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Chiral separation of basic drugs by capillary electrophoresis with carboxymethylcyclodextrins

Janusz Zukowski[a], Vern De Biasi[b], Alain Berthod[c,*]

[a]*GlaxoSmithKline, Analytical Chemistry, New Frontiers Science Park (North), Harlow, Essex CM19 5AD, UK*
[b]*GlaxoSmithKline, Analytical Chemistry and Chemical Technology, Via Fleming 2, 37135 Verona, Italy*
[c]*Laboratoire des Sciences Analytiques, CNRS, Université de Lyon 1 Bat. CPE-308, 69622 Villeurbanne Cedex, France*

Abstract

Capillary electrophoresis (CE) with carboxymethylated β- or γ-cyclodextrins was used to achieve the rapid enantiomeric separation of a set of basic drugs. The enantiomers of 12 chiral amino-containing pharmaceutical compounds belonging to various therapeutic categories were analyzed by CE using an uncoated 60 cm×75 μm I.D. silica capillary. Several experimental parameters such as the nature, concentration and pH of the buffer, nature and concentration of the anionic cyclodextrin and temperature were studied in order to optimize the enantiomeric separation. The variation of the solute partition coefficient for the chiral selector, the enantioselectivity and resolution factors are used to assess the quality of the chiral separation. It is shown that the solute affinity for the chiral selector is not related to its enantioresolution factor. None of the two cyclodextrin selectors used was able to separate the whole set of basic drugs.

Keywords: Enantiomer separation; Pharmaceutical analysis; Basic drugs

1. Introduction

Chirality has become a key parameter in the development of pharmaceuticals, biological molecules and agrochemicals [1]. The very efficient separating power of capillary electrophoresis (CE) was soon used to perform enantioseparations. Furthermore, CE uses low amounts of chiral selectors. These qualities explain the exploding interest encountered in the use of CE for chiral separations. An electronic literature search (ChemFinder®) with the words: ''capillary electrophoresis enantioseparation'' returned 294 references in 1 year of time between mid-1999 and mid-2000. Numerous review articles and books appeared on the field [2–5]. Cyclodextrins (CDs) became well established as powerful tools for the chiral discrimination of enantiomers by means of CE [6–8].

α-, β-, or γ-CDs are used as neutral chiral additives or as pseudo stationary phase in CE. Randomly substituted anionic or cationic CDs are chiral selectors that move faster or slower than the electroosmotic flow (EOF) [8]. Charged CDs are gaining much interest: they are able to perform fast chiral separations at low concentrations and they can resolve neutral racemates [9]. Anionic CDs are the most popular charged chiral selectors. They are mainly carboxylate or sulfate CD derivatives [2,4,5,8,9]. Carboxymethyl CDs were the first anionic chiral selectors introduced in CE by Terabe

*Corresponding author. Address until June 2002: c/o Professor D.W. Armstrong, Iowa State University, Department of Chemistry, Gilman Hall, Ames, IA 50011-3111, USA. Fax: +33-472-431-078.

E-mail address: berthod@univ-lyon1.fr (A. Berthod).

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et al. [10]. Carboxymethyl β- (CM-β-CD) or γ-CDs (CM-γ-CD) are neutral selectors at pH values lower than 4 and anionic at pH values higher than 5.5.

Numerous parameters effect chiral separations by CE. The experimental enantioselectivity factor can vary considerably upon small experimental changes. Several parametric studies appeared [11,12]. They show the complexity of a rapid optimization of the enantioseparation of a particular racemic pair.

Basic solutes have a tendency to interact with surface silanols. This produces broad and tailing peaks in both HPLC and CE analyses. This problem was partially addressed by adding triethylamine to the mobile phase or the running buffer [13]. Studies with cationic β-CD [3,14], molecular and sulfated anionic β-CD [15] showed ways to improve the separation and enantioresolution of chiral basic compounds. This problem is addressed in this work using carboxylated pH-sensitive CD chiral selectors. The separation of a set of 12 pharmaceutical amine-containing racemates by CE using CM-β- and CM-γ-CDs was studied varying the experimental conditions. The size of the chiral selectors and their concentrations, the nature of the buffering electrolytes and their concentrations, the pH and the temperature were the studied parameters.

2. Experimental

2.1. Apparatus

A P/ACE 5510 capillary electrophoresis apparatus (Beckman, Palo Alto, CA, USA) equipped with a capillary UV detector working at 200 nm was used for all measurements. Sample injections were done by pressurization (N_2, 2 s). An uncoated fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA), 60 cm (50 cm inlet to UV window)×75 μm I.D., was conditioned by pressurizing for 10 min with 1 *M* NaOH prior to the first use. Before each run, it was washed for 2 min with 0.1 *M* NaOH, then for 2 min with the appropriate buffer solution. The capillary inlet was connected with the positive (anode) electrode (outlet=negative cathode). The applied voltage was 20 kV for all experiments. The data were processed with the Millennium 32 software (Waters, Milford, MA, USA).

2.2. Reagents

Sodium hydrogenphosphate and dihydrogenphosphate, phosphoric acid, sodium borate, boric acid and sodium hydroxide were obtained from Lancaster (Morecambe, UK). Water was obtained from a Milli-Q system (Millipore Bedford, MA, USA). The buffer solutions (pH 8, 7 or 6) were prepared by mixing appropriate amounts of sodium hydrogenphosphate, sodium dihydrogenphosphate and phosphoric acid (or sodium borate and boric acid). The substituted CD was weighed directly into the background electrolyte.

CM-β-CD and CM-γ-CD were obtained from Cyclolab (Budapest, Hungary). They were pure β (seven glucopyranoside units) or γ (eight units) CDs with an average degree of substitution of three carboxymethyl groups per CD ring randomly attached. The average molecular masses were taken as 1309 for the CM-β-CD and 1471 for the CM-γ-CD. Table 1 lists the pharmaceutical compounds analyzed. The 12 basic drugs were obtained from Sigma–Aldrich–Fluka (l'Isles d'Abeau Chesnes, France) and used as received. Approximately 1 g/l mother solutions were prepared in methanol–water (50:50) and diluted to ~50 μg/ml (20 times dilution) with the appropriate buffer solution prior to injection.

3. Results and discussion

3.1. Solute selection

The capabilities of cyclodextrins used as chiral selectors in separation are well established. In liquid and gas chromatography they have been demonstrated to separate enantiomers containing aromatic structure [16–18]. Carboxymethyl-derivatized CDs are anionic compounds above pH 5. They have a significant electrophoretic mobility in opposition to the electroosmotic flow. They have a separation window much larger than neutral CDs. Cationic solutes will have greater chances of equilibrium interactions with anionic CDs [19]. Consequently, the selected solutes were compounds of pharmaceutical interest containing aromatic structures and amine groups. Table 1 shows the chemical structure

Table 1
The pharmaceutical racemates used as test solutes

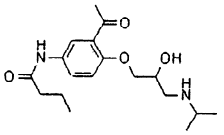
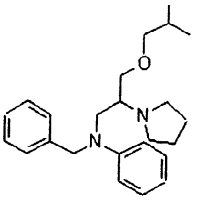
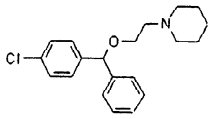
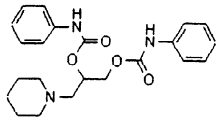
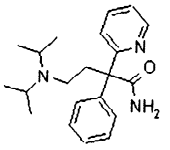
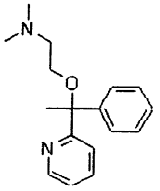
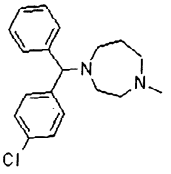
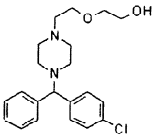
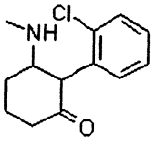
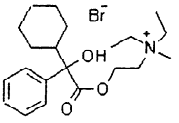
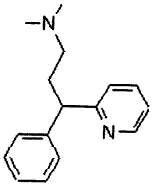
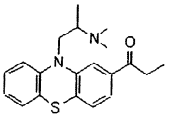
Solutes	Formula	M_r	pK_a	Therapeutic category
Acebutolol	 $C_{18}H_{28}N_2O_4$	336.4	(9)	β-Adrenergic blocker
Bepridil	$C_{24}H_{34}N_2O$ 	366.5	(10)	Calcium channel blocker, antianginal
Cloperastine	 $C_{20}H_{24}ClNO$	330	(10)	Antitussive
Diperodon	 $C_{22}H_{27}N_3O_4$	397.5	(10)	Local anesthetic
Disopyramide	 $C_{21}H_{29}N_3O$	339.5	10.2	Antiarrhytmic Class IA
Doxylamine	 $C_{17}H_{22}N_2O$	270.4	(10)	Antihistaminic

Table 1. Continued

Solutes	Formula	M_r	pK_a	Therapeutic category
Homochlorcyclizine	 $C_{19}H_{23}ClN_2$	315	(10)	Serotonin antagonist
Hydroxyzine	 $C_{21}H_{27}ClN_2O_2$	375	(10)	Anxiolitic
Ketamine	 $C_{13}H_{16}ClNO$	237.7	7.5	Anesthetic
Oxyphenonium bromide	 $C_{21}H_{34}BrNO_3$	428.4	Always cationic	Anticholinergic
Pheniramine	 $C_{16}H_{20}N_2$	240.4	(10)	Antihistaminic
Propiomazine	 $C_{20}H_{24}N_2OS$	340.5	(10)	Sedative, hypnotic

of the 12 pharmaceutical compounds with their therapeutic category. Oxyphenonium bromide contains a quaternary ammonium group so it is a cationic solute at any pH value. Nine compounds contain tertiary amine groups and two compounds, i.e. acebutolol and ketamine, contain secondary amine groups. It is known that tertiary amines are stronger bases than secondary amines. However, solvation effects can significantly change the dissociation constant values. Table 1 gives the pK_a values of the basic drugs. Most of the values are between parentheses indicating that they are estimated values [20]. In any case, the whole set of solutes but ketamine (pK_a=7.5), is in a cationic form within the 6–8 pH range of the study. Oppositely, the carboxylic groups of the CD selectors have a pK_a value between 4 and 5. They are always negatively charged in the 6–8 working pH range. The primary interaction force between the solutes and the CM-CD selectors will be the electrostatic attraction.

3.2. Chiral separation performances

Tables 2 and 3 list the results obtained with the CM-β-CD and CM-γ-CD chiral selectors, respectively. The tables list the retention times of the isomers, α, the enantioselectivity factor, R_s, the resolution factor and K, the solute partition coefficient. The enantioselectivity factor is the ratio of the retention factors, k'_2/k'_1. The resolution factor, R_s, is measured directly on the electropherogram as the ratio of the distance between the two peak maxima divided by the average peak width at base. Assuming that the anionic CD selectors behave similarly to anionic micelles, K, the pseudo partition coefficient, is calculated as [10]:

$$K = \frac{V_m}{V_s} \cdot \frac{(t_r - t_{eof})}{\left(1 - \frac{t_r}{t_{CD}}\right) \cdot t_{eof}} \quad (1)$$

with t_r, t_{eof} and t_{CD} the enantiomer retention time, the dead time (electroosmotic flow) and the retention time of the negatively charged chiral CD selector, respectively. The V_m/V_s ratio of the aqueous phase volume over the CD phase volume was taken as 39 (97.5/2.5) assuming the 2.5% (w/v) CD concentration occupied 2.5% of the mobile phase volume (i.e. the CD solvated density is assumed to be 1 g/ml). Even if this assumed value was not correct, all the listed K values would be erroneous by a constant term and as such it would still be valid to compare them.

The 12 racemates were tested at three different pH values obtained with two different buffer compositions, and two chiral selectors gave rise to the 144 experiments listed in Tables 2 and 3. Ninety-one of these experiments were able to separate to some extent the enantiomers of the 12 racemates studied. This number corresponds to 63% of successful hits. Fig. 1 shows the results of Tables 2 and 3 arranged in term of resolution factor. Three classes were defined: $R_s=0$ is the unsuccessful class (37% of the 144 experiments), $0.1<R_s<1.4$ is the class of partially resolved enantiomers (43%) and $R_s>1.5$ is the class of the baseline resolved enantiomers (20%). The bars of Fig. 1 correspond to the size of the CD cavity and the nature of the buffer electrolyte.

3.3. β- or γ-CD selector?

The difference between CM-β-CD and CM-γ-CD is the size of their hydrophobic cavity. The β-CD and γ-CD are made of seven or eight glucopyranoside units, respectively. The diameter of the internal cavity is 0.78 and 0.95 nm, respectively [21]. Fig. 1 does not show a clear difference in the capability of one CD selector or the other. A small advantage in successful enantioseparations goes to the CM-γ-CD (48 on 72 experiments or 67%). It is not statistically different from the CM-β-CD successful separations (43 over 72 or 60%). However, Tables 2 and 3 show that the chemical structure of the racemate has a key role in the enantiorecognition by CD selectors. Disopyramide, doxylamine and ketamine are much better separated or separated only by CM-β-CD. Oppositely, bepridil, cloperastine, diperodon and hydroxyzine are much better separated or separated only by CM-γ-CD. Doxylamine and disopyramide have both an ortho-pyridine and a benzene ring directly attached to the stereogenic center. Pheniramine has the same structural characteristic and is also well optically separated by the CM-β-CD selector. Similarly for the CM-γ-CD, cloperastine and hydroxyzine have both a benzene ring and a

Table 2
Separation of the pharmaceutical compounds with CM-β-CD chiral selector (2.5%, w/v) at 25°C

Solute	pH	Borate buffer					Phosphate buffer				
		t_1	t_2	α	R_s	K	t_1	t_2	α	R_s	K
Acebutolol	8	7.35				5	5.71				5
	7	6.70				5	6.19				5
	6	9.05				30	8.80				5
Bepridil	8	12.67				260	8.80				250
	7	11.10				260	10.19				220
	6	12.25	11.14	1.009	0.5	280	19.75	19.93	1.016	0.4	300
Cloperastine	8	13.73				1060	9.51				1200
	7	11.94				930	11.13				900
	6	13.05				850	23.74	23.93	1.012	0.4	2000
Diperodon	8	11.35				100	8.09				100
	7	9.82	9.85	1.011	0.4	90	9.22				100
	6	10.39	10.45	1.019	0.7	80	12.91	13.01	1.020	0.5	50
Disopyramide	8	*7.04*	*7.16*	*1.017*	1.2	−3	*5.39*	*5.55*	*1.030*	1.3	−3
	7	*6.54*	*6.63*	*1.014*	1.2	−3	*5.81*	*5.96*	*1.026*	1.3	−5
	6	*6.91*	*7.03*	*1.017*	1.2	−3	8.81	9.2	1.045	1.5	5
Doxylamine	8	11.44	11.80	1.086	1.6	110	8.12	8.32	1.078	1.5	110
	7	10.11	10.38	1.079	1.5	110	9.18	9.45	1.093	1.5	100
	6	10.99	11.31	1.084	1.5	110	18.16	19.07	1.090	1.9	180
Homochloro-cyclizine	8	13.84	14.00	1.024	1.4	1500	9.53	9.62	1.021	1.2	1300
	7	12.01	12.13	1.023	1.4	1200	11.20	11.32	1.024	1.4	1100
	6	13.21	13.35	1.023	1.4	1200	23.35	23.76	1.026	1.5	1800
Hydroxyzine	8	13.89				1700	9.54				1400
	7	11.85				800	11.09				800
	6	12.79				550	22.28				700
Ketamine	8	10.98				80	7.82				80
	7	8.66	8.78	1.064	1.1	40	7.92	7.96	1.022	0.3	40
	6	8.89	9.05	1.098	1.4	30	12.50	12.89	1.088	1.3	40
Oxyphenozium	8	12.40	12.81	1.081	1.2	200	9.02				340
	7	10.80	11.22	1.102	1.2	200	10.47				300
	6	12.05	12.60	1.14	1.2	230	22.40	23.5	1.076	1.4	700
Pheniramine	8	12.18	12.61	1.089	1.6	180	8.70	8.81	1.035	1.3	200
	7	10.66	11.06	1.101	1.6	170	9.30	10.12	1.110	1.5	200
	6	11.60	11.99	1.088	1.6	160	17.95	19.03	1.192	1.7	160
Propiomazine	8	13.43	13.55	1.020	1.3	600	9.28				600
	7	11.61	11.70	1.019	1.3	500	10.85	10.95	1.021	0.8	500
	6	12.64	12.75	1.020	1.4	430	18.36	18.66	0.8	1.4	210

t_1 and t_2 are the elution times of the first and second enantiomer, α is the enantioselectivity factor, R_s is the resolution factor, K is the first enantiomer partition coefficient for the CM-β-CD. No data means $t_2 = t_1$, $\alpha = 0$, $R_s = 0$. Italicized values = the solute eluted before t_{eof} (cationic character), α was taken as t_2/t_1. Experimental conditions: 50 cm × 75 μm I.D. capillary; pressurized 2 s injection of 50 μg/ml solution, 20 kV; buffer concentration, 0.1 M; CM-β-CD concentration, 2.5% or 0.019 M; 25°C.

para-chloro phenyl group directly attached to the chiral center. Homochlorocyclizine, which has the same structural arrangement, is much better optically resolved by the CM-γ-CD selector than by the CM-β-CD. It is not possible to proclaim a particular CD better than the other. From our experiments we can observe that the molecular structure of a given enantiomeric pair can be well recognized by a CD and not by the other. Conversely it can be recognized by both CDs or by none. In our set of racemates and with our experimental conditions, neither CDs were able to separate the acebutolol enantiomers.

Table 3
Separation of the pharmaceutical compounds with CM-γ-CD chiral selector (2.5%, w/v) at 25°C

Solute	pH	Borate buffer					Phosphate buffer				
		t_1	t_2	α	R_s	K	t_1	t_2	α	R_s	K
Acebutolol	8	6.78				−7	5.44				−7
	7	6.40				−7	5.89				−8
	6	7.32				−7	8.83				−9
Bepridil	8	9.57				50	7.67	7.85	1.103	0.8	50
	7	8.81	9.06	1.141	1.6	45	8.42	8.65	1.120	1.5	50
	6	11.10	11.48	1.135	2.9	35	15.17	15.90	1.137	3.0	60
Cloperastine	8	10.34	10.64	1.101	1.5	90	8.16	8.33	1.073	0.9	90
	7	9.55	9.78	1.091	1.5	90	9.03	9.24	1.083	1.4	90
	6	8.81	12.33	1.093	1.5	60	19.44	20.37	1.105	2.1	130
Diperodon	8	8.76	8.93	1.119	1.3	20	6.78	6.89	1.130	1.2	20
	7	7.94	8.08	1.147	1.3	20	7.22	7.46	1.292	1.1	15
	6	9.05	9.17	1.112	1.3	10	12.54	12.95	1.161	1.8	20
Disopyramide	8	6.00				−14	4.99				−10
	7	5.74				−14	5.29				−14
	6	6.63				−13	7.69				−2
Doxylamine	8	6.82				−7	5.58				−5
	7	6.44				−7	5.96				−7
	6	7.46				−7	8.58				4
Homochloro-cyclizine	8	12.16	12.26	1.021	0.4	1000	9.53	9.58	1.016	0.3	1300
	7	11.12	11.22	1.023	0.5	900	10.69	10.77	1.021	0.3	1300
	6	14.73	14.90	1.026	0.7	200	22.16	22.49	1.026	0.6	1400
Hydroxyzine	8	11.62	11.74	1.029	1.1	300	8.81	8.85	1.015	0.3	200
	7	10.09	10.26	1.053	1.1	150	9.68	9.70	1.006	0.4	200
	6	12.64	12.88	1.053	1.4	80	18.09	18.63	1.063	1.5	170
Ketamine	8	12.08				800	9.21				400
	7	8.91				50	8.41				50
	6	8.75				3	10.70	11.17	1.470	0.6	7
Oxyphenozium	8	9.60	9.69	1.052	0.5	50	7.78	7.86	1.048	0.3	60
	7	9.06	9.18	1.061	0.6	50	8.69	8.82	1.059	0.3	60
	6	11.97	12.18	1.060	0.8	50	19.18	19.78	1.071	1.3	100
Pheniramine	8	*6.67*	*6.75*	*1.012*	1.4	−8	*5.44*	*5.50*	*1.011*	0.9	−7
	7	*6.27*	*6.34*	*1.011*	1.1	−8	*5.79*	*5.85*	*1.011*	1.0	−9
	6	*7.16*	*7.29*	*1.018*	1.4	−8	*8.23*	*8.42*	*1.024*	1.4	−10
Propiomazine	8	11.94	12.46	1.116	2.2	600	8.81	9.23	1.143	1.6	200
	7	10.30	10.83	1.163	2.0	200	9.64	10.18	1.166	1.8	170
	6	12.86	13.68	1.168	3.2	100	22.54	25.40	1.197	3.0	700

t_1 and t_2 are the elution times of the first and second enantiomer, α is the enantioselectivity factor, R_s is the resolution factor, K is the first enantiomer affinity constant for the CM-γ-CD. No data means $t_2 = t_1$, $\alpha = 0$, $R_s = 0$. Italicized values = the solute eluted before t_{eof} (cationic character), α was taken as t_2/t_1. Experimental conditions: 50 cm × 75 μm I.D. capillary; pressurized 2 s injection of 50 μg/ml solution, 20 kV; buffer concentration, 0.1 M; CM-γ-CD concentration, 2.5% or 0.017 M; UV detection 200 nm, 25°C.

3.4. Interactions with the selector and chiral recognition

3.4.1. Partition coefficients

Tables 2 and 3 list the pseudo-partition coefficient of the racemates calculated using Eq. (1). The coefficient K is an indicator of the strength of the solute–CD interactions. It covers all types of interactions: hydrophobic, electrostatic or inclusion complexation. The highest coefficients with the two CDs were obtained for homochlorocyclizine. It means probably that this positively charged molecule cumu-

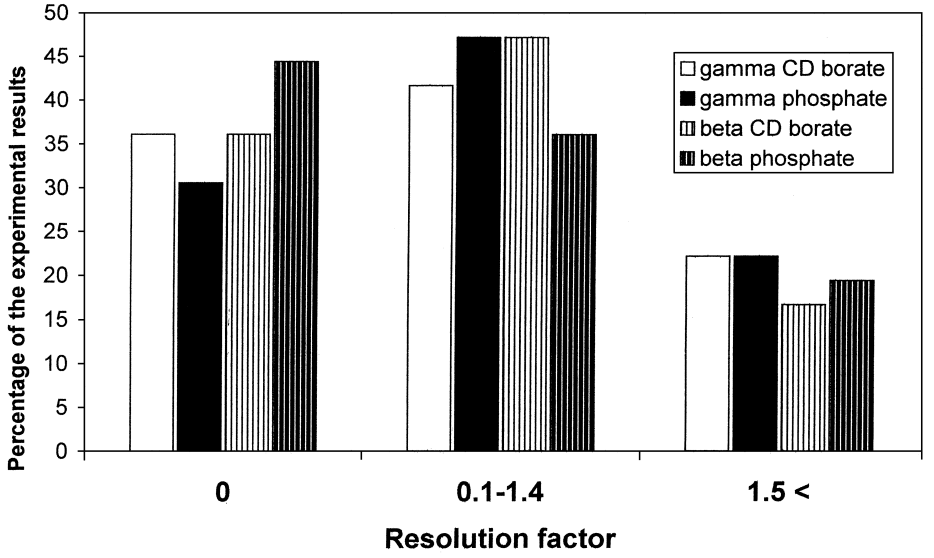


Fig. 1. Histogram of the results obtained with the set of 12 basic drugs. $R_s = 0$: unsuccessful results, $0.1 < R_s < 1.4$: partial separation, $R_s \geq 1.5$: baseline separation.

lates electrostatic interactions between the negative carboxylate groups of the substituted CDs and inclusion complexation. Cloperastine and hydroxyzine, the two racemates structurally similar to homochlorocyclizine, have a high partition coefficient with CM-β-CD only (Table 2). Likely, they do not form a tight inclusion complex with CM-γ-CD which reduces the corresponding K coefficient (Table 3). The lowest coefficients were negative values obtained for disopyramide. A negative K value means that the solute keeps a positive character with the experimental conditions, i.e. it elutes before t_{eof}, the elution time for neutral compounds. The change in the ionization state of ketamine has a dramatic effect on its partition coefficient with the γ-CD selector (Table 3). At pH 8, the neutral ketamine molecule seems able to make an inclusion complex with the γ-CD cavity (K values of 800 or 400 in pH 8 borate or phosphate buffers, respectively). At pH 6, ketamine is positively ionized ($pK_a = 7.5$, Table 1), and as such should add the electrostatic attraction for the negative carboxymethyl moieties to the inclusion complexation. However, the partition coefficient of ketamine decreased by two orders of magnitude ($K = 3$ or 7 in pH 6 borate or phosphate buffers, respectively), and the conformational change induced by its ionization and/or electrostatic interactions appears to preclude the inclusion complex formation.

3.4.2. Interaction strength and enantiorecognition

Clearly, the magnitude of the partition coefficient of a compound and its enantioresolution factor are not related. Some compounds with a high affinity for the CD chiral selector are not enantiorecognized ($R_s = 0$), e.g. cloperastine and hydroxizine with β-CD (Table 2), ketamine at pH 8 with γ-CD (Table 3). Other compounds with a very low affinity for the CD selectors may be well enantioseparated, e.g. disopyramide and ketamine pH 6 with β-CD (Table 2) or bepridil, cloperastine, diperodon and pheniramine with γ-CD (Table 3). Also some compounds with high K values have high R_s values (doxylamine, pheniramine or propiomazine with β-CD and cloperastine or propiomazine with γ-CD) and compounds with low K values are not separated (acebutolol for both selector, disopyramide or doxylamine for γ-CD). The K value is an inclusive measure of the magnitude of the interaction energy of the chiral molecule with the CD receptor. Part of this energy corresponds to achiral interactions and another part corresponds to chiral interactions. Our results show that these two parts are completely independent. Similar results were obtained with CD

in chiral GC; the solute retention factors were completely independent from the enantioselectivity factors [22].

3.4.3. Weak interactions and high enantiorecognition

It is interesting to note that the enantiomers of disopyramide and pheniramine are separated by the β-CD and γ-CD, respectively, when they have negative partition coefficients (data in italics in Tables 2 and 3). Since these racemates moved faster than the electroosmotic flow and their enantiomeric separations indicate that efficient interactions with the chiral selectors took place, it means that these positive molecules interacted rapidly with a great number of CD selectors. These interactions with the negatively charged chiral selectors moving slower than the eof were obviously fast. However, they were selective enough to give an efficient chiral separation. Fig. 2 illustrates this observation. Pheniramine has a partition coefficient of about 200 with the CM-β-CD selector. Its two enantiomers are baseline separated in 12 or 19 min, depending on the buffer electrolytes (Fig. 2, top). The efficiencies are in the 60 000–70 000 plate range (10 000 plates only for the first enantiomer with phosphate buffer). Fig. 2, bottom, shows the same separations with the CM-γ-CD selector for which pheniramine has a partition coefficient value of −8 (Table 3). The separation is done in less than 8 or 9 min. The peak efficiencies are in the 100 000 or 50 000 plate range for the borate or phosphate buffer, respectively (height equivalent to a theoretical plate, HETP = 5 or 10 μm, respectively) proving fast interactions with the chiral selectors.

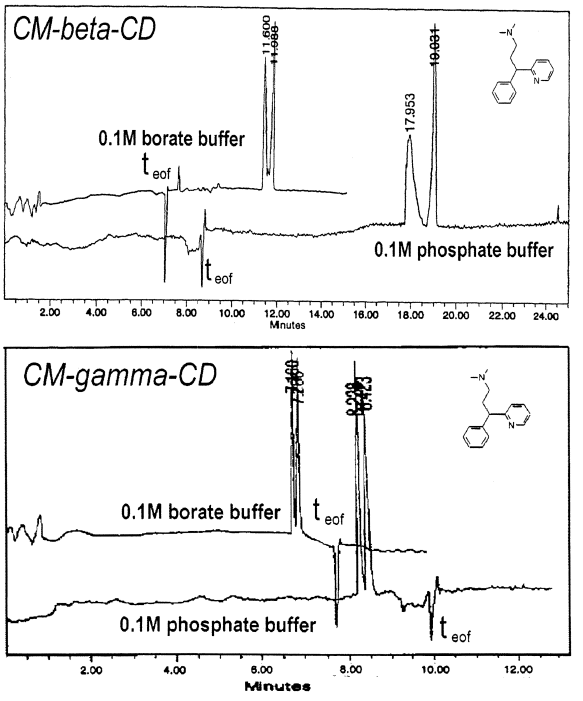


Fig. 2. Electropherograms of pheniramine separated with CM-β-CD (top) and CM-γ-CD (bottom) with borate and phosphate buffers. A 20 kV, 25°C, 2 s injection by pressurization, 50 cm × 75 μm I.D. uncoated capillary, 200 nm UV detection.

3.5. Buffer ion effects

3.5.1. pH and retention times

Tables 2 and 3 show the effect of the buffer ion on the separation. The electroosmotic flow induced by the phosphate ions is much more dependent on the pH value than that obtained with borate ions. This is because at constant concentrations, this alternation in analyte retention is due to changes in the ionic strength when the dihydrogenphosphate ions change to hydrogen phosphate ions between pH 6 and 8 (pK_a $H_2PO_4^-/HPO_4^{2-} = 7.2$). At constant ionic strength, the electroosmotic flow would be independent of the buffer type [23]. The retention times of the analytes are between 15 and 50% lower with phosphate buffer at pH 8 for the same CD. They have the same order of magnitude at pH 7. With phosphate buffer at pH 6, the solute retention times are between 20 and 200% longer than these with borate buffer at the same pH (Tables 2 and 3).

3.5.2. Partition coefficient

Oppositely, the buffer type does not seem to have a significant effect on the solute partition coefficient. Most experimental K values listed in Tables 2 or 3 are within ±20% with the borate or the phosphate buffers. The higher difference in K values is observed for oxyphenozium (CM-β-CD, Table 2) with an average K value of 200 with borate buffer and $300 < K < 700$ with phosphate buffer.

3.5.3. Efficiency

The efficiencies obtained with the borate buffer

were slightly better than the corresponding efficiencies obtained with the phosphate buffer. Fig. 2 illustrates the case of pheniramine. With the CM-γ-CD (negative *K* values), the peak efficiencies obtained with the borate buffer are in the 100 000 plate range, twice the ones obtained with the phosphate buffer. With the CM-β-CD selector, the efficiencies measured on the second eluting peak are similar with the two buffer ions; severe broadening is observed on the first eluting pheniramine peak with phosphate buffer (Fig. 2, top). The efficiency difference seems to be generally more pronounced at pH 7 and 8.

Unexpected peak distortions and/or broadening were occasionally observed with some test solutes, e.g. first peak of pheniramine with CM-β-CD and phosphate buffer at pH 6 (Fig. 2, top). These peak distortions are likely due to small heterogeneities in the chiral selector composition [23]. The CM substituted CDs have an average substitution number of three anionic groups per CD ring and these anionic groups are attached randomly to the CD unit. Part of the chiral selector may have differing interactions with one or the two enantiomers of given solutes giving rise to this an anomalous peak shape.

3.5.4. Buffer concentration

Few experiments were done with a 50 and a 150 m*M* buffer concentrations (data not shown). It is known that the buffer concentration acts directly on the magnitude of the electroosmotic flow, a higher buffer concentration giving a lower eof and vice versa [23]. The solutes retention times were changed accordingly: a higher buffer concentration produces higher retention times. However, the partition coefficients were unaffected by the buffer concentration changes and the enantioselectivity and resolution factors were also unaffected.

3.6. Other parameters

3.6.1. Chiral selector concentration

Beside 2.5% (w/v), CM-γ-CD concentrations of 1 and 3.75% (w/v) were used at pH 7 with phosphate and borate buffers. Table 4 lists the results obtained with the phosphate buffer at pH 7. The retention times increased with the ionic CD concentration due to the increased ionic strength that in turn decreased the electroosmotic flow [4,23]. For many compounds, e.g. bepridil, homochlorocyclizine, hydroxyzine, oxyphenozium, pheniramine, the 1% w/v (6.8 m*M*) γ-CD concentration was not high enough to obtain enantiorecognition (Table 4). Increasing the CD concentration from 2.5 (17 m*M*) to 3.75% (25.5 m*M*) also had little effect on the enantioresolution factors obtained and on the partition coefficients of the solute. Taking in account the experiment duration, 2.5% w/v was selected as the optimum concentration of the ionic chiral selector for our set of pharmaceuticals and our experimental conditions. Similar conclusions were obtained with the borate buffer (data not shown).

3.6.2. Effect of temperature

It has been established that low temperatures allowed a better chiral recognition by CD selectors [4,14,16,17]. Table 4 lists the results obtained with CM-γ-CD and phosphate buffer at pH 7 and 15°C. As expected, the enantioresolution factors are strongly enhanced by the cooling. The R_s value of bepridil was 1.5 at 25°C and 3.6 at 15°C. For homochlorocyclizine a five-fold enhancement is observed from 0.2 at 25°C to 1.1 at 15°C. For all other compounds a significant resolution factor enhancement was observed (Table 4). This enhancement is partly due to thermodynamic, i.e. increased solute enantioresolution factors and partition coefficients (Table 4). It is also due to kinetic effects, i.e. increased efficiency. A 10°C decrease in temperature increases the mobile phase viscosity, which decreases the band broadening by diffusion. This viscosity effect doubles the plate numbers.

However, in the lowering of the temperature, a deleterious effect is observed on experimental run times as the change in viscosity also decreases the electroosmotic flow. All retention times at 15°C are between 50 and 80% longer than the corresponding times at 25°C. For example, the retention time of the first enantiomer of bepridil was 9.31 min at 25°C, whereas at 15°C it was 15.05 min. Thus it would be of value to work at lower temperatures only where partial enantioresolution at room temperature were obtained.

Table 4
Separation of the pharmaceutical compounds with the CM-γ-CD chiral selector at different concentrations (in %, w/v, or g/100 ml) and temperatures (°C), pH 7

Solute	[γ-CD] (%)	25°C					15°C, 3.75% (w/v) γ-CD				
		t_1	t_2	α	R_s	K	t_1	t_2	α	R_s	K
Acebutolol	1	4.71				−30					
	2.5	5.89				−8					
	3.75	6.34				−2	9.95				−0.2
Bepridil	1	6.46				70					
	2.5	8.42	8.65	1.120	1.6	50					
	3.75	9.31	9.56	1.093	1.5	45	15.05	15.60	1.108	3.6	50
Cloperastine	1	6.16	6.26	1.114	0.5	90					
	2.5	9.03	9.24	1.083	1.4	90					
	3.75	9.93	10.21	1.084	1.4	75	16.42	17.24	1.128	1.7	90
Diperodon	1	5.47	5.57	1.592	1.3	12					
	2.5	7.22	7.46	1.292	1.1	15					
	3.75	8.05	8.20	1.104	1.4	17	13.09	13.41	1.098	1.6	25
Disopyramide	1	4.43				−40					
	2.5	5.29				−14					
	3.75	5.67				−7	8.52				−7
Doxylamine	1	4.55				−35					
	2.5	5.96				−7					
	3.75	6.70				1	10.20				1
Homochloro-	1	7.25				900					
cyclizine	2.5	10.69	10.77	1.021	0.3	1300					
	3.75	11.69	11.76	1.013	0.2	1000	19.68	19.96	1.029	1.1	1300
Hydroxyzine	1	6.92				350					
	2.5	9.68	9.70	1.006	0.4	200	17.01	17.52	1.075	0.5	120
	3.75	10.51	10.79	1.072	0.3	120					
Ketamine	1	7.39				1400					
	2.5	8.41				50					
	3.75	8.95				40	12.17				14
Oxyphenozium	1	5.86				50					
	2.5	8.69	8.82	1.059	0.3	60					
	3.75	9.43	9.65	1.079	0.4	50	15.35	15.79	1.082	0.5	60
Pheniramine	1	*4.55*				−35					
	2.5	*5.79*	*5.85*	*1.011*	1.0	−9					
	3.75	*6.27*	*6.37*	*1.016*	1.3	−3	*9.58*	*9.96*	*1.040*	1.6	−3
Propiomazine	1	6.77	6.99	1.144	1.0	270					
	2.5	9.64	10.18	1.166	1.8	170					
	3.75	10.80	11.28	1.114	1.9	170	18.20	19.26	1.130	2.1	220

t_1 and t_2 are the elution times of the first and second enantiomer, α is the enantioselectivity factor, R_s is the resolution factor, K is the first enantiomer partition coefficient for the CM-γ-CD. No data means $t_2 = t_1$, $\alpha = 0$, $R_s = 0$. Italicized values = the solute eluted before t_{eof} (cationic character), α was taken as t_2/t_1. Experimental conditions: 50 cm × 75 μm I.D. capillary; pressurized 2 s injection of 50 μg/ml solution, 20 kV; buffer concentration, 0.1 *M*, pH 7; CM-γ-CD concentration indicated; UV detection 200 nm.

4. Conclusion

CM-β- and -γ-CDs are useful anionic chiral selector for the capillary electrophoresis separation of basic chiral compounds. The molecular structure of the racemate to be separated should be considered with the selection of the CD to be used. A chlorinated phenyl group would suggest to use a γ-CD when a pyridyl substituent seems better recognized by a β-CD selector. Unfortunately, there are no absolute rules and some experimentation will remain necessary. In the case of amino compounds such as the pharmaceuticals investigated in this work, the operation at the lower pH value yielded better results

overall. It ensured a positive ionization of the amine drugs and a negative ionization of the carboxylic groups of the CD selectors. When a partial separation of the enantiomers is obtained, lowering the temperature will increase significantly the resolution factor at the cost of a long duration of the separation. It was shown recently, not specifically for basic compounds, however, that the mixing of neutral and anionic chiral CDs could greatly enhance chiral separations of enantiomers [24,25].

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