ELSEVIER

Journal of Chromatography A, 922 (2001) 303–311

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Non-aqueous capillary electrophoretic separation of enantiomeric amines with (−)-2,3:4,6-di-*O*-isopropylidene-2-keto-L-gulonic acid as chiral counter ion

Ylva Carlsson[a], Mikael Hedeland[b], Ulf Bondesson[a,b], Curt Pettersson[a,*]

[a]*Division of Analytical Pharmaceutical Chemistry, Uppsala University, Biomedical Centre, Box 574, SE-751 23 Uppsala, Sweden*
[b]*Department of Chemistry, National Veterinary Institute (SVA), SE-751 89 Uppsala, Sweden*

Received 28 November 2000; received in revised form 17 April 2001; accepted 26 April 2001

Abstract

(−)-2,3:4,6-Di-*O*-isopropylidene-2-keto-L-gulonic acid [(−)-DIKGA] has been introduced as a chiral counter ion in non-aqueous capillary electrophoresis. High enantioresolutions ($R_S \geq 3$) were obtained for amines, e.g., pronethalol, labetalol and bambuterol. Methanol containing NaOH and (−)-DIKGA was used as the background electrolyte. The counter ion concentration and the nature of the injection medium were found to affect the chiral separation. Covalent coating of the fused-silica capillary reduced the electro-osmotic flow resulting in improved enantioresolutions.

Keywords: Non-aqueous capillary electrophoresis; Enantiomer separation; Background electrolyte composition; Amines; Diisopropylideneketogulonic acid; Labetalol

1. Introduction

Chiral mobile phase additives (CMPA) have been used for several years in liquid chromatography for direct separation of enantiomers [1]. The separation is based on formation of reversible diastereomeric complexes. Enantioselective retention can be obtained by different formation constants of the diastereomeric complexes in the mobile phase and/or different affinity of the diastereomeric complexes for the stationary phase. A chiral selector dissolved in the mobile phase can also be adsorbed to the stationary phase giving rise to a dynamically coated chiral stationary phase (CSP) [1,2].

In capillary electrophoresis (CE) direct enantiomeric separation is only promoted by enantioselective interaction with a chiral selector in the background electrolyte (BGE), i.e. stereoselective formation of diasteromeric complexes. CE exhibit some advantages over high-performance liquid chromatography (HPLC) such as higher efficiency, which gives higher resolution and also in many cases, faster analysis [3]. A chiral selector often promotes high enantioselectivity for a limited number of solutes, but may for a large number of compounds with similar structure give rise to low enantioselectivity making it difficult or impossible to obtain an enantioseparation in HPLC. A chiral selector applied in CE,

*Corresponding author. Tel.: +46-184-714-340.
E-mail address: curt.pettersson@farmkemi.uu.se (C. Pettersson).

0021-9673/01/$ – see front matter
PII: S0021-9673(01)00925-6

should due to the high separation efficiency, give a more general enantiomeric separation system than in HPLC, provided the separation is based on the same mechanism. Equilibration time at changes between different mobile phase conditions, e.g., selectors or selector concentration is not necessary in CE, which makes it easy to evaluate new chiral selectors as well as to optimize the separation. Furthermore, the operation in CE is less expensive owing to lower consumption of solvents and chiral selectors than in HPLC.

Most of the chiral selectors applied in CE have previously been used as CMPAs in HPLC, e.g., quinine [4], (+)-(*S*)-10-camphorsulphonic acid [1], albumin [5], α_1-acid glycoprotein [6], cellobiohydrolase I [7,8] and cyclodextrins [9].

Enantioselective separation systems using a chiral counter ion in CE as well as in HPLC have been based on organic solvents to promote a high degree of ion-pair formation, due to lower dielectric constants. Quinine has been used in CE for separation of enantiomers of *N*-3,5-dinitrobenzoyl amino acids and carboxylic acids. The BGE was quinine dissolved in methanol containing ammonium acetate [10]. Bjørnsdottir et al. have achieved enantioresolution of 15 alkaline drugs with (+)-(*S*)-10-camphorsulphonate dissolved in acetonitrile [11]. Non-aqueous media in capillary electrophoresis was first exploited by Walbroehl et al. in 1984 [12] for the separation of a number of quinoline-type compounds. Several advantages have been ascribed to organic solvents compared to pure water as electrophoretic separation media, e.g., more selective solvation properties, different selectivities for protolytes due to shifts in dissociation constants and the possibility to analyze water insoluble compounds [13].

The aim of this study was to introduce (−)-2,3:4,6-di-*O*-isopropylidene-2-keto-L-gulonic acid [(−)-DIKGA] as a chiral selector for amines in non-aqueous capillary electrophoresis. A further objective was to compare enantioseparations in CE with the previously observed chiral separations with (−)-DIKGA in HPLC [14]. This investigation may elucidate enantioselective equilibria promoted by (−)-DIKGA. The importance of counter ion concentration and the injection medium on the chiral separation was also studied.

The possibility to suppress the electro-osmotic flow (EOF) and improve the enantioseparation in the non-aqueous BGE by chemical modification of the capillary was investigated.

2. Materials and methods

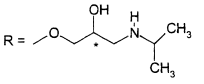
2.1. Chemicals

The chiral counter ion (−)-DIKGA was obtained from Fluka (Buchs, Switzerland) and methanol (HPLC grade) was from Fischer scientific (Loughborough, UK).

3-aminopropyltriethoxysilane (98%), γ-methacryloxypropyltrimethoxysilane (98%), mesityloxide, *rac*-propranolol hydrochloride, (*R*)- and (*S*)-propranolol hydrochloride were purchased from Sigma (St Louis, MO, USA). *rac*-Atenolol hydrochloride, *rac*-alprenolol benzoate, *rac*-*p*-hydroxyalprenolol hydrochloride, *rac*-metoprolol tartrate and *rac*-oxprenolol hydrochloride were generous gifts from Astra Hässle (Mölndal, Sweden). *rac*-Bupivacaine hydrochloride and *rac*-remoxipride hydrochloride were gifts from Astra Arcus AB (Södertälje, Sweden) and *rac*-bambuterol hydrochloride was a gift from Astra Draco (Lund, Sweden). Toluene, acetone, NaOH and HCl (all analytical-reagent grade) were from Merck (Darmstadt, Germany). The coating chemicals acrylamide (100%), ammonium persulfate and *N′,N′,N,N*-tetramethylethylenediamine (TEMED) were obtained from Bio-Rad Labs. (Hercules, CA, USA). *rac*-Pronethalol hydrochloride was purchased from ICI (Macclesfield, UK) and *rac*-labetalol hydrochloride (*RR*/*SS* and *RS*/*SR*) were from Glaxo (Greenford, UK). Ethanol (96%) was obtained from Kemetyl (Stockholm, Sweden). All other reagents used were of analytical grade or better. The water used was purified in a Milli-Q Water system (Millipore, Bedford, MA, USA). Solute structures are shown in Fig. 1.

2.2. Equipment

The CE instrument used in the experiments was a Beckman P/ACE system 2050 with UV detection (Beckman, Fullerton, CA, USA) and a System Gold 7.11 unit for instrument control and data collection. Fused silica capillaries (50 μm I.D.×365 μm O.D.)



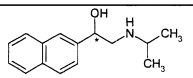
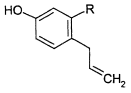
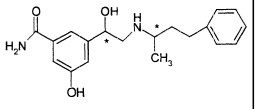
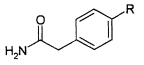
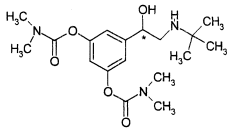
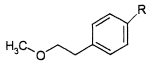
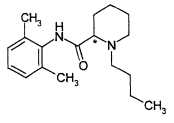
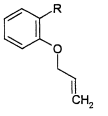
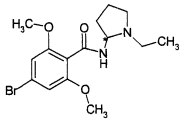
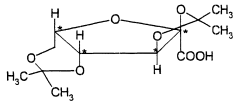
Substance	Formula	Substance	Formula
1. Alprenolol		7. Pronethalol	
2.p-OH-Alprenolol		8. Labetalol	
3. Atenolol		9. Bambuterol	
4. Metoprolol		10. Bupivacaine	
5.Oxprenolol		11. Remoxipride	
6. Propranolol		12. (-)-2,3:4,6-di-O-isopropyl-idene-2-keto-L-gulonic acid (DIKGA).	

Fig. 1. Solutes (Nos 1–11) and counter ion structures (No. 12).

were obtained from MicroQuartz (Munich, Germany). A vacuum suction device, Piab Lab Vac (Täby, Sweden) and a Constametric 4100 MS HPLC pump (Spectra-Physics, CA, USA) were used for the capillary coating.

2.3. Procedures

2.3.1. Capillary coating procedure

The capillaries were cut at a length of 48 cm and approximately 0.2 cm of the polyimide coating was burned off for the detection window 8 cm from the outlet end of the capillary. In order to minimize sample carry over, approximately 0.1 cm of the polyimide coating was burned off at the inlet end. Before use, the new uncoated capillaries were conditioned with NaOH (0.1 *M*), water, HCl (0.1 *M*) and water, 5 min each (20 p.s.i.; 1 p.s.i.=6894.76 Pa). For the polyacrylamide-coated capillaries, a coating procedure previously published [15] was used. The aminopropyl-coated capillaries were coated according to [16] with some modifications. Before the coating procedure the capillaries were conditioned with water, NaOH (0.1 *M*), water, HCl (0.1 *M*), water again, acetone and finally with dried toluene. 3-Aminopropyltriethoxysilane was mixed with dried toluene (1:9). This reagent was flushed into the capillary for 15 min by vacuum suction, and after that, the capillaries were placed in an oven at 100°C for 12 h. Finally, the capillaries were flushed

with dried toluene, acetone and water. The coated capillaries were stored in water until use.

2.3.2. Electrophoretic procedures

The electrophoresis was carried out at 20 kV at ambient temperature and the amines were detected at 214 nm on the cathodic side. Between analyses, the capillaries were flushed with electrophoresis medium for 3 min (20 p.s.i.). The samples were injected by pressure at 0.5 p.s.i. for 5 s. Mesityloxide was used as a marker of the electro-osmosis.

The BGE was prepared as follows: (−)-DIKGA was weighed and dissolved in appropriate volumes of methanol containing NaOH, and degassed by an ultrasonic bath before use. The samples were dissolved in methanol at a concentration of 0.1–0.5 m*M* and degassed before analysis unless otherwise stated. In the cases where the total concentration of (−)-DIKGA were altered, the ratio between (−)-DIKGA and NaOH was held constant (5:2).

The resolutions (R_s) were calculated as $2*(t_{mig2}-t_{mig1})/(w_{t1}+w_{t2})$, where t_{mig2} and t_{mig1} are the migration times of enantiomer 2 and 1, respectively, and w_{t1} and w_{t2} are the base widths of the peaks between the tangents in time units. As the peak widths are difficult to calculate with high precision at low degrees of enantioseparation, the resolutions are also presented as Kaiser factors in Table 1. The Kaiser factor was calculated as f/g. A straight line was drawn between the two peak maxima; g is defined as the distance from this line to the extended baseline through the valley between the two peaks, f is the distance from the same line to the valley [17].

3. Results and discussion

The present chiral separations are based on a reversible complex formation between the chiral counter ion (−)-DIKGA and enantiomeric amines, i.e. formation of diastereomeric ion-pairs. The electrophoretic mobilities of free enantiomeric amines are equal whereas the uncharged diastereomeric ion-pairs have no electrophoretic mobility. Thus, the enantiomeric mobility difference ($\Delta\mu$) is based on differences in the equilibrium constants for ion-pair formation, the mobility of the free forms of the solute and the concentration of the selector, cf. enantioselective model of Wren et al. [18].

It is generally agreed that a ''three-point interaction'' is necessary between at least one of the enantiomers and the chiral selector in order to obtain enantioselective complexation [19]. The structure of (−)-DIKGA is given in Fig. 1. The counter ion has a

Table 1
Separation of amines with (−)-DIKGA as a chiral selector

Substance	A (uncoated)[a]				B (polyacrylamide-coated)[b]				C (aminopropyl-coated)[c]			
	$\mu_{eff_1}\cdot10^4$ ($cm^2/V\,s$)	$\mu_{eff_2}\cdot10^4$ ($cm^2/V\,s$)	R_s	Kaiser f (f/g)	$\mu_{eff_1}\cdot10^4$ ($cm^2/V\,s$)	$\mu_{eff_2}\cdot10^4$ ($cm^2/V\,s$)	R_s	Kaiser f (f/g)	$\mu_{eff_1}\cdot10^4$ ($cm^2/V\,s$)	$\mu_{eff_2}\cdot10^4$ ($cm^2/V\,s$)	R_s	Kaiser f (f/g)
Alprenolol	1.36	1.35	N.D.[d]	0.03	1.35	1.34	N.D.	0.12	1.68	1.68	N.D.	0.13
p-Hydroxyalprenolol	1.19	1.19	N.D.	0.08	1.22	1.21	N.D.	0.28	1.52	1.51	N.D.	0.16
Atenolol	1.10	1.08	0.91	0.75	1.15	1.13	1.12	0.86	1.47	1.45	1.03	0.75
Bambuterol	1.35	1.32	1.72	1.00	1.38	1.35	1.76	1.00	1.64	1.61	1.71	1.00
Labetalol (*RR*/*SS*)	0.74	0.70	2.69	1.00	0.76	0.71	3.24	1.00	1.02	0.96	3.41	1.00
Metoprolol	1.25	1.23	1.03	0.80	1.32	1.30	1.22	0.61	1.63	1.61	1.17	0.88
Pronethanol	1.08	1.03	3.13	1.00	1.43	1.36	3.40	1.00	1.69	1.62	3.85	1.00
Propranolol	1.21	1.20	N.D.	0.05	1.25	1.24	N.D.	0.29	1.47	1.46	N.D.	0.19
Oxprenolol	1.54	–	N.D.	0.00	1.56	–	N.D.	0.00	1.79	–	N.D.	0.00
Bupivacaine	1.47	–	N.D.	0.00	1.41	1.40	N.D.	0.11	1.73	1.72	N.D.	0.11
Remoxipride	0.87	–	N.D.	0.00	0.93	–	N.D.	0.00	1.15	–	N.D.	0.00

Conditions: 100 m*M* (−)-DIKGA and 40 m*M* NaOH in methanol. Separation voltage: 20 kV. The values are and average of three injections, except for pronethalol, which is an average of 13 injections.

[a] μ_{eof} uncoated, $8.02\cdot10^{-5}$ $cm^2/V\,s$.

[b] μ_{eof} acrylamide, $2.17\cdot10^{-5}$ $cm^2/V\,s$.

[c] μ_{eff} aminopropyl, $-2.84\cdot10^{-5}$ $cm^2/V\,s$.

[d] N.D., not determined due to the difficulty to calculate R_s at low degree of enantioseparation.

rigid structure with polar functions in the vicinity of the carboxylic group. Simultaneous multipoint interactions with enantiomeric amines might thus be possible. (−)-DIKGA has low UV absorbing properties which facilitate UV detection of the enantiomeric amines without having to use the partial filling technique [7] or indirect detection [20].

As found in HPLC [14] addition of NaOH to the background electrolyte proved to be important in order to enhance the ionisation of (−)-DIKGA, giving rise to an increased ionic interaction between solute and selector. Furthermore, this addition increases the conductivity of the electrophoretic medium, which was important in order to obtain symmetrical peaks. However, no external buffer system was added to the solution in order to avoid high currents. Presence of buffer ions in the BGE would also give rise to competitive non-stereoselective ion-pair formation, reducing the chiral separation. The proportion of ionised (−)-DIKGA was held constant by keeping the ratio between (−)-DIKGA and NaOH fixed (5:2), using methanol as the separation medium.

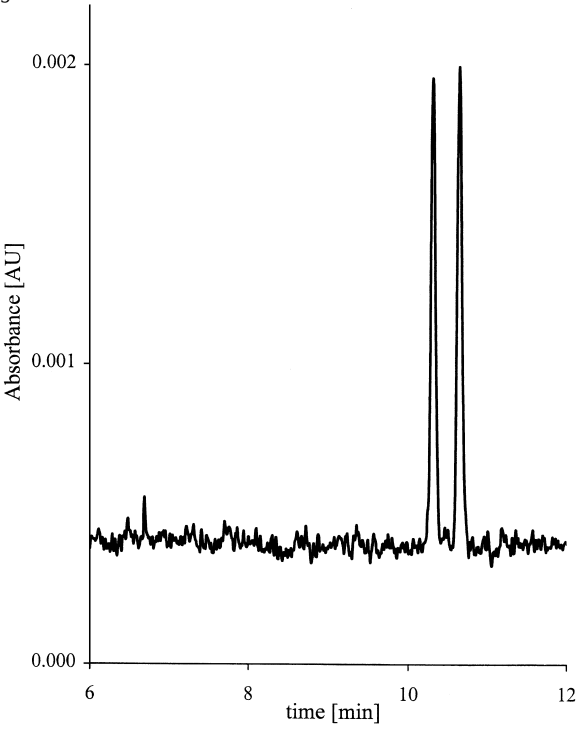


Fig. 2. Separation of (*RR*/*SS*) labetalol. Uncoated silica capillary. Conditions: BGE: 100 m*M* (−)-DIKGA and 40 m*M* NaOH in MeOH. Sample dissolved in MeOH at a concentration of 0.1 m*M*. Separation voltage+20 kV, λ_{abs} 214 nm.

3.1. Enantioseparation of amines using (−)-DIKGA as a chiral selector

Successful enantioseparations of amines such as β-adrenoceptor antagonists (β-blockers) (Nos. 1–8 in Fig. 1), an anti-asthmatic (bambuterol, No. 9), a local anesthetic (bupivacaine, No. 10) and a neuroleptic drug (remoxipride, No. 11) with (−)-DIKGA (No. 12) as a chiral selector were performed. The electrophoretic results are given in Table 1, column A. Enantioresolution was observed for all the β-blockers except for oxprenolol. A complete resolution of (*RR*)- and (*SS*)-labetalol within 11 min is demonstrated in Fig. 2. The highest enantioresolution (Kaiser factor or R_s) was observed for amino alcohols with the asymmetric centre attached directly to an aromatic ring (labetalol, bambuterol Fig. 1, Nos. 8 and 9) and pronethalol (No. 7) an amine with the alkanolamine chain attached to naphthalene, Table 1. The separation was impaired if the alkanolamine chain was bound via a $-OCH_2-$ group to the aromatic ring, cf. propranolol (Fig. 1, No. 6) and pronethalol (No. 7). Amino alcohols with a substituent in the *ortho*-position to the alkanolamine chain (alprenolol, *p*-hydroxyalprenolol and oxprenolol, Fig. 1, Nos 1, 2 and 5) gave no or low enantioresolution. However, a detailed discussion of the influence of solute structure on the enantioselectivity, (i.e. the difference in complexation constants for the two enantiomers) solely based on $\Delta\mu$ observed for different amines is not possible as this parameter ($\Delta\mu$) also depends on differences in mobilities for the free solutes as has been stressed by Wren et al. [18].

The possibility of obtaining resolution of all four isomers of labetalol, which has two asymmetric centers (Fig. 1) was tested. Racemic mixtures of (*RR*)/(*SS*)-labetalol (Fig. 2, Table 1 column C) as well as (*RS*)/(*SR*)-labetalol (R_s=4.2) were possible to separate, whereas a mixture of the four stereoisomers of labetalol only gave three peaks in the electrophorogram, Fig. 3. Thus, one pair of diastereoisomers remained unresolved.

The enantiomers of oxprenolol and remoxipride

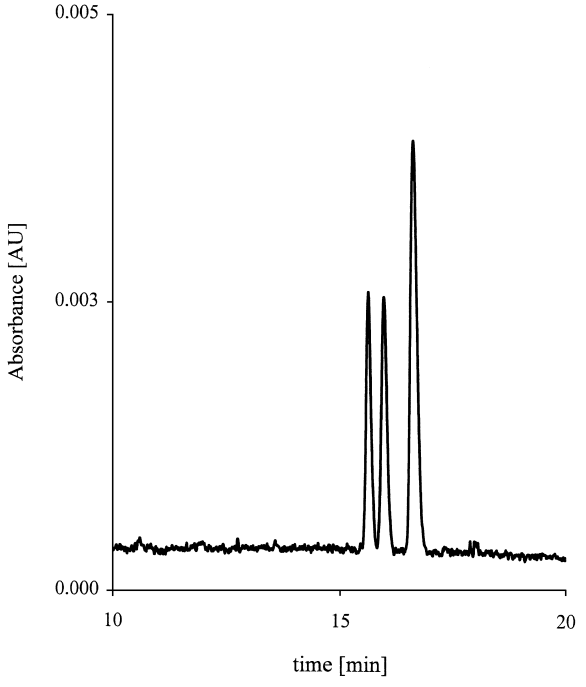


Fig. 3. Separation of (*RR*/*SS*) and (*RS*/*SR*) labetalol. Aminopropyl-coated capillary. Conditions as in Fig. 2.

(Fig. 1, Nos. 5 and 11, respectively) were previously separated in chiral ion-pair chromatography using (−)-DIKGA as the counter ion [14]. However, they did not separate using the same selector in CE, Table 1. This indicates that different distribution to the stationary phase of diastereomeric ion-pairs is the enantioselective retention mechanism in liquid chromatography. However, it should be stressed that no optimization of the separation conditions in CE for oxprenolol and remoxipride was performed in this study.

3.2. Covalent coating of the capillaries

A high EOF has a detrimental effect on resolution when it has the same migration direction as the analytes. One method to overcome this problem is to dynamically coat the capillary with detergents added to the background electrolyte. Bjørnsdottir et al. [11] used low concentrations of Tween 20 for this purpose in separations of basic drugs with (*S*)-camphorsulfonate as chiral counter ion in acetonitrile. They obtained improved separations due to lowered EOF, but with poor repeatability.

Another way of modifying the EOF and to suppress wall adsorption of solutes is to covalently coat the capillaries. For example, a neutral coating (polyacrylamide) [15] and a positively charged coating (aminopropyl) [16], have earlier been used for these purposes in aqueous CE systems.

In the present study, these two coatings were adopted in non-aqueous CE in order to evaluate their ability to suppress the high positive EOF, using methanol as the electrophoresis medium. The positive EOF was lower in the polyacrylamide-coated capillaries and the direction of the EOF was reversed for the aminopropyl-coated capillaries compared to the uncoated ones (Fig. 4). These findings are in accordance with the earlier observations in aqueous systems [15,16]. A decrease in the absolute value of the electro-osmotic flow was observed for all capillary coatings at an increased content of (−)-DIKGA. This was probably due to the commonly observed effect of increased ionic strength on the EOF.

The positive effect of a decreased EOF on resolution was obvious for some of the solutes (Table 1, columns A–C). Slight tendencies of enantioseparation of the local anesthetic bupivacaine were observed on both the polyacrylamide and the amino-

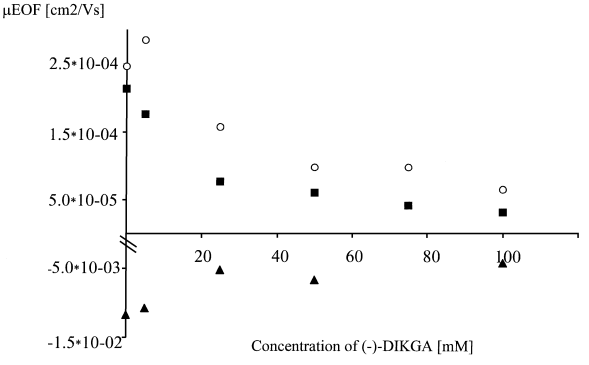


Fig. 4. The electro-osmotic mobility as a function of concentration (−)-DIKGA. Square=polyacrylamide-coated capillary, triangle=aminopropyl-coated capillary, circle=uncoated capillary. The concentration of (−)DIKGA was varied between 5 and 100 m*M*. The proportion of ionized (−)-DIKGA was held constant by keeping the ratio between (−)-DIKGA and NaOH fixed (5:2). Mesityloxide dissolved in MeOH was used as a marker of the electro-osmotic flow. The remainder conditions were the same as in Fig. 2.

propyl-coated capillaries, whereas there were no signs of separation on the uncoated capillary. Furthermore, the resolution (R_s) increased from 3.13 to 3.85 for pronethalol on the aminopropyl-coated capillary compared to the uncoated one. Another example of the effect of decreased EOF on enantioseparation is demonstrated for (*RR*/*SS*) labetalol in Fig. 5. Unfortunately, an unexpected decrease in efficiency was observed on the coated capillaries, Fig. 6. This phenomenon largely counteracted the positive effect on resolution that could theoretically be obtained by a lowered EOF. The decreased mobilities on the coated capillaries give rise to longer diffusion times, which partly might contribute to band broadening. However, it was not possible to experimentally verify this hypothesis due to the great uncertainty in the increase in band broadening.

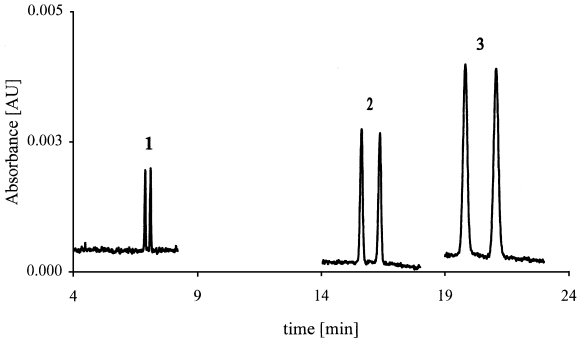


Fig. 5. Separation of (*RR*/*SS*) labetalol on the different capillaries. Conditions as in Fig. 2. No. 1 corresponds to the uncoated capillary, No. 2 to the polyacrylamide-coated capillary and No. 3 to the aminopropyl-coated capillary.

3.3. Counter ion concentration

An increase in (−)-DIKGA concentration decreased the observed mobility for the analytes (e.g., labetalol, bambuterol and pronethalol) owing to an increased degree of ion-pair formation and a lowered EOF (polyacrylamide-coated capillary). As could be expected, this was accompanied by a raise in $\Delta\mu$ and R_s as shown for bambuterol, Fig. 7. The proportion of ionized (−)-DIKGA in the background electrolyte was held constant by adding (−)-DIKGA and NaOH at a fixed ratio (5:2).

3.4. Injection medium

Analysis of low concentrations is often required in determinations of the enantiomeric purity of a product, which makes sharpening of the injection zone useful. The peak performance of the enantiomers of bambuterol was investigated as this solute was dissolved in pure methanol and methanol containing 100 m*M* (−)-DIKGA and 40 m*M* NaOH (identical to the separation medium), Fig. 8. The nature of the injection medium proved to have a great impact on separation efficiency. The peaks were considerably sharper when the solute was dissolved in pure methanol with low conductivity. This phenomenon can probably be explained by electrophoretic stacking and/or affinity compression of the injection zone.

4. Repeatability and long-term stability

The repeatability of the migration times for pronethalol in the system with 100 m*M* (−)-DIKGA and 40 m*M* NaOH in methanol on the coated capillaries was evaluated within and between days. There was a good precision for the systems both within (n=10) and between days (n=3) (see Tables 2 and 3). The relative standard deviations in mobilities and $\Delta\mu$ for the coated capillaries are similar to the uncoated ones (0.7–1.5%). For the aminopropyl-coated capillary, the third day is 2 months later, and the results are still comparable with the

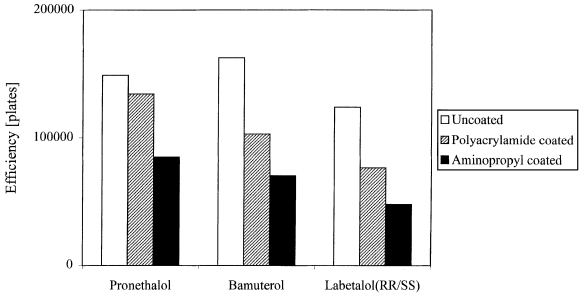


Fig. 6. Differences in efficiency. Differences in efficiency (N) on the uncoated (white) the polyacrylamide (striped) and the aminopropyl-coated (black) capillaries. Pronethalol (n=13), bambuterol (n=2) and labetalol (n=3). Conditions as in Fig. 2.

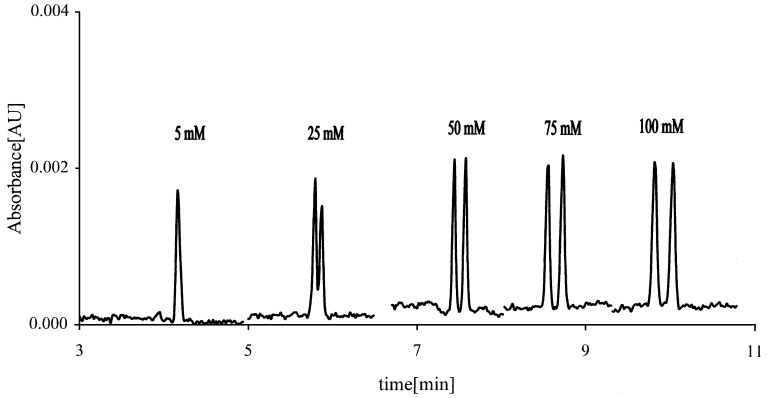


Fig. 7. *rac*-Bambuterol at different concentrations of (−)-DIKGA. The concentration of (−)-DIKGA was varied between 5 and 100 m*M*. The remainder conditions were the same as in Fig. 2.

earlier ones, but with a small tendency towards increased mobility (3% higher μ_{OBS}), probably due to a decreased reversed electro-osmotic flow.

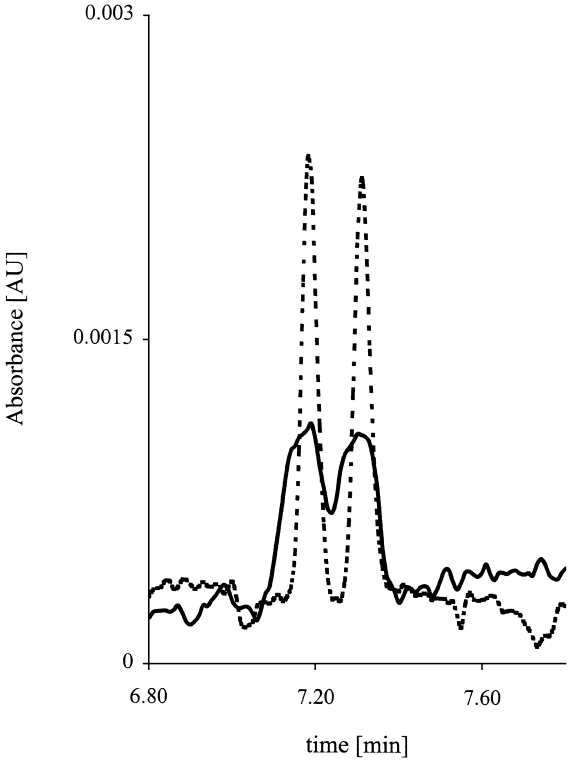


Fig. 8. Different injection media. Bambuterol dissolved in MeOH (dotted) and in BGE (line). Conditions as in Fig. 2.

5. Conclusion

A non-aqueous capillary electrophoresis system for chiral separations of amines with (−)-DIKGA as the selector has been developed. Enantioseparation of several different pharmacologically active amines (e.g., metoprolol, bambuterol, pronethalol, and bupivacaine) were performed. The results indicate that stereoselective complex formation is possible to obtain between the solute and the selector.

Covalent coating of the capillaries with the neutral polyacrylamide and the positively charged aminopropyl gave decreased and reversed electro-osmosis respectively, using methanol containing (−)-DIKGA and NaOH (5:2) as the background electrolyte. This had a positive effect on enantioresolution. An increased content of (−)-DIKGA in the BGE decreased the absolute value of the electro-osmotic flow in the capillaries. This was generally accompanied by an increased enantioresolution.

A significant peak sharpening effect due to electrophoretic stacking and/or affinity compression was shown when the solutes were dissolved in low conducting pure methanol instead of the background electrolyte. The repeatability of the system was excellent. The relative standard deviations in the mobilities and $\Delta\mu$ for the coated capillaries were similar to the uncoated ones (0.7–1.5%). The mobilities of the enantiomers of labetalol had only increased by 3% after 2 months on the same aminopropyl-coated capillary.

Table 2
Repeatability of $\Delta\mu$ and μ_{obs}

Capillary	μ_{obs_1}(cm^2/V s) $\cdot 10^4 \pm$RSD (%)	μ_{obs_2}(cm^2/V s) $\cdot 10^4 \pm$RSD (%)	$\Delta\mu$ (cm^2/V s) $\cdot 10^4 \pm$RSD (%)
Uncoated	2.03±1.2	1.96±1.3	6.84±1.2
Polyacrylamide	1.87±0.8	1.81±0.8	6.62±1.5
Aminopropyl	1.29±0.7	1.22±0.7	6.85±0.9

Conditions: BGE: 100 m*M* (−)-DIKGA and 40 m*M* NaOH in methanol. Separation voltage: 20 kV, solute: pronethalol, $n=10$.

Table 3
Stability expressed as mobility (μ_{obs}) and mobility difference ($\Delta\mu$)

Capillary	Day 1			Day 2			Day 3		
	$\mu_{obs_1}\cdot 10^4$ (cm^2/V s)	$\mu_{obs_2}\cdot 10^4$ (cm^2/V s)	$\Delta\mu \cdot 10^4$ (cm^2/V s)	$\mu_{obs_1}\cdot 10^4$ (cm^2/V s)	$\mu_{obs_2}\cdot 10^4$ (cm^2/V s)	$\Delta\mu \cdot 10^4$ (cm^2/V s)	$\mu_{obs_1}\cdot 10^4$ (cm^2/V s)	$\mu_{obs_2}\cdot 10^4$ (cm^2/V s)	$\Delta\mu \cdot 10^4$ (cm^2/V s)
Polyacrylamide	1.65	1.57	8.05	1.65	1.59	6.54	1.70	1.64	6.57
Aminopropyl	1.32	1.25	6.82	1.42	1.36	6.29	1.28[a]	1.21[a]	6.85[a]

Conditions: The same as in Table 2.
[a] For aminopropyl the third day is 2 months later (on the same capillary).

References

[1] C. Pettersson, G. Schill, J. Chromatogr. 204 (1981) 179.
[2] V.A. Davankov, V.R. Kurganov, Chromatographia 17 (1983) 686.
[3] H. Nishi, S. Terabe, J. Chromatogr. A 694 (1995) 245.
[4] C. Pettersson, J. Chromatogr. 316 (1984) 553.
[5] C. Pettersson, T. Arvidsson, A.-L. Karlsson, I. Marle, J. Pharm. Biomed. Anal. 4 (1986) 221.
[6] J. Hermansson, J. Chromatogr. 298 (1984) 67.
[7] L. Valtcheva, J. Mohammad, G. Pettersson, S. Hjertén, J. Chromatogr. 638 (1993) 263.
[8] M. Hedeland, R. Isaksson, C. Pettersson, J. Chromatogr. A 807 (1998) 297.
[9] J. Debowski, D. Sybilska, J. Jurczak, J. Chromatogr. 237 (1982) 303.
[10] A.M. Stalcup, K.H. Gahm, J. Microcol. Sep. 8 (1996) 145.
[11] I. Bjørnsdottir, S.H. Hansen, S. Terabe, J. Chromatogr. A 745 (1996) 37.
[12] Y. Walbroehl, J. Jorgensen, J. Chromatogr. 315 (1984) 135.
[13] K. Sarmini, E. Kenndler, J. Chromatogr. A. 792 (1997) 3.
[14] C. Pettersson, C. Gioeli, Chirality 5 (1993) 241.
[15] S. Hjertén, J. Chromatogr. 347 (1985) 191.
[16] M. Thorsteinsdóttir, R. Isaksson, D. Westerlund, Electrophoresis 16 (1995) 557.
[17] R. Kaiser, Chromatographie in der Gasphase, I, Bibliographisches Institut, Mannheim, 1960, p. 35.
[18] S.A.C. Wren, R.C. Rowe, J. Chromatogr. 603 (1992) 253.
[19] C.E. Dalgliesh, J. Chem. Soc. (1952) 3940.
[20] S. Hjertén, K. Elenbring, F. Kilar, J. Li-Liao, A.J.C. Chen, C.J. Siebert, M. De-Zhu, J. Chromatogr. 403 (1987) 47.