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High-performance liquid chromatographic study of the interactions between immobilized β-cyclodextrin polymers and hydrophobically end-capped polyethylene glycols

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Abstract

The formation of inclusion complexes between polyethylene glycols (PEGs) bearing hydrophobic ends (naphtyl and phenyladamantyl) and β-cyclodextrin polymers (polyβ-CD) immobilized onto silica particles was studied by high-performance liquid chromatography (HPLC). It was shown that hydrophobic interactions were involved in the retention mechanism of these compounds, since retention volumes decreased when organic solvents were added to the mobile phase while it was the contrary in the presence of salts. Moreover, the association could be reversed by adding a competitor (hydroxypropylβ-cyclodextrin) to the mobile phase. A theoretical model permitted the evaluation of affinity constants of 1:1 complexes formed between the modified PEGs and the immobilized polyβ-CD which depended on the type of hydrophobic groups grafted to the PEG.

Keywords: β-Cyclodextrin; Polyethylene glycol

1. Introduction

β-cyclodextrin (β-CD) belongs to a family of torus-shaped cyclic oligosaccharides composed of seven α-1,4 linked D-glucopyranose units. While the outside of the molecule is hydrophilic, its inner cavity is hydrophobic. Thus β-CD can form inclusion complexes (host–guest complexes) with hydrophobic molecules or with hydrophilic ones which possess hydrophobic moities [1,2].

Due to its optical activity, β-CD can distinguish between the enantiomers of chiral molecules. Therefore, β-CDs are extensively used as stationary phases in gas chromatography [3] as well as stationary or mobile phase additives in liquid chromatography [4–6] and capillary electrophoresis [7].

In previous studies, original associating systems were obtained by mixing, in aqueous solution, hydrophobically end-capped polyethylene glycols (PEG) and water soluble β-cyclodextrin polymers [8,9]. It was shown that the formation in solution of inclusion complexes between the PEG terminal groups and the β-cyclodextrin polymer induced polymolecular associations and led to high viscosity solutions.

In the present study, a polymer of β-cyclodextrin (polyβ-CD) was immobilized onto silica particles. Its ability to form host–guest complexes with hydrophobically modified PEGs was investigated by high-performance liquid chromatography (HPLC). If such systems lead to reversible inclusion complexes at the surface of silica particles, original regenerable chromatography supports could be elaborated.

Thus, the aim of this paper is to report the

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retention behaviour of UV-absorbing naphtyl and phenyladamantyl PEGs on polyβ-CD supports. Firstly, hydrophobically modified PEGs were injected in pure water mobile phase. Then organic solvents, salts and finally a competitor (hydroxypropylβ-cyclodextrin) were added into the eluent. Moreover, affinity constants between immobilized polyβ-CD and hydrophobic PEGs were evaluated.

2. Materials and methods

2.1. Chemicals

Diol-bonded silica (LiChrosorb Diol, particle size: 5 μm; porosity: 100 Å; specific area: 300 m^2/g; 4.2 μmol of hydroxy group per m^2) and LiChrosorb RP-8 (particle size: 10 μm) were commercial products from Merck (Darmstadt, Germany). The following reagents were purchased from Aldrich (St Quentin Fallavier, France): 1,4-diisocyanatobutane, triethylamine, dibutyltin dilaurate (DBLT), 4-dimethylaminopyridine (DMAP), NaCl, hydroxypropylβ-cyclodextrin (MW=1500 g/mol, *ds*=0.8), monomethoxypolyethylene glycol 5000 (MPEG) and finally polyethylene glycol 4500 (PEG2B). Star polyethylene glycol 20 000 (PEG4B) comes from Shearwater Polymers (Enschede, The Netherlands). All solvents were obtained from SDS (Peypin, France). Water was purified with a MilliQ RG system (Millipore, Bedford, MA, USA).

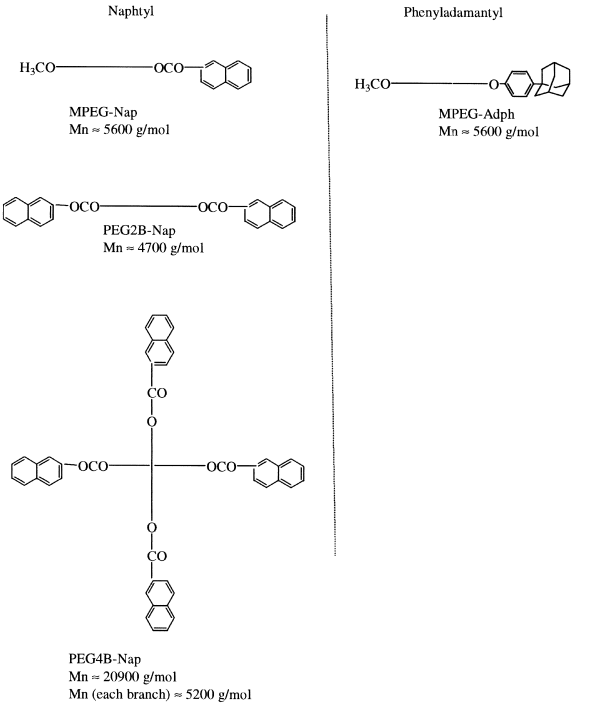
2.2. HPLC instrumentation

All chromatographic measurements were performed using a Waters 501 HPLC pump (Milford, MA, USA) and a Shimadzu SPD-6A variable-wavelength UV detector (Kyoto, Japan). Naphtyl- and phenyladamantyl-modified PEGs were respectively monitored at 240 nm and 276 nm. Samples were injected by a Rheodyne (Berkeley, CA, USA) Model 7125 sampling valve with a 20-μl loop. Chromatograms were recorded with a Kipp and Zonen chart-recorder (type BD 41). The flow-rate was set at 1 ml/min. Both columns (1: reference LiChrosorb Diol and 2: the polyβ-CD modified diol silica) were 100×4.6 I.D, packed at high pressure by using an equipment from Touzart and Matignon (Courtaboeuf, France).

2.3. Polymers

β-cyclodextrin polymers (polyβ-CD) were prepared by crosslinking β-CD with epichlorohydrin under strongly alkaline conditions. Reaction was stopped before the gelation point in order to obtain water-soluble polymers. The synthesis and characterization of these polymers were described in detail elsewhere [10]. Their molecular mass was evaluated by size exclusion chromatography in water (TSK G3-4000 SW column: M_n=30 000, MW=68 000, I_p=2.26) and the β-CD content of polymers was determined by ^{1}H NMR (45 wt.%).

Polyethylene glycols bearing one, two and four naphtyl or phenyladamantyl terminal groups (Scheme 1) were synthesized as described previously [11]. Naphtyl end-capped PEGs (PEG-Nap) were easily obtained by reaction of OH terminal functions with 2-naphtoylchloride in dichloroethane. In the case of phenyladamantyl PEGs (PEG-Adph), a two



Scheme 1. Structural formula of hydrophobically-modified PEGs.

steps modification of the initial polymers was performed using 4-toluenesulfonylchloride, followed by nucleophilic displacement by adamantylphenolate.

2.4. Preparation of polyβ-CD modified silica

The grafting of polyβ-CD to diol-modified silica was performed by a two steps reaction (Scheme 2). 1,4-diisocyanatobutane was first linked to diol functions (reaction 1). Then, the β-CD polymer was coupled to unreacted isocyanate groups (reaction 2).

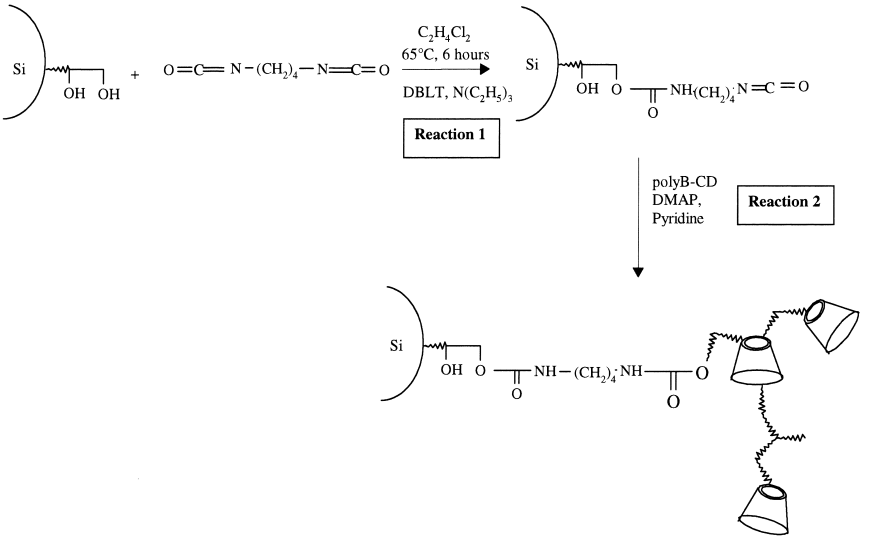
In the first step, diol silica particles (1 g), dried overnight under vacuum at 70°C, were suspended in 10 ml of anhydrous $C_2H_4Cl_2$. The suspension was heated at 65°C under a nitrogen atmosphere before adding 25 μl of triethylamine (HCl scavenger), 25 μl of dibutyltin dilaurate DBLT (catalyst) and 1.6 ml of 1,4-diisocyanatobutane. After 6 h at 65°C, the reaction mixture was filtered on a 0.45-μm cellulose membrane and washed several times with $C_2H_4Cl_2$. In these conditions, 1.3 meq of diisocyanatobutane were bound per gram of silica (elementary analysis data). Moreover, the formation of urethan bonds was shown by infrared spectroscopy ($\nu_{O\text{-}CO\text{-}NH}$=1703 cm^{-1}). Although crosslinking could occur during reaction 1, it appeared on infrared spectra that isocyanate groups ($\nu_{O=C=N}$=2273 cm^{-1}), able to react with polyβ-CD (reaction 2), were still present at the end of reaction 1.

In the second step, 1 g of isocyanate-modified silica and 0.21 g of DMAP were added to a solution of polyβ-CD (2.5 g dried overnight at 70°C under vacuum) in dry pyridine (77 ml). The suspension was mixed at room temperature during 48 h, filtered on a 0.45-μm cellulose membrane and washed with pyridine and ethanol. By this way, infra red spectra showed the total disappearance of isocyanate functions at 2273 cm^{-1}.

3. Results and discussion

3.1. Formation of inclusion complexes between hydrophobically end-capped PEGs and immobilized polyβ-CD

As shown previously [8,9] polymolecular associations between β-cyclodextrin polymers (polyβ-CD) and hydrophobically modified polyethylene glycols were observed when both polymers were in aqueous solution. However, such guest–host interactions might be hindered by the immobilization of one polymer, here the polyβ-CD, on a rigid surface. Thus the ability of polyβ-CD silica particles to form inclusion complexes with naphtyl and



Scheme 2. Modification of diol silica particles by polyβ-CD using a diisocyanate reagent.

Table 1
Retention factor of unmodified MPEG and hydrophobically end-capped MPEG on the reference LiChrosorb Diol column (column 1) and polyβ-CD column (column 2)[a]

Polymer	MPEG	PEG2B	PEG4B	MPEG-Nap	MPEG-Adph
Column 1 *k*	0.1	0.1	0	1.5	2.1
Column 2 *k*	0.3	0.2	0	20.9	Not eluted

[a] Mobile phase: water; flow-rate: 1 ml/min; injected volume: 20 μl; solute concentration: 2 g/l for MPEG-Nap, 10 g/l for MPEG-Adph (UV detection at respectively 240 nm and 276 nm) and 1.5 g/l for unmodified PEGs (refractometric detection).

phenyladamantyl end-capped PEGs was first examined, using water as a mobile phase. Table 1 indicates retention factors (*k*) of initial PEGs (MPEG, PEG2B, PEG4B) and hydrophobically functionalized PEGs on the LiChrosorb Diol reference column (column 1) and polyβ-CD column (column 2).

It appears that interactions of unmodified PEGs, MPEG-Nap and MPEG-Adph with diol silica (column 1) are negligible. Further, when injected on polyβ-CD silica (column 2), the initial PEGs were also eluted near to the dead volume, while the hydrophobic PEGs were highly retained or not eluted (MPEG-Adph). These results clearly demonstrate that the formation of inclusion complexes between the immobilized polyβ-CD and hydrophobic PEGs was efficient. Moreover, the involvement of methoxy end groups in the retention mechanism of MPEG-Nap and MPEG-Adph on column 2 can be considered as negligible since MPEG was not retained ($k=0.3$) by polyβ-CD supports. Thus, it can be concluded that the high retention of MPEG-Nap and MPEG-Adph, really resulted from the inclusion of hydrophobic ends into β-CD cavities. Additionally, it can be noted that phenyladamantyl derivatives were more retained than the naphtyl-MPEG. This is consistent with affinity constants reported in the literature for the formation of inclusion complexes between β-CD and 1-adamantylcarboxylic acid [12] or naphthalene [13].

Besides, PEG2B-Nap and PEG4B-Nap with respectively two and four hydrophobic end groups, were not eluted from the polyβ-CD column indicating that interactions increased with the number of hydrophobic moities. It also confirms that it is the naphtyl end which is engaged in the retention mechanism of MPEG-Nap.

3.2. Effects of organic modifiers on the retention of PEG derivatives

The effect of organic solvents such as acetonitrile or *N*,*N*-dimethylformamide (DMF) on interactions between immobilized polyβ-CD and hydrophobic PEGs was investigated. It appears in Fig. 1, that retention volumes (V_r) of MPEG derivatives decreased when organic modifiers were added to the mobile phase, confirming that hydrophobic interactions were involved in the retention mechanism of these compounds. Similarly PEG2B-Nap and PEG4B-Nap which were irreversibly adsorbed by the polyβ-CD support with water as a mobile phase, began to be eluted in the presence of organic solvents (respectively with 10 and 15% of acetonitrile).

As shown in Fig. 1, the retention of MPEG-Adph

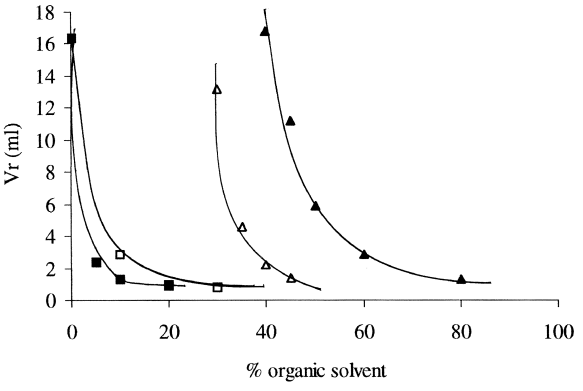


Fig. 1. Injection of modified-MPEG on polyβ-CD support (column 2): retention volume versus percent of organic solvent in the aqueous mobile phase. Flow rate: 1 ml/min; injected volume: 20 μl; solute concentration: 2 g/l for MPEG-Nap and 10 g/l for MPEG-Adph (UV detection at respectively 240 nm and 276 nm). □: MPEG-Nap, △: MPEG-Adph (empty: acetonitrile, full: DMF).

was much lower in the presence of acetonitrile than with DMF in the eluent, while the opposite effect was observed for naphtyl derivatives. For a better understanding of these phenomena, a similar study was performed on a reversed-phase column (250× 4.6 I.D. LiChrosorb RP-8). It appears, in Table 2, that results obtained with the LiChrosorb RP-8 column were similar to those with polyβ-CD supports. It means that the retention order is maintained and the respective effects of DMF and acetonitrile on MPEG-Nap and MPEG-Adph are the same.

Thus, the opposite retention behaviours observed for MPEG-Adph and MPEG-Nap on the polyβ-CD support in the presence of DMF or acetonitrile probably result from their different solubility in both solvents.

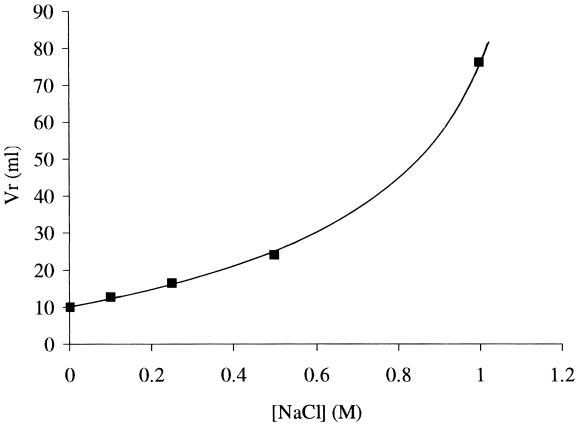


Fig. 2. Injection of naphtyl-modified MPEG on polyβ-CD support (column 2): retention volume as function of NaCl concentration (same chromatographic conditions as in Fig. 1).

3.3. Effects of salt on the retention of PEG derivatives

NaCl was added to the eluent to study the effects of salts on interactions between polyβ-CD and hydrophobically end capped PEGs. As shown in Fig. 2, the retention of MPEG-Nap increased with salt concentration. This result was in good agreement with the involvement of hydrophobic interactions in the retention mechanism of this compound. In the case of MPEG-Adph, 25% of acetonitrile had to be added to saline solutions because this solute was not eluted in purely aqueous mobile phase. Nevertheless, the presence of this solvent inhibited the effect of NaCl, leading to a constant retention even by increasing salt concentration.

Table 2
Retention volumes of naphtyl and phenyladamantyl end-capped MPEG on reversed-phase column (Lichrosorb RP-8) as function of percent of organic solvent in the mobile phase[a]

% Solvent	MPEG-Nap		MPEG-Adph	
	DMF	CH_3CN	DMF	CH_3CN
25	27.2	Not eluted		
30	22.4	Not eluted		
35		52.5		
40	18.5	7.5	Not eluted	43.6
45		3.9	Not eluted	11.1
50	16	2.85	Not eluted	4.7
55		2.7	Not eluted	3.3
80			57.6	

[a] Same chromatographic conditions as in Table 1.

3.4. Effects of complex competitor on the retention of PEG derivatives

As described in the first part of this paper, host–guest interactions between the polyβ-CD support and hydrophobic PEGs were reduced in the presence of organic modifiers while they were intensified by salts, due to their hydrophobic character. The reversibility of the association was also investigated after adding a competitor, hydroxypropylβ-cyclodextrin (HPβ-CD), to the mobile phase. As expected, host–guest interactions with the immobilized cyclodextrin polymer decreased when the concentration of HPβ-CD in the eluent increased (Fig. 3).

Much more pronounced effects were produced by the competitive reagent than the organic modifiers. For instance, MPEG-Adph which was irreversibly adsorbed on polyβ-CD supports in pure water, was eluted at 13 ml with 30% of acetonitrile (corresponding to 5.8 *M*) in the eluent, while only 4 m*M* of HPβ-CD was necessary to obtain a similar elution volume.

These curves were used to calculate complexation constants between modified PEG solutes and β-CD molecules according to the theoretical model developed for the quantitative treatment of affinity chromatography [14–16].

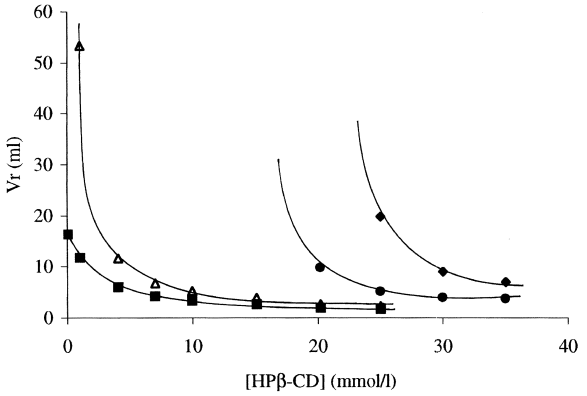


Fig. 3. Injection of modified-PEGs on polyβ-CD support (column 2): retention volume as function of hydroxypropylβ-cyclodextrin concentration (same chromatographic conditions as in Fig. 1) ■: MPEG-Nap, ●: PEG-2B-Nap, ♦: PEG4B-Nap, △: MPEG-Adph.

3.5. Determination of complexation constants between modified PEGs and β-CD

As demonstrated by Chaiken in competitive affinity chromatography, the retention volumes of solutes depend on a set of equilibrium Eqs. (1) and (2) which describe the interactions between the solute A, the immobilized ligand X, and the soluble ligand L in the eluent. The simplest model assumes that only 1:1 complexes are formed and that there is no interaction between the mobile ligand (L) or the ligand–solute complex (AL) with the immobilized ligand X at the surface. Then, the interactions between the solute A and the ligand (X or L) can be defined by two equilibria:

$$A + X \Leftrightarrow AX \quad K_{AX} = \{AX\}/([A]\{X\}) \tag{1}$$

$$A + L \Leftrightarrow AL \quad K_{AL} = [AL]/([A][L]) \tag{2}$$

Where K_{AX} and K_{AL} are the binding constants and the braces {} represent the surface concentrations in mol/m^2.

According to the model, a linear relationship is obtained when $1/(V_Z - V_o)$ is plotted versus the total ligand concentration [L] in the mobile phase.

$$\frac{1}{V_Z - V_0} = \frac{1}{Q_X K_{AX}} + [L]\frac{K_{AL}}{Q_X K_{AX}} \tag{3}$$

In this equation Q_X represents the accessible amount of immobilized ligand and V_o the dead volume. V_Z is the limit value of the retention volume (V_R) extrapolated to zero sample concentration. Retention volumes, indeed, depend on the amount of solute injected on the column, according to Eq. (4) [16].

$$\sqrt{\frac{1}{V_R - V_0}} = \sqrt{\frac{1 + [L]K_{AL}}{Q_X \times K_{AX}}} + [A]\sqrt{\frac{K_{AX}}{Q_X(1 + [L]K_{AL})}} \tag{4}$$

Thus V_Z and Q_X values can be determined by plotting $\sqrt{1/V_R - V_0}$ versus solute concentration. V_Z is obtained by extrapolation of the line to zero concentration and Q_X is derived from the slope and *Y*-intercept of the same line

$$\left(Q_X = \frac{1}{(\text{slope} \times \text{ordinate intercept})}\right)$$

According to Eq. (3), the binding constants K_{AL} and K_{AX} can be deduced from the linear dependency of $1/(V_Z - V_0)$ versus [L]. K_{AL} is the slope to ordinate intercept ratio and K_{AX} is determined from the *Y*-intercept and Q_X value.

The model was applied to our macromolecular system using monofunctional PEG solutes (A= MPEG-Nap or MPEG-Adph). X and L, in Eqs. (1) and (2), referred respectively to the immobilized polyβ-CD and hydroxypropylβ-cyclodextrin (HPβ-CD) in the mobile phase. As shown in Fig. 4, linear relationships were observed for both solutes when

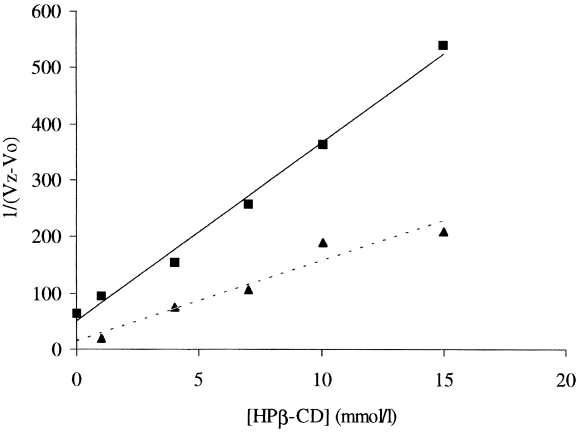


Fig. 4. Injection of modified MPEG on polyβ-CD support (column 2) with hydroxypropylβ-CD in the mobile phase: application of the theoretical model ■: MPEG-Nap, ▲: MPEG-Adph.

the reciprocal of $(V_Z - V_0)$ was plotted against the HPβ-CD concentration, demonstrating that the theoretical model could be applied and that 1:1 complexes were actually involved between hydrophobically modified MPEGs and polyβ-CD. Affinity constant K_{AL} and K_{AX} were determined as described above from the lines in Fig. 4, using $Q_X = 4.9 \times 10^{-5}$ mol. Values, reported in Table 3, showed that complexation constants were similar in solution (K_{AL}) and on surface (K_{AX}). Moreover, it appears that interactions with the cavity of β-cyclodextrin were stronger for PEG-Adph than for PEG-Nap. In the case of naphtyl derivatives, constants are in good agreement with values given in the literature for the formation of inclusion complexes between naphthalene and β-CD (from 190 to 850 l/mol according to the analytical method [8,13]). The values obtained for the phenyladamantyl groups were lower than the literature data reported for the complexation of adamantane carboxylic acid by β-CD [12]. However, the structure and charge of both moities were different.

Besides, in order to study the influence of the number of hydrophobic groups, PEG2B-Nap and PEG4B-Nap were injected on the polyβ-CD column with hydroxypropylβ-cyclodextrin in the mobile phase. The evolution of their retention was noted in Fig. 3. As for MPEG-Nap, a decrease of retention was observed with addition of hydroxypropylβ-cyclodextrin to the mobile phase. Moreover, PEG4B-Nap was retained more than respectively PEG2B-Nap and MPEG-Nap. However theoretical models described for monovalent and multivalent macromolecular solutes [16] were not suitable to fit curves, showing that hydrophobic ends did not interact simultaneously with the support.

Table 3
Affinity constants of hydrophobically end-capped MPEGs and β-cyclodextrin cavities in solution (K_{AL}) and on the surface of silica (K_{AX}) with $Q_X = 4.9 \times 10^{-5}$ mol

	K_{AL} (l/mol)	K_{AX} (l/mol)
MPEG-Nap	630	400
MPEG-Adph	1000	1400

4. Conclusion

Therefore, high-performance liquid chromatography appeared to be a satisfactory method to observe and to characterize polymolecular interactions between hydrophobic PEGs and polyβ-cyclodextrin immobilized on silica particles.

It was demonstrated that polymers interacted by formation of 1:1 complexes between the hydrophobic moiety beared by the PEG and the cavity of β-cyclodextrin. These interactions are hydrophobic since the retention volume decreased by addition of organic solvent in the aqueous mobile phase and on the contrary increased by addition of salts.

Moreover, in the presence of a competitor in the mobile phase, affinity constants were evaluated by a theoretical model. Values are in good agreement with literature data.

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