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Use of chiral and achiral ion-pairing reagents in combination with cyclodextrins in capillary electrophoresis

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Abstract

The suitability of chiral and achiral acidic ion-pairing reagents to effect chiral separation in capillary electrophoresis in the presence of cyclodextrins (CDs) has been demonstrated previously. This method was applied to a great variety of analytes. Besides alkylsulfonates, alkanoic acids are able to improve chiral resolution in combination with CD. The dependence of the separation on alkyl chain length is described. The use of the cationic ion-pairing reagent quinine, is a powerful tool in influencing enantiomeric separation of acidic and basic analytes. Methods of direct and indirect UV detection are used in quinine containing running buffers. The influences of pH value, quinine and CD concentration on the separation factors are reported. L-Hyoscyamine showed the best capability to improve the enantioseparation of propranolol. © 1998 Elsevier Science B.V.

Keywords: Enantiomer separation; Ion-pairing reagents; Cyclodextrins; Organic acids; Cyclodrine

1. Introduction

Cyclodextrins are widely used as additives to the mobile phase and above all as chiral stationary phases (CSP) for enantiomeric separations in high-performance liquid chromatography (HPLC). Separations of ionic analytes with ion-pairing reagents (IPRs) are well known from HPLC [1–8]. Chiral IPRs also have the ability to separate racemates by forming diastereomeric ion pairs. These diastereomeric ion pairs are temporarily bonded strongly or weakly to the stationary phase or show a different solvatation in the mobile phase [9]. The basis for forming an ion pair is an apolar medium (represented in HPLC by the reversed-phase). In capillary electrophoresis (CE) this requirement is not given a priori. CD have apolar cavities and can include compounds into their central cavity. The stability of this inclusion complex depends on the interaction between the compound and the cavity. Based on the assumption that the inclusion of a diastereomeric ion pair can occur in the apolar CD cavity, their supply in combination with a second chiral selector (the IPR) seems to be as an interesting possibility for enantiomeric separations by CE.

Diastereomeric ion pairs in nonaqueous medium in CE have already been described [10,11]. In this study the usefulness of various anionic, cationic chiral and achiral IPRs with various cyclodextrins for enantiomeric separations by CE was investigated.

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2. Experimental

2.1. Instrumentation

CE was performed using a P/ACE 2100 capillary electrophoresis instrument (Beckman, Fullerton USA) equipped with an on-column UV-detector. GOLD software (Beckman) was used for data acquisition.

Fused-silica capillaries (eCAP capillary tubing 37/30 cm×50 μm I.D.×375 μm O.D. (Beckman) and neutrally coated capillary (eCAP neutral capillary 37/30 cm×50 μm I.D.×375 μm O.D. (Beckman) were used.

2.2. Chemicals

Hydroxypropyl-β-CD, methyl-β-CD, β-CD, γ-CD were obtained from Wacker (Munich, Germany). $Na(CH_3COO)$, NaH_2PO_4, Na_2HPO_4 and NaOH were purchased from Laborchemie Apolda (Apolda, Germany). Alkanoic acids were obtained from Bergakademie Freiberg (Germany). Alkane sulfonic acids were obtained from Serva (Heidelberg, Germany). Sodium cyclamate was a friendly gift from Ankerwerk (Rudolstadt, Germany). Water was doubly distilled.

Cyclopentolate was from cyclopentolate 0.5% eye drops (Alcon Thilo, Freiburg, Germany). Acetymandelic acid, doxylamine, homatropine–HBr, pheniramine maleate, β-phenyllactic acid and prilocaine–HCI were purchased from Sigma (St. Louis, MO, USA). Atrolactic acid, 3-hydroxy-4-methoxymandelic acid (3-Hy-4-MO-MA), 4-hydroxy-3-methoxymandelic acid (4-Hy-3-MO-MA), 3-(4-hydroxyphenyl)lactic acid, 2-methoxy-2-phenylacetic acid, tropic acid were from Aldrich (Steinheim, Germany), *p*-brommandelic acid, 2-phenylbutyric acid, 2-phenylpropionic acid from Fluka (Neu-Ulm, Germany), α-bromphenylacetic acid, mandelic acid from Merck-Schuchardt (Hohenbrunn near Munich, Germany).

Analytes were obtained from various factories. We thank the following companies for friendly support: biperidine–HCl (Merckle, Blaubeuren, Germany), brompheniramine (Kreussler Pharma, Wiesbaden, Germany), bupivacaine (Astra, Södertalje, Sweden), butamirate (Zyma, Munich, Germany), butetamate (Chemische Werke Hommel, Munich, Germany), clemastine hydrogenfumarate (Sandoz, Basel, Switzerland), α-(1-hydroxycyclopentyl)-phenylacetic acid and cylodrine (Ankerwerk), dipiproverine (Isis-Chemie, Zwickau, Germany), disopyramide (Albert-Roussel, Wiesbaden, Germany), terbutaline sulfate (Merckle), phenprocoumone (Hoffmann–La Roche, Grenzach-Wyhlen, Switzerland), propranolol–HCl (Isis-Chemie).

2.3. Methods

Conditions are described in the legends of the figures and tables. The analytes were dissolved in doubly distilled water or 2-propanol (1 mg/ml). Samples were injected hydrodynamically (0.5 p.s.i., 3 s). The uncoated capillary was rinsed with 0.1 *M* NaOH for 2 min prior to each analysis.

3. Results and discussion

3.1. Anionic ion-pairing reagents

Previous investigations into CE with an aqueous milieu [12,13] showed better enantiomeric separations with CD by adding camphersulphonic acid (CSA) as the ion-pairing reagent. Chiral separation of analytes containing a dialkylaminoethanol structure could be achieved by adding (*S*)-(+)-CSA to a β-CD containing buffer solution. The increased ion strength of the buffer solution was excluded as reason for the separation observed. The formation of diastereomeric ion pairs cannot be the reason for the chiral separation, because enantiomeric separation is also achieved with (+)- and (−)-CSA in the molar ratio 1:1 [14]. This result led us to investigate the usability of achiral ion-pairing reagents (alkane sulfonic acids, sodium cyclamate). Alkane sulfonic acids and sodium cyclamate cause an improvement in selectivity and resolution of the chiral separation in the same way (Fig. 1).

These ion-pairing reagents could be applied to influence the chiral separation of a great variety of other basic and acidic analytes (Table 1).

It seemed to be promising to examine the ability of alkanoic acids to affect chiral resolution in combination with cyclodextrins. Indeed, alkanoic

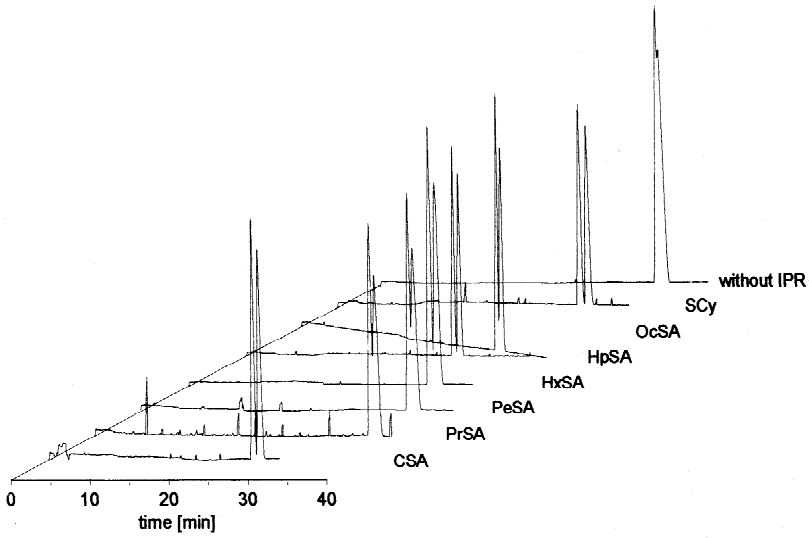

Fig. 1. Electropherograms of cyclodrine with different buffer additives 100 m*M* PB, 1.8% β-CD, 40 m*M* alkanoic acid, pH 2; 214 nm; 35°C; 12 kV; uncoated capillary 57/50 cm×75 μm.

acids can influence the electrophoretic behaviour of basic analytes in the presence of CD (Table 1). The application of achiral ion-pairing reagents (alkanoic- and alkanesulfonic acids, sodium cyclamate) can effect better separations (see Table 1). Interestingly a decrease of separation factors was observed with some analytes.

Depending on the structure of the ion-pairing reagent different separation factors are obtained (see [14]). The best separations are gained by adding CSA, sodium cyclamate and hexanesulfonic acid to the buffer.

Universally valid rules for chiral separation depending on the analyte structure, the structure of IPR and CD-type are not recognizable.

The separation factor ($\alpha = t_2/t_1$) depends on the alkyl chain lengths of the alkanesulfonic acid [14] and the alkanoic acids (Fig. 2). It is assumed that the more hydrophobic alkanoic acids with longest alkyl chains are better included in the CD cavity, resulting in a stronger displacement of the analyte from the CD cavity. The altered position of the guest molecule in the CD leads to a change of chiral recognition.

3.1.1. Influence of pH value, CSA and CD concentrations

The investigations mentioned above were performed at pH 2 in an uncoated capillary. The ionic interaction is assumed to be diminished by increasing the pH value, thus causing a poorer separation. The separation factors (e.g. for cyclodrine) were decreased with increased pH, supporting the above assumption (Fig. 3). However, due to the electrosmotic flow (EOF) at higher pH values the change of the separation factors is not only related to the pH dependence of the interaction with the IPR.

To compare the pH dependence the investigations were repeated in a coated capillary, where the EOF was suppressed. A decreasing separation factor by increasing the pH was confirmed, showing that this effect is due to the weaker interaction with IPRs (see Fig. 3).

A comparison of selectivity between the uncoated and the coated capillary is not possible because of the different lengths and inner diameters of the two capillaries.

The separation factor passes through an optimum depending on the CSA concentration both in the uncoated and the coated capillary. The reduction of migration times observed in the presence of CD with increasing CSA concentrations seems to be unusual. A different curve form is given when plotting the migration times against the CSA concentrations without addition of CD to the buffer solution. At a CSA concentration of about 40 m*M* there is no

Table 1
Influence of various ion-pair reagents (IPR) on separation of basic and acidic analytes in the presence of CD

Analyte	Cyclodextrin	IPR	
Basic analytes	(100 m*M* PB+1.8% CD+40 m*M* IPR (pH 2); 12 kV; 35°C; uncoated capillary 57/50 cm; 75 μm		
Cyclodrine	β-CD	CSA, PrSA, PeSA, HxSA, HpSA, OcSA, SCy	+
	Me-β-CD	CSA	+
Cyclopentolate	β-CD	CSA, PrSA, PeSA, HxSA, HpSA, OcSA, SCy	+
	Me-β-CD	CSA	+
Butetamate	β-CD	CSA, PrSA, PeSA, HxSA, SCy	+
Prilocaine	Me-β-CD	CSA	−
Bupivacaine	Me-β-CD	CSA, HxSA	−
Disopyramide	Me-β-CD	CSA	−
Brompheniramine	β-CD	HxSA	+
Doxylamine	β-CD	CSA, PrSA, PeSA, HxSA, HpSA, SCy	−
Basic analytes	(100 m*M* PB+1.8% CD+40 m*M* IPR (pH 7.2); 12 kV; 25°C; uncoated capillary 57/50 cm; 75 μm)		
Biperidene	β-CD	HxA, HpA, OcA	−
	γ-CD	OcA	+
Brompheniramine	β-CD	HxA, HpA, OcA	−
	HP-β-CD	OcA	+
Bupivacaine	Me-β-CD	HxA, HpA, OcA	−
	γ-CD	OcA	+
Cyclodrine	β-CD	HxA, HpA, OcA	+
	HP-β-CD	HxA, HpA, OcA	+
Cyclopentolate	β-CD	HxA, HpA, OcA	+
	HP-β-CD	HxA, HpA, OcA	+
	Me-β-CD	OcA	+
	γ-CD	HpA	+
Dipiproverine	HP-β-CD	HxA, HpA, OcA	−
	Me-β-CD	HxA, HpA, OcA	+
Homatropine	β-CD	HxA, HpA	−
	HP-β-CD	HxA, HpA, OcA	−
	Me-β-CD	HxA, HpA, OcA	−
Pheniramine	β-CD	HpA, OcA	−
	γ-CD	OcA	+
Terbutaline	β-CD	HxA, HpA, OcA	−
	HP-β-CD	OcA	−
	Me-β-CD	OcA	−
Acidic analytes	(100 m*M* PB+1.8%-CD+40 m*M* IPR (pH 6.9); 12 kV; 35°C; uncoated capillary 57/50 cm; 75 μm)		
α-(1-Hydroxycyclo-	β-CD	CSA, HxSA	+
pentyl)-	Me-β-CD	CSA	−
phenylacetic acid	γ-Cd	CSA	−
Phenprocoumone	Me-β-CD	CSA	+
	HP-β-CD	CSA	+

Abbreviations: CSA, camphersulfonic acid; PrSA, propanesulfonic acid; PeSA, pentanesulfonic acid; HxSA, hexanesulfonic acid; HpSA, heptanesulfonic acid; OcSA, octanesulfonic acid; SCy, sodium cyclamate; HxA, hexanoic acid; HpA, heptanoic acid; OcA, octanoic acid. Separation factor: +...increased, −...decreased)

change in the mobility of the analytes. Considering these results the alteration of migration times seems to be partly based on the interaction between ion-pairing reagent and CD. The separation is improved with increased concentrations of CD presumably caused by an enhanced interaction of the analyte with CD.

Selectivity decreased with increasing temperatures. The more unstable cyclodextrin inclusion complex on the one hand and the diminished inter-

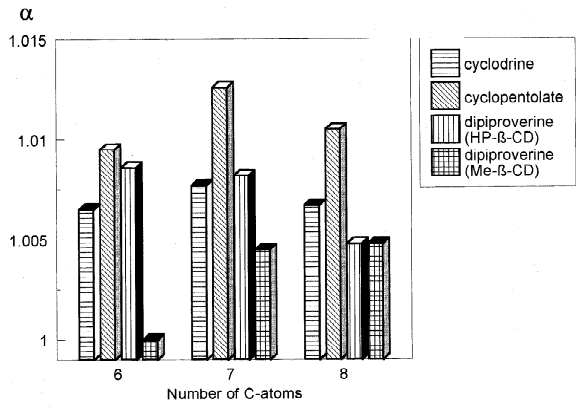

Fig. 2. Dependence of the separation factor α on the alkyl chain length of alkanoic acid 100 m*M* PB, 1.8% HP-β-CD, 40 m*M* alkanoic acid, pH 7.2; 214 nm; 35°C; 12 kV; uncoated capillary 57/50 cm×75 μm.

action between IPR, analyte and CD on the other is thought to be responsible for that.

3.2. Cationic ion-pairing reagents for separation of acidic and basic analytes

Quinine is a well known ion-pairing reagent in HPLC. The ability of quinine to affect chiral separations in CE was examined by trying to resolve various acids (Table 2). Due to the UV absorbance of quinine, detection of the analytes was difficult or impossible. To avoid this problem a method — analogous to the counter-current flow-method (see Valtcheva et al. [15] and Chankvetadze et al. [16]) was chosen, where only quinine-free buffer was placed in the detection window. The analytes migrated to the anode at the capillary outlet, where the detection window was located. The quinine containing buffer was rinsed close to the detection window into the capillary. The quinine molecules migrated back to the capillary inlet, so that they did not reach the detector. No EOF was allowed, since it would move the analyte and quinine in the same direction to the cathode. Therefore a coated capillary was used to suppress the EOF.

By adding quinine to a β- and γ-CD containing buffer the separation of acids was achieved (Table 2). No separation was observed without quinine.

Likewise in the investigations with acidic ion-pairing reagents the sole responsibility of the increased buffer ion strength for the separation was excluded by replacing quinine with NaCl at the same ion strength.

Cationic ion-pairing reagents are able to influence the chiral separation of cationic analytes, but is it not an ion-pairing mechanism. The separation of propranolol can be improved, if the buffer system contains (*S*)-hyoscyamine and β-CD (Fig. 4), HP-β-CD or γ-CD.

In contrast to the acidic ion-pairing reagents a slow migration is shown by using L-hyoscyamine. At a pH value of 2.5 the analyte and the IPR exist in charged form and under the influence of an electric field both substances migrated in the same direction. The direct detection of propranolol in the presence of the IPR was possible at a wavelength of 280 nm, where the absorbance maximum of propranolol [(*S*)-hyoscyamine shows only a weak absorbance].

Further investigations were carried out with

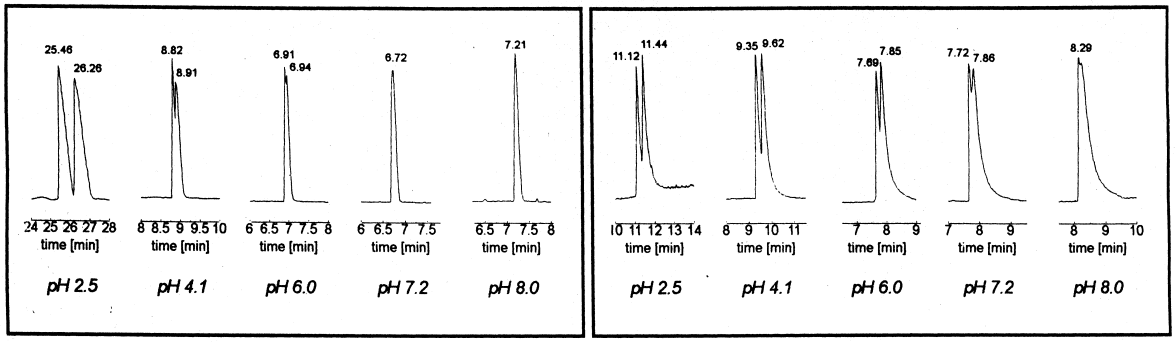

Fig. 3. Separation of cyclodrine in dependence on the pH value in an uncoated capillary (left) and a coated capillary (right) 100 m*M* PB, 1.8% β-CD, 40 m*M* (*R*)-(−)-CSA; 214 nm; 35°C; 12 kV.

Table 2
Influence of quinine on separation of acidic analytes in the presence of various CDs

Analyte	β-CD	Me-β-CD	HP-β-CD	γ-CD
Acetylmandelic acid	−	−	−	
Atrolactic acid	+	−	−	+
p-Brommandelic acid	+	−	+	+
α-Bromphenylacetic acid	+			+
α-(1-Hydroxycyclopentyl)-phenylacetic acid	−	−	−	+
3-Hydroxyγ-4-methoxymandelic acid	+	−	−	+
4-Hydroxy-3-methoxymandelic acid				+
3-(4-Hydroxyphenyl)lactic acid	−	−	−	
Mandelic acid	+			+
2-Methoxy-2-phenylacetic acid				
2-Phenylbutyric acid	+	−	−	+
β-Phenyllactic acid	−	−	−	
2-Phenylpropionic acid	−	−	−	
Tropic acid		−	−	

Separation factor: +...increased, −...decreased, ...no change).
Buffer: B1:100 m*M* AcB+15 m*M* CD+40 m*M* quinine–HCl (pH 5). B2: 100 m*M* AcB (pH 5) neutral coated capillary (44/37 cm×50 μm) voltage: −15 kV; temperature: 25°C; detection: 214 nm.

quinine in combination with β-CD and γ-CD for various cationic analytes, which were not directly detectable in the quinine-containing buffer solution like propranolol (amphetaminil, bamethane, brompheniramine, bupivacaine, dipiproverine, etilefrine, homatropine, isoprenaline, norfenefrine, norpseudoephedrine, pheniramine, pholedrine and terbutaline).

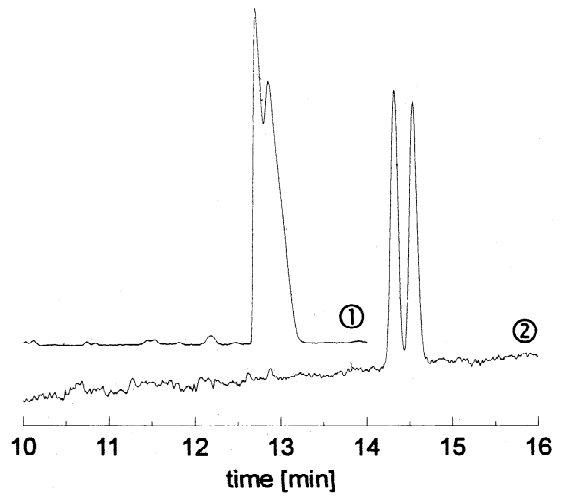

Fig. 4. Electropherograms of propranolol 50 m*M* PB+20 m*M* Me-β-CD; pH 2.5 (1)+10 m*M* L-hyoscyamine–HBr (2) 280 nm; 25°C; 12 kV; uncoated capillary 37/30 cm×50 μm.

Quinine shows a UV maximum at a wavelength of 333 nm. None of the cationic analytes investigated have absorbance maxima at this wavelength and the method of indirect UV-detection, used in HPLC and in CE [17–20] as well, is practicable. The detection of the separated analytes was possible as negative peaks. In this system quinine is both the chiral selector in combination with CD and the UV-absorbing part in the buffer. In all the electropherograms there is a positive system peak with the same migration time as that of quinine in an analoguos buffer system without quinine. Chiral separations of the other basic analytes were not influenced through quinine, considering the results on the separation of propranolol with hyoscyamine as IPR.

3.3. Influence of pH value, quinine and CD concentrations

The concentration of both quinine (Fig. 5) and CD influence the selectivity of the separation of the acidic analytes. An optimum CD concentration exists for some of the acids (Fig. 6). Such behaviour of the separation factor using CD as chiral selector has

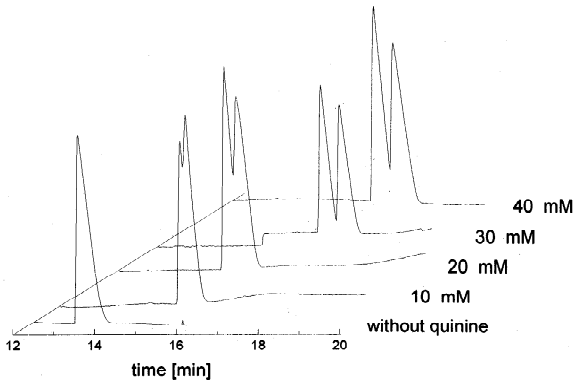

Fig. 5. Electropherograms of 2-phenylbutyric acid with different concentrations of quinine 100 m*M* acetate buffer (AcB)+15 m*M* γ-CD+quinine–HCl (pH 5.0); 214 nm; 25°C; −15 kV; neutrally coated capillary 44/37 cm×50 μm.

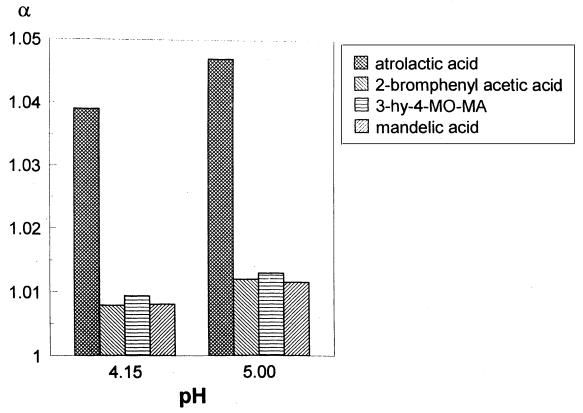

Fig. 7. Dependence of the separation factor α on the pH value 100 m*M* acetate buffer (AcB)+15 m*M* γ-CD+40 m*M* quinine–HCl; 214 nm; 25°C; −15 kV; neutrally coated capillary 44/37 cm×50 μm.

been described several times [21–24]. Due to the weaker ionic interactions of the analytes with the quinine a pH value of 5.0 (Fig. 7) is favoured for the enantiomeric separation. A further reduction of the pH value affects the neutral form of the analytes and they are not detectable. Because of the weak solubility of quinine, investigations at higher pH values were not possible.

3.4. Separation mechanism

The existence of diastereomeric ion pairs, which could explain the better separations by different inclusions of these diastereomers in the CD cavity, is excluded by using achiral ion-pairing reagents. If the separation mechanism is assumed to be related to an enhanced inclusion of the analyte in the CD by ion-pairing with achiral IPR, the inclusion of both enantiomers would be increased to the same extent, because no enantioselective ion-pairing with only one enantiomer of the analyte is imaginable. An increased inclusion would lead to higher migration times, however a shortening of migration times was observed by adding IPR. It was only by using γ-CD that increased migration times were obtained. The CD cavity may be too small for pure analytes and only the inclusion of the diastereomeric complex leads to a chiral discrimination. The reduction of migration times rather indicates a displacement of the analyte from the CD cavity, raising the question as to how the enantioselectivity of the interaction of CD and analyte can increase simultaneously. Probably, the IPR alters the position of the analyte molecule by its own inclusion in the CD cavity, so that the chiral C atom could reach a better position for its chiral recognition.

A nonenantioselective formation of ion pairs leading to better inclusion of the guest molecule in the apolar form of an ion pair is not imaginable either due to the change of selectivity for the separation of anionic analytes in the presence of

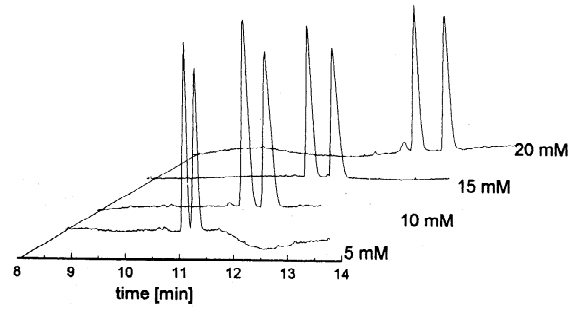

Fig. 6. Electropherograms of 2-phenylbutyric acid with different concentrations of γ-CD 100 m*M* acetate buffer (AcB)+γ-CD+40 m*M* quinine–HCl, pH 5.0; 214 nm; 25°C; −15 kV; neutrally coated capillary 44/37 cm×50 μm.

anionic IPR and the influence of cationic IPR on the separation of cationic analytes. This fact also suggests the displacement of the analyte from the CD cavity by the IPR.

The true location of analyte and IPR in CD could not be established to date so the real mechanism of separation cannot be characterized more precisely.

4. Conclusions

The use of anionic/cationic chiral and achiral ion-pairing reagents seems to be a promising new method of separating acidic and basic enantiomers in CE. The separation of the analytes depends on the type of CD and IPR used, the pH value and the concentrations of IPR and CD. Further investigations into these problems are under way.

Acknowledgements

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