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Comparative study of the complex forming ability and enantioselectivity of cyclodextrin polymers by CE and ^{1}H NMR

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ABSTRACT

The interactions between nine drugs (baclofen, bupivacaine, chlorpheniramine, ketoconazole, paliperidone, promethazine, propranolol, risperidone and verapamil) and six cyclodextrins (α-CD, β-CD, γ-CD, HP-β-CD, HP-γ-CD and Me-β-CD) or six polymers of cyclodextrins (polyα-CD, polyβ-CD, polyγ-CD, polyHP-β-CD, polyHP-γ-CD and polyMe-β-CD) were studied by affinity capillary electrophoresis and/or ^{1}H NMR at pH 2.5. An exhaustive qualitative study was performed through the determination of the retardation factor. Then, four compounds and both β-CD and polyβ-CD were selected for the quantitative study of the interactions at pH 2.5 and 7.0. By comparing the results obtained with the β-CD and polyβ-CD, it appears that the apparent binding constants are up to five times higher with the polymer. The 2D-NMR results seem to indicate that the structure of the polymeric network favours the inclusion of the guest in the hydrophobic cavity of the CD units. Moreover, the poly-CDs have shown very high enantioselective abilities at both pH.

1. Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides formed by glucopyranose units linked by α(1,4) bonds. The common α-CD, β-CD and γ-CD are composed of 6, 7 and 8 glucose units, respectively. CDs are cone-shaped and present a hydrophobic environment in the interior of the cavity while the outer surface remains hydrophilic. These properties make them complexing agents of first interest since they can include a great variety of molecules of appropriate polarity and size (Duchêne, 1987). Their pharmaceutical applications rely mainly on their abilities to enhance the solubility, the stability and the bioavailability of drug molecules (Loftsson & Duchêne, 2007). Furthermore, CDs are widely used as chiral selectors for the enantioresolution of drugs in separation techniques as high-performance liquid chromatography (HPLC) (Subramanian, 1994) or capillary electrophoresis (CE) (Chankvetadze, 1997).

Covalent polymer networks containing cyclodextrins are also of great interest, because (i) their crosslinked macromolecular structure allows a cooperative action between neighbouring cyclodextrins cavities, or between the cavities and the polymeric network in the complexation of bulky and poorly water soluble drugs; (ii) depending on the degree of polymerization and crosslink density, they may exist under an enhanced water soluble form compared to their parent cyclodextrins (especially in the case of β-CD), or in the shape of swellable hydrogels; (iii) due to their polymeric nature, they may be separated from a liquid medium either by filtration or by membrane techniques. As a consequence, cyclodextrin polymers in which CD moieties are structurally incorporated to the network present a high potential in pharmaceutical and biomedical applications (Van de Manakker, Vermonden, Van Nostrum, & Hennink, 2009).

Although originally designed as materials for food applications (Solms, 1964) or separation chromatography (Crini & Morcellet, 2002), the first CD containing polymer networks for pharmaceutical purposes were prepared in the 1980s (Fenyvesi, 1988; Szeman, Fenyvesi, & Szejtli, 1987). They resulted from the chemical crosslinking between CDs and epichlorohydrin (ECH) (Renard, Barnathan, Deratani, & Sébille, 1997a, 1997b) and showed good complexation properties towards low molecular-weight drugs (i.e. butylparaben, hydrocortisone, cinnarizine and furosemide). These systems were then improved in order to increase their drug loading capacities through the incorporation of anionic or cationic

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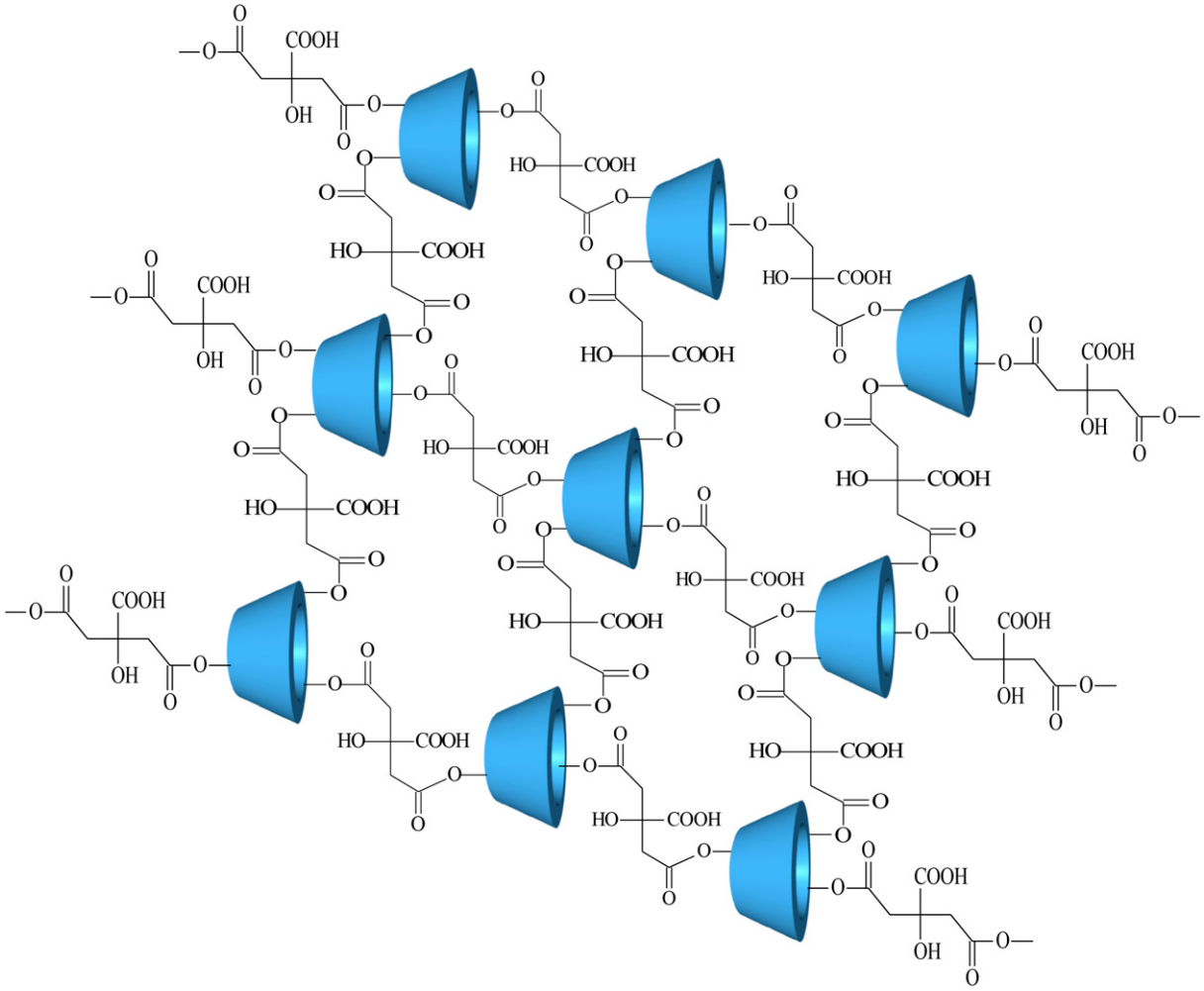

Fig. 1. Schematized structure of poly-CDs issued from the polyesterification between citric acid and native or CDs derivatives.

groups. For example, Li (Li, Xiao, Li, & Zhong, 2004) synthesized positively charged networks by adding choline chloride in the polymer precursor solution, that yielded a quaternary ammonium containing network presenting a reduced toxicity towards erythrocytes. Besides, Fenyvesi (Fenyvesi, Ujhazy, Szejtli, Putter, & Gan, 1996) obtained ECH-crosslinked β-CD networks containing carboxymethyl groups that could be efficiently loaded with cationic compounds, such as the cetylpyridinium chloride antimicrobial agent. Other bifunctionnal crosslinking agents were applied in the literature, such as diepoxides (Cserhati, Fenyvesi, & Szejtli, 1992; Rodriguez-Tenreiro, Alvarez-Lorenzo, Rodriguez-Perez, Concheiro, & Torres-Labandeira, 2006, 2007), diisocyanates (Binello, Robaldo, Barge, Cavalli, & Cravotto, 2008; Ma & Li, 1999; Tang et al., 2006) and anhydrides (Berto et al., 2007; Girek, Shin, & Lim, 2000; Girek, Kozlowski, Koziol, Walkowiak, & Korus, 2005; Gu, Tsai, & Tsao, 2006). In 2001, Martel (Martel, 2001; Martel, Ruffin, Weltrowski, Lekchiri, & Morcellet, 2005) reported the use of polycarboxylic acids as crosslinking agents, such as citric, 1,2,3,4-butanetetracarboxylic and polyacrylic acids. These polymers obtained through polyesterification have been characterized by Fourier transform infrared spectroscopy and size exclusion chromatography (Martel et al., 2005). This family of polymers presented the advantage of employing environmentally friendly and non toxic reagents, and presented an anionic character through a one pot reaction. As a consequence, these polymers whose structure is schematized in Fig. 1 presented improved complexation capacities towards doxycycline (Bakkour et al., 2006), albendazole (Joudieh, Bon, Skiba, Martel, & Skiba Lahiani, 2009), and provided enhanced and sustained release of ciprofloxacin, once they were immobilized onto biomedical devices such as vascular prostheses (Laurent et al., 2011) or hydroxyapatite bone substitutes (Leprêtre et al., 2009).

Quantification of the interactions between the drug and the cyclodextrins based systems is a recurrent question in the literature. Therefore, many techniques may be used to assess the apparent stability constants of drug/CD complexes. Among them, UV–visible or fluorescence spectrometry, chromatography, isothermal titration calorimetry, affinity capillary electrophoresis (ACE) and nuclear magnetic resonance spectroscopy (NMR) are the most used and are alternatives to the more time and material-consuming phase solubility methods (Bednarek, Bocian, & Michalska, 2008; Brun Malta et al., 2008; Chankvetadze, Endresz, Schulte, Bergenthal, & Blaschke, 1996; Chankvetadze et al., 1999; Chankvetadze, Pintore, et al., 2000a; Chankvetadze, Burjanadze, et al., 2000b; Danel et al., 2006, 2011; Hellriegel et al., 2001; Marçon et al., 2009; Misiuk & Zalewska, 2009; Rojas-Aguirre et al., 2012; Zeng, Ren, Zhou, Yu, & Chen, 2011; Zhou, Thompson, Reamer, Miller, et al., 2003a; Zhou, Thompson, Reamer, Lin, et al., 2003b).

In this paper, we investigate the complexing and enantioselective properties of cyclodextrin polymers obtained from the reaction between citric acid and six different cyclodextrins (polyα-CD, polyβ-CD, polyγ-CD, polyHP-β-CD, polyHP-γ-CD and polyMe-β-CD) towards a large panel of nine drugs: baclofen, bupivacaine, chlorpheniramine, ketoconazole, paliperidone,

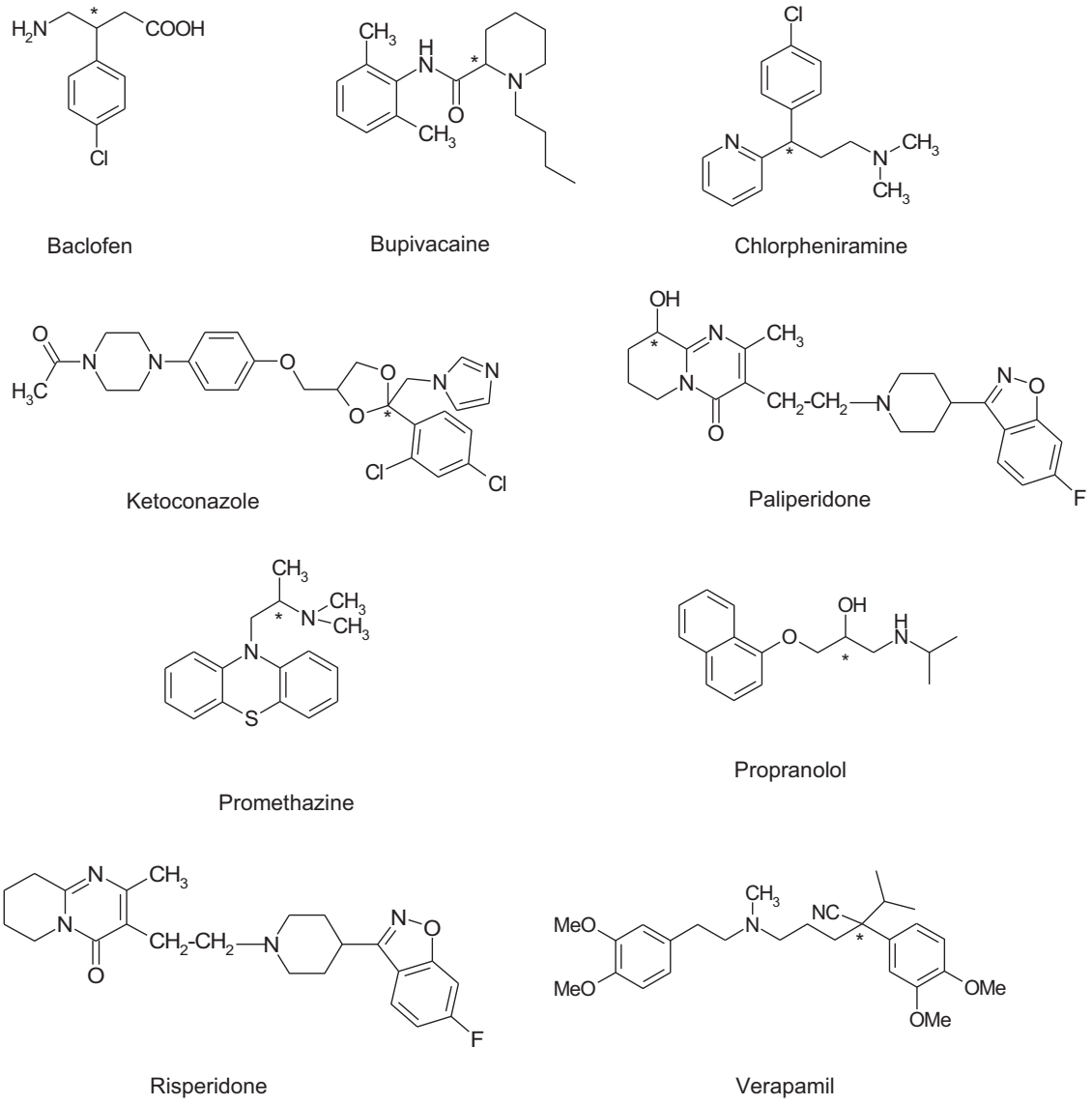

Fig. 2. Structure of the studied compounds.

promethazine, propranolol, risperidone and verapamil (Fig. 2). To emphasize the interest of these polymers, the study was performed at the same time with the corresponding native or modified CDs (α-CD, β-CD, γ-CD, HP-β-CD, HP-γ-CD and Me-β-CD). The study was performed using both ACE and NMR. ACE, which is the fastest and less reagent-consuming technique, was used first to screen the binding properties of the 12 CDs or poly-CDs by considering the qualitative parameter Rf which is the retardation factor of the migration in presence or absence of CDs in the background electrolyte. Secondly, the apparent and averaged binding constants *K* were determined, through the Scott's method (Scott, 1956), for four selected drugs with the β-CD and polyβ-CD at pH 2.5 and 7.0. It is worth mentioning that the poly-CD remains uncharged at pH 2.5 whereas it is anionic at neutral pH (due to the carboxylate functions of the crosslinking agent). Thus, the effect of the charge of the poly-CD on the complexation will be discussed. In the same time, since the ACE experiments were performed with the racemic mixture of each drug, the enantioselective abilities of the poly-CDs were briefly reported. If enantioseparation occurred, the apparent binding constants were simultaneously calculated for both enantiomers. Last, the one and two-dimensional NMR spectroscopy, more time and reagent consuming technique, was implemented for selected complexes and has provided additional information on the structure of the complexes.

2. Experimental

2.1. Chemicals

Risperidone and paliperidone were a gift from Janssen-Cilag (Issy-les-Moulineaux, France). Baclofen, bupivacaine, chlorpheniramine, ketoconazole, propranolol and verapamil were purchased from Sigma–Aldrich (Saint-Quentin Fallavier, France). The promethazine chlorhydrate was purchased from the Cooper (Melun, France). Phosphoric acid (85%, w/w), sodium hydroxide, triethylamine, sodium dihydrogen phosphate and disodium hydrogen phosphate were purchased from Merck (Nogent-sur-Marne, France). Deuterium oxide (100%) was purchased from Euriso-top (Gif sur Yvette, France).

2.2. Cyclodextrins

α-CD, γ-CD and HP-γ-CD were purchased from Wacker Chimie (Lyon, France). β-CD, HP-β-CD and Me-β-CD were supplied by

Roquette (Lestrem, France). The modified cyclodextrins, HP-β-CD, HP-γ-CD and Me-β-CD represent multicomponent mixtures with molar substitution (MS) 0.75–0.95, 0.5–0.8 and 0.5 per anhydroglucose unit, respectively. Their molar concentrations were calculated taking into account their averaged molecular weight.

Cyclodextrin polymers (poly-CDs) were prepared as previously reported (Martel et al., 2005) by solubilization of citric acid as crosslinking agent, sodium hypophosphite as catalyst and CDs in respective weight ratio 10 g/3 g/10 g in 100 mL of water. After water removal, the solid mixture was then cured at 140 °C during 30 min under vacuum. Water was then added and the resulting suspension was filtered and the filtrate dialysed against water using 12–14 kDa membranes. Finally the CD polymers were recovered after freeze drying. The average molecular weight of the poly-CDs was 50–100 kg mol^{-1} measured by size exclusion chromatography and their CD content was considered to be 50% of the weight of the polymer, as confirmed by proton NMR studies (Bakkour et al., 2006).

2.3. Capillary electrophoresis

ACE experiments were performed on a Beckman P/ACE MDQ Capillary Electrophoresis system, including an on-column diode-array UV-detector. The whole system was driven by the 32 Karat software (Beckman Coulter, Villepinte, France) package for system control, data collection and analysis. It was equipped with a 50.2 cm (40 cm effective length) × 50 μm I.D. untreated fused-silica capillary (Composite Metal Services, Ilkley, UK). The capillary was mounted in a cartridge and thermostated at 298 K ± 0.1 K (otherwise specified) and the applied voltage was 20 kV. Hydrodynamic injections were made with a 5 s injection time at 1.0 psi pressure. Analytes were detected using the diode-array detector between 190 and 300 nm. New capillaries were flushed for 20 min with 0.1 M sodium hydroxide (NaOH) (P = 25 psi) and 10 min with water (P = 25 psi). Each day the capillary was flushed successively with NaOH (5 min, 25 psi), water (5 min, 25 psi), and then with background electrolyte (BGE) (3 min, 25 psi). Between each run, it was treated with NaOH (1 min, 25 psi), water (1 min, 25 psi) and BGE (2 min, 25 psi). The 50 mM phosphate buffer pH 2.5 was prepared from a phosphoric acid solution adjusted to the pH by addition of triethanolamine. The 50 mM phosphate buffer pH 7 was prepared by mixing appropriate amounts of NaH_2PO_4 and Na_2HPO_4. The sample solutions at 100 mg L^{-1} were obtained from methanolic solutions at 1 g L^{-1} by dilution in a ten times less concentrated buffer to favour stacking.

The apparent binding constants were calculated from the mobilities of the analytes determined in seven BGE containing 0, 2.5, 5.0, 7.5, 10.0, 12.5 and 15.0 mM of β-CD or 0, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 mM of polyβ-CD (the molar concentrations of the poly-CD are the molar concentrations in CD taking into account that the CD moieties represent 50% in weight of the poly-CDs (Bakkour et al., 2006)). Each experiment was run in triplicate.

2.4. Nuclear magnetic resonance

The one-dimensional NMR experiments were carried out on a Bruker AVANCE 300 spectrometer operating at 300.09 MHz for proton and equipped with a BBI probe. The two-dimensional NMR experiments were realized on a Bruker AVANCE 500 with a TXI probe operating at 500.13 MHz for proton and 125.8 MHz for carbon. Five hundred microlitre of solutions were introduced into a standard 5 mm NMR tubes and the experiments were realized at 300 K.

Attributions of the ^1H signals of analyte and CDs were realized by standard NMR experiment spectra: COSY (homonuclear scalar correlation ^1H–^1H), HSQC (heteronuclear correlation ^1H–^{13}C) and HMBC (long range correlation ^1H–^{13}C). ROESY (homonuclear dipolar correlation ^1H–^1H) experiments spectra were recorded with a mixing time of 500 ms.

The apparent binding constant of the risperidone/polyβ-CD complex was studied at pH 2.5. The 50 mM phosphate buffer was prepared in D_2O and the pH was adjusted to 2.5 using triethylamine. The risperidone and polyβ-CD concentrations of initial solutions were 5 and 50 mM, respectively. To be in accordance with the Scott's model and take into account that the equilibrium molar concentration of the CD is equal to the total introduced CD concentration, the concentration of the CD in the complexed form must be insignificant. The [polyβ-CD]/[risperidone] ratios were progressively increased from 5 to 25. The ROESY experiment was performed for a ratio equal to 1 (the concentrations of both risperidone and polyβ-CD were 4.5 mM)

3. Results and discussion

3.1. Preliminary study

Since the determination of the binding constants for this wide variety of analyte–CD complexes (9 pharmaceutical compounds and 12 CD compounds) would be too time consuming, the complexation was first studied by taking into account the migration retardation factor Rf. The Rf was computed from the migration times of the analyte with or without CD in the BGE ($Rf = tm_{(with\ CD)}/tm_{(without\ CD)}$) and it may be qualitatively correlated to the interaction. For this preliminary study, the pH of the BGE was set to 2.5, the analytes and CDs are then cationic and neutral, respectively, considering that below pH = 4, all the carboxylic groups of the CDs polymers are in the COOH form. The apparent and averaged binding constants were further established for selected analytes and CDs.

3.1.1. Choice of the CD concentration

Preliminary experiments were performed with the β-CD and its polymeric analogue polyβ-CD at four concentrations (1.1 mM, 2.2 mM, 4.4 mM and 8.8 mM) in order to select the concentration which allows to obtain rather short analysis times and Rf factors sufficiently different from 1.

Whatever the analyte, the interaction with the CDs is proved by the increase of migration times when poly-CDs are added to the BGE. For example, the migration times of risperidone in presence of CD at 1.1, 2.2, 4.4 or 8.8 mM are 8.02, 8.53, 9.35 and 10.42 min for β-CD and 13.50, 28.62, 41.58 and greater than 90 min for polyβ-CD (the migration time of risperidone in absence of CD is 7.19 min.)The concentration of 2.2 mM appears to be a good compromise between sufficient Rf values and short analysis times. Then, this concentration was selected to estimate the Rf factor of all complexes in order to qualitatively investigate the complexing abilities of the monomeric and polymeric CDs. The impact of the viscosity change on the Rf values is neglected. The viscosities of the BGE without CD or with 2.2 mM of β-CD or 2.2 mM of polyβ-CD were evaluated using the Hagen–Poiseuille law as described by François et al. (François, Zhang, Varenne, & Gareil, 2006).The η/η_0 ratios, usually used to correct the electrophoretic mobilities, are closed to 1 (1.011 for β-CD and 1.021 for polyβ-CD) and then it could be omitted.

3.1.2. Effect of the nature of the CD

The migration retarding factors Rf obtained with the native or modified CDs (α-CD, β-CD, γ-CD, HP-β-CD, HP-γ-CD and Me-β-CD) and their polymeric analogues (polyα-CD, polyβ-CD, polyγ-CD, polyHP-β-CD, polyHP-γ-CD and polyMe-β-CD) are presented in Tables 1a and 1b.

Firstly, by studying the Rf values obtained for the native or modified monomeric CDs, it appears that the nature of the CD (and

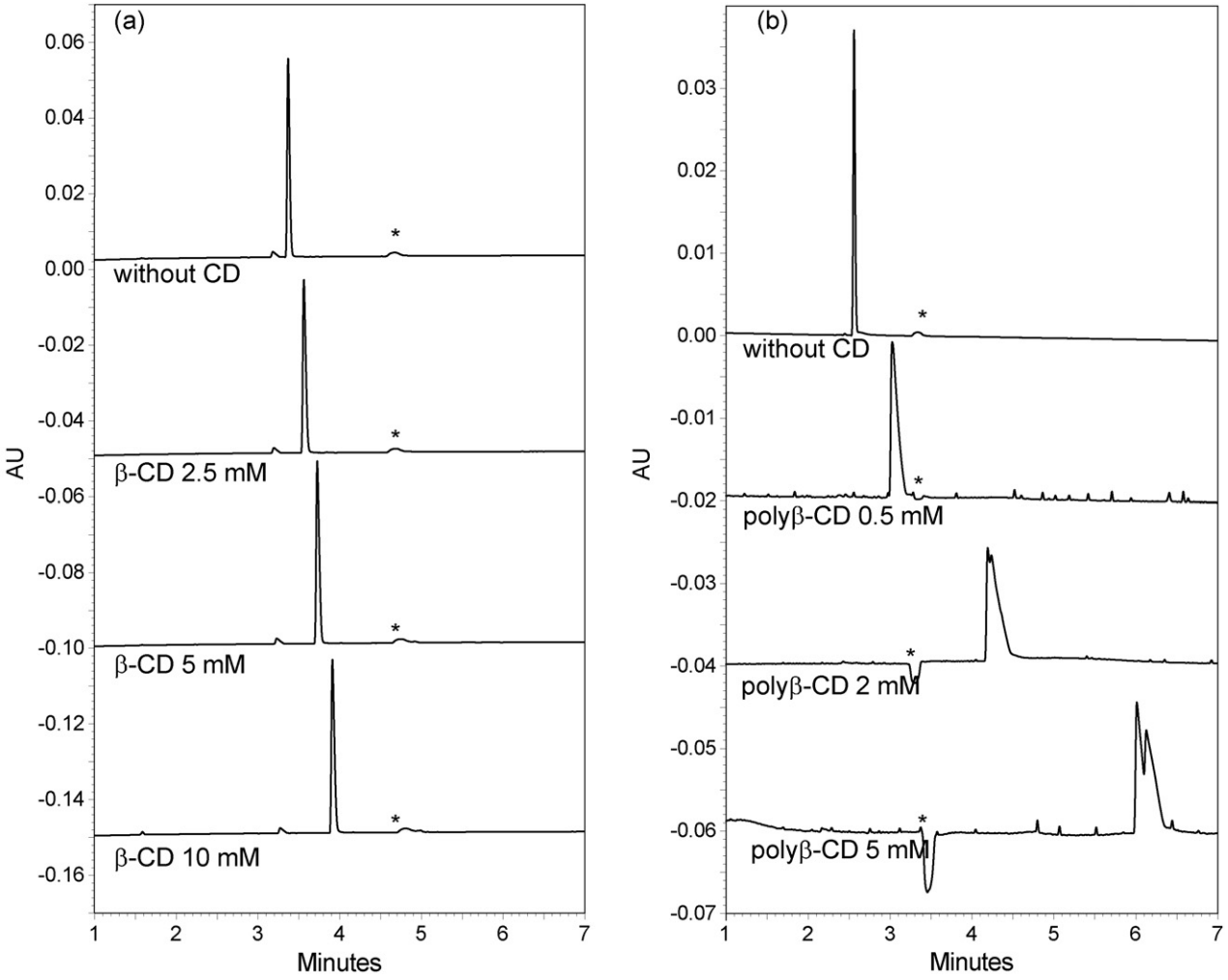

Fig. 3. Electrophoretic behaviour of propranolol in absence or in presence of β-CD (a) or polyβ-CD (b) at various concentrations (BGE, 50 mM phosphate buffer pH 7.0).

especially the size of their hydrophobic cavity) has a great importance on the complexation of the analytes.

Concerning the α-CD, its performance on the complexation is largely dependent of the size of the analytes. The highest Rf value is obtained for the smallest compound Baclofen (Rf is 2.19) whereas Rf values are closed to 1 for the other compounds for which the αCD cavity size seems too small to favour inclusion.

Whatever the structure of the analyte, the Rf values obtained with the γ- and HP-γ- CDs are smaller than those obtained with all others studied CDs (except for promethazine). The cavity size of both CDs should be too large to form stable complexes: the analytes does not fit tightly in the hydrophobic cavity and cannot strongly interact, by hydrophilic interactions, with the outer rim of CD. With both CDs, the highest Rf values are obtained for promethazine which presents a bulky phenothiazine ring (Rf are 1.12 with the γ- and HP-γ-CDs). The Rf values observed with HP-β-CD show that the hydroxypropylation of the β-CD, v which causes an extension of the cavity depth (Adam et al., 2002), does not favour inclusion of the compounds since the calculated Rf are lower than those obtained with the β-CD (except for ketoconazole). Thus, the highest complexing abilities on this series of compounds seem obtained for the β-CD and Me-β-CD (the methylation slightly affects the cavity size but slightly enhances its hydrophobic character).

Secondly, by comparing the Rf values obtained for the six native or modified CDs and their polymeric analogues, its appears that the use of the CDs polymers causes, in all cases (except for baclofen), an enhancement of the Rf values. In half of cases, this enhancement is superior to 40%. It is worth mentioning that the Rf values provide qualitative information. Thus, the determination of the binding constants is essential to really compare the complexing abilities of both forms of CDs. This study was performed using the β-CD and polyβ-CD, the pair of CDs displaying the highest Rf values.

3.2. Determination of apparent binding constants

This second part of the study was performed using the β-CD and poly-β-CDs since both CDs have displayed good complexation abilities towards our series of compounds. Four compounds were selected as representative of the series: paliperidone, propranolol, risperidone and verapamil. To investigate the influence of the pH on the complexation, the study was performed at acidic pH 2.5 and neutral pH 7.0.

Benesi–Hildebrand's method (Benesi & Hildebrand, 1949) and Scott's modified equation (Scott, 1956) allow, for analyte/CD complex of assumed 1:1 stoichiometry, the calculation of apparent binding constants from the three following linear equations obtained after mathematical rearrangements (*y*-reciprocal (1), double-reciprocal (2) and *x*-reciprocal (3)):

$$\frac{[CD]}{R_i - R_f} = \frac{1}{R_c - R_f}[CD] + \frac{1}{(R_c - R_f)K} \quad (1)$$

Table 1a
Migration times and retarding factors of analytes in presence of 2.2 mM of native α-, β- and γ-CDs or 2.2 mM of poly-CDs in the BGE.

Compound	$t_{(without\ CD)}$ (min)	α-CD		Polyα-CD		β-CD		Polyβ-CD		γ-CD		Polyγ-CD	
		t_1 (min) t_2 (min)	Rf[a]	t_1 (min) t_2 (min)	Rf[a]	t_1 (min) t_2 (min)	Rf[a]	t_1 (min) t_2 (min)	Rf[a]	t_1 (min)t_2 (min)	Rf[a]	t_1 (min) t_2 (min)	Rf[a]
Baclofen	7.75	15.85 17.03	2.19	18.28	2.36	9.21	1.19	8.50	1.10	7.81	1.01	8.04	1.04
Bupivacaine	10.03	10.18	1.01	12.20 12.26	1.22	10.88	1.08	11.78	1.17	10.22	1.02	10.78	1.07
Chlorpheniramine	5.42	7.89	1.46	18.23	3.36	6.47 6.69	1.23	13.14	2.42	5.48	1.01	6.67	1.23
Ketoconazole	8.15	11.78	1.45	29.27	3.59	11.03	1.35	>120	>14	9.03	1.11	61.10 66.36	8.14
Paliperidone	10.17	10.36	1.02	16.45	1.67	11.58	1.14	18.53	1.82	10.31	1.01	12.18	1.20
Promethazine	8.41	8.91	1.06	17.51 18.35	2.18	12.09 12.34	1.47	18.42	2.19	9.40	1.12	15.38 16.24	1.93
Propranolol	8.92	9.28	1.04	11.07 11.13	1.24	10.41	1.17	15.25 16.01	1.79	9.12	1.02	11.80	1.32
Risperidone	7.19	7.30	1.02	15.68	2.18	8.53	1.18	28.62	3.98	7.32	1.02	9.03	1.26
Verapamil	12.33	12.93	1.05	19.31 20.20	1.64	13.88 14.23	1.15	74.76	6.06	12.79	1.04	17.30 18.31	1.48

t_1 and t_2 are the migration times of the first and second detected enantiomer, respectively. Electrophoretic conditions: fused-silica capillary, 50.2 cm (effective length, 40 cm) × 50 μm i.d., long-end, normal polarity; BGE, 50 mM phosphate buffer pH 2.5; anodic injection, 1 psi pressure for 5 s; temperature 298 K; voltage, 20 kV.

[a] Rf (= $tm_{(with\ CD)}/tm_{(without\ CD)}$) is calculated for the second enantiomer (if enantioseparation occurs).

Table 1b
Migration times and retarding factors of analytes in presence of 2.2 mM of HP-β-, HP-γ- and Me-β-CDs or 2.2 mM of poly-CDs in the BGE.

Compound	$t_{(without\ CD)}$ (min)	HP-β-CD		Polyhp-β-CD		HP-γ-CD		Polyhp-γ-CD		Me-β-CD		PolyMe-β-CD	
		t_1 (min) t_2 (min)	Rf[a]	t_1 (min) t_2 (min)	Rf[a]	t_1 (min) t_2 (min)	Rf[a]	t_1 (min) t_2 (min)	Rf[a]	t_1 (min) t_2 (min)	Rf[a]	t_1 (min) t_2 (min)	Rf[a]
Baclofen	7.75	8.12	1.05	8.34	1.08	7.78	1.00	8.12	1.05	8.12	1.05	8.79	1.13
Bupivacaine	10.03	10.18	1.01	10.98	1.09	10.19	1.02	11.42	1.14	10.51	1.05	11.65 11.71	1.17
Chlorpheniramine	5.42	5.75	1.06	6.98 7.11	1.31	5.50	1.01	6.21	1.15	6.44	1.19	7.22	1.33
Ketoconazole	8.15	11.31 11.52	1.41	25.12	3.08	8.83	1.08	16.03 17.01	2.09	15.17 15.43	1.89	70.45	8.64
Paliperidone	10.17	10.75	1.06	12.92	1.27	10.30	1.01	11.86	1.17	11.42	1.12	13.32	1.31
Promethazine	8.41	10.42	1.24	18.14	2.16	9.25 9.38	1.12	16.03 17.01	2.02	10.71 11.17	1.33	24.60 25.15	2.99
Propranolol	8.92	9.08	1.02	11.18 11.35	1.27	9.18	1.03	12.83 12.89	1.45	9.71 9.79	1.10	11.92 12.11	1.36
Risperidone	7.19	7.39	1.03	9.06	1.26	7.25	1.01	8.66	1.20	7.68	1.07	8.51	1.18
Verapamil	12.33	13.19	1.07	21.38 23.33	1.89	12.71	1.03	16.95 17.36	1.41	16.32	1.32	104.58 128.23	10.40

[a] Rf (= $tm_{(with\ CD)}/tm_{(without\ CD)}$) is calculated for the second enantiomer (if enantioseparation occurs).

$$\frac{1}{R_i - R_f} = \frac{1}{(R_c - R_f)K}\frac{1}{[CD]} + \frac{1}{(R_c - R_f)} \quad (2)$$

$$\frac{(R_i - R_f)}{[CD]} = K(R_i - R_f) + K(R_c - R_l) \quad (3)$$

where K is the apparent binding constant, [CD] is considered to be the total concentration since the complexed CD concentration must be insignificant, R_i is the experimental response observed and R_f and R_c are the responses of the analytes in their free and complexed forms, respectively. In our study, the three linear equations were used since differences due to relative uncertainties of the variables before and after transformation for plotting can occur (Rundlett & Armstrong, 1997; Plätzer et al., 1999). Moreover, deviations from linearity can be more easily observed using x- or double-reciprocal plot than the y-reciprocal plot (Rundlett & Armstrong, 2001).

It is worth mentioning that the binding constants are apparent because the concentrations are considered (instead of activities) and averaged when the complexation occurred with modified CDs of averaged degree of substitution.

The studied responses in ACE or NMR spectroscopy are the electrophoretic mobility (μ_i) or the chemical shift (δ_i), respectively.

The apparent mobility (μ_{app}) is the sum of the electrophoretic mobility (μ_i) and the electroosmotic mobility (μ_{eof}) and can be calculated as follows:

$$\mu_{app} = \mu_i + \mu_{eof} = \frac{l \cdot L}{t \cdot V}$$

μ_{eof} was calculated from the migration time of the marker substance (ethanol).

3.2.1. Affinity capillary electrophoresis

To determine the binding constants, the evolution of the mobility of the compound is studied at various CD concentrations. According to the complex studied, the ranges of the CD or poly-CD concentrations needed to be adapted in order to be sufficiently high to involve significant mobility changes and sufficiently low to avoid the saturation phenomenon. In all cases, the CD ranges for the polyβ-CD are much lower than those used for the β-CD. The four compounds studied are cationic at pH 2.5 and pH 7.0. Then, the complexation with neutral or anionic CDs involves a decrease of their mobility. As depicted in Fig. 3 for propranolol, the increase of the migration times of the four compounds when CDs are added in the BGE is much more significant with the polyβ-CD than with the β-CD. It is worth mentioning that, at pH 7.0, the increase of the poly-CD concentration causes the shift of the electrophoretic signal of the cationic compounds after the EOF signal. This can be only observed with the polymer since it is in anionic form at pH 7.0, due to the deprotonation of the citrate crosslinks.

The apparent and averaged binding constants K were determined for the four complexes previously cited at acidic and neutral pH using the three linear models. The r values with the y-, double- and x-reciprocals were greater than 0.99 and proved the adequacy of the model for the 1:1 complexes. Whatever the linear model used, results were similar. The K values determined with the y-reciprocal equation are reported in Table 2. The results obtained for risperidone and paliperidone with the β-CD at pH 2.5 are in accordance with our previously published results (91 instead of 108 M^{-1} and 167 instead of 165 M^{-1}, respectively) (Danel et al., 2008). At neutral pH, the results are also of same order in both studies whereas the pH is slightly different (pH 7.0 instead of pH 7.4): 216 instead of 221 M^{-1} for risperidone and 193 instead of 259 M^{-1} for paliperidone (Danel et al., 2008). It is worth mentioning that the apparent binding constants for the guest/polyβ-CD complexes could be determined in most cases for both enantiomers directly when an enantioseparation phenomenon occurs. The enantioselective properties of the poly-CDs are discussed in the last Section 3.4.

Table 2
Apparent and averaged binding constants determined by CE (K in M^{-1}) for the complexes formed between paliperidone, propranolol, risperidone or verapamil with the native β-CD or polyβ-CD at pH 2.5 and 7.0.

	pH 2.5		pH 7.0	
	β-CD	Polyβ-CD	β-CD	Polyβ-CD
Paliperidone	167 (165[b])	437	193 (259[b])	574/602[a]
Propranolol	157	247/273[a]	144	517/537[a]
Risperidone	91 (108[b])	562	216 (221[b])	729
Verapamil	308/350[a]	1513/1643[a]	410	1198/1332[a]

Mean values calculated from three experiments. Relative standard deviations lower than 5% with β-CD and than 10% with polyβ-CD.

[a] The first and second values correspond to the first and second detected enantiomer.

[b] Value obtained in our previous study in close experimental conditions at pH 2.5 or 7.4.

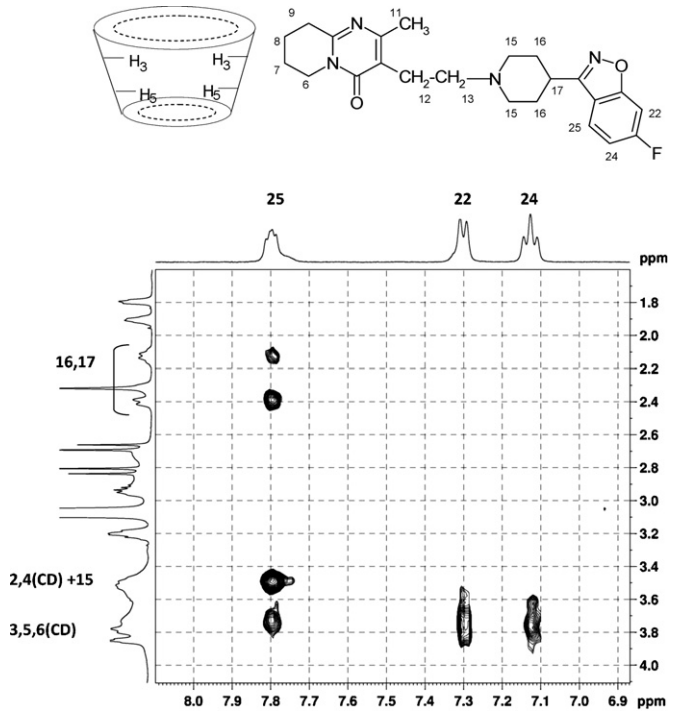

Fig. 4. Partial ROESY spectrum of the risperidone/polyβ-CD complex at pH 2.5 ([risperidone] = [polyβ-CD] = 4.5 mM). Structure and assigments of the risperidone and the schematic CD cavity.

By comparing the results obtained with the β-CD and the polyβ-CDs, it appears that the K values strictly increase using the polymer instead of its monomeric form: from 65% to 517% depending on the studied compounds. For example, at pH 2.5, the K values for the risperidone/β-CD or risperidone/polyβ-CD complexes are 91 or 562 M^{-1} and for verapamil, they are 308 or 1513 and 1643 M^{-1} (these two values corresponding to the first and second detected enantiomer). The influence of the pH on the complexation is significant. Except for verapamil, the K values determined for the polyβ-CD at pH 7.0 are 30% to 100% higher than those determined at pH 2.5. For propranolol, the K values are 247 and 517 M^{-1} at pH 2.5 and 7.0, respectively. These increases of the binding constants may be related to the presence of ionic interactions between the carboxylate functions of the poly-CD and the cationic protonated compounds. As a general rule, the high complexation abilities of the poly-CDs may be attributed to a synergy effect between hydrophobic interactions (inclusion in the CD moieties), hydrophilic interactions with the polymer network and electrostatic interactions (when the polymer is under its anionic form).

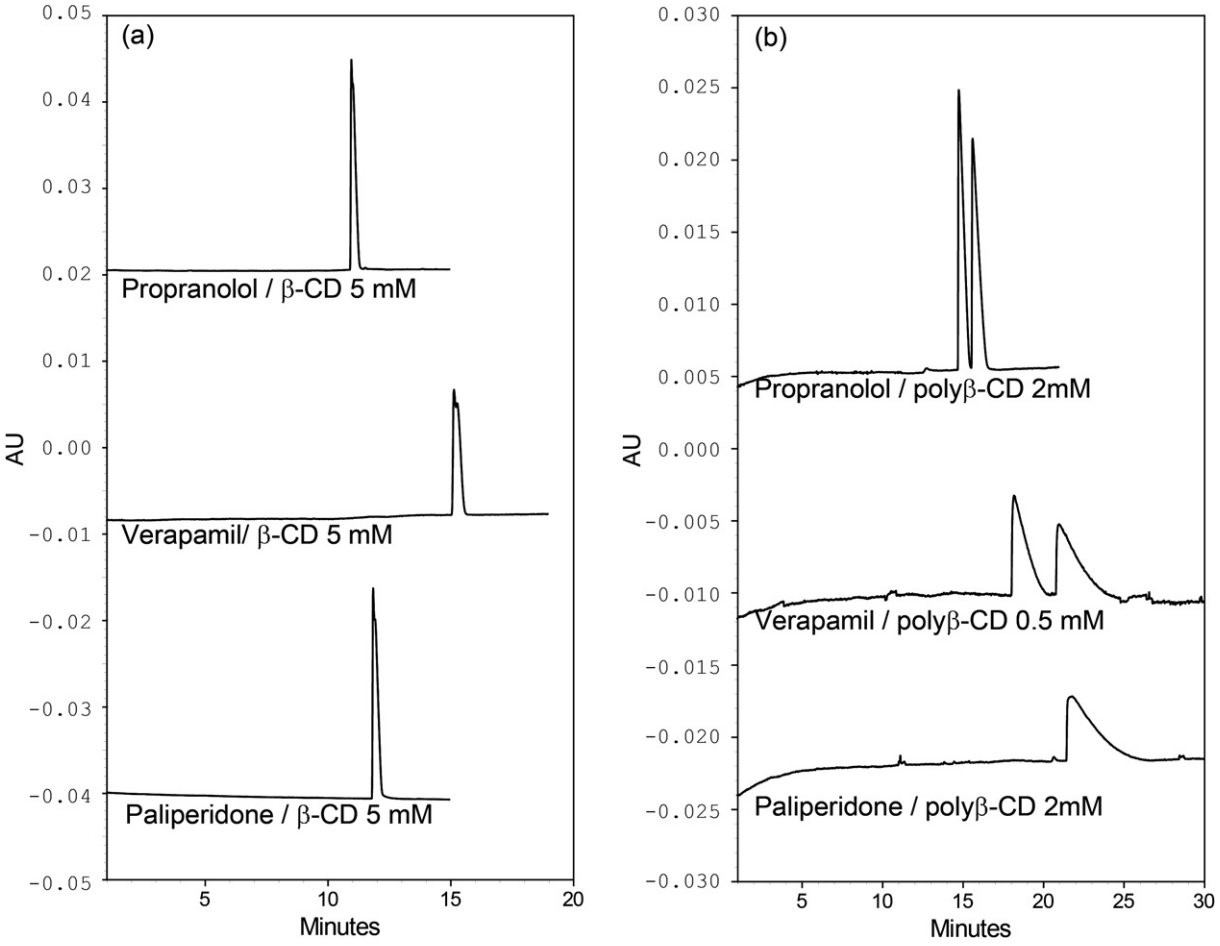

Fig. 5. Electropherograms of propranolol, verapamil and paliperidone in presence of β-CD (a) or polyβ-CD (b) in the 50 mM phosphate buffer at pH 2.5.

3.2.2. ^1H NMR spectroscopy

Since NMR is much more reagent and time consuming than ACE, the NMR study was only performed for one compound, risperidone. This NMR study was performed at pH 2.5 in order to facilitate the comparison between the β-CD and polyβ-CD properties. At pH 7.0, the polyβ-CD becomes anionic although its monomeric analogue remains uncharged. By comparing the spectra of the risperidone in absence or in presence of the polyβ-CD, it appears that the aromatic protons present the more significant chemical shift differences ($\Delta\delta$) (see supplementary data). The $\Delta\delta$ obtained for the ratio [polyβ-CD]/[risperidone] equal to 15 are 27 Hz, 19 Hz and 37 Hz for H_{22}, H_{24} and H_{25}, respectively. This observation should indicate a spatial proximity of this aromatic part of the risperidone with the polyβ-CD. The linear Scott's plot obtained ($r^2 > 0.99$) allows to evaluate the apparent binding constant at 775 M^{-1}. Since, the K value obtained for the complex risperidone/β-CD was 124 M^{-1} (Danel et al., 2008), the polymerization of the β-CD induces an improvement of the complexing abilities of 525% Even if differences of the K values obtained via ^1H NMR and ACE occur, the influence of the polymerization of β-CD is similar whatever the used technique (increase of 517% in the ACE study). The high complexing abilities of the polymer at pH 2.5 cannot be attributed to additional electrostatic interactions (as seen at pH 7.0 in the ACE study) but to the structure of the polymeric network either by the establishment of new hydrophilic interactions with the crosslinking agent citric acid either by favouring the inclusion maintenance in the cavities.

3.3. Study of the structure of the risperidone/polyβ-CD complex

The structure of the complex formed between the risperidone and the polyβ-CD was investigated at pH 2.5 by two-dimensional frame Overhauser effect spectroscopy since ROESY permits to identify the homonuclear dipolar interactions ^1H–^1H. The partial ROESY spectrum obtained is displayed in Fig. 4 and reveals both intra- and inter-molecular interactions. The intramolecular interactions between the aromatic H_{25} and the protons H_{15}, H_{16} and H_{17} of the piperidinyl ring are clearly proved. The other cross-peaks observed are attributed to intermolecular interactions between the H_{22}, H_{24} and H_{25} of the aromatic ring and the H_3 and H_5 of the CD unit and prove the spatial proximity of the aromatic protons of risperdone with both protons located inside the hydrophobic cavity of the CDs. The ROESY spectra obtained for the risperidone/β-CD (see supplementary data) and risperidone/polyβ-CD complexes are similar. No dipolar interaction between the protons of risperidone and the protons of the carboxylic functions of the reticulating agent of the polyβ-CD was detected. Thus, among our previous hypothesis it seems that the structure of the polymeric network favours the inclusion of the guest in the hydrophobic cavity of the CD units due to additional steric constraints.

3.4. Enantioselective properties of the poly-CDs

The preliminary study of the migration retardation factors Rf of the nine pharmaceutical compounds with the six poly-CDs and

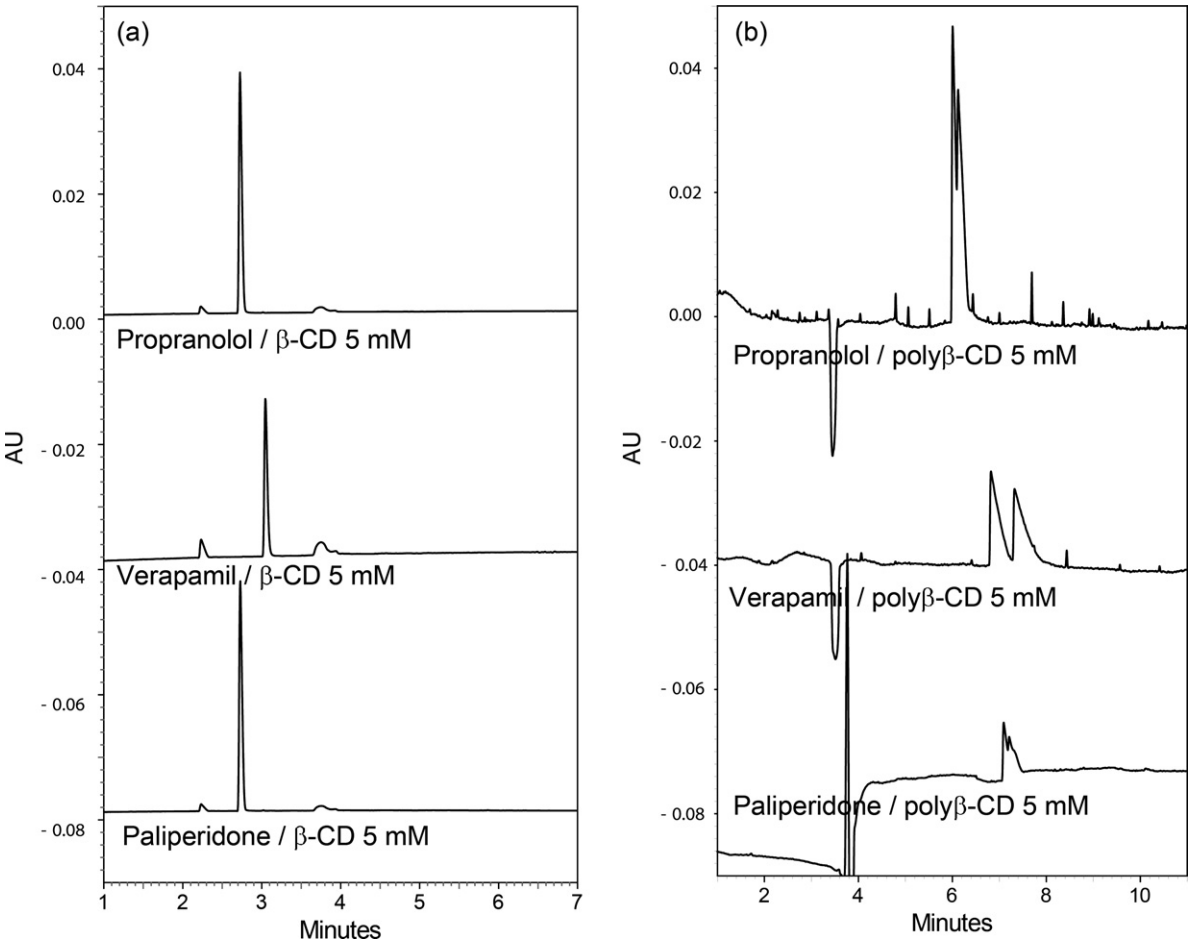

Fig. 6. Electropherograms of propranolol, verapamil and paliperidone in presence of β-CD (a) or polyβ-CD (b) in the 50 mM phosphate buffer at pH 7.0.

their corresponding non-polymeric form can give us information on the enantioselective abilities of the poly-CDs. Indeed, by studying the Tables 1a and 1b, two migration times are displayed for some compounds corresponding to the first and second detected enantiomer, respectively. Generally, for a same concentration, the poly-CDs display more enantioselectivity than the corresponding non polymeric form. In some cases, the use of the poly-CDs (especially for polyβ-CD) involves a loss of enantioselectivity. However, this is observed for complexes displaying a rather high retardation factor (Rf superior to 2); it may be envisaged that enantioselectivity could be observed for more appropriate poly-CD concentrations. Indeed, the previous analyses of the three chiral compounds (paliperidone, propranolol and verapamil) in presence of polyβ-CD display the high enantioselective properties of this polymer (versus the β-CD) since in most of cases enantioseparations were observed and had permitted the direct determination of the *K* values for both enantiomers. The high enantioselective abilities of the polyβ-CD are unambiguously displayed for the three compounds in Figs. 5 and 6. For similar or lower concentrations, the polyβ-CD induces partial or complete enantioresolutions whereas no enantioselectivity occurs with its non polymeric form. It is worth mentioning that resolutions superior to 1.5 are obtained for the enantiomers of verapamil at acidic and physiological pH (without any optimization of the various electrophoretic parameters).

4. Conclusion

The study of the retardation factors Rf has proved the very high complexing abilities of the six poly-CDs since the Rf values obtained for the poly-CDs are, in most cases, much higher than those obtained for their corresponding native or modified CD. These preliminary qualitative results have been confirmed by the determination of the apparent and averaged binding constants: the *K* values are up to five times higher with the poly-CDs. All the information collected by ACE and NMR seem to point out the major role of the polymeric network which should favour, through additional steric constraints, the hydrophobic inclusion of the drugs in the CD-cavities. Then, these non-toxic poly-CDs present interesting complexing properties towards a large panel of drugs and should be involved in multiple pharmaceutical applications (as solubilizing agent, in controlled drug delivery...). Moreover, the poly-CDs have shown very high enantioselective abilities and could also be used as efficient chiral selectors, particularly in CE.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2012.11.095.

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