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ORIGINAL PAPER

Formation and Characterization of Stable Fluorescent Complexes Between Neutral Conjugated Polymers and Cyclodextrins

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Abstract Solubilisation and stabilization of conjugated polymers, CPs, in aqueous media remains a challenge for many researches trying to extend the biological and environmental applications of this kind of polymers. A number of different alternatives have been considered to address this problem, which are mostly based on the enhancement of the macromolecule polarity, by appending hydrophilic side chains on the polymer backbone. In this work we have investigated a new strategy in which water solubilization is reached by external addition of classical cyclodextrins (α-, β- and γ-CDs) to a solution of non-polar CPs. This strategy allows working with such polymers eliminating the need to synthesize new water-soluble species. The polymer selected for the study was poly-[9,9-bis(6′-bromohexyl-2,7-fluorendyil)-co-alt-(benzene-1,4-diy)], $PFPBr_2$, a polyfluorene previously synthesized in our laboratory. Results show that $PFPBr_2$ forms fluorescent complexes in aqueous media with β-CD and γ-CD, and much less efficiently with α-CD, probably due to the small size of its cavity. The new $PFPBr_2$/CD complexes are stable in time and in a large range of pH, however, at high concentration and temperature, they tend to aggregate and precipitate. In order to increase stabilization and minimize polymer aggregation, complexes were encapsulated inside the pores of silica glasses fabricated using the sol-gel process, obtaining transparent and fluorescent hybrid matrices which were stable in time and temperature. In addition, immobilization of the complexes allows an easy manipulation of the material, thus offering promising applications in the development of biological and chemical sensors.

Keywords Conjugated polymers · Polyfluorene · Cyclodextrins · Fluorescence · Sol-gel matrix

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Introduction

Conjugated polymers (CPs) are polymers with delocalized π-electron systems, showing many interesting and useful properties, including strong absorption and high efficiencies in both photoluminescence and electroluminescence [1, 2]. Because of these properties they have been receiving significant attention over the last years as versatile active components in luminescent optoelectronic devices and fluorescent sensory materials [1–5]. One of the difficulties in working with CPs is their low solubility, especially in water, which reduces luminescence yields because the formation of large visible aggregates that precipitate from the solution, severely limiting their biological and environmental applications [6]. Consequently, many efforts are being made in this direction to develop strategies to improve the poor aqueous solubility of these macromolecules [7, 8]. Moreover, in the construction of optoelectronic devices, these improvements would prevent the emission of toxic volatile substances, which take place over a large surface area during processing of the material [9].

A common strategy employed to increase aqueous solubility of CPs is based on enhancement of the macromolecule polarity, by appending hydrophilic side chains on the main chain of polymer [10–20]. Most of water-soluble conjugated polymers possess pendant polar groups as carbohydrates or

charged groups such as carboxylate, sulfonate, phosphonate and ammonium groups. However, these conjugated polymers, named conjugated polyelectrolytes (CPEs), have a strong tendency to aggregate in aqueous media due to their hydrophobic aromatic backbone, resulting in a reduction in the photoluminescence quantum yield as a consequence of their fluorescence self-quenching, which is not desirable for many biological applications. To overcome this drawback different approaches are being carried out through the addition of ionic and non-ionic surfactants or biomolecules, in order to reduce water CPEs aggregation [21, 22]. In a recent work, Evans et al. have encapsulated CPEs in a silica matrix [23]. Within this matrix, CPEs were caged and retained in pores which protected them from aggregation, being accessible to small molecules diffusing into the matrix. With this methodology, they obtained nanocomposites which have been used for solid-state sensors applications to detect nitroaromatic explosives. The suitable porosity of these hybrid materials and the capacity of being processed in aqueous media open the possibility of being also used as sensors in biological media.

Other strategy to decrease the polymer intrinsic aggregation and improve water-solubility includes encapsulating the backbone of the conjugated polymer with suitable macrocycles. Polyrotaxanes are polymers with a novel molecular architecture where cyclic molecules are threaded onto the polymer [24, 25]. These architectures show specific characteristics, having a strong effect on the optical and electronic properties of the polymer and thus offering an alternative to the production of new materials for diverse applications. Cyclodextrins (CDs) are the macrocycle of choice for the majority of polyrotaxanes [7, 26, 27]. They are cyclic D-glucopyranose of several units: six (alpha), seven (beta) and eight (gamma), which form inclusion complex in aqueous solution with various molecules (guests) with suitable characteristics of polarity and dimension of toroidal cavity. This cavity within CDs is hydrophobic and less polar than the surrounding water molecules, thus the main driving forces involved in CDs inclusion complexes are nonpolar interactions, such as hydrophobic and van der Waals interactions, and the chemical and spectral properties of the guest (lipophilic molecules) are usually modified upon inclusion into the CD cavity [28–30]. It has been reported that the encapsulation of conjugated polymers by cyclodextrins derivatives prevents π-π interactions between the polymer chains, increasing water solubility and photo and electroluminescence efficiencies [7, 31, 32]. Recently, Farcas et al. have reported the synthesis and characterization of polyrotaxane with inclusion of β-CD and γ-CD into backbone of a polyfluorene (PF), a class of CP that exhibits stable blue emission with high value of quantum yield [33, 34]. Properties of the synthesised polyrotaxane were characterized and compared with the reference polymer. The new polymer showed a good solubility in polar/non-polar solvent mixtures (v/v, 1/1) but its solubility in water increased only slightly (ca. 6 % by weight in hot water) as compared to the reference PF, completely insoluble in water. Other strategy has been recently developed which describes a new method to stabilize PCs in aqueous solution. In this case, polymeric aggregates are encapsulated in block copolymer micelles, forming stable fluorescent nanoparticles [35]. This strategy allows working with non-polar CPs in aqueous media, eliminating the need to synthesize new water-soluble CPs and extending the range of applications of neutral CPs to biological sensing.

Based on the above works, we have investigated a new strategy in which water solubilization is reached by external addition of cyclodextrins to a solution of non-polar CPs. The selected polymer was poly-[9,9-bis(6′-bromohexyl-2,7-fluoren-dyil)-co-alt-(benzene-1,4-diy)], $PFPBr_2$, (Fig. 1) a PF completely insoluble in water, previously synthesized in our laboratory [36]. With this purpose we have tried two approaches: First, taking into account that CDs are able to form host-guest complexes with hydrophobic molecules, we have explored the interaction of $PFPBr_2$ with α-CD, β-CD and γ-CD as well as the stability and nature of the structures formed. Second, we have encapsulated such complexes inside the pores of a silica matrix fabricated using the sol-gel process, in order to increase stabilization and minimize polymer aggregation. Characterization was made analysing the polymer-backbone intrinsic fluorescence with steady-state fluorescence spectroscopy and fluorescence microscopy. Results show, on one hand, that $PFPBr_2$ spontaneously interacts with β-CD and γ-CD forming water-soluble fluorescent complexes, which are stable in time and in a large range of pH. On the other hand, encapsulation of these complexes within the sol-gel matrix does not affect their fluorescent properties but increases their thermal stability, avoiding further aggregation and precipitation of complexes. Note that such complexes are not polyrotaxane, but pseudopolyrotaxane, since CDs are not threaded onto the polymer by a synthesis process.

Materials and Methods

Reagents

The neutral conjugated polymer poly-[9,9-bis(6′-bromohexyl-2,7-fluoren-dyil)-co-alt-(benzene-1,4-diy)], ($PFPBr_2$, Fig. 1), Mw=10.0 $kgmol^{-1}$, PDI=2.0 (based on polyfluorene calibration[37]) having 35 monomer units (fluorine-phenylene) per polymer chain, was obtained by Suzuki coupling reaction with Pd(II) as catalyst and characterized in our laboratory, as was previously described [36, 37]. α-Cyclodextrin, β-cyclodextrin and γ-cyclodextrin (CDs,

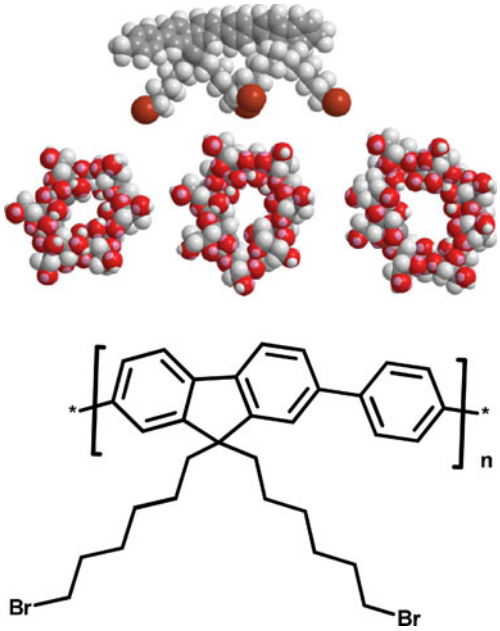

Fig. 1 *Top* Molecular structures of conjugated polyfluorene $PFPBr_2$ and α-, β- and γ-CDs, rendered on the same scale. *Bottom* $PFPBr_2$ chemical structure

Fig. 1) were purchased from Fluka (Spain). Tetraethylorthosilicate (TEOS) was obtained by Sigma-Aldrich (Spain) and chloroform (analytical grade) was provided from Merck (Spain). Other chemicals were of analytical or spectroscopic reagent grade. A sodium phosphate buffer (50 mM, pH7.3) was prepared with deionised doubly distilled water.

Preparation of the $PFPBr_2$/CDs Complexes

Stock solutions of $PFPBr_2$ (7.1×10^{-5}M, in repeating unit) was prepared in chloroform. The preparation of the $PFPBr_2$/CDs complexes was made as follows: different weighed amounts of CD were added to 280 μL aliquots of the stock solution of $PFPBr_2$ in chloroform, in separated flasks which were covered with aluminium foil. All the samples were vigorously stirred for 30 min at room temperature in the dark and, afterwards, they were dried submitted to rotaevaporation. Finally, every flask was re-suspended on 2 mL of phosphate buffer (50 mM, pH7.3), stirred during 2 min and stored in the dark at 4 °C before use. For all samples, the final $PFPBr_2$ concentration in buffer was maintained constant at 10 μM, while CD concentrations were varied between 0 mM and 6 mM.

Immobilization in Sol-gel Matrix

Silica stock solution was prepared mixing 4.46 mL TEOS, 1.44 mL of H_2O miliQ and 0.04 mL HCl 0.6 M in a closed vessel. The mixture was stirred during 1 h and was kept at −20 °C. Alcohol was subsequently removed by means of rotaevaporation. Afterwards, 700 μL of the $PFPBr_2$/CD complexes in buffer were mixed with 700 μL of the silica solution in a disposable polymethylmethacrylate cuvette. Gelation occurs readily after mixing. After 1 h, monoliths, having a size of ~9×9×12 mm, were washed with phosphate buffer three times and were wet aged in 0.5 mL of the same buffer at 4 °C during 24 h. Cuvettes were covered with parafilm and stored in fridge before use.

Steady-State Fluorescence Measurements

Fluorescence spectra were performed in a PTI-QuantaMaster spectrofluorometer interfaced with a Peltier cell. Excitation wavelength at 370 nm for $PFPBr_2$ was utilized. The experimental samples (sol-gel monoliths and buffer solutions) were placed in 10×10 mm path length quartz cuvettes. Background intensities were always checked and subtracted from the sample when it was necessary.

Fluorescence Microscopy Measurements

Images of the fluorescent aggregates were obtained using a Nikon Eclipse TE2000-U inverted microscope equipped with a Nikon Digital Sight DS-1QM/H and Nikon Digital Camera DXM1200C. Data acquisition was monitored successively by manually format and data processing with NIS-Elements AR 2.30 software.

Dynamic Light Scattering Measurements (DLS)

The size of the aggregates was also explored by dynamic light scattering techniques, using a Malvern Zetasizer Nano-ZS instrument, equipped with a monochromatic coherent 4 mW Helium Neon laser (λ=633 nm) as light source, with 173° scattering angle of lecture for size measurements. All measurements were performed in disposable cuvettes. Reported values were the average of approximately 50 measurements. Measurements were realized in triplicate at 25 °C.

Results and Discussion

Complex Formation Between $PFPBr_2$ and CDs

The synthesized conjugated polyfluorene $PFPBr_2$ was readily soluble in organic solvents with intermediate polarity, such as chloroform. In this solvent the polymer exhibited an absorption maximum around 370 nm, showing a strong fluorescence emission in the blue, with two maximum peaks at 410 nm and 430 nm, as was previously described [36] (Fig. 2). In contrast, when $PFPBr_2$ was incorporated in aqueous solution no fluorescence signal was observed,

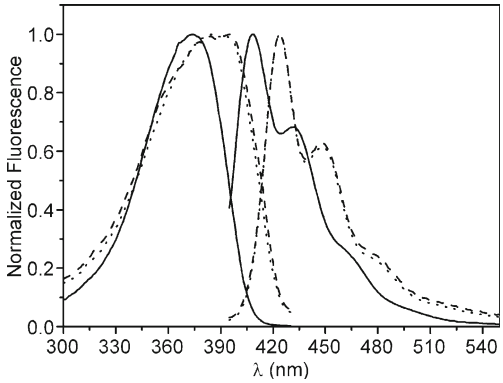

Fig. 2 Normalized fluorescence excitation and emission spectra of $PFPBr_2$ (10 μM) in chloroform (*solid*), and incorporated in β-CD (4 mM) (dashed) and γ-CD (4 mM) (*dotted*)

which is indicative of the lack of solubility of the polymer in this medium. To improve this solubility, as a first strategy, increasing concentrations of different CDs (α-, β- and γ-CD) were added to a water solution containing $PFPBr_2$ (10 μM, in terms of repeating unit). The addition of the cyclodextrin, up to 4 mM, did not produce any change in the spectrum, and no fluorescence was observed. A yellow precipitate was found at the bottom of the cuvette which was associated to non-soluble $PFPBr_2$ aggregates. When a similar experiment was made but adding $PFPBr_2$ on the aqueous solution of CDs, the same result was obtained. These results suggest that complexes between CDs and $PFPBr_2$ are not spontaneously formed in aqueous media, probably due to the high hydrophobilicity of the polymer which readily aggregates and precipitates before reaching cyclodextrins.

To facilitate the contact between $PFPBr_2$ and CDs, and as a second strategy, weighed amounts of cyclodextrins were put in contact with a solution containing $PFPBr_2$ in chloroform, as is described in Material and Methods. Afterwards, the sample was dried under vacuum to obtain a dry film at the bottom of the flask. This was later resuspended and stirred in phosphate buffer and added to a cuvette to make the fluorescence measurements. The first evidence of the interaction between $PFPBr_2$ and cyclodextrins was the observation of a fluorescence signal upon excitation at 370 nm. Excitation and emission fluorescence spectra were then recorded for the three cyclodextrin systems. While in presence of α-CD fluorescence intensity of $PFPBr_2$ was very low (data not shown), a substantial fluorescence enhancement was detected for the other two samples. Figure 2 shows that the fluorescence spectra were almost similar to those obtained in the organic solvent, but with a clear shift to the red followed by a higher resolution in the vibrational structure of the emission spectrum. This behaviour is close to that observed for a cationic conjugated polyelectrolyte derivative (HTMA-PFP) in aqueous solvents [11, 22] and is indicative of a reduction in the number of degrees of freedom of the polymer chains and hence a decrease in the number of conformations present in the excited state. These results suggest the presence of fluorescent complexes in aqueous media which are mostly formed by interaction of $PFPBr_2$ with β-CD and γ-CD and with very low probability with α-CD. The fact that the fluorescence spectra of $PFPBr_2$/β-CD and $PFPBr_2$/γ-CD are similar in shape and position could mean that physico-chemical interaction between polymer and cyclodextrin has the same nature for both CDs.

To better explore the ability of cyclodextrins to solubilize $PFPBr_2$ in water, we record the fluorescence spectra of the polymer at a constant concentration (10 μM in terms of repeating unit) and increasing concentrations of β-CD and γ-CD (α-CD was ruled out because of the lower fluorescence signal). The shape and position of the spectra were similar in the range of concentrations studied, without shifts or changes in their vibrational structure (data not shown). The addition of CDs at concentrations lower than 6 mM, produced a progressive fluorescence enhancement (Fig. 3), evidencing the formation of complexes between $PFPBr_2$ and cyclodextrins. The fact that the van der Waals radius for bromine is around 2 Å, suggests that the complex could be formed by the insertion of the hydrophobic side chains of

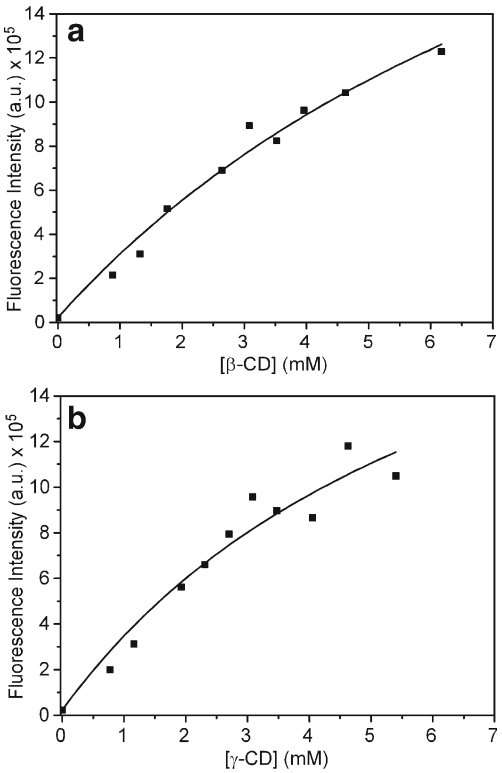

Fig. 3 Influence of the β-CD (**a**) and γ-CD (**b**) concentration on the fluorescence intensity of $PFPBr_2$ (λ_{exc}=370 nm, λ_{ems}=420 nm, [$PFPBr_2$]=10 μM, in terms of the repeating unit). *Solid line* fits show the non-linear regression analysis

$PFPBr_2$ within the cavity of CDs. At cyclodextrin concentrations above 6 mM a strong increase in the turbidity of the sample was observed, evidencing the formation of supramolecular aggregates. We have estimated the fluorescent quantum yield of the complexes, comparing it with the quantum yield obtained previously in chloroform in our laboratory (Φ=0.8), using quinine sulfate. Although, it was difficult to determine with precision the optical density of the samples, because of the sample light scatter, it is possible to conclude that both complexes show similar quantum yields, around 7–8 times lower than the value determined in chloroform.

The above results were used to estimate the association constants (K) of the $PFPBr_2$ */CD,* complexes, by using non-linear least-squares fitting to Eq. 1 [38]. Note that, given the protocol used to the complex formation, the final concentration of free cyclodextrin available in solution to complex with polymer could be lower than that initially added. Taking it into account, the association constants obtained from this equation are referred to as "apparent constants" and are used only to compare the affinity of $PFPBr_2$ by both CDs:

$$\frac{I}{I_0} = 1 + \left(\frac{I_{max}}{I_0} - 1\right)\frac{[CD]_0 K}{1 + [CD]_0 K} \quad (1)$$

I_0 and I represent the fluorescence intensities in the absence and in presence of CD, respectively, $[CD]_0$ denotes the initially added concentration of cyclodextrin and I_{max} is the limiting intensity of fluorescence obtained when all $PFPBr_2$ molecules are complexed. The solid line in Fig. 3 represents the fit of data to Eq. 1, which yields values of K= 92±32 $M^{-1}$and K=143±68 M^{-1} for the apparent association constants of β-CD and γ-CD, respectively. The slightly higher value estimated for γ-CD, although is within the error margin, is reasonable taking into account the higher size of its cavity (see below).

The fact that the turbidity of the samples increases drastically at higher cyclodextrin concentrations, indicate that, depending on the cyclodextrin concentration at least two different types of structures are formed. At low cyclodextrin concentrations small complexes between $PFPBr_2$ and β-CD or γ-CD could take place, probably via hydrophobic interaction between the host CD cavity and the side chains of the polymer unit, while its hydrophobic backbone is exposed to the water molecules. These complexes seem to be soluble and stable in aqueous media. Taking into account the minimum internal diameter of the CD cavity (4.4, 5.8 and 7.4 Å for α-CD, α-CD and β-CD, respectively [39]), and the van der Waals diameter for bromine (around 2 Å), it could be also possible the formation of complexes between α-CD and $PFPBr_2$, however this complex was formed with very low probability. We think that the explanation of this result is related to the high hydrophobicity of $PFPBr_2$. It has been reported that for extremely hydrophobic polymers, as $PFPBr_2$, formation of complexes with cyclodextrins becomes practically impossible because the threading kinetics is too slow [40]. With our strategy of formation, the molecular contact between polymer and CD is favored, accelerating the inclusion process. However, not all the polymer chains are able to interact with CDs; only those which are closer to them. The rest, probably hide from water contact by forming aggregates with each other, and precipitate. As bigger is the CD cavity, as higher is the probability of $PFPBr_2$ to reach it, forming complexes (although the complex should be thermodynamically less stable because the van der Waals interactions drop with increasing intermolecular distances). Therefore, in spite of α-CD has a diameter similar to that of Br, the poor solubility of

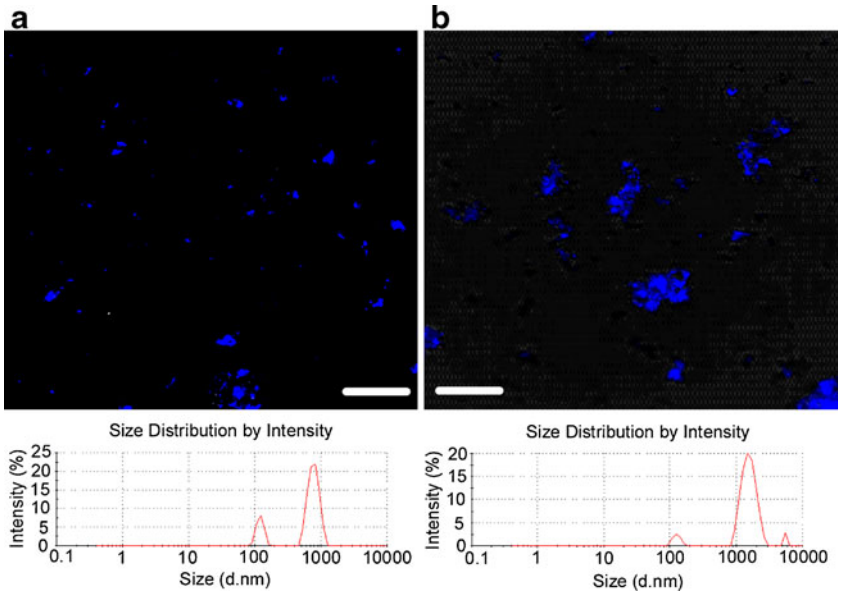

Fig. 4 Fluorescence microscopy images (*top*) and size distribution (intensity distributiuon) determined by DLS (*bottom*) of $PFPBr_2$/β-CD (**a**) and $PFPBr_2$/γ-CD (**b**) aggregates at room temperature (scale bar=50 μm)

the polymer slows down the CD threading kinetics and polymer aggregates and precipitates before complex formation takes place. It also could explain why γ-CD has an apparent association constant slightly higher than β-CD. In addition, given the internal diameter of β-CD, we cannot rule out that this cavity can accommodate more than one side chain belonging to different polymers. We tried to confirm theses hypothesis by ^{1}H NMR experiments, using a Bruker AVANCE 500 spectrometer, maintaining the same concentrations used in fluorescence; however, given the low concentration of polymer respect to that of CD, the polymer signal was inappreciable respect that of cyclodextrin and therefore, no conclusion could be obtained from this result (data not shown).

At higher concentrations of CD (> 4 mM), large aggregates are formed which could coexist with the small complexes. The existence of such aggregates has been confirmed by fluorescence microscopy and DLS. The top of Fig. 4 shows the images visualized for $PFPBr_2$ in presence of β- and γ-CD. For both cyclodextrins, fluorescent aggregates showing heterogeneous size were detected, being slightly larger for the $PFPBr_2$/γ-CD system. During the image acquisition, an increase in the size of aggregates was observed, which was attributed to the heating of the sample induced by irradiation. The bottom of Fig. 4 shows the size distribution (intensity distribution) of the aggregates determined by DLS. Aggregates presented high polidispersity, especially for the β-CD complexes, which made the determination of the size very

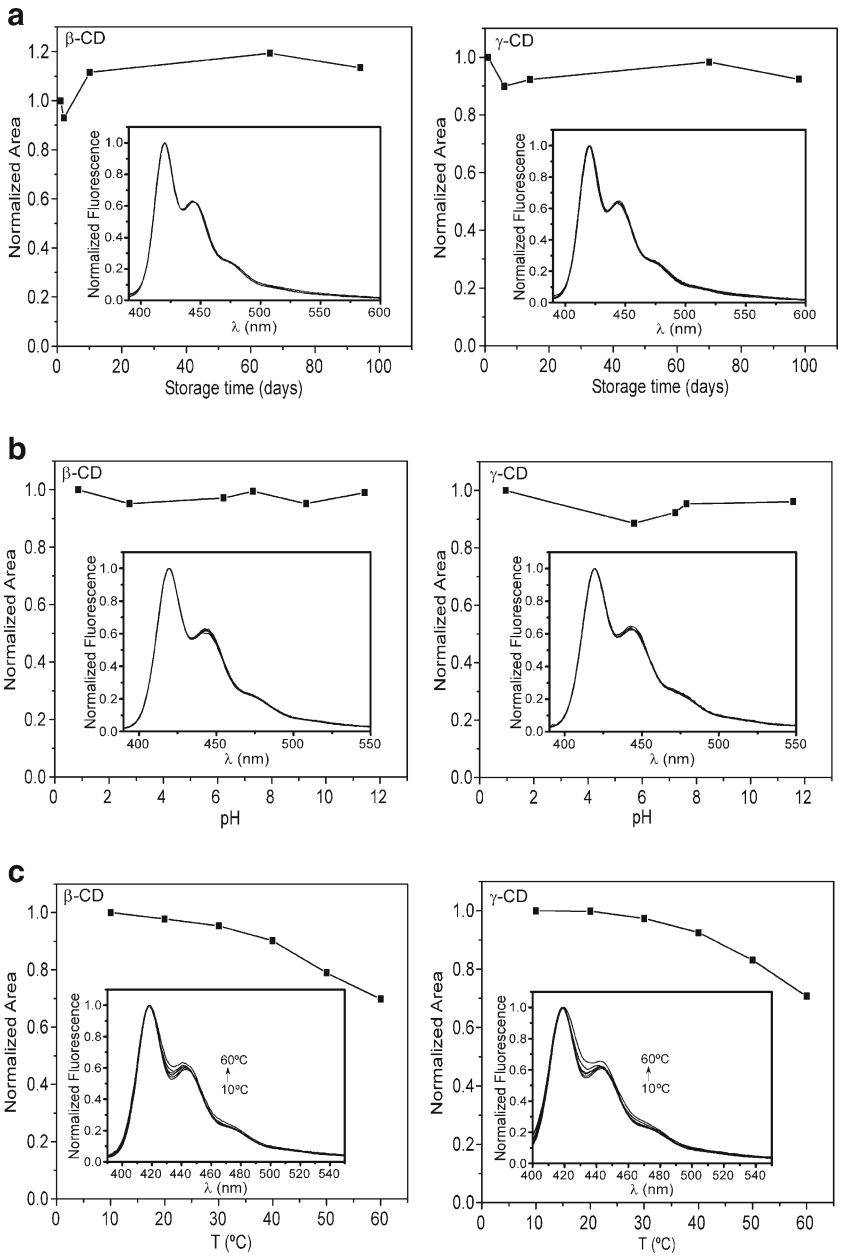

Fig. 5 Effect of storage time (**a**), pH (**b**) and temperature (**c**) on the stability of the $PFPBr_2$/β-CD and $PFPBr_2$/γ-CD complexes, measured as the area under the non-normalized spectrum (λ_{exc}=370 nm). Inset: normalized fluorescent emission spectra of the complexes recorded as a function of storage time (**a**), pH (**b**) and temperature (**c**). [PFPBr2]=10 μM; [β and γ-CD]=4 mM

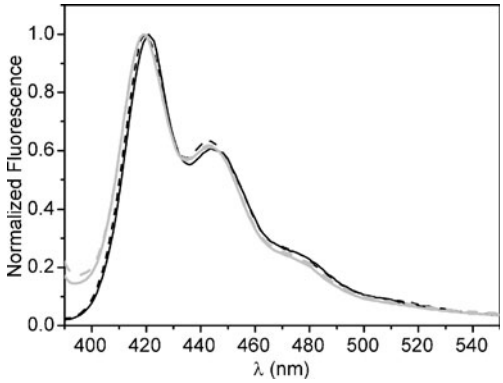

Fig. 6 Normalized fluorescence emission spectra of the $PFPBr_2$/β-CD complex (*solid lines*), $PFPBr_2$/γ-CD complex (*dashed lines*) in solution (*black lines*) and immobilized in a sol-gel matrix (*grey lines*)

difficult. However, it could be concluded from these experiments that there was a population of aggregates with hydrodynamic diameters between 80 nm and 200 nm, a second population with sizes around 1–2 μm, (slightly smaller for β-CD) and a very small population, which is not observed in β-CD complexes, with higher diameters (4–6 μm). The nature of these aggregates is not clear, but the more probable is that the higher concentration of CD increases the number of $PFPBr_2$/CD complexes, which start to interact with each other through π-π interactions between their rigid aromatic backbones, giving rise to the formation of aggregates. However, we cannot rule out that aggregate formation also can proceed through hydrogen bonding between hydroxyls of CD fragments. In fact, several works have reported that both, cyclodextrins and cyclodextrin complexes can self-associate in aqueous media to form large aggregates resulting in turbidity of aqueous cyclodextrin solutions [41, 42].

Stability Assays

Stability of the $PFPBr_2$/CD complexes was explored as a function of storage time, pH and temperature. We consider that a methodical study of this parameter is important for future applications, such as stimuli-response or controlled release. Stability was assessed monitoring the fluorescence spectra of the complexes and plotting the area of each spectrum as a function of time (days after preparation), pH or temperature. Fluorescent spectra were recorded at room temperature, with exception of the thermal stability experiments.

Figure 5a shows the stability as a function of storage time for samples which were prepared and kept in the dark, at 4 °C, for up to 2 months. During this period of time small fluctuations of the fluorescence intensity were detected for both $PFPBr_2$/β-CD and $PFPBr_2$/γ-CD complexes but, in general, the behaviour was stable. In addition, the shape and position of the fluorescence maxima were not altered with the time (inset in Fig. 5a). These results suggest that, at the selected conditions, the complexes are stable and water-soluble, and that after their initial formation additional aggregation is not occurring.

Stability of the complexes as a function of pH was evaluated by addition the appropriate amounts of HCl (0.31 M) and NaOH (0.28 M) to aqueous samples containing $PFPBr_2$/CD complexes. Figure 5b displays the fluorescence intensity determined at the different pHs (from pH= 0.9 to pH=12) for both $PFPBr_2$/β-CD and $PFPBr_2$/γ-CD complexes as well as the normalized fluorescence spectra recovered for each sample (inset). Results show that addition of acid or basic solution practically did not modify the intensity, the shape or the position of the fluorescence emission spectrum, indicating that the complexes are completely stable in the range of pH studied. Given that the pKa of the cyclodextrin hydroxyls is close to 12, these results suggest that the formation of fluorescent aggregates previously described probably is not taking place through hydrogen bonding between hydroxyls of CD fragments but through interactions between the aromatic polymer backbones.

Thermal stability of the complexes formed was evaluated recording the emission spectrum of the $PFPBr_2$/β-CD and $PFPBr_2$/γ-CD complexes at different temperatures (Fig. 5c). Thermal scans were measured, simultaneously, for the two samples. The increase of temperature from 10° to 60 °C induced a similar effect in both systems. Fluorescence

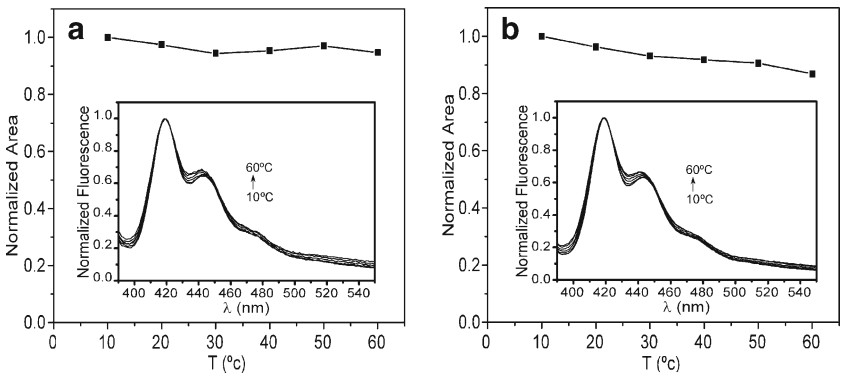

Fig. 7 Effect of temperature on the stability of the $PFPBr_2$/β-CD (**a**) and $PFPBr_2$/γ-CD (**b**) complexes immobilized in a sol-gel matrix, measured as the area under the non-normalized spectrum (λ_{exc}=370 nm). Inset: normalized fluorescent emission spectra of the complexes recorded at different temperatures. [PFPBr2]=10 μM; [β and γ-CD]=4 mM

intensity showed a slight decrease up to around 40 °C, which have been also reported for other conjugated polymers [43]. Above this temperature, a more pronounced effect on the fluorescence intensity was observed probably caused by the aggregation of the complexes induced by temperature and the subsequent deposition of the aggregates formed. These changes in intensity were accompanied by a barely perceptible blue shift of the emission spectrum followed by a lower resolution in the vibrational structure, suggesting an increase in the degree of freedom of the polymer chains due to the rise of temperature, but not the dissociation of the inclusion complex.

Immobilization of the $PFPBr_2$/CD Inclusion Complexes in Sol-gel Matrix

The new complexes were immobilized in a porous silicate glass matrix, fabricated using the sol-gel process, as is described in Methods. Within these materials, the immobilized macromolecules are individually caged and retained in pores that protect them from aggregation, providing an environment similar to that of the aqueous solution [44–46]. In addition, the sol-gel matrices show excellent optical transparency which allows to characterize the macromolecules within the nanopores through fluorescence spectroscopy. Fluorescence spectra of the immobilized complexes were directly recorded from the sol-gel monolith and are shown normalized in Fig. 6. The fact that the emission spectra of the immobilized complexes practically coincide with those recorded in phosphate buffer is indicative of the suitability of the immobilization process for both, $PFPBr_2$/β-CD and $PFPBr_2$/γ-CD complexes. Results also suggest that encapsulation of the complexes within the pore does not reduce the degree of freedom of the polymer chains since the vibrational structure of the spectrum is not increased, probably because the size of the pore is quite higher than that of the complex. However, a slight blue-shift is observed in the spectrum which could be attributed to a small conformational change of the polymer chains occurring during the immobilization process. The leaching of the complexes from the matrix was practically negligible and samples were stable during, at least, 2 months (data not shown).

Thermal stability of the immobilized complexes was explored monitoring the fluorescence spectra of the complexes and plotting the area of each spectrum as a function of temperature, from 10° to 60 °C (Fig. 7). Fluorescence spectra were recorded simultaneously for both samples, $PFPBr_2$/β-CD and $PFPBr_2$/γ-CD complexes entrapped in the sol-gel matrix and are shown, normalized, in the inset of Fig. 7. Results show that, for the two immobilized complexes, the fluorescence intensity was practically stable with temperature, especially for the $PFPBr_2$/β-CD complexes, in contrast to that occurring in solution (Fig. 5c). This behaviour can be explained because the encapsulation of the complexes within the pores of the silica matrix reduces the possibility of aggregation as well as the eventual deposition on these structures at the bottom of the flask. However, the mobility of the complexes does not seem to be restricted within the pore since, as in solution, the vibrational structure of the emission spectra decreases with temperature.

Conclusions

The conjugated polymer $PFPBr_2$, completely insoluble in aqueous media, has been solubilised in buffer solution through the formation of fluorescent complexes with β-CD and γ-CD, which show practically the same behaviour. Taking into account the chemical structures of polymer and cyclodextrins, and the fact that fluorescence data fit well to Eq. 1, it seems very probable that the complexes are formed by insertion of the hydrophobic side chains of $PFPBr_2$ within the cavity of CDs. Results show that complexes are stable in time and in a large range of pH, however, at high concentration and temperature, they tend to aggregate and precipitate, forming amorphous supramolecular structures. Immobilization of these complexes in a silica sol-gel matrix does not affect their fluorescent properties but increases their thermal stability. This is because, within these materials, the complexes should be retained in pores that protect them from aggregation and eventual precipitation, but that do not restrict their conformational freedom. In addition, the successful incorporation of the fluorescent complexes in the porous inorganic matrix allows an easy manipulation of the material, thus offering new opportunities in the development of biological and chemical sensors, via quenching or energy transfer mechanisms, as well as for biphotonic applications which should be possible due to the interesting properties of the fluorenyl moiety.

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