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Chiral separations of basic and acidic compounds in modified capillaries using cyclodextrin-modified capillary zone electrophoresis

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ABSTRACT

Chiral separations of basic drugs and organic acids can be achieved using cyclodextrins or cyclodextrin derivatives as chiral selectors in surface-modified capillaries. The influence of temperature and selector concentration on separation performance was investigated in specially modified capillaries. Separations of basic compounds at acidic pH were performed in capillaries modified by either dynamic or permanent coating by adsorption of non-ionic hydroxylic polymers. Separations of acidic compounds could be significantly improved by use of thermally immobilized poly(vinyl alcohol) or adsorbed cationic polymers as surface coatings. The influence of non-ionic and cationic coatings on separations of acidic compounds was compared with regard to resolution, separation efficiency and analysis time.

INTRODUCTION

In capillary zone electrophoresis (CZE), enantiomeric separations can be achieved in buffers that contain chiral additives. The diastereomeric analyte–selector complexes formed are characterized by a significant difference in charge density and molecular geometry which leads to changes in electrophoretic mobilities compared with those of the free chiral analytes. Differences in the stability constants of diastereomeric analyte–selector complexes formed lead to different migration times that are characteristic for the enantiomers. Especially cyclodextrins (CDs) have been used successfully as complexing chiral agents for the separation of racemic compounds [1–15]. Sufficiently large enantionselectivities can be achieved in CE for different types of chiral analyte molecules when different types and derivatives of cyclodextrins as chiral buffer additives are added to the buffers in adequate concentrations. Only low enantioselectivities can typically be achieved in the aqueous separation matrices of CE and optimization of the separation efficiency is therefore essential in order to achieve the necessary resolution in separations of analytical relevance. In CZE separations analyte–surface interaction causes band broadening and peak tailing. The related loss in efficiency can be expected from theory and is especially well known to be detrimental from the CZE of proteins.

Various procedures for "dynamic" and "permanent" surface modifications have been proposed for deactivating the acidic fused-silica surface with regard to analyte interactions [16–29]. By such procedures a considerable improvement in separation efficiency and especially also tailing-free peak shapes can be achieved.

In the field of chiral separations of basic compounds of pharmaceutical interest it has been demonstrated that "dynamic" deactivation of the capillary walls by adsorptive modification with non-ionic hydroxylic polymers such as poly(vinyl alcohol) or hydroxyethylcellulose leads to

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a significant improvement in resolution with unchanged enantioselectivity [3,29]. By addition of such hydroxylic polymers in low concentrations (0.05%, w/w) to acidic buffers, separation efficiencies can be enhanced significantly ($N >$ 200 000), especially at higher field strength ($E \approx$ 40–60 kV/m) [29].

Modifications of the fused-silica surfaces are always associated with simultaneous suppression or even reversal of the electroosmotic flow (EOF) in the capillary. Depending on the direction of electromigration of the charged analyte species, the suppression or reversal of the EOF may lead to a retardation or acceleration of a certain separation which may not generally be of analytical advantage.

Modification or deactivation of the surface can be achieved by either "dynamic" or "permanent" coatings. In dynamic modification procedures the surface-modifying agents, mostly either non-ionic or ionic surfactants or polymers, are contained in the buffer and are adsorbed from there on the surface. These "modifiers" can also be deposited on the capillary walls by repetitive rinsing with solutions of these compounds before each separation and may then not be contained in the buffer during the actual separation. This method may be important if UV-absorbing modifiers are to be applied. Permanent coatings can either be covalently bonded to the surface or can be adsorbed to the surface with subsequent physical or chemical immobilization procedures such as cross-linking. It seems obvious that capillary coatings may reduce especially the adsorption of cationic analytes on the fused-silica surface, which is acidic owing to suppressed analyte–wall coulombic interactions. If analyte adsorption on the capillary walls is attributed to coulombic interactions, the influence of capillary coatings on CE separations of anionic (*i.e.*, acidic) analytes could be expected to be less significant than for separations of cationic (*i.e.*, basic) compounds.

In this work, the influence of surface modification by a previous rinsing and by permanent coating on chiral separations of acidic organic compounds was investigated. Thermally immobilized poly(vinyl alcohol) (PVA), introduced by Gilges and Schomburg [30,50] for the separation of proteins at neutral and basic pH, and strongly adsorbed cationic polymer coatings were compared with regard to separation efficiency and modification of EOF in the separation of chiral organic acids. The separations were executed in buffer systems modified by different cyclodextrins at neutral pH.

In a previous paper [29] we reported on separations of racemic basic drugs of the tocainide type in dynamically deactivated capillaries using γ-cyclodextrin as a chiral discriminating additive. In continuation of this work, the influence of different system parameters such as type of cyclodextrin and derivative and selector concentration on the separations of the mentioned basic chiral molecules was also investigated under the conditions of optimized surface modification.

EXPERIMENTAL

Instrumentation

All experiments were executed in laboratory-made equipment with UV detection (Spectra-Physics Model UV 100), time-controlled hydrodynamic sample introduction by connection of the capillary outlet to a vacuum pump (DC 12/08 P; Fürgut, Altrach, Germany), a high-voltage source connected to the inlet (HCN 35-35 000; FUG, Rosenheim, Germany) and active cooling of the capillary by a thermostated water circuit. Raw data analysis was performed using modified COLACHROM computer software [31].

Capillaries

Fused-silica capillaries of 50 μm I.D. and 0.36 mm O.D. from Polymicro Technologies (Phoenix, AZ, USA) or Microquartz (Munich, Germany) were used. The detection window was generated by burning according to Lux *et al.* [32] or by scratching of the polyimide with a scalpel in the case of a permanent inner surface coating.

The thermally immobilized PVA coating was generated according to Gilges and Schomburg [30,50] by forcing a 10% (w/w) solution of PVA (M_r 50 000, 99+% hydrolyzed) in water through a capillary with subsequent removal of the solu-

tion by nitrogen pressure. The final immobilization of the polymer film is achieved by heating the capillary to 140°C under nitrogen flow.

Chemicals

The following cyclodextrins were obtained from Wacker Chemie (Munich, Germany): γ-cyclodextrin, hydropropyl-γ-cyclodextrin (HP-γ-CD; molecular substitution 0.6), α-cyclodextrin, hydroxypropyl-α-cyclodextrin (HP-α-CD; molecular substitution 0.6), β-cyclodextrin, hydroxypropyl-β-cyclodextrin (HP-β-CD; molecular substitution 0.9), hydroxyethyl-β-cyclodextrin (HE-β-CD; molecular substitution 1.0), methyl-β-cyclodextrin (degree of substitution 1.8); heptakis(2,6-di-O-methyl-β-cyclodextrin) was synthesized according to Boger *et al.* [51]. Poly(vinyl alcohol) (M_r 77 000–79 000) and the cationic polymer Polybren were from Aldrich (Steinheim, Germany) and hydroxyethylcellulose (middle viscosity 1) from Fluka (Buchs, Switzerland). Buffers were prepared with orthophosphoric acid (pH 3) or NaH_2PO_4 (pH 7) from Fluka by adjustment of the pH with 3 *M* aqueous sodium hydroxide and were filtered through 0.45-μm Minisart RC 25 membrane filters (Sartorious, Göttingen, Germany) prior to use.

Samples

Tocainide and analoguous basic compounds were kindly supplied by Astra-Hässle (Sweden) and 2,4-dichlorophenoxypropionic acid (DCPP) by BASF (Germany). (*R*)-3-Phenyllactic acid, (*S*)-3-phenyllactic acid, (*R*)-3-phenylbutyric acid, (*S*)-3-phenylbutyric acid and racemic 2-phenylproprionic acid were purchased from Sigma (St. Louis, MO, USA) and racemic mandelic acid from Fluka.

Rinsing procedures

Rinsing steps were applied either to adsorb surface-modifying agents or to regenerate the separating buffer system.

Dynamic deactivation for the separation of basic compounds at pH 3 was performed as follows. After installation, the capillary was rinsed for about 15 min (30 capillary volumes) with triply distilled water and then for 15 min with the buffer not yet containing the polymeric surface modifier. Then the separation buffer containing a modifier (in addition to the chiral selector) was introduced by rinsing for 1 min with a volume about twice that of the capillary. After each separation the capillary was rinsed for 1 min with buffer not containing modifier with a volume again about twice that of the capillary. Then the buffer containing a modifier for the actual separation was introduced again.

Surface deactivation by previous rinsing with cationic Polybren was used for the separation of acidic chiral compounds at pH 7. After installation, the capillary was rinsed for about 15 min (30 capillary volumes) with triply distilled water. Before each separation the capillary was rinsed for 2 min with a solution of 0.05% Polybren in water, then the separation buffer free from Polybren was introduced.

With thermally immobilized PVA coatings for capillary conditioning rinsing steps were not required and the separation buffer was replaced after each separation.

RESULTS AND DISCUSSION

Chiral separations of basic compounds

In previous work, certain compounds which are analogues of the antiarrhythmic agent tocainide were separated with high separation efficiency in buffer media with γ-CD as a chiral discriminating agent. The optimum separation efficiencies, which were as high as $N > 200\,000$, and the corresponding resolution could only be achieved in dynamically deactivated capillaries using PVA or hydroxyethylcellulose as buffer additive. Especially at high field strength a considerable increase in separation efficiency in terms of numbers of theoretical plates or resolution was obtained [29]. In continuation of this work, we investigated the influence of some important system parameters on enantioselectivity and resolution under the conditions of such optimized surface modification.

Influence of ring size of cyclodextrin on enantioselectivity. CZE separations of four selected tocainide analogues and ephedrin with α-, β-

and γ-CD as chiral selectors are shown in Fig. 1. The enantioselectivity and resolution data evaluated from these separations are given in Table I. It was found that α- and β-CDs are better suited for the chiral separation of those analytes which contain a less bulky substituted aromatic group (analytes 4 and 5). Analytes containing a bulkier aromatic substituent (1, 2 and 3) have longer migration times and are separated at higher enantioselectivity with γ-CD as selector. These results are consistent with a model of intermolecular interaction between analyte and selector which involves inclusion of the aromatic group in the hydrophobic cavity of the CD molecule. Analytes containing less bulky aromatic groups fit better into the smaller cavity of α-CD, whereas the larger diameter cavity of γ-CD is better suited for the inclusion of bulkier groups. This was observed. For the separation in Fig. 1B, β-CD could only be applied at lower

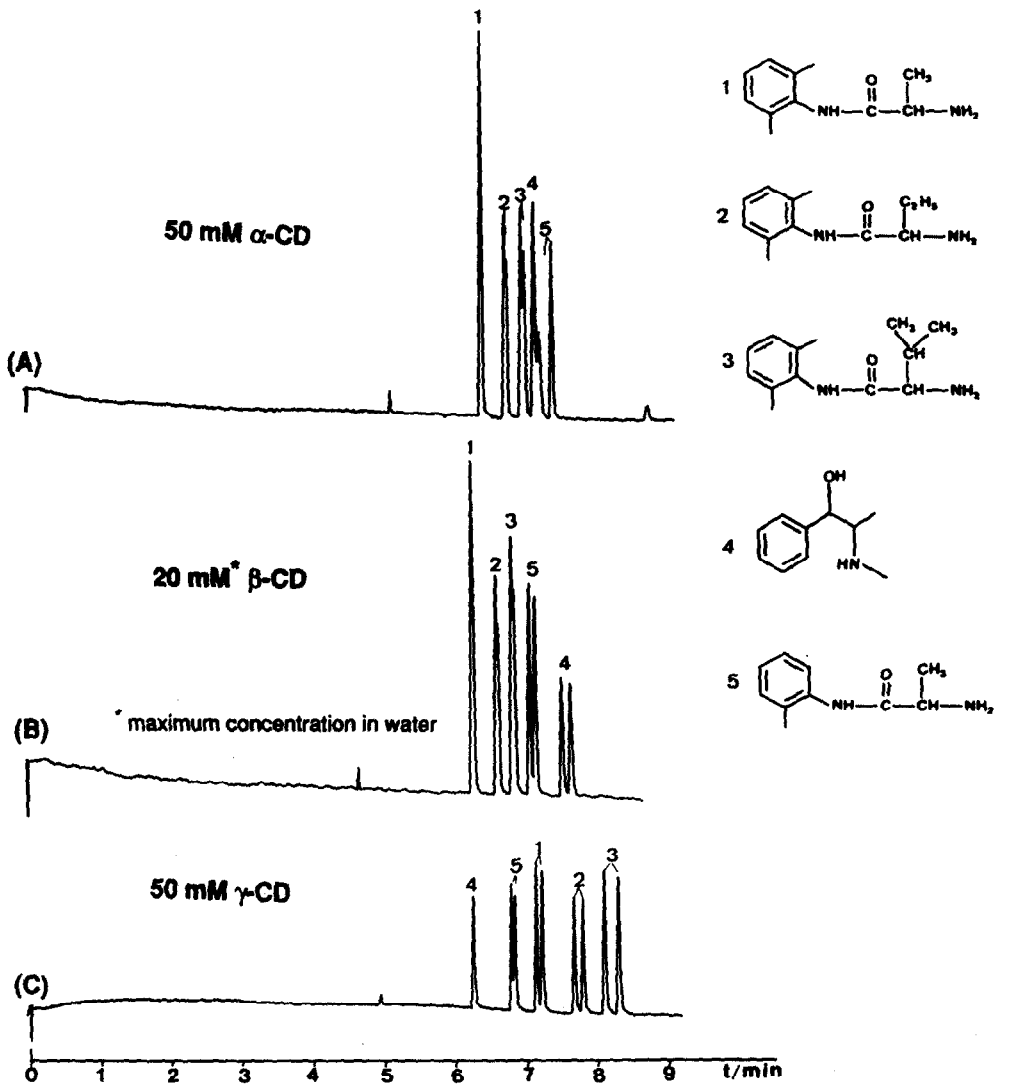


Fig. 1. Influence of ring size of cyclodextrin on the separation of five racemic bases. Capillary, 56.4 cm total length, 43.0 cm effective length, 50 μm I.D., 0.36 mm O.D.; voltage, +35 kV, 30 μA; buffer, 40 m*M* sodium phosphate (pH 3.0); (A) 50 m*M* α-CD; (B) 20 m*M* β-CD; (C) 50 m*M* γ-CD; 0.05% PVA; temperature, 20°C; sampling, vacuum Δ*P* −85 mbar, 3 s; detection, UV at 210 nm; samples, 0.02 mg/ml of each racemic compound dissolved in water.

TABLE I

INFLUENCE OF RING SIZE OF CYCLODEXTRIN ON SELECTIVITY AND RESOLUTION

Data were evaluated from the separations in Fig. 1. Selectivity α was calculated from $\alpha = \mu_1/\mu_2$ and resolution from $R_s = \Delta t/2(\sigma_1 + \sigma_2)$, where μ = electrophoretic mobility corrected by EOF, Δt = migration time difference and σ = peak variance.

Analyte	50 m*M* α-CD		20 m*M* β-CD		50 m*M* γ-CD	
	α	R_s	α	R_s	α	R_s
1	–	–	–	–	1.011	1.36
2	1.005	–	1.005	–	1.017	2.23
3	1.007	–	1.004	–	1.025	2.63
4	1.007	–	1.020	1.34	–	–
5	1.032	3.52	1.010	1.12	1.007	0.87

concentrations than those of α- and γ-CD because of its limited solubility in the aqueous buffer. Therefore, the separation obtained with β-CD cannot be directly compared with those obtained with α- or γ-CD as selectors because the enantioselectivity depends on the concentration of the selector in the buffer [33].

Partially methylated cyclodextrins as selectors. It is well known but also surprising that methylated β-CDs show an increased solubility in water compared with the underivatized β-CD. The enantionselectivities achievable in CZE separations are also changed by methylation. In Fig. 2, CE separations of the basic chiral test compounds obtained with two differently O-methylated cyclodextrins are compared with that obtained with the underivatized β-CD. Because of their better water solubilities, higher concentrations of the methylated cyclodextrins in the buffer could be chosen so that the chiral resolution in these separations could also be enhanced. Baseline resolution for all five analytes in a single run was achieved using heptakis(2,6-di-O-methyl)-β-cyclodextrin (see Fig. 2C). A product which was claimed to be heptakis(2,6-di-O-methyl)-β-cyclodextrin has already been applied as chiral selector by Schutzner and Fanali [33]. The methylated cyclodextrin derivative applied for the separation in Fig. 2C had been synthesized in our laboratory and proved to be better suited for this separation than a product which was purchased from Wacker-Chemie (see Fig. 2B). For the Wacker product a degree of substitution $DS = 1.8$ was specified. High-temperature GC and HPLC analyses of these cyclodextrin derivatives indicated that both products are mixtures of methylated cyclodextrin derivatives with different degrees of substitution [34]. The product we synthesized contained about 80% of heptakis(2,6-di-O-methyl)-β-cyclodextrin. The commercial product was less uniform with regard to methylation, it contained a much larger number of species and must have had a lower degree of methylation than specified.

The influence of the uniformity of commercially available methylated cyclodextrins on chiral CE separations was also studied by Nielen [15], who compared two commercially available products which were also claimed to be heptakis(2,6-di-O-methyl)-β-cyclodextrin. He also found different enantioselectivities at the molar selector concentrations in the buffer. Our HPLC and high-temperature GC analyses revealed that commercial products of derivatized cyclodextrins are mostly mixtures of derivatives with differing degrees of methylation [35]. For this reason, it is difficult to determine the accurate concentration of a certain cyclodextrin derivative which is effective as a selector in the CE buffer. CE results obtained with cyclodextrin products of different commercial origin have to be compared with caution.

Influence of selector concentration on resolution. The influence of selector concentration on the chiral separation of those basic compounds selected as test analytes can be seen from Figs. 3 and 4. For these experiments 0.05% (w/w) hydroxypropylmethylcellulose instead of PVA was added to the buffer for dynamic wall deactivation, and suppression of EOF also occurred. This modifier was first used by Lindner *et al.* [25] but for the separation of proteins. The resolution of the chiral compounds increases considerably towards higher γ-CD concentrations (see Figs. 1A and B and 4A). The influence of the selector concentration on migration time of the faster migrating enantiomer is shown in Fig. 4B. In Fig. 4C the dependence of selectivity and separa-

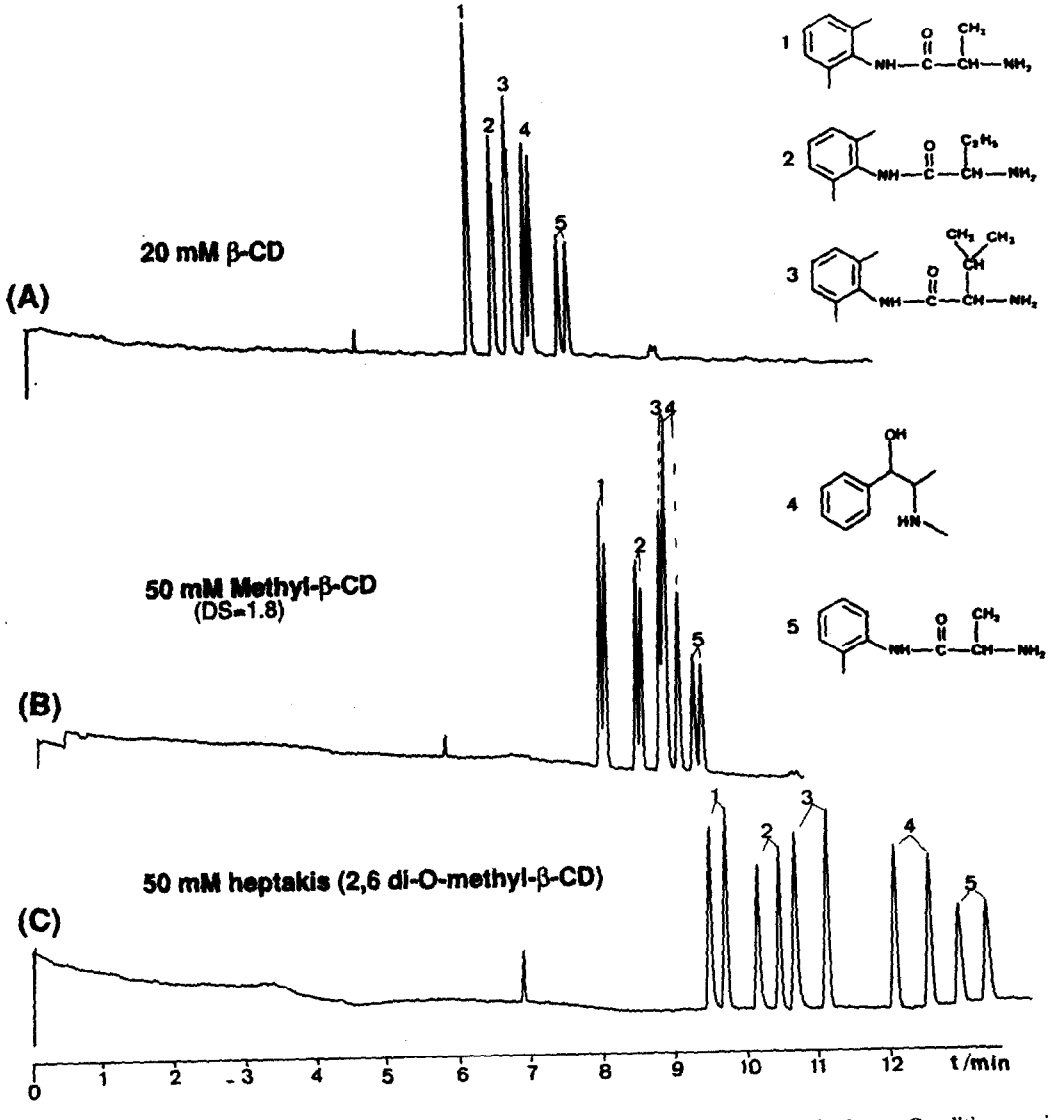


Fig. 2. Partially methylated β-cyclodextrins as selectors in chiral separations of basic drugs. Conditions as in Fig. 1.

tion efficiency on the selector concentration is plotted. Selectivity increases and the separation efficiency, in terms of numbers of theoretical plates, N, decreases with increasing selector concentration. The increase in enantioselectivity at higher selector concentrations is more significant than the decrease in efficiency. Therefore, better resolution at high selector concentrations is achieved.

Selectivities were calculated from

$$\alpha = \mu_2/\mu_1 \quad (1)$$

efficiency from

$$N = (t_r/\sigma)^2 \quad (2)$$

and resolution from

$$R_s = \Delta t/2(\sigma_1 + \sigma_2) \quad (3)$$

where μ = observed mobility (suppressed EOF), Δt = migration time difference of the enantiomers and σ = peak variance.

Chiral separations of organic acids

Several recent papers have also dealt with the separation of chiral organic acids using cyclodextrins as chiral additives [36–38]. We have investi-

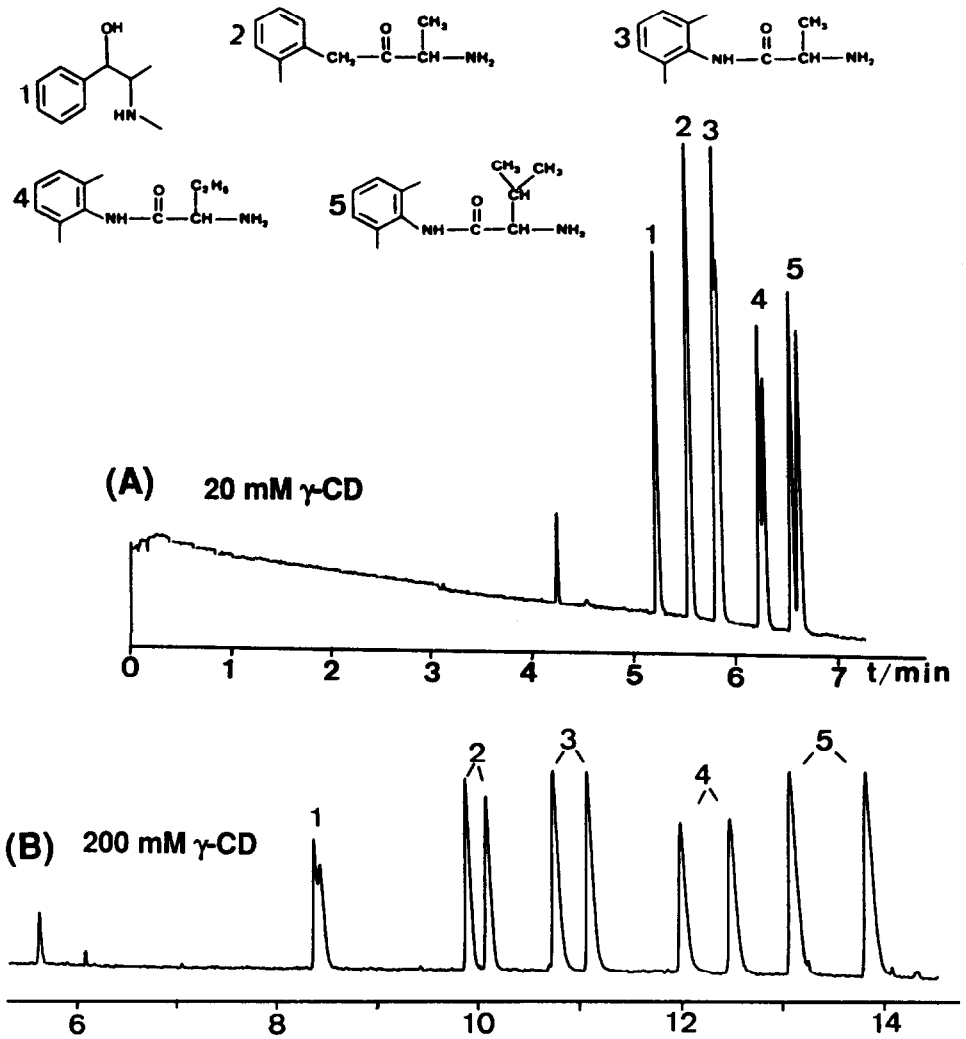
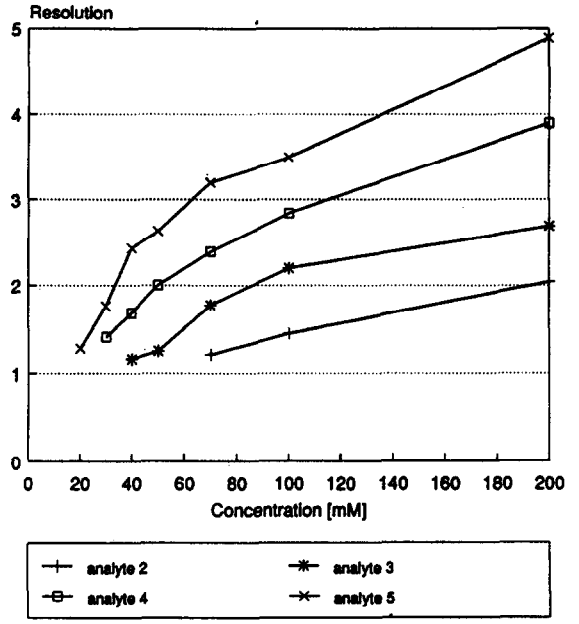


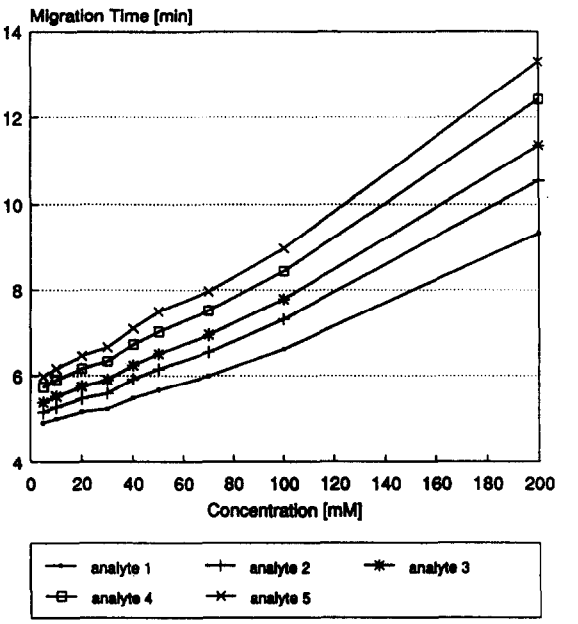
Fig. 3. Influence of selector concentration on chiral separations of basic drugs. Buffer, 40 m*M* sodium phosphate; (A) 20 m*M* γ-CD; (B) 200 m*M* γ-CD; 0.05% (w/w) hydroxypropylmethylcellulose; voltage, 35 kV; (A) 35 μA; (B) 22 μA; other conditions as in Fig. 1.

gated chiral separations of organic acids also under conditions of specially modified surfaces. Surface coatings are desirable in CE separations of acids for modifying the EOF to perform faster analytical separations, in order to improve the reproducibility and to suppress analyte–surface interactions.

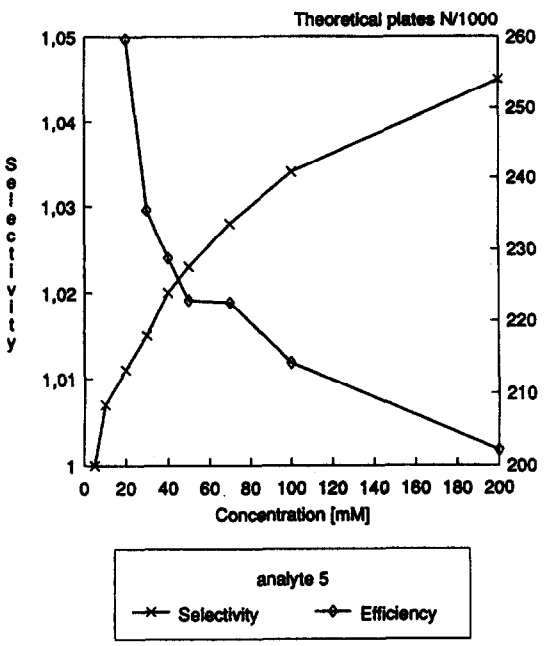
Contamination of uncoated capillaries. In CE separations at neutral or basic pH performed in bare fused-silica capillaries using derivatized cyclodextrins as buffer additives, we observed that the performance of separation deteriorates after only a few runs (see Fig. 5). We assume that cyclodextrins or impurities of similar chemical structure contained in the products applied are adsorbed on the bare fused-silica surface and give rise to analyte interactions, resulting in peak broadening. This assumption is supported by the observation that a contaminated capillary could be reconditioned by a simple rinsing step with aqueous or ethanolic NaOH solutions. In CE separations of these acidic analytes under similar conditions but without cyclodextrin additives no deterioration of separation performance occurs. With chiral separations of basic compounds which are separated at acidic pH a similar



(a)



(b)



(c)

Fig. 4. (A) Influence of selector concentration on resolution. Resolution was calculated according to eqn. (3). Conditions and samples as in Fig. 3. Analyte: + = 2; * = 3; □ = 4; × = 5. (B) Influence of selector concentration on migration time. Analyte: ■ = 1; + = 2; * = 3; ◧ = 4; × = 5. (C) Influence of selector concentration on (×) selectivity and (◇) efficiency for analyte 5.

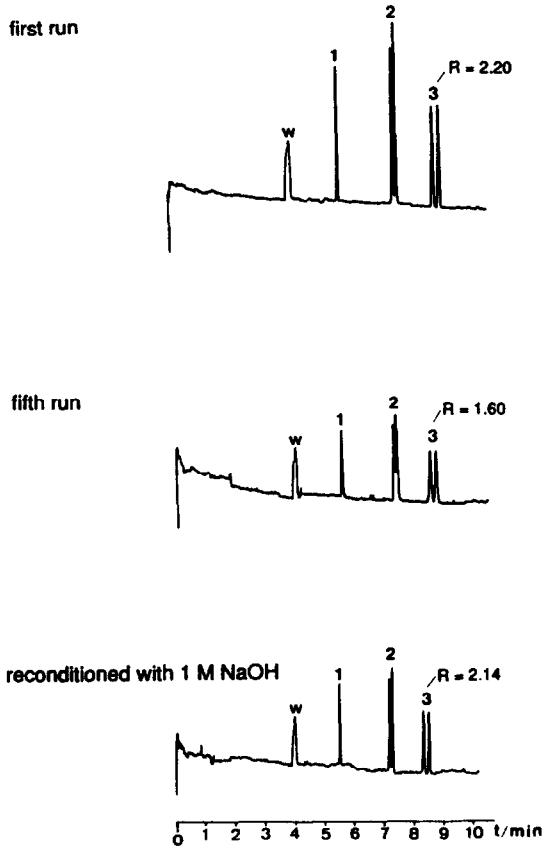


Fig. 5. Separation of organic acids in an unmodified capillary at neutral pH. Capillary contamination with cyclodextrin buffer and reconditioning by rinsing with 1 *M* NaOH. Capillary, 43.1 cm effective length, 56.5 cm total length, 50 μm I.D., 0.36 mm O.D. (Polymicro Technologies); buffer, 40 m*M* sodium phosphate (pH 7.0); 50 m*M* hydroxypropyl-γ-CD (molecular substitution 0.6); voltage, +35 kV, 55 μA; temperature, 20°C; sampling, vacuum Δ*P* −85 mbar, 2 s; detection, UV at 210 nm. Samples, 0.02 mg/ml of each racemic compound dissolved in water; 1 = D,L-ibuprofen; 2 = D,L-2,4-dichlorophenoxypropionic acid; 3 = D,L-3-phenylbutyric acid; w = water.

influence of surface contamination could not be found. This observation can be explained by the stronger dissociation of the SiOH groups at high pH, which may increase the adsorptivity of the fused-silica surface.

Influence of coatings and different types of selectors. Surface modification procedures involving adsorption or chemical bonding of cationic surfactants or polymers to the fused-silica surface have been applied to decrease or reverse the EOF in CE [39–49]. Especially in CE of small inorganic anions these surface coatings have been successfully used to perform fast analytical separations.

A comparison of CE separations of chiral organic acids performed in an unmodified capillary or in a capillary preconditioned with the cationic polymer Polybren (hexadimethrin bromide) is shown in Fig. 6. In unmodified capillaries the high cathodic EOF is effective against the direction of migration of the low-mobility anions (see Fig. 6A). The effective mobility ($\mu_{anion} + \mu_{EOF}$) of these anions is directed to the

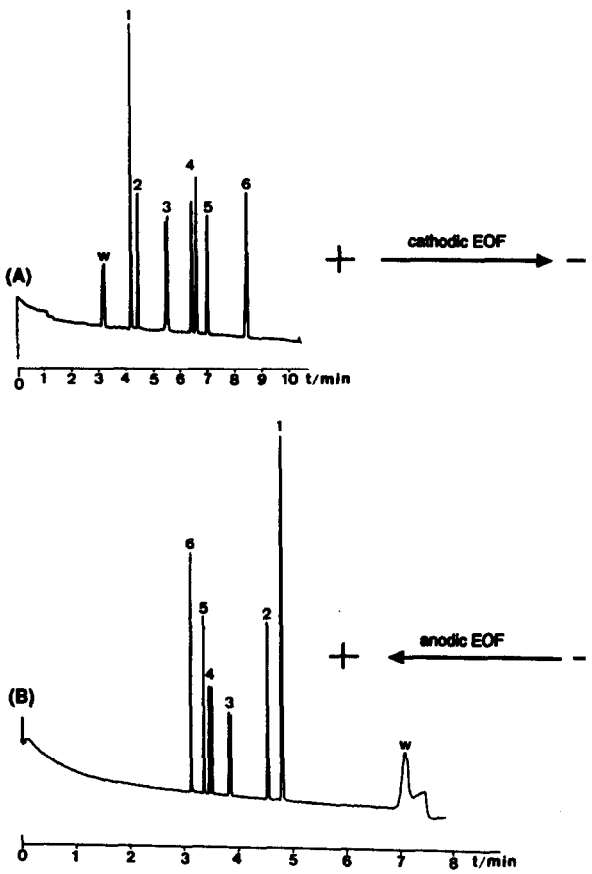


Fig. 6. Chiral CE separation of organic acids in an unmodified capillary and in a capillary preconditioned with Polybren. Buffer, 50 m*M* hydroxypropyl-α-CD (molecular substitution 0.6); voltage, (A) +35 and (B) −35 kV; other conditions as in Fig. 5. 1 = D,L-2,4-dichlorophenoxypropionic acid; 2 = D,L-ibuprofen; 3 = D,L-3-phenylbutyric acid; 4 = D,L-3-phenyllactic acid; 5 = D,L-mandelic acid; 6 = D,L-2-phenylpropionic acid; w = water, EOF marker.

cathode, and they therefore appear at the capillary outlet after the neutral marker for the EOF. Anions with low mobilities (μ_{anion}) appear at the detector outlet before analytes with higher mobilities because of the strong cathodic EOF. Rinsing of the capillary with Polybren reverses the surface charge and the zeta potential. The EOF is also reversed and now directed to the anode (see Fig. 6B) in the same direction as the vectorial mobility of the anions, resulting in a reversed migration order. The magnitude of the effective mobility of the fast anions (analytes 3–6) is increased, resulting in shorter migration times in a separation for which the voltage source polarity was reversed. The analysis time is significantly reduced under these conditions and excellent peak shapes are obtained. This is unexpected because adsorption of the anionic analytes on the cationic surface due to coulombic interactions was assumed.

The separation efficiency is also increased especially for the fast ions in the capillary with a cationic surface. This increase in efficiency can be explained by the higher effective mobility of

TABLE II

CHIRAL SEPARATIONS OF ORGANIC ACIDS USING DIFFERENT DERIVATIZED CYCLODEXTRINS IN UNMODIFIED CAPILLARIES

Resolution was calculated from eqn. 3. Conditions as for separation in Fig. 6A. R_s = resolution; t_1 = migration time of the first migrating enantiomer. HP = Hydroxypropyl; HE = hydroxyethyl.

Analyte	HP-α-CD		α-CD		HP-β-CD		γ-CD		HE-β-CD		HP-γ-CD	
	R_s	t_1 (min)	R_s	t_1 (min)	R_s	t_1 (min)	R_s	t_1 (min)	R_s	t_1 (min)	R_s	t_1 (min)
DCPP	–		–		1.40	7.61	–		1.15	7.11	1.36	4.15
3-Phenylbutyric acid	1.45	5.72	–		–		[a]	8.84	–		2.22	8.97
3-Phenyllactic acid	2.46	6.71	1.39	5.51	1.10	9.74	–		[a]	8.93	–	
Mandelic acid	–		–		2.20	24.01	2.10	18.4	2.00	19.55	4.02	17.85
2-Phenylpropionic acid	–		–		1.04	12.37	–		[a]	11.17	–	

[a] Resolution too small to be calculated.

TABLE III

CHIRAL SEPARATIONS OF ORGANIC ACIDS USING DIFFERENT DERIVATIZED CYCLODEXTRINS IN CAPILLARIES PRECONDITIONED WITH POLYBREN

Conditions as for separation in Fig. 6B. Symbols and abbreviations as in Table II.

Analyte	HP-α-CD		α-CD		HP-β-CD		γ-CD		HE-β-CD		HP-γ-CD	
	R_s	t_1 (min)	R_s	t_1 (min)	R_s	t_1 (min)	R_s	t_1 (min)	R_s	t_1 (min)	R_s	t_1 (min)
DCPP	–		–		[a]	4.91	[a]		1.42	4.53	[a]	3.64
3-Phenylbutyric acid	1.51	3.85	–		–		[a]	4.07	–		0.68	3.40
3-Phenyllactic acid	1.96	3.49	1.13	3.03	[a]	4.30	–		[a]	3.95	–	
Mandelic acid	–		–		[a]	3.39	1.30	3.26	[a]	3.21	1.46	2.87
2-Phenylpropionic acid	–		–		[a]	3.93	–		–		–	

[a] Resolution too small to be calculated.

these ions and according to the dependence of the separation efficiency on mobility:

$$N = (\mu_{analyte} + \mu_{EOF})V/2D \quad (4)$$

where V = voltage, D = diffusion coefficient of the analyte, $\mu_{analyte}$ = electrophoretic mobility of the analyte and μ_{EOF} = electroosmotic mobility. When the EOF is reversed by Polybren as wall modifier the resolution is increased for 3-phenylbutyric acid with hydroxypropyl-α-cyclodextrin as selector.

The resolution of the enantiomers of 3-phenyllactic acid is slightly decreased, because the increase in efficiency is not as effective as the decrease in the mobility differences of the enantiomers. The effective electrophoretic mobility is strongly increased especially for this fast ion in the case of EOF reversal.

The resolution of chiral acids may be either enhanced or reduced with a cationic wall modification depending on the apparent mobility of the analytes because of the reciprocal influence of the migration time difference and peak broadening (variance σ) on resolution (eqn. 3). An increase in effective mobility causes a reduced migration time difference of the enantiomers but also a higher efficiency (reduced σ).

The apparent mobility of the analyte is dependent on the interaction with the selector. The influence of the adsorbed cationic coatings on the separation of the anionic analytes is therefore different for various cyclodextrins as selectors (see Tables II and III). The analysis time and resolution must be optimized for each analyte by selection of a suitable cyclodextrin derivative and by surface coating.

The influence of non-ionic hydroxylic surface modification on the separation of acids was investigated in capillaries coated with thermally immobilized PVA. The dynamic surface modification method which could be successfully applied to the separation of basic compounds by addition of PVA to the buffer does not lead to reproducible results at neutral or basic buffer pH.

A chiral separation of the test mixture of acidic chiral compounds performed at neutral pH in a capillary coated with thermally immobilized PVA was compared with separations performed in unmodified or cationic capillaries (see Fig. 7). With thermally immobilized PVA as an non-ionic hydroxylic coating the EOF is strongly reduced (compare Fig. 7B and A). The electrophoretic mobilities of the anions in the direction of the anode are predominant and the migration order of the analytes is reversed in comparison with separations performed in unmodified capillaries. The magnitude of the effective mobility, or migration time, is smaller as for separations performed in Polybren-coated capillaries, but a significant enhancement of efficiency is also

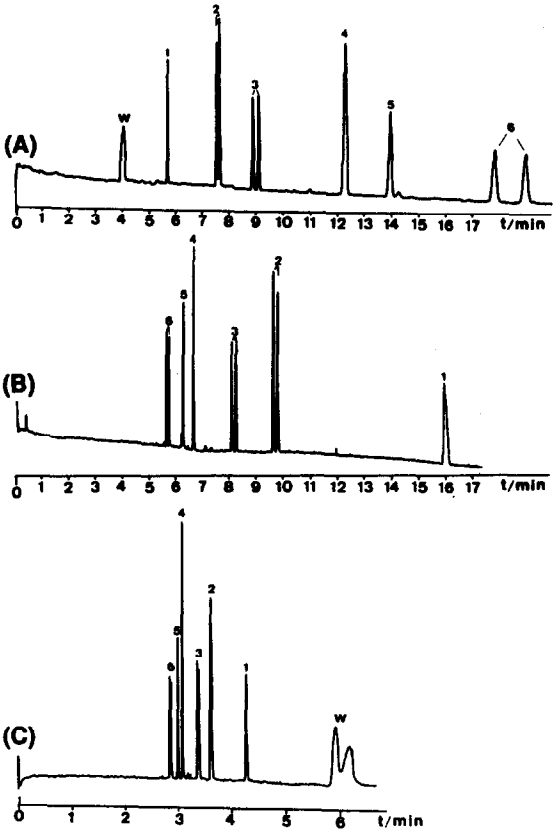


Fig. 7. Influence of a non-ionic- or a cationic-modified capillary on chiral separations of organic acids. A = bare fused-silica; B = thermally immobilized PVA; C = capillary preconditioned with 0.05% (w/w) Polybren in water. Voltage: (A) +35 kV, 55 μA; (B) −35 kV, 50 μA; (C) −35 kV, 52 μA. Samples: 1 = D,L-ibuprofen; 2 = D,L-2,4-dichlorophenoxypropionic acid; 3 = D,L-3-phenylbutyric acid; 4 = D,L-3-phenyllactic acid; 5 = D,L-2-phenylpropionic acid; 6 = D,L-mandelic acid; w = water. Other conditions as in Fig. 5.

observed. For these separations hydroxypropyl-γ-cyclodextrin was used as a chiral selector; the data derived from these separations are given in Table IV. The separation efficiencies for D,L-2,4-dichlorophenoxypropionic acid using capillaries coated with thermally immobilized PVA are significantly improved compared with those obtained in bare fused-silica capillaries (compare Fig. 7A and B). This observation cannot be explained as above by an effect of altered mobility of the analytes, because the effective mobility of these analytes is lower in the non-ionic modified capillary so that the opposite influence on efficiency is to be expected. The increase in efficiency in the PVA-coated capillary can therefore be explained by suppression of analyte–surface interactions in a similar manner to that for the chiral separation of bases. Interaction of anions with the acidic, negatively charged surface cannot, of course, be attributed to coulombic interactions. Other interaction mechanisms especially of the hydrophobic type have to be considered. A coating of the capillary may also cover inhomogeneities on the capillary surface such as micro-fissures and may therefore lead to an improvement of separation efficiency. Further experiments will be focused on these aspects.

Another practical feature of the capillary coat-

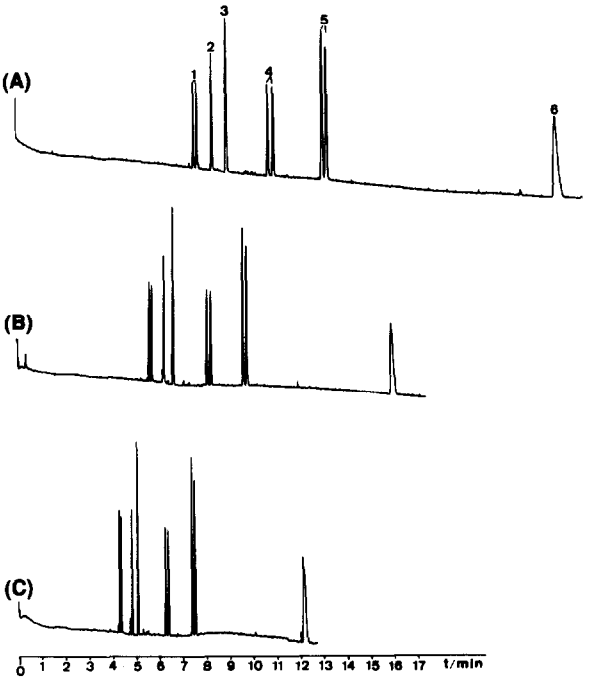


Fig. 8. Influence of temperature on chiral separations of organic acids performed in a capillary coated with thermally immobilized PVA. Samples: 1 = D,L-mandelic acid; 2 = D,L-2-phenylpropionic acid; 3 = D,L-3-phenyllactic acid; 4 = D,L-3-phenylbutyric acid; 5 = D,L-2,4-dichlorophenoxypropionic acid; 6 = D,L-ibuprofen. Conditions as in Fig. 7B. (A) 5°C; (B) 20°C; (C) 35°C.

TABLE IV

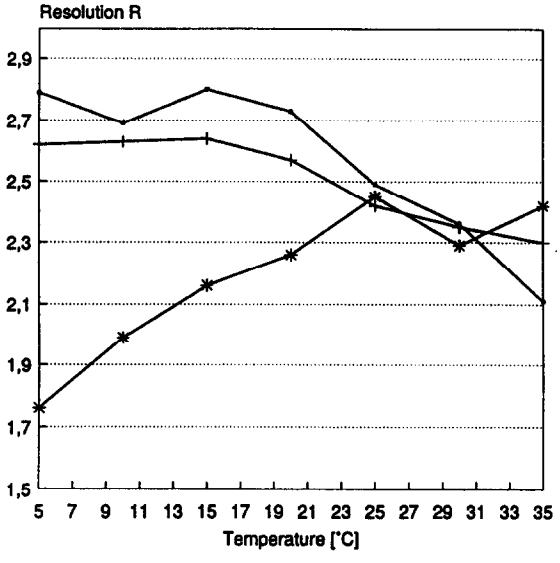
INFLUENCE OF CAPILLARY COATINGS ON CHIRAL SEPARATIONS OF ORGANIC ACIDS

Comparison of separations performed in an unmodified capillary, in a cationic-modified capillary and in a capillary coated with non-ionic hydroxylic PVA. Data evaluated from separations in Fig. 7.

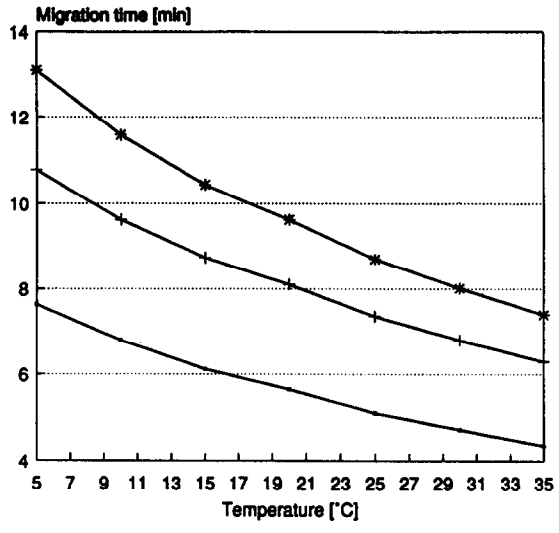
Capillary	Analyte	t_1 (min)	t_2 (min)	N_1 (theoretical plates)	N_2 (theoretical plates)	R_s	μ_{EOF} (10^{-9} $m^2/V \cdot s$)
Bare fused silica	DCPP	7.63	7.73	188 000	160 000	1.36	28
	Phenylbutyric acid	8.97	9.18	136 000	160 000	2.22	28
	Mandelic acid	17.85	18.99	69 000	65 000	4.02	28
Thermally immobilized PVA	DCPP	9.62	9.78	313 000	287 000	1.36	1.6
	Phenylbutyric acid	8.10	8.26	294 000	260 000	2.57	1.6
	Mandelic acid	5.66	5.77	328 000	318 000	2.73	1.6
Adsorbed Polybren coating	DCPP	3.64	3.66	–	–	[a]	−19
	Phenylbutyric acid	3.40	3.42	247 000	300 000	0.68	−19
	Mandelic acid	2.87	2.90	313 000	278 000	1.46	−19

[a] Resolution too small to be measured.

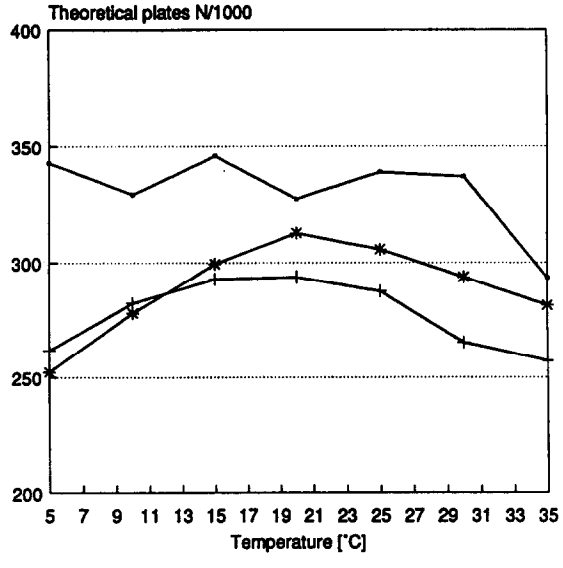
ings treated here is that contamination of the capillary walls by cyclodextrin buffers, which occurs in unmodified capillaries, is avoided. No rinsing or etching steps need to be applied to recondition the surface. However, with the adsorbed Polybren coating the capillary has to be preconditioned before each run by a rinsing step with Polybren solution to obtain reproducible EOF and migration times in series of runs. The cationic polymer cannot be added to the run



(a)



(b)



(c)

Fig. 9. (a) Influence of temperature on resolution of chiral separations of organic acids. Conditions as in Fig. 8. (b) Influence of temperature on migration time. (c) Influence of temperature on efficiency. ■ = Mandelic acid; + = 3-phenylbutyric acid; * = 2,4-dichlorophenoxypropionic acid. Capillary: thermally immobilized PVA.

buffer because of its UV absorbance at the detection wavelength of 210 nm.

Influence of temperature. The influence of temperature on chiral separations of organic acids can be assessed from the separations in Fig. 8. These measurements were again performed in capillaries coated with thermally immobilized PVA using hydroxypropyl-γ-cyclodextrin as chiral selector. An increase in temperature causes a decreased migration time owing to the temperature dependence of electrophoretic mobility. In Fig. 9 the influence of temperature on separation performance is demonstrated in more detail. For mandelic and 3-phenylbutyric acid the resolution decreases slightly with increasing temperature but for 2,4-dichlorophenoxypropionic acid the resolution increases at higher temperature. The influence of temperature on efficiency is not very pronounced. It should be noted that the relative standard deviation of the measurements of the number of theoretical plates is about 5–10%. For these separations it is advantageous to execute the measurements at elevated temperatures of up to 35°C because the analysis times are shorter and the performance of separation is not impaired.

CONCLUSIONS

Numerous basic drugs and organic acids could be baseline separated in cyclodextrin-modified CZE under optimized conditions. The performance of chiral separations is strongly influenced by alteration of the surface coating, variation of the type and concentration of selector and by the capillary temperature. Adsorbed Polybren as a cationic coating and thermally immobilized PVA as a permanent hydroxylic non-ionic coating are well suited to improve the resolution in analytical chiral separations of organic acids. In cationic-modified capillaries the EOF is reversed and the separation efficiencies may become much higher. In the PVA-coated capillaries the EOF is strongly reduced at pH 7 and the separation efficiencies of organic acids are also improved. The analysis time could be reduced by the use of these coatings for chiral separations of acidic compounds.

REFERENCES

1 S. Terabe, *Trends Anal. Chem.*, 8 (1989) 129.
2 J. Snopek, I. Jelinek and E. Smolkova-Keulemansova, *J. Chromatogr.*, 452 (1988) 571.
3 J. Snopek, H. Soini, M. Novotny, E. Keulemansova and I. Jelinek, *J. Chromatogr.*, 559 (1991) 215.
4 S. Fanali, *J. Chromatogr.*, 474 (1989) 441.
5 S. Fanali and P. Bocek, *Electrophoresis*, 11 (1990) 757.
6 S. Fanali, *J. Chromatogr.*, 545 (1991) 437.
7 M.J. Sepaniak, R.O. Cole and B.K. Clark, *J. Liq. Chromatogr.*, 15 (1992) 1023.
8 S.A.C. Wren and R.C. Rowe, *J. Chromatogr.*, 603 (1992) 235.
9 T.E. Peterson and D.J. Trowbridge, *J. Chromatogr.*, 603 (1992) 298.
10 R. Kuhn, F. Stoeckun and F. Erni, *Chromatographia*, 33 (1992) 32.
11 T. Ueda, F. Kitamura, R. Mitchell, T. Metcalf, Th. Kuwana and A. Nakamoto, *Anal. Chem.*, 63 (1991) 2979.
12 A. Pluym, W. van Ael and M. de Smet, *Trends Anal. Chem.*, 11 (1992) 27.
13 H. Nishi, T. Fukuyama and S. Terabe, *J. Chromatogr.*, 553 (1991) 503.
14 A. Guttmann, A. Paulus, A. Cohen, N. Grinberg and B. Karger, *J. Chromatogr.*, 448 (1988) 41.
15 M.W.F. Nielen, *Anal. Chem.*, 65 (1993) 885.
16 S. Hjertén, *J. Chromatogr.*, 347 (1985) 191.
17 J. Kohr and H. Engelhardt, *J. Microcol. Sep.*, 4 (1992) 233.
18 M. Huang, W.P. Vornink and M.L. Lee, *J. Microcol. Sep.*, 4 (1992) 233.
19 G.J.M. Bruin, J.P. Chang, R.H. Kuhlmann, K. Zegers, J.C. Kraak and H. Poppe, *J. Chromatogr.*, 471 (1989) 429.
20 W. Nasbeth and Z. El Rassi, *J. Chromatogr.*, 559 (1991) 376.
21 J.K. Towns and F.E. Regnier, *J. Chromatogr.*, 516 (1991) 69.
22 J.A. Bullocj and L.C. Yan, *J. Microcol. Sep.*, 3 (1991) 241.
23 A. Emmer, M. Jansson and J. Roerade, *J. High Resolut. Chromatogr.*, 14 (1991) 738.
24 J.E. Wiktorowicz and J.C. Colburn, *Electrophoresis*, 11 (1990) 769.
25 H. Lindner, W. Hellinger, A. Dirschlmayer, M. Jaquemar and B. Puschendorf, *Biochem. J.*, 283 (1992) 467.
26 J.K. Towns and F.E. Regnier, *Anal. Chem.*, 63 (1991) 1126.
27 J.K. Towns, J. Bao and F.E. Regnier, *J. Chromatogr.*, 559 (1992) 227.
28 M. Gilges, H. Husmann, M.H. Kleemiss, St.R. Motsch and G. Schomburg, *J. High Resolut. Chromatogr.*, 15 (1992) 452.
29 D. Belder and G. Schomburg, *J. High Resolut. Chromatogr.*, 15 (1992) 686.
30 M. Gilges and G. Schomburg, presented at the *17th International Symposium on Column Liquid Chromatography, Hamburg, Germany, 1993.*

31 E. Ziegler, D. Henneberg and G. Schomburg, *Anal. Chem.*, 42 (1970) 51A.
32 J.A. Lux, U. Häusig and G. Schomburg, *J. High Resolut. Chromatogr.*, 13 (1990) 374.
33 W. Schutzner and S. Fanali, *Electrophoresis*, 13 (1992) 687.
34 H. Husmann, E. Hübinger, A. Deege, F. Kobor and G. Schomburg, presented at the *15th International Symposium on Capillary Chromatography, Riva del Garda, Italy, 1993.*
35 G. Schomburg, in preparation.
36 A. Nardi, A. Elisseev, P. Bocek and S. Fanali, *J. Chromatogr.*, 638 (1993) 247.
37 Y.Y. Rawjee, D.U. Staerk and G. Vigh, *J. Chromatogr.*, 635 (1993) 291.
38 M.W.F. Nielen, *J. Chromatogr.*, 637 (1993) 81.
39 S. Terabe, K. Ishiwaka, A. Otsuka, A. Tsuchiya and S. Ando, presented at the *26th International LC Symposium, Kyoto, Japan, 1983.*
40 K.D. Altria and C.F. Simpson, *Anal. Proc.*, 23 (1986) 453.
41 K.D. Altria and C.F. Simpson, *Anal. Proc.*, 25 (1988) 85.
42 T. Tsuda, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 10 (1987) 622.
43 X. Huang, J. Luckey, M.J. Gordon and R.N. Zare, *Anal. Chem.*, 61 (1989) 766.
44 P. Jandik and W.R. Jones, *J. Chromatogr.*, 546 (1991) 431.
45 W.R. Jones and P. Jandik, *J. Chromatogr.*, 546 (1991) 445.
46 J.K. Towns and F.E. Regnier, *J. Chromatogr.*, 516 (1990) 69.
47 J.T. Smith and Z. El Rassi, *J. High Resolut. Chromatogr.*, 15 (1992) 573.
48 C.S. Lee, C.T. Wu, T. Lopes and B. Patel, *J. Chromatogr.*, 559 (1991) 133.
49 St.R. Motsch and G. Schomburg, presented at the *5th International Symposium on High Performance Capillary Electrophoresis, Orlando, FL, USA, 1993.*
50 M. Gilges and G. Schomburg, in preparation.
51 J. Boger, R.J. Corcoran and J.-M. Lehn, *Helv. Chim. Acta*, 61 (1978) 2190.