be calculated from the analysis of the anisotropy versus β-CD concentration according to this model. We have fitted the measured data by a non-linear least-squares procedure, that has the advantage over linear methods, as the Scatchard one, of giving the same statistical weight to all the experimental points (solid lines in Fig. 9). The fitted parameters thus obtained in each case (K_{ap}, r_f^0 and r_b^0) have been collected in Table 2, together with the binding constants reported for the complexes with β-CD.

For the neutral dibenzofurans (DBF, DBFOH), the apparent constants with both polymers are almost one order of

Table 2
Binding constants, $K \times 10^{-3}$ (L mol^{-1}), of the DBF and its derivatives to β-CD polymers (T = 25.0 °C)

	DBF	DBFOH	DBFC
β-CDPL			
K	17 ± 2	8.4 ± 0.3	3.9 ± 0.4
r_f^0	0.005 ± 0.002	0.0177 ± 0.0009	0.0316 ± 0.0009
r_b^0	0.0761 ± 0.0008	0.1303 ± 0.0006	0.086 ± 0.001
β-CDPH			
K	9 ± 2	13.0 ± 0.8	1.37 ± 0.08
r_f^0	0.013 ± 0.005	0.024 ± 0.002	0.034 ± 0.002
r_b^0	0.159 ± 0.004	0.195 ± 0.001	0.394 ± 0.009
β-CD			
K	1.8 ± 0.2 [21]	2.95 ± 0.04 [24]	2.12 ± 0.05 [22]

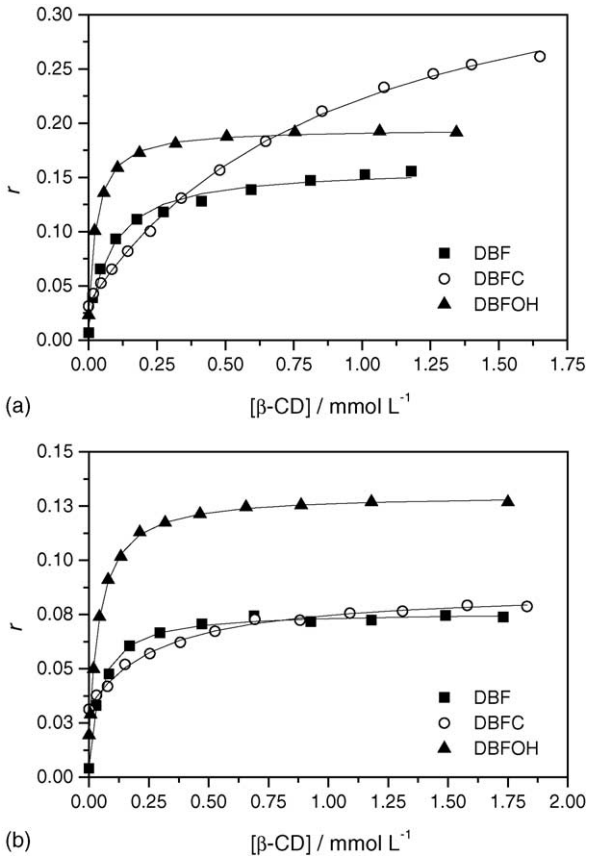


Fig. 9. Fluorescence anisotropy of DBF 1.56×10^{-5} M, DBFC 2.42×10^{-5} M and DBFOH 2.76×10^{-5} M in the presence of (a) β-CDPH, and (b) β-CDPL. Solid lines are the fittings to Eq. (3).

magnitude higher than for the unimeric β-CD. DBF displays a slightly improved affinity for the β-CDPL compared to that of the β-CDPH, whereas the opposite effect is observed with DBFOH. The lowest constants are obtained, with any of the two polymers, with the anionic DBFC. For β-CDPH, the binding constant is even lower than with β-CD, whereas for the β-CDPL is almost twice this value.

These results raise several issues: (i) why the affinity of the neutral dibenzofurans is higher for the polymer than for the β-CD; (ii) why the anionic derivative is comparatively so poorly trapped by the polymer; (iii) the differences between apparent binding constants for each derivative with both polymers.

There are clearly two potential binding zones in the polymer, i.e., the β-CD cavity and the cross-linker chains, so it can be adduced that the cross-linker might act as an additional binding site, thus increasing the amount of trapped DBF-X. In order to quantify the affinity of the guest by the linkers, we have carried out titration experiments of DBFOH with PVA and PEG. These polymers are linear and consequently form random coils in solution, but in a certain sense resemble the microstructure of the glyceryl cross-linkers and represent two extreme cases: PVA, with one hydroxyl group at every other carbon in the chain, is more hydrophilic than the glyceryl unit, whereas PEG, is less hydrophilic. We have calculated the binding constants by anisotropy titration in the same way but, in this case, the concentration basis has been the molecular weight of the repeat unit (46 g mol^{-1} for PVA, by taking into account the acetylation degree, and 44 g mol^{-1} for PEG). The fittings to Eq. (3) produce apparent constants of 12 ± 2 and 18 ± 3 L mol^{-1} for PEG and PVA, respectively, i.e., poor binding affinities. This is noticed in the low values of the measured anisotropy and also in the minute changes that can be seen in the fluorescence or absorption spectra (not shown). These constants do not seem high enough to justify the values obtained with the β-CDPs in the case of DBF and DBFOH.

One possible explanation to the enhanced binding, given by Xu et al. [37] for pyrene complexes with β-CDPs, could be the cooperative "clamshell" binding of two CDs of the same polymer to the guest. In principle, the spacer length in our polymers suffices for two CDs to envelop a molecule of dibenzofuran (Table 1). However, it is known that these DBFs form stable 1:1 complexes with β-CD, in contrast to pyrene, which gives a 1:2 stoichiometry with this CD. It seems unlikely that the system reaches a higher stability by sharing the guest between two CDs, with the consequent loss in entropy that comes from the rearrangement of the linker units to form the clamshell. On the other hand, the spectral behavior, both in absorption and in fluorescence, is qualitatively very similar to that with β-CD, what suggests a similar environment of the guest.

The work of Werner et al. on the interactions of CDPs with fluorescent probes derived from naphthalene provides evidence that the binding sites in the polymers are more hydrophobic than the binding sites on the CD monomers, suggesting a role of the glyceryl linkers in the binding [39]. These would contribute with a non-inclusional interaction that adds to the enclosure of the guest within the CD cavities. In a later work of these authors with pyrene and CDPs they conclude that, at least for this compound, the binding is largely non-inclusional, in agreement with a more open binding environment compared to that of CD [35]. Although it is clear that the presence of the linkers contribute to the stability of the complex, the hypothesis of the non-inclusional binding seems difficult to be applied in our case, especially if we consider the low association constants with the linear polymers and the likeness between the spectral features in the complexes with β-CD and with β-CDP.

We sense that the enhancement in the affinity for the β-CDP must be related with the diffusion process of the guest molecule through the polymer. This point has not been addressed in the cited works, but it is evident that when the guest binds to the macromolecule it must penetrate in a gel-like medium, either until reaching the cavity or until remaining retained in a different place. In this sense, the inclusion process of the guest occurs with more kinetic impediments than the reaction with a single β-CD in solution. The partial substitution by glyceryl linkers at the positions C2, C3 and C6 of the CD (as an average, almost one third of the OH)

enlarge the binding site regions so that the linkers can rearrange around the included guest, wrapping and furnishing it with a more hydrophobic surface. This increases the intrinsic binding constant. In addition, this hinders the release of the guest upon inclusion, so that the glyceryls act as a stopper, diminishing the rate constant of the inverse process. Kinetic studies or time resolved fluorescence spectroscopy could help to confirm this hypothesis.

As far as the relatively low constants measured for DBFC are concerned, they must be imputed to the negative charge of the carboxylate group. For instance, in a study of bisphenol A with an insoluble EP β-CD polymer, it has been reported that the adsorption capacity decreases dramatically at pHs at which ionization of the guest occurs [40]. This same phenomenon has also been observed in the anionic naproxen [41]. The environment inside the polymer, in spite of the glyceryl linkers and the non-reacted OHs of the β-CD, is less polar than water. The charge of DBFC implies a solvation shell around the carboxylate that must permeate the polymer network attached to the DBF moiety. In a previous work we have proven that the carboxylate group remains hydrated outside the β-CD cavity [23], so that the linkers attached to the CD can destabilize the binding. In this way, the inclusion will be much more probable at the surface of the polymer or its proximity, where the CDs can have the appropriate orientation, or even they can accommodate to form the complex. This agrees with the similar values of the association constant of β-CDPL and that of β-CD. With β-CDPH, on the other hand, with an average hydrodynamic radius of 19 nm, the surface/volume ratio of the particle is lower, and the number of available outer cavities that can be filled diminishes, following the same trend than that of the binding constant. Nevertheless, the presence of an isoemissive point in the fluorescence spectra, not found with the β-CD, suggests the apparition of another emitting species, so the occurrence of binding sites different to those of the cavity cannot be discarded, as Werner et al. have pointed out [39].

The remaining question, regarding the differences between the measured constants for the neutral derivatives, is somewhat more difficult to explain. In the mentioned studies with β-CDPs and pyrene, it has been proved that the binding increases with respect to that with β-CD, existing a correlation between the apparent constant with the EP/β-CD ratio [37]. This also seems to be the case with DBF and DBFOH. The spectral features are nearly the same for each dibenzofuran with both polymers, although for the case of DBF with β-CDPL the constant is higher, almost twice than for β-CDPH. On the other hand, DBFOH binds more strongly to the large β-CDPH than to β-CDPL, the difference being less marked. A possible explanation can be given in terms of the density of the polymer network and the relative hydrophobicity of the guest compared to that of the polymer inside. For β-CDPH, the amount of EP is nearly 58%, which implies a high degree of swelling by penetration of the solvent inside the network, whereas in the case of β-CDPL, the EP network is denser (36% EP). The deep penetration of the hydrophobic DBF is more favored in the small β-CDPL than in the water-like cages formed inside the network of β-CDPH. This hindered diffusion provokes a higher proportion of vacant cavities, which is reflected in a lower apparent constant (although considerably higher than that of β-CD). In the case of DBFOH, more polar due to the hydroxyl group, the penetration in the gel-like environment is easier and the proportion of filled CDs increases, what can justify the higher value of the constant compared to that of DBF.

4. Conclusions

The results presented in this work show a synergic effect in the binding of neutral dibenzofurans by cyclodextrin polymers. Although the cavity of β-CD is compatible in size with dibenzofurans, and forms stable 1:1 inclusion complexes with this family of guest molecules, the significant increase of the apparent binding constants points to an additional favorable effect of the glyceryl cross-linking bridges, most probably through the improvement of the non-polar character of the cavities. The poorer affinity of the anionic DBF derivative for the polymers seems to confirm this point. In any case, the binding constants of the polymers with the neutral dibenzofurans are considerably higher than those of the unimeric β-CD, irrespective of the size or composition of the polymer. This fact, added to the much improved water solubility of the polymeric CDs, suggests promising applications with analytical purposes, such as sample preconcentration, or in the field of water purification, among others.

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