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Chiral supercritical fluid chromatography on porous graphitic carbon using commercial dimethyl β-cyclodextrins as mobile phase additive

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Abstract

Using dimethylated-β-cyclodextrin mixtures (MeCD) as chiral selectors in CO_2-polar modifier mobile phase and porous graphitic carbon as solid-phase, chiral supercritical (or subcritical) fluid chromatography was performed. The adsorbed quantity of MeCD onto the porous graphitic carbon (Hypercarb) was measured for various chiral selector concentrations using the breakthrough method with evaporative light scattering detector. The effects of MeCD concentration in the mobile phase, the nature of the polar modifier, the outlet pressure, the column temperature and the nature of the commercial MeCD mixture on the retention and the enantioselectivities were studied. For a given solute, the enantioselectivity is greatly dependent on the commercial MeCD mixture used. The retention mechanism was also studied. From the data, we find that the dominant mechanism for the chiral discrimination is the diastereoisomeric complexation in the mobile phase.

Keywords: Supercritical fluid chromatography; Chiral selectors; Porous graphitic carbon; Enantiomer separation; Cyclodextrins; Dimethyl β-cyclodextrins

1. Introduction

Chiral stationary phase (CSP) or (and) chiral mobile phase additive (CMPA) can be used for enantiomeric separations. The latter approach was introduced in the 1970s [1–3] in liquid chromatography (LC) and is now commonly employed in electromigration methods [4,5]. Besides, since 1985 [6], supercritical fluid chromatography (SFC) was proved to be a convenient method for chiral separations using CSP, recently reviewed [7] or CMPA [8–10].

With its unique adsorption properties [11,12], the porous graphitic carbon (PGC) can be used for chiral separations in LC if a chiral selector is added in the mobile phase or adsorbed onto the stationary phase [13–21].

Only few investigations have been done using SFC on PGC with CMPA [8,10,22,23]. Preliminary results have recently been presented [22,23] concerning packed column SFC on PGC using CO_2-methanol mobile phase and methylated β-cyclodex-

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trin (MeCD) as the chiral selector. Kinetics of adsorption and desorption of MeCD were studied [23]. The retention times and enantioselectivities were found to be stable and reproducible (RSD=1.1 and 0.3%, respectively). Using the same chromatographic system, it was observed that enantiomeric separation is dependent on the composition of the commercial MeCD used as chiral selector [22,23]. In fact, MeCD are complex mixtures [24–29], which can be different from one supplier to another. Chiral discrimination in LC or capillary electrophoresis can vary largely as a function of the number of methyl groups and their positions on the cyclodextrin cavity [22,30].

In this work, the influence of some chromatographic parameters on the chiral separation of eight test solutes (Fig. 1) was studied using 80:20 or 95:5 (v/v) CO_2-polar modifier mobile phase with MeCD. A short study of chiral recognition mechanism is also presented.

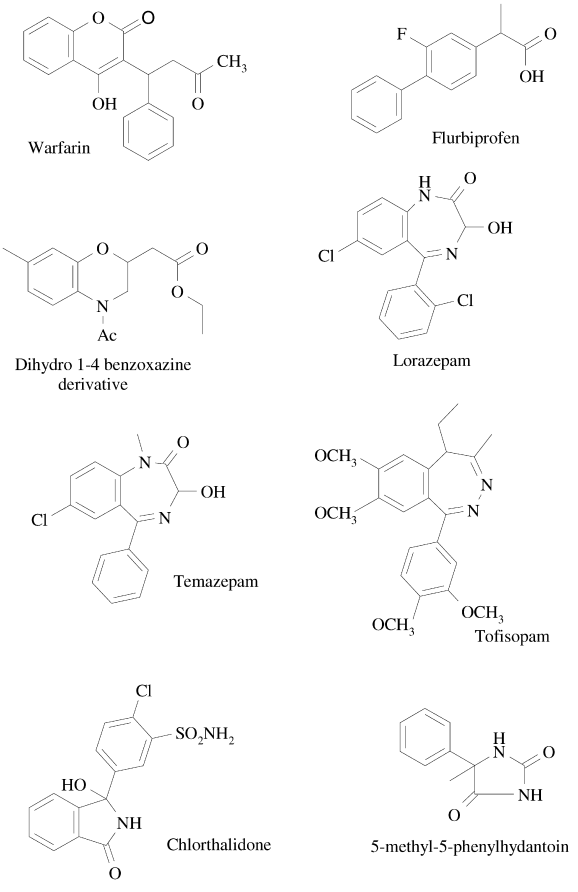

Fig. 1. Structure of solutes.

2. Experimental

2.1. Chromatography

SFC analyses were conducted with a model SF_3 Gilson (Villiers Le Bel, France) apparatus including CO_2 pump, modifier pump, mixing tee and pressure regulator. A Rheodyne (Berkeley, CA, USA) model 7125 injector with a 20-μl sample loop was used. The column was placed in a column oven set at 41°C. The column outlet pressure was 110 bars. The flow-rate was 3 ml min^{-1}. For solute detection, UV detector (Model 9565, Varian, Les Ulis, France) equipped with a high pressure cell was set at 220 nm. Adsorption and desorption kinetics were obtained using breakthrough method [31] with Evaporative Light Scattering Detector (ELSD) model Sedex 55 (Sedere, Alfortville, France) equipped with a 5 cm×50 μm I.D. silica restrictor as SFC interface. ELSD settings were: photomultiplier 5, evaporative temperature 40°C, nebulizer 40°C and 0.5 bar of nitrogen gas.

The column was Hypercarb 7 μm (100×4.6 mm I.D.) or 5 μm (100×2.1 mm I.D.) from Hypersil (Runcorn, UK). This stationary phase is a porous graphitic carbon support with 100% carbon, 250 Å pore size and 110 m^2 g^{-1} surface area [11,12].

No differentiation will be made between what is sometimes called subcritical fluid chromatography (SubFC or sSFC) and SFC because ''transitions'' between these ''defined'' states are often undetectable chromatographically and the instrumentation used is identical.

2.2. Chemicals

Carbon dioxide was industrial grade (purity 99.7%, Air Liquide, Paris, France) and other solvents were of analytical grade. The racemic benzoxazine derivative was synthesised by S. Mayer [32]. The test solutes were dissolved in methanol (100 mg l^{-1}). MeCD ''A'' mainly used was from Ikeda Corporation (Japan) and was characterised in a

previous work (DMβCD A in Ref. [27]). The degree of substitution (DS) was 2.1. As far as other MeCD used in Section 3.8 are concerned, MeCD ''B'' (DS=2.3) was from Ikeda Corporation, MeCD ''D'' (DS=1.7) from Wacker (Lyon, France) and MeCD ''E'' (DS=2.1) from Ringdex (Paris, France). Permethylated-β-CD was from Sigma (St Louis, USA). The MeCDs were dissolved in methanol, but all the concentrations are expressed as MeCD concentration in the mobile phase (CO_2+methanol). Since the solubility of MeCD is limited to 15 mmol l^{-1} in methanol and because MeCD is dissolved in methanol in the first instance, the corresponding maximum concentration of MeCD in 95:5 and 80:20 CO_2-methanol mobile phase will be 0.75 and 3 mmol l^{-1}, respectively. No problems due to possible MeCD precipitation in eluent were observed.

3. Results and discussion

In order to obtain acceptable retention times for all the eight test solutes using PGC column without any chiral selector in the mobile phase, a 80:20 or 95:5 CO_2-methanol mobile phase (41°C, 110 bars) was used. However, benzoxazine derivative and temazepam can be eluted using both conditions. This will allow a comparison of retention and chiral selectivity by using 80:20 or 95:5 CO_2-methanol eluent containing MeCD.

3.1. Adsorption of the MeCD onto the PGC

The use of MeCD as chiral mobile phase additive in SFC required determination of their behavior with respect to the PGC stationary phase. This can be determined by the shape of their adsorption isotherm.

The breakthrough method [31] was used to determine the MeCD quantity adsorbed onto the PGC stationary phase as a function of MeCD concentration in the mobile phase. The use of ELSD proved to be a simple and convenient detection system for these studies because MeCDs contain no chromophore and do not allow UV detection. When MeCD is present in the effluent, solid particles, which scattered the light, are formed in the detector. The signal is proportional to MeCD quantity leaving the column. In order to evaluate the influence of methanol percentage in the mobile phase, 80:20 and 95:5 CO_2: methanol mixtures were used (Fig. 2a). The maximum quantity adsorbed onto the column were from 4.5 10^{-5} to 5.8 10^{-5} mole. At these conditions, it will be assumed that a monolayer coverage of the surface is formed and that Langmuir isotherms are obtained. Such model was successfully used in LC [33]. Consequently, for a given MeCD concentration in the mobile phase, the rate of adsorption (V_a) and the rate of desorption (V_d) will be [34]:

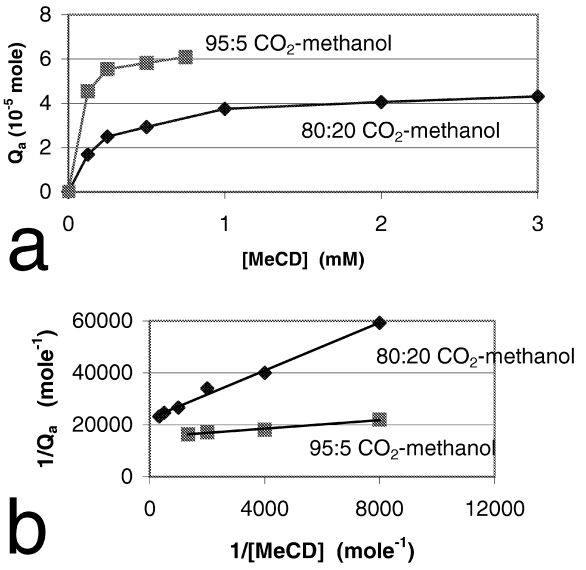

Fig. 2. (a) MeCD quantities adsorbed on PGC (Q_a) as a function of MeCD concentration in the mobile phase. Q_a values were determinated using breakthrough method. (b) Plot of $1/Q_a$ as a function of 1/[MeCD] according to Eq. (4). Conditions: Column Hypercarb (100×4.6 mm D.I.), flow-rate 3 ml min^{-1}, temperature 41°C, outlet pressure 110 bars.

$$V_a = k_a\,[\text{MeCD}]\,(Q_{max} - Q_a) \quad (1)$$

$$V_d = k_d Q_a \quad (2)$$

Where Q_{max} and Q_a are the capacity of monomolecular adsorption layer (maximum adsorbed quantity of MeCD (mole) and the quantity of MeCD adsorbed (mole), respectively. k_a and k_d are rate constants.

At the equilibrium:

$$k_d Q_a = k_a\,[\text{MeCD}]\,(Q_{max} - Q_a) \quad (3)$$

Table 1
Capacity of monomolecular adsorption layer (Q_{max}) and K_a values from Eq. (4) obtained using PGC 100×4.6 I.D. column

CO_2:methanol	Q_{max} (mol)	K_a
95:5	$6.32\ 10^{-5} \pm 0.14\ 10^{-5}$	18 400±1700
80:20	$4.42\ 10^{-5} \pm 0.20\ 10^{-5}$	4840±350

and by defining $K_a = k_a/k_d$, we obtained:

$$1/Q_a = (1/(K_a\,[\text{MeCD}]\,Q_{max})) + 1/Q_{max} \quad (4)$$

Using this equation, a linear relationship was obtained (Fig. 2b) and, consequently, the Langmuir isotherm model was valid. The values of K_a and Q_{max} were calculated for 80:20 and 95:5 CO_2:methanol eluents (Table 1). K_a is a measure of the strength of adsorption. The K_a and Q_{max} values were increased by a factor 3.8 and 1.4, respectively, using 95:5 eluent in comparison with the 80:20 eluent. Consequently, the surface coverage will be lower using the latter mobile phase. The capacity of the PGC stationary phase (Q_a) decreases with increasing methanol content in the eluent. This is due to competitive adsorption of methanol as described in RPLC [33].

The adsorbed MeCD quantity onto PGC was also measured for various polar modifiers (Fig. 3) using 80:20:2 (v:v:mmol) CO_2-polar modifier-MeCD. The adsorbed quantities were of the same order of magnitude (±30%). Concerning alcohols, the values decreased as a function of the carbon chain length and elution strength [35].

Because MeCDs exist both in the mobile phase and adsorbed to the stationary phase, the MeCD concentration may influence enantiomeric separations.

3.2. Influence of MeCD concentration on chiral selectivity

The retention and enantioselectivity of the solutes were studied using CO_2-methanol-MeCD mobile phases (Figs. 4 and 5). As expected, the retention decreased as a function of increased MeCD concentration for most of the solutes as exemplified in Fig. 4a. A low concentration of MeCD (0.125 m*M*) induces a strong reduction in retention. However, chlorthalidone and methyl-phenylhydantoin are exceptions because retention increased and (or) addition of MeCD in the mobile phase does not induce a large reduction in retention (Fig. 4b). Such exceptions have been noted in normal [36] and reversed-phase liquid chromatography (RPLC) [33,37] using methylated or native β-cyclodextrins as CMPA. Using PGC and MeCD, the retention mechanism has to be studied and this will be discussed later.

The enantioselectivities increase gradually with the MeCD concentration (Fig. 5). As high enantioselectivities and low retention factors are obtained, it is more attractive to work with high MeCD concentration (Fig. 6). Another reason for using

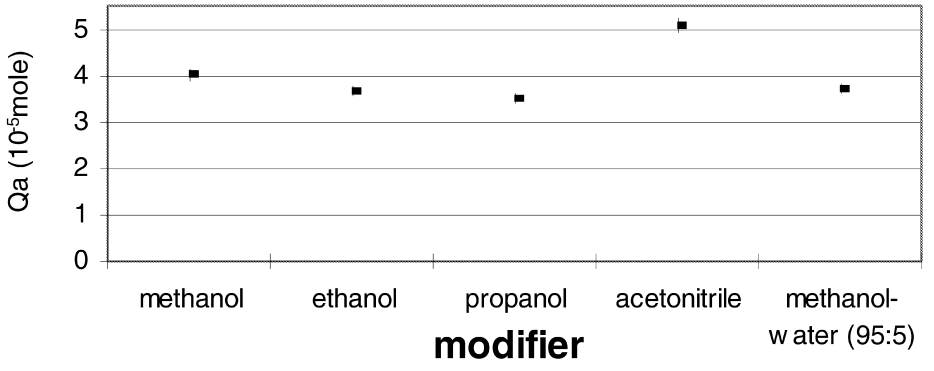

Fig. 3. Influence of the nature of the polar modifier on MeCD quantity adsorbed (Q_a) on PGC. Conditions: mobile phase CO_2-polar modifier-MeCD (80:20:2, v:v:m*M*), column Hypercarb (100×4.6 mm D.I.), flow-rate 3 ml min^{-1}, temperature 41°C, outlet pressure 110 bars.

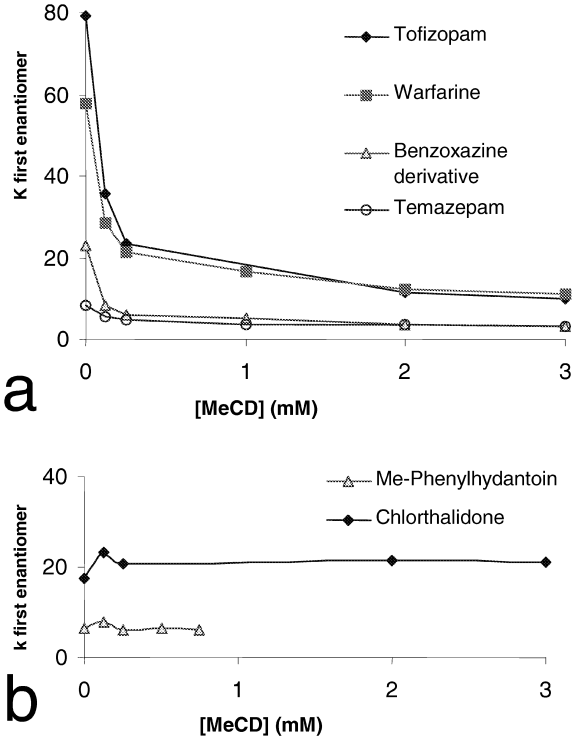

Fig. 4. Plots of retention factor of the first eluted enantiomer as a function of MeCD concentration. Mobile phase: 95:5 (methylphenylhydantoin) or 80:20 (other solutes) CO_2-methanol + MeCD. Other conditions as in Fig. 3.

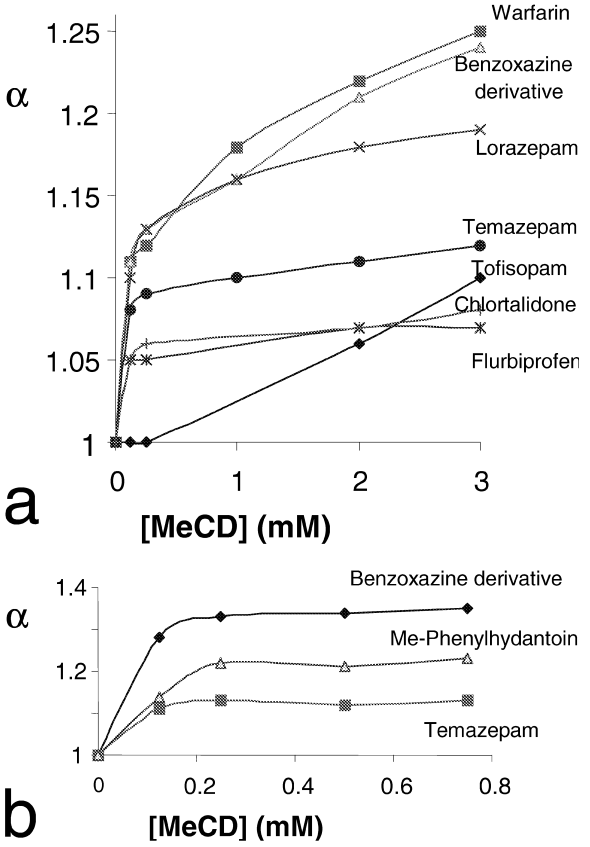

Fig. 5. Plots of enantioselectivity as a function of MeCD concentration in mobile phase. Mobile phase: 80:20 (a) or 95:5 (b) CO_2-methanol + MeCD. Other conditions as in Fig. 3.

higher concentrations of the CMPA is the increase in column efficiency (Fig. 6). For the following studies, 80:20:2 and 95:5:0.5 (v:v:m*M*) CO_2-polar modifier-MeCD mobile phases were used.

3.3. Influence of the methanol percentage in the mobile phase

Benzoxazine derivative and temazepam were eluted using 80:20 and 95:5 (v:v) CO_2-methanol with 0.125 or 0.25 m*M* MeCD (Fig. 4). As expected, retention times were higher using 95:5 than 80:20 (v:v) CO_2-methanol containing the same MeCD concentration. Besides, MeCD concentration has a much greater influence in 95:5 than in 80:20 eluent. For example, when MeCD concentration increases from 0 to 0.125 m*M*, retention factor of the benzoxazine derivative decreased from 103 to 10 using 95:5 CO_2-methanol and from 23 to 8 using 80:20 CO_2-methanol.

As far as enantioselectivities are concerned, the values are identical with 80:20 and 95:5 mobile phases for temazepam. On the contrary, benzoxazine derivative enantioselectivity is higher using low methanol content: for example, 1.33 and 1.13 values (Fig. 5) are obtained with 95:5:0.25 and 80:20:0.25 CO_2-methanol-MeCD, respectively. This can be explained by the competitive interaction between the solutes and methanol with the cyclodextrin cavity, on one hand, and by an increase of mobile phase polarity, on the other hand. In fact, high mobile phase polarity is more favourable for encapsulation of the solute in the hydrophobic cavity of the MeCD, as it was shown in liquid chromatography [38,39].

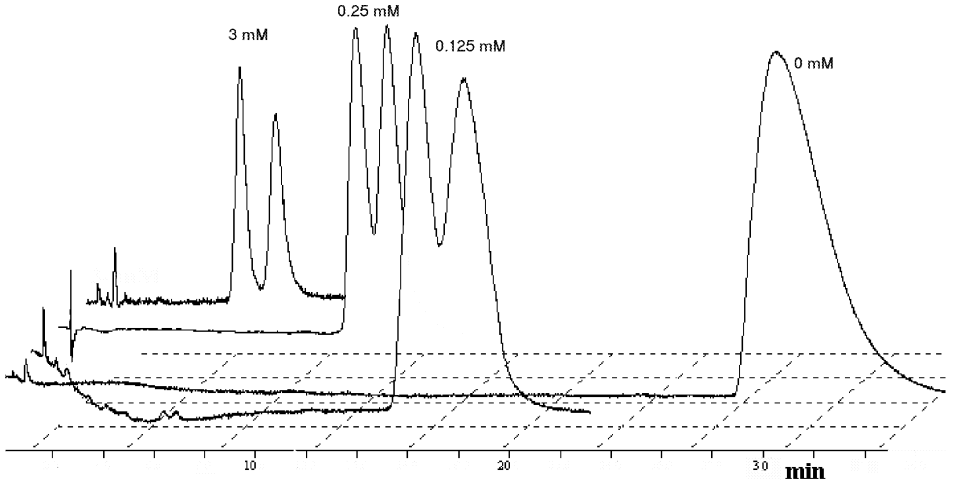

Fig. 6. Influence of MeCD concentration on enantiomeric separation of warfarin. Mobile phase: 80:20 CO_2-methanol+MeCD. Other conditions as in Fig. 3.

Taking into account these observations, solute-stationary phase-solvent–cyclodextrin interactions have to be studied in order to explain enantioselectivity variations from one solute to another as a function of the modifier rate.

3.4. Influence of the nature of the polar modifier

The nature of the organic solvent influences the cyclodextrin inclusion constants in RPLC [40] as well as in normal-phase liquid chromatography [41]. This is dependent to a certain extent on the solvent-cyclodextrin stability constant. Retention factors and enantioselectivities obtained using PGC and 80:20:2 or 95:5:0.5 CO_2-*X*-MeCD, where *X* was methanol, ethanol, *n*-propanol or acetonitrile, are reported in Table 2. In most cases, acetonitrile or methanol provides highest retentions. Clearly, retention decreased as a function of the chain length of the alcohols. For most solutes, higher enantioselectivities are obtained using acetonitrile or methanol as polar modifier (Table 2). Enantiomer separations can be obtained using the adequate polar modifier (e.g. acetonitrile) in less than 12 min (Fig. 7).

3.5. Influence of the outlet pressure

The influence of outlet pressure was studied from 95 to 250 bars using 80:20:2 or 95:5:0.5 CO_2-methanol-MeCD. When the pressure increases, only a slight decrease in retention was noted as expected in SFC with a polar modifier. As far as enantioselectivities are concerned, for most solutes, a low pressure was more favourable but this was dependent on the solute structure (Fig. 8). A decrease in complex formation constant has been noted [42] as a function of the pressure for 2-anilinonaphthalene-6-sulphonic acid. A similar effect can take place in chiral SFC for some solutes. A pressure of 110 bars seems to be a good choice for most solutes.

3.6. Influence of column temperature

Temperature has a great effect on the enantiomeric separations [43–49]. Usually, the natural logarithms of the retention factors or enantioselectivities against the reciprocal of absolute temperature (Van't Hoff plots) are plotted and are linear. The influence of column temperature was studied from −5 to 82°C. The most important fact is that a linear plot is almost obtained for four solutes (Fig. 9) since others gave non-linear plots (results not shown). Such unusual behaviours were also observed in SFC [47] or RPLC [48]. Besides, the presence of multiple types of retention mechanisms leads to non-linearity of the

Table 2
Influence of polar modifier on retention factor (k_1, first eluted enantiomer) and enantioselectivity. Column: Hypercarb (100×4.6 mm I.D.), flow-rate 3 ml min^{-1}, temperature 41°C, outlet pressure 110 bars

Mobile phase	Solute		Polar modifier			
			Methanol	Ethanol	*n*-propanol	Acetonitrile
80:20:2	Tofizopam	k_1	11.6	9.0	7.1	9.9
(v:v:m*M*)		α	1.06	1.04	1.00	1.40
CO_2-polar	Warfarin	k_1	12.3	8.9	7.7	13.8
modifier-MeCD		α	1.22	1.30	1.23	1.42
	Benzoxazine	k_1	3.6	2.2	1.5	2.6
	derivative	α	1.21	1.20	1.20	1.24
	Lorazepam	k_1	11.1	9.6	6.6	17.9
		α	1.18	1.22	1.19	1.38
	Flurbiprofen	k_1	7.9	4.6	2.2	18.6
		α	1.07	1.03	1.00	1.06
	Temazepam	k_1	3.5	2.9	–*	3.4
		α	1.11	1.12	–*	1.00
	Chlorthalidone	k_1	21.4	20.0	14.4	>66.0
		α	1.07	1.10	1.08	–*
95:5:0.5	Benzoxazine	k_1	6.9	5.6	4.6	9.0
(v:v:m*M*)	derivative	α	1.34	1.38	1.20	1.43
CO_2-polar	Temazepam	k_1	15.1	16.45	13.9	12.7
modifier-MeCD		α	1.12	1.11	1.00	1.07
	Me-phenyl	k_1	6.3	7.0	1.2	39.9
	hydantoin	α	1.21	1.26	1.00	1.34

*, Not determined.

Van't Hoff plots [48]. This will be discussed in the next part.

Clearly, in the conditions used, the chiral selector is present both in mobile phase and stationary phase. The enantiomer undergoing a stronger interaction with the MeCD adsorbed on the PGC is retarded in the stationary phase but the same enantiomer undergoing a stronger interaction with the MeCD in the mobile phase is accelerated in the mobile phase leading to overall compensation of enantioselectivity [43,49]. Then, enantiomeric complexation is tem-

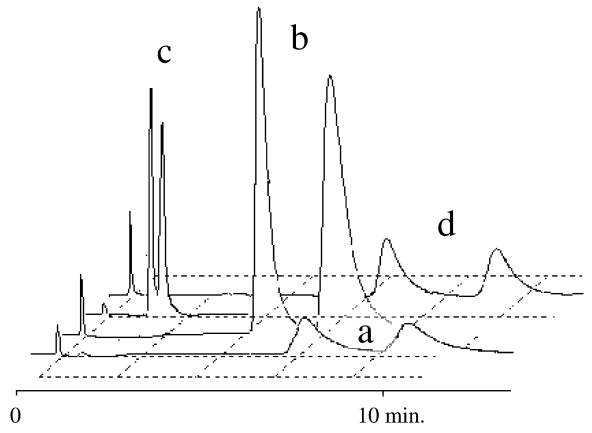

Fig. 7. Enantiomeric separations of warfarin (a), tofizopam (b) benzoxazine derivative (c) and lorazepam (d) using acetonitrile as polar modifier. Mobile phase: 80:20:2 (v/v/m*M*) CO_2-acetonitrile-MeCD. Other conditions as in Fig. 3.

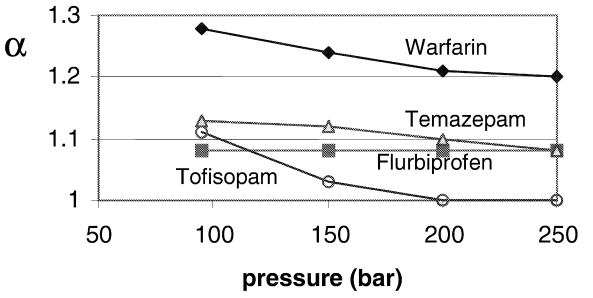

Fig. 8. Influence of outlet pressure on enantioselectivity for some solutes. Mobile phase: 80:20:2 (v/v/m*M*) CO_2-methanol-MeCD. Other conditions as in Fig. 3.

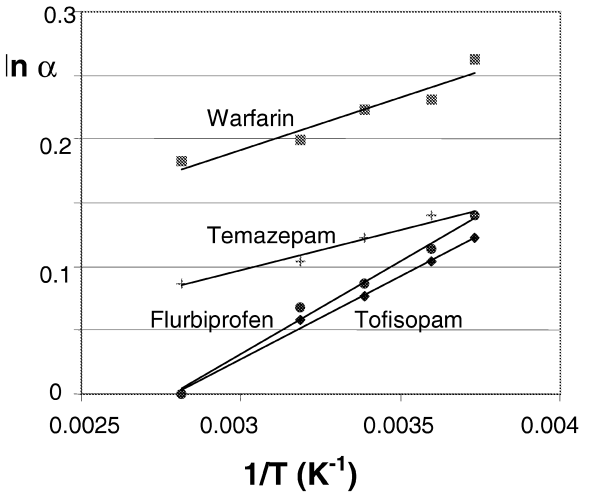

Fig. 9. Plot of ln α as a function of $1/T$ (Van't Hoff plot). Mobile phase: 80:20:2 (v/v/mM) CO_2-methanol-MeCD. Other conditions as in Fig. 3.

perature dependent and compensation of enantioselectivity can arises when enthalpic and entropic contributions to chiral recognition are equal [43–47]. From Van't Hoff plots in Fig. 9, enthalpy controlled enantioselectivities are obtained. Isoenantioselective temperature for flurbiprofen and tofisopam was probably 82°C. This phenomena was not observed for warfarin and temazepam because their isoenantioselective temperatures were well above the working temperature of the chromatographic system.

From a practical point of view, temperature affects column efficiency of the chromatographic system (Fig. 10). Resolution was highest at 41°C.

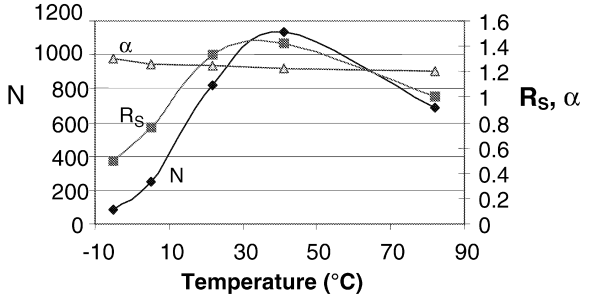

Fig. 10. Influence of column temperature on enantioselectivity, number of theorical plate (N) and resolution (R_S) obtained for warfarin. Mobile phase: 80:20:2 (v/v/mM) CO_2-methanol-MeCD. Other conditions as in Fig. 3.

3.7. Preliminary studies of chiral recognition mechanism

Depending on the localisation of the chiral complex, enantiomeric recognition can occur in the mobile phase (this concerns the majority of applications), onto the stationary phase or in both phases simultaneously [33]. In the present work, the chiral selector is present in the mobile phase but also adsorbed onto the stationary phase (Table 1). Consequently, one can assume that chiral recognition may occur in the mobile and/or the stationary phases.

To study chiral recognition, one can presume, at least in a first attempt, that the solutes can be adsorbed onto the stationary phase and be complexed by MeCD in the mobile phase (this assumption will be verified if the theoretical model is valid). By introducing equations for the distribution and complexation constants, apparent retention factor (k) was obtained using the following equation assuming that only one species (neutral) of molecule G is present in the mobile phase and that 1:1 complexes are produced [33,37,40,50–54]:

$$k = (k_G + k_{GCD}K_{CD}\,[\text{MeCD}])/(1 + K_{CD}\,[\text{MeCD}]) \quad (5)$$

or by simple rearrangement:

$$k = ((k_G - k')/K_{CD}\,[\text{MeCD}]) + k_{GCD} \quad (6)$$

where k_G is retention factor of solute G without MeCD in mobile phase, k_{GCD} is retention factor of the G-MeCD complex, K_{CD} is the formation constant for the inclusion complex and [MeCD] is the MeCD concentration in the mobile phase.

Considering that adsorption of complex is negligible ($k_{GCD} \approx 0$), it was obtained:

$$k = k_G/(1 + K_{CD}\,[\text{MeCD}]) \quad (7)$$

or

$$1/k = (1/k_G) + (K_{CD}\,[\text{MeCD}]/k_G) \quad (8)$$

The linearity of $1/k$ as a function of [MeCD] (or $[\text{MeCD}]^n$ for complex stoichiometry n) was often experimentally found in LC work [33,40,52,55–59]. From experimental k values, a plot of $1/k$ versus [MeCD] was not linear in the range studied (Fig. 11). However, for most solutes, the model was quite

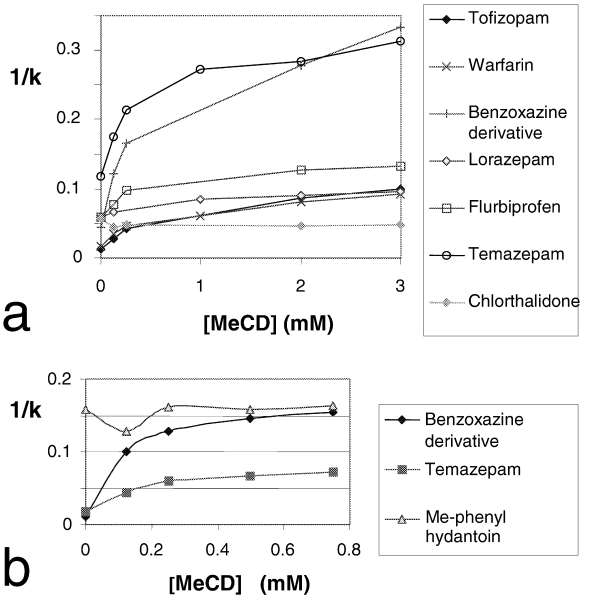

Fig. 11. Plots of $1/k$ as a function of MeCD concentration according to Eq. (8). k was the retention factor of the first eluted enantiomer. Mobile phase: 80:20:2 (a) and 95:5:0.5 (b) (v/v/mM) CO_2-methanol-MeCD. Other conditions as in Fig. 3.

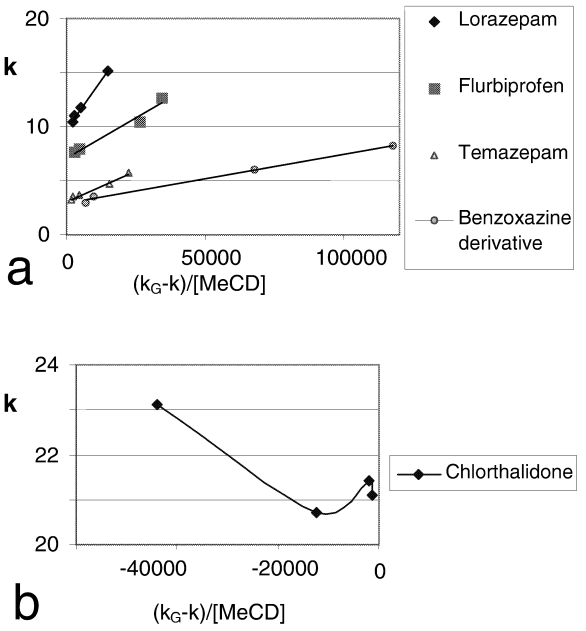

Fig. 12. Plots of $(k_G - k)/[\text{MeCD}]$ as a function of k according to Eq. (6). k was the retention factor of the first eluted enantiomer. Mobile phase: 80:20:2 (v/v/mM) CO_2-methanol-MeCD. Other conditions as in Fig. 3.

linear in the range of MeCD concentration from 0.25 to 3 mM and from 0.25 to 0.75 mM using 80:20 and 95:5 CO_2-methanol eluent, respectively. One can conclude that, for these solutes, a change in retention mechanism occurred at a 0.25-mM MeCD concentration.

In fact, for such low chiral selector concentration, adsorption of the complex G-MeCD became significant and Eq. (6) has to be used. In that case, a plot of k versus $(k_G - k)/[\text{MeCD}]$ would give a straight line with a slope $1/K_{CD}$ and intercept k_{GCD} [37,40,53]. For tofizopam, warfarin, benzoxazine derivative, lorazepam, flurbiprofen and temazepam, the linearity was satisfactory as exemplified in Fig. 12a. Consequently, the chiral recognition mainly occurs in the mobile phase, although the adsorption of the complex G-MeCD is not negligible. The formation constant for the inclusion complex, K_{CD}, and the retention factor of the complex G-MeCD, k_{GCD}, were estimated using Eq. (6) and values are reported in Table 3. Chiral separation is due to differences in the formation constants of the two enantiomers (K_{CD} of the first eluted enantiomer $> K_{CD}$ of the second enantiomer) and differences in the adsorption of the complex (k_{GCD} of the first eluted enantiomer $< k_{GCD}$ of the second). These factors influence retention of enantiomers in a subtractive (tofizepam) or additive manner (other solutes in Table 3).

Although the chiral recognition mainly occurs in the mobile phase for the six above-mentioned solutes, a minor chiral recognition onto stationary phase may be considered especially at low MeCD concentration. In fact, a slow removal of chiral selector by achiral eluent (CO_2 with methanol only) with a slow decrease in enantioselectivity was observed in the preliminary results [23]. It was stated [41] that such phenomena indicate that chiral recognition occurs, at least partially, on the adsorbed chiral layer. However, for tofizopam, warfarin, benzoxazine derivative, lorazepam, flurbiprofen and temazepam, complexation occurs mainly in the mobile phase.

As far as chlorthalidone and methyl-phenylhydantoin are concerned, a linear plot was not obtained as exemplified in Fig. 12b. This suggests that, for both solutes, chiral recognitions do not occur mainly in the mobile phase and (or) complex stoichiometry is not 1:1. Such results were also obtained in some LC

Table 3
Retention factor of solute without MeCD in mobile phase k_G and calculated values of retention factor of the complex *G*-MeCD (k_{GCD}) and formation constant K_{CD} (m*M*) of the inclusion complexes using Eq. (6) and correlation coefficient (*r*). For each solute, the first and second lines correspond to the first and second eluted enantiomers respectively. Column Hypercarb (100×4.6 mm I.D.), flow-rate 3 ml min^{-1}, temperature 41°C, outlet pressure 110 bars

Mobile phase	Solute	k_G	k_{GCD}	K_{CD}	*r*
80:20:2	Tofizopam	79.4	8.2	13.24	0.992
(v:v:m*M*)		79.4	9.2	13.80	0.990
CO_2-methanol-	Warfarin	57.8	10.1	12.79	0.999
MeCD		57.8	12.4	10.92	0.995
	Benzoxazine	22.9	2.9	17.6	0.998
	derivative	22.9	3.6	15.75	0.998
	Lorazepam	17.0	9.8	2.77	0.990
		17.0	11.8	1.86	0.948
	Flurbiprofen	17.0	7.1	6.45	0.962
		17.0	7.6	5.61	0.930
	Temazepam	8.5	3.1	8.81	0.993
		8.5	3.4	7.24	0.988
95:5:0.5	Benzoxazine	102.9	6.4	217.96	0.980
(v:v:m*M*)	derivative	102.9	8.7	182.64	0.980
CO_2-methanol-	Temazepam	58.6	13.7	35.57	0.950
MeCD		58.6	15.4	30.40	0.973

work [37,53]. It was suggested in RPLC [37] that, if retention of solutes is greater at low concentration of MeCD than those determinated without MeCD in mobile phase, resolution of enantiomers must be governed mainly by the inclusion of the solute in the MeCD adsorbed onto the stationary phase. When MeCD concentration increases, the influence of complexation in the mobile phase increased and consequently the retention decreased. In normal-phase liquid chromatography using silica and per-methylated-cyclodextrins (PMCD) [36], it was shown that retention increased as a function of PMCD quantity and consequently chiral separation is caused by chiral recognition on the dynamically generated stationary phase. More studies are required for better understanding of the retention mechanisms of chlorthalidone and methyl-phenylhydantoin complexes on PGC.

3.8. Influence of the MeCD composition

Inclusion of a solute might be dependent on the number of methyl groups and their position on the ring of the cyclodextrin. In fact, commercial di-methylated cyclodextrins are complex mixtures and number and (or) position of methyl groups can vary to a large extent [26–29]. In previous works [22,23], it was shown that chiral separation of tofisopam and benzoxazine derivative is dependent on the commercial MeCD mixture used. Enantioselectivities obtained with the MeCD previously used in this work (''A'') and other MeCD mixtures (''B'', ''D'', and ''E'') are reported in Table 4. Permethylated β-cyclodextrin (''C'') was also used for comparison but no enantioselectivity was obtained for the test solutes. Clearly, the composition of MeCD mixture is of crucial importance for a successful enantiomeric separation and this is dependent on the solute structure. For example, MeCD ''D'' is well adapted for tofisopam since MeCD ''A'' is not suitable (Table 4). On the contrary, MeCD ''A'' is well adapted for benzoxazine derivative since MeCD ''D'' is not suitable. Similar results were obtained for amphetamine derivatives in chiral capillary electrophoresis [22] and for amino acid derivatives in liquid chromatography using selectively methylated β-cyclodextrin-bonded phases [30]. From results in Table 4, to chose the MeCD for a specific separation is not easy. Are differences in enantioselectivities, from one MeCD mixture to another, due to variation

Table 4
Influence of the nature of MeCD mixture on enantioselectivity. Column Hypercarb (100×2.1 mm I.D.), flow-rate 3 ml min^{-1}, temperature 41°C, outlet pressure 110 bars

Mobile phase	Solute	MeCD				
		(A)	(B)	PMCD[a]	(D)	(E)
80:20:2	Tofizopam	1.05	1.00	1.00	**1.14**	1.00
(v:v:m*M*)	Warfarin	**1.23**	1.16	1.00	1.15	1.06
CO_2-methanol-MeCD	Benzoxazine derivative	**1.19**	1.17	1.00	1.00	1.05
	Lorazepam	1.15	**1.18**	1.00	1.00	1.07
	Flurbiprofen	1.08	1.07	1.00	**1.27**	1.00
	Temazepam	**1.08**	1.05	1.00	**1.08**	1.00
	Chlorthalidone	**1.10**	1.07	1.00	1.00	1.00
95:5:0.5	Benzoxazine derivative	**1.38**	1.00	1.00	1.22	1.14
(v:v:m*M*)	Temazepam	1.07	1.10	1.00	**1.15**	1.07
CO_2-methanol-MeCD	Me-phenylhydantoin	**1.23**	1.07	1.00	1.00	1.00

[a] PMCD: permethylated β-cyclodextrin.

in apparent formation constant (K_{CD}) and (or) the apparent retention factor of the complex *G*-MeCD (k_{GCD})? More studies have to be made using various pure methylated cyclodextrins.

4. Conclusions

The addition of MeCD in SFC mobile phase allows efficient chiral separations using PGC as stationary phase. Good enantioselectivities were obtained with 80:20:2 or 95:5:0.5 (v/v/m*M*) CO_2-polar modifier-MeCD, with moderate column temperature (41°C) and outlet pressure (110 bars). Acetonitrile or methanol can be used as polar modifier; the choice is dependent on the solute. One important fact is that enantioselectivities are dependent on the commercial MeCD mixture used. As far as retention mechanism is concerned, chiral recognition occurs mainly in the mobile phase except for chlorthalidone and methyl-phenylhydantoin.

A major advantage of the use of MeCD in the mobile phase is that, using the same achiral column, a screening of various chiral selectors can be made. Perhaps, this versatile system will be a good tool for rational studies in order to progress for a better comprehension of the retention and chiral recognition by methylated cyclodextrins. Besides, joint use of various cyclodextrin additives, as in LC [33,60–62], would allow new perspectives for analysis of a wide range of solutes using a single achiral column.

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