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Chiral separation of amines with *N*-benzoxycarbonylglycyl-*L*-proline as selector in non-aqueous capillary electrophoresis using methanol and 1,2-dichloroethane in the background electrolyte

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Abstract

N-Benzoxycarbonylglycyl-*L*-proline (*L*-ZGP) has been introduced as a chiral selector for enantioseparation of amines in non-aqueous capillary electrophoresis. Methanol mixed with different proportions of dichloromethane, 1,2-dichloroethane or 2-propanol containing *L*-ZGP and ammonium acetate was used as the background electrolyte. Enantioseparation of different types of pharmacologically active amines was performed, e.g. the local anaesthetic bupivacaine and the β-adrenoceptor blocking agent pindolol. Addition of the solvents (dichloromethane, 1,2-dichloroethane or 2-propanol) gave an improved chiral separation partly due to a distinct decrease in the electroosmotic flow. The use of 1,2-dichloroethane in the background electrolyte gave higher precision in migration time (RSD 2.2%) compared to the systems containing dichloromethane. An enantiomeric separation of mepivacaine was performed within 72 s by use of short-end injection with an effective capillary length of 8.5 cm.

Keywords: Non-aqueous capillary electrophoresis; Enantiomer separation; Short-end injection; Injection methods; Amines; Benzoxycarbonylglycyl-proline

1. Introduction

Since the first publication on non-aqueous capillary electrophoresis (NACE) appeared in 1984 [1], interest in this mode of separation has grown [2,3]. The use of non-aqueous media offers possibilities to regulate selectivity by changing the solvent properties. Bjørnsdottir and Hansen [4] observed selectivity changes for primary, secondary and tertiary amines when methanol (MeOH), a solvent with hydrogen donating and accepting properties, was replaced by acetonitrile (ACN), which only can act as a hydrogen acceptor. These changes were suggested to depend on different alkalinities of the amines in the different solvents due to different solvation of the solutes.

The use of background electrolytes (BGEs) based on organic solvents often gives lower currents than aqueous BGEs. Valko et al. have shown that the

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current in ethanol–acetonitrile (50:49) containing 1% acetic acid and 20 m*M* ammonium acetate (NH_4Ac) was only one-fourth to one-third as high as in aqueous buffer (water–acetic acid (99:1) with 20 m*M* NH_4Ac) [5]. The lower current in non-aqueous media permits use of higher field strength and wider capillaries without problems with Joule heating. However, many of the solvents used in NACE have fairly low boiling points (high volatility) compared to water (Table 1), which reduce the maximum applicable running current.

The use of NACE also makes it possible to analyse lipophilic compounds insoluble in water. Recently, hydrophobic oligomers have been separated using chloroform or dichloromethane in the BGE [6]. Electrophoretic migration in mixtures containing as high as 95% chlorinated solvent with 10 m*M* perchloric acid added to the BGE was reported.

The influence of different solvents used in NACE on the electroosmotic flow (EOF) has been studied by Valko et al. [7]. In that study, no EOF was observed in pure dichloromethane. The authors claim that this was due to the absence of hydrogen donating and/or accepting properties of dichloromethane. Thus, dissociation of the silanol groups on the capillary surface was not considered possible.

The majority of chiral separations in capillary electrophoresis (CE) are still performed in aqueous media or aqueous media modified with non-aqueous solvents such as MeOH or ACN. In general, the chiral selectors used in NACE have been adopted from aqueous CE systems, or have been used as chiral mobile phase additives (CMPAs) in HPLC. Since the dielectric constants in non-aqueous solvents are often lower than in aqueous solvents (Table 1) the electrostatic interactions become stronger. Thus, a better environment for ion-pair formation between a solute and the chiral selector is achieved in non-aqueous media. Bjørnsdottir et al. have shown enantioseparation of β-amino alcohols using (+)-(*S*)-10-camphorsulphonate as chiral counter-ion in ACN [8]. In aqueous medium, no separation was observed for the enantiomers. Stalcup and Gahm achieved enantioresolution of *N*-3,5-dinitrobenzoyl amino acids and other carboxylic acids using quinine dissolved in MeOH as chiral selector [9]. (−)-2,3:4,6-Di-*O*-isopropylidene-2-keto-*L*-gulonic acid ((−)-DIKGA) in methanolic BGE has been used for enantioseparation of pharmacologically active amines [10] and recently, (*R*)- and (*S*)-3,5-dinitrobenzoyl-leucine have been used to separate the enantiomers of mefloquine and cinchona alkaloid derivatives in NACE using the partial filling technique [11]. Other examples of charged selectors have also been presented. Wang et al. have used anionic β-cyclodextrins for enantioseparation of pharmacologically active amines using formamide, *N*-methylformamide or *N*, *N*-dimethylformamide in the BGE [12]. (+)-18-Crown-6-tetracarboxylic acid has been used for enantioseparation of primary amines in formamide [13]. Recently, Busby et al. have separated basic enantiomers using octakis(2,3-*O*-dimethyl-6-*O*-sulfo)-γ-cyclodextrin in methanolic BGE [14].

The *N*-blocked dipeptide *N*-benzoxycarbonylglycyl-*L*-proline (*L*-ZGP) has previously been applied as a CMPA in normal-phase HPLC [15]. Dichloromethane, 1,2-dichloroethane, 2-propanol (2-PrOH), ACN and MeOH have been used as mobile phase solvents and LiChrosolv Diol, Nucleosil CN or porous graphitic carbon (Hypercarb) as solid phases.

The aim of this study was to introduce *L*-ZGP as a chiral selector in non-aqueous capillary electrophoresis. Furthermore, the influence of 2-PrOH, dichloromethane and 1,2-dichloroethane as additives to the BGE on the separation and EOF was studied. A further objective was to compare the chiral resolution obtained in NACE with that earlier observed in HPLC.

Table 1
Solvent properties [16]

Solvent	t_{boil} (°C)[a]	η (kg/ms)[b]	ϵ[c]	ϵ/η
H_2O	100.00	1.137	78.36	69
MeOH	64.55	0.593	32.66	55
ACN	81.60	0.374	35.94	96
2-PrOH	82.24	2.773	19.92	7.2
CH_2Cl_2	39.64	0.449	8.93	20
$C_2H_4Cl_2$	83.48	0.887	10.37	12

[a] 1 atm (=101 325 Pa).
[b] 15 °C.
[c] 25 °C.

2. Materials and methods

2.1. Chemicals

The chiral counter ion *L*-ZGP (''puriss'' grade≥ 99%) was obtained from Sigma (St. Louis, MO, USA). The solvents methanol, 2-propanol and dichloromethane (all HPLC-grade) were from Fischer Scientific (Loughborough, UK) and 1,2-dichloroethane (''purum'' grade) was obtained from Fluka (Buchs, Switzerland). Ammonium acetate was purchased from Sigma. Mesityl oxide, *rac*-propranolol hydrochloride, *rac*-pindolol, *rac*-sotalol hydrochloride, (*R*)-propranolol hydrochloride, (*S*)-propranolol hydrochloride, (*R*)-atenolol hydrochloride and (*S*)-atenolol hydrochloride were purchased from Sigma. *rac*-Atenolol hydrochloride, *rac*-alprenolol benzoate, *rac*-*p*-hydroxyalprenolol hydrochloride, *rac*-metoprolol tartrate, *rac*-tocainide hydrochloride and *rac*-oxprenolol hydrochloride were generous gifts from Astra Hässle (Mölndal, Sweden). *rac*-Bupivacaine hydrochloride, *rac*-mepivacaine hydrochloride, *rac*-prilocaine hydrochloride and *rac*-remoxipride hydrochloride were gifts from Astra Läkemedel (Södertälje, Sweden). *rac*-Bambuterol hydrochloride and *rac*-terbutaline sulfate were gifts from Astra Draco (Lund, Sweden). *rac*-Pronethalol hydrochloride was purchased from ICI (Macclesfield, UK). *rac*-Labetalol hydrochloride (*RR*/*SS* and *RS*/*SR*) and *rac*-salbutamol sulphate were from Glaxo (Greenford, UK). *rac*-Isoprenaline hydrochloride was obtained from Apoteket (Stockholm, Sweden) and terodiline hydrochloride was from Kabi-Vitrum (Stockholm, Sweden). (+)-ψ-Ephedrine hydrochloride and (−)-ψ-ephedrine hydrochloride were from Serva (Heidelberg, Germany). NaOH and HCl (both analytical-reagent grade) were supplied by Merck (Darmstadt, Germany). 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). Counter-ion and solute structures are shown in Fig. 1a and b.

2.2. Equipment

The CE instrument was a $HP^{3D}CE$ equipped with UV-diode array detection from Hewlett-Packard (Waldbronn, Germany). The data were recorded with the HP Chemstation software version A 07.01. Fused silica capillaries (50 μm I.D.×365 μm O.D.) were obtained from MicroQuartz (Munich, Germany).

2.3. Procedures

The electrophoresis was carried out at 30 kV and the temperature was set at 25.0 °C unless otherwise stated. The solutes were detected at the wavelengths 214, 254 and 272 nm on the cathodic side. About 0.2 cm of the polyimide coating was burned off on both ends of the capillary in order to avoid sample carry-over. The capillary length was 68.5 cm, with an effective length of 60.0 cm unless otherwise stated.

Before first use, the capillaries were flushed with 0.1 *M* sodium hydroxide, water, 0.1 *M* hydrochloric acid, water and methanol (15 min and 1 bar for each step). The samples were injected by pressure for 5 s (35 mbar). Between analyses, the capillaries were flushed with the BGE for 3 min (1 bar). Mesityl oxide was used as a marker of the electroosmosis.

The BGE was prepared as follows: *L*-ZGP was weighed and dissolved in appropriate volumes of the solvent or solvent mixture (presented as %, v/v) containing NH_4Ac. The solutes were dissolved in pure MeOH at a concentration of 0.1–0.5 m*M*. No further efforts to dry the chemicals (from their water content) were done before dissolution.

The minimum capillary length is often fixed on commercial instruments. One way of reducing the length further is to inject from the end of the capillary nearest the detector, which minimizes the effective capillary length to 8.5 cm for this instrument. This technique, known as short-end injection [17], was practised in the present study.

It is difficult to precisely calculate peak resolution (R_s) for enantiomers with low chiral resolution, therefore the Kaiser factor (Kf) was used instead of R_s in these cases. The chiral resolution expressed as Kf 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 [18].

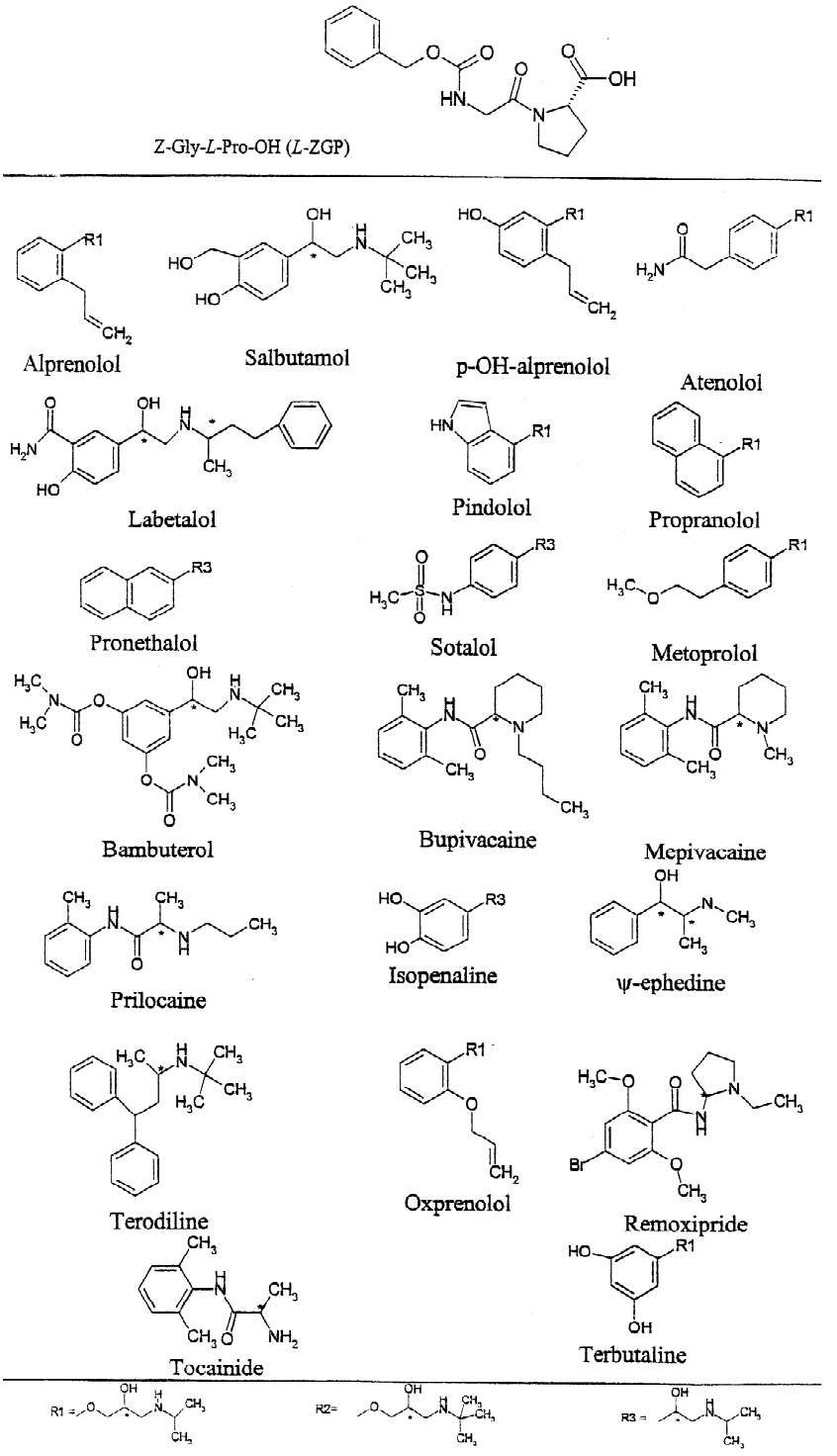

Fig. 1. Structures of counter ion (a) and (b) solutes.

3. Results and discussion

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 chiral discrimination involving only complexation with the selector [19]. These interactions are not restricted to attractive interactions but can include repulsions as well (e.g. steric repulsion).

The selector *L*-ZGP (Fig. 1a) is an *N*-blocked dipeptide with a rigid structure. The carboxylic group can participate in hydrogen bonding or electrostatic interaction with amines, and the amide and the carbamate group can give additional interactions, e.g. hydrogen bonding. In the present study, it was for the first time shown that *L*-ZGP can be used as a chiral selector in NACE. NH_4Ac was added to the BGE in order to ionize *L*-ZGP and to obtain enough conductivity to avoid electrodispersion. The choice of NH_4Ac was made according to its solubility in the BGE. A further advantage using the volatile NH_4Ac would be for future on-line partial filling CE–MS. One of the substances that exhibited enantioselective formation of diastereomeric complexes with the chiral selector was the β-blocking agent pindolol (0.1 m*M*) (Fig. 2). A high signal-to-noise ratio was obtained despite the high background absorbance by *L*-ZGP (125 m*M*).

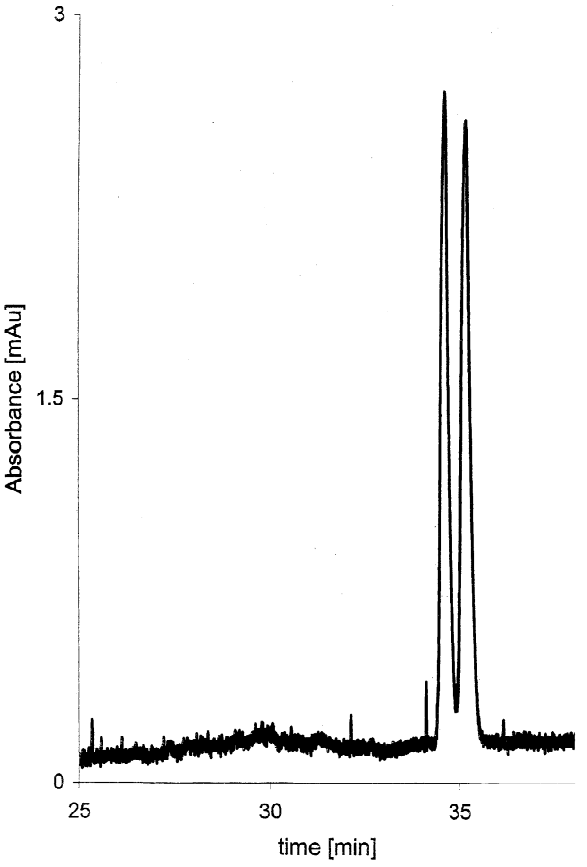

Fig. 2. Chiral separation of pindolol. BGE: 125 m*M* *L*-ZGP and 50 m*M* NH_4Ac with 55% 1,2-dichloroethane in MeOH. For other conditions, see Section 2.3.

3.1. Influence of selector concentration on chiral resolution and EOF

The influence of counter-ion concentration (between 50 and 250 m*M*) on the relative mobility difference (RMD), Kf, $\Delta\mu$ (electrophoretic mobility difference of enantiomers) and EOF was studied in a BGE containing MeOH and NH_4Ac (Fig. 3a and b). The proportion between *L*-ZGP and NH_4Ac was held constant at a molar ratio of 5:2. A low concentration of NH_4Ac was used in order to minimize non-stereoselective ion-pairing with the selector and the solutes as well as to avoid high currents.

An increased chiral resolution (RMD and Kf) was observed for all substances at increasing concentrations of *L*-ZGP (Fig. 3a). However, the values of $\Delta\mu$ for the enantiomers were rather constant for mepivacaine and bupivacaine (Fig. 3b). According to the chiral separation model of Wren et al., these results indicate that $\Delta\mu$ of these solutes had reached the maximum, and did not change in the investigated concentration interval [20]. However, the $\Delta\mu$ for prilocaine reached its maximum at 100 m*M* *L*-ZGP. The RMD followed the same trend as Kf at increasing concentration of selector. Thus, the decrease in the EOF was the main contributing source of the increased Kf (Fig. 3b). At the highest concentrations of *L*-ZGP, prilocaine and bupivacaine had a tendency to a decreased Kf. This effect has not been elucidated in detail, but might be due to lower efficiency caused by the lower EOF (increased time of diffusion).

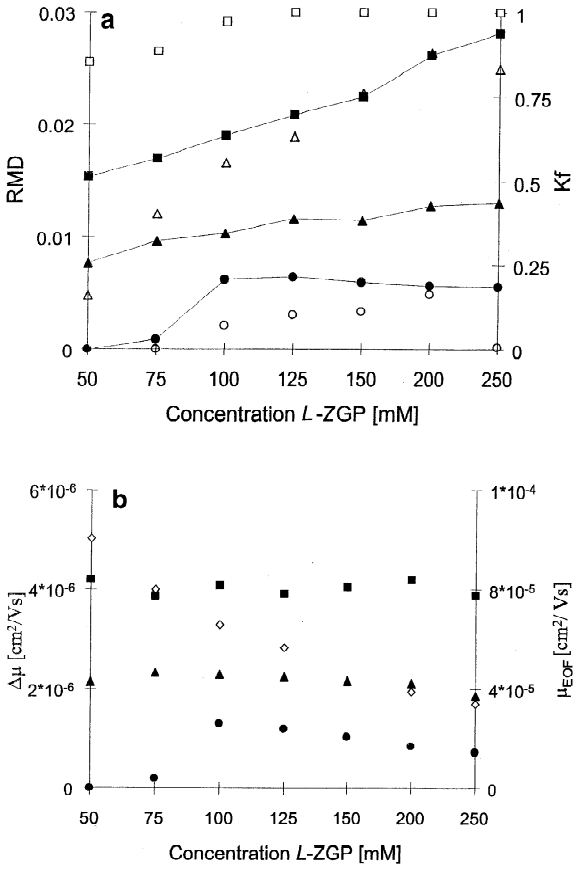

Fig. 3. (a) Enantioresolution and selector concentration. BGE: Different concentrations of *L*-ZGP (50–250 m*M*) and NH_4Ac (20–100 m*M*) at a ratio of 5:2 in MeOH. ■, Mepivacaine RMD; ▲, bupivacaine RMD; ●, prilocaine RMD; □, mepivacaine Kf; Δ, bupivacaine Kf; ○, prilocaine Kf. For other conditions, see Section 2.3. (b) Mobility difference, electroosmosis and selector concentration. Conditions as in (a). ■, Mepivacaine △μ; ▲, bupivacaine △μ; ●, prilocaine; □, electroosmosis.

3.2. Influence of solvent properties on EOF

The high velocity of the EOF in aqueous and non-aqueous systems is often a problem when separating substances with similar characteristics (low $\Delta\mu$), e.g. enantiomers. The EOF can be suppressed in different ways in NACE. Bjørnsdottir and Hansen used dynamic coating by addition of low concentrations of Tween 20 to the BGE consisting of ACN [4]. They obtained improved separations but poor repeatability. Wang et al. used addition of the cations tetramethylammonium and tetrabutylammonium [12]. They also suppressed the EOF, but the additives competed with the solute for the binding to the chiral selector, which decreased the chiral separation at higher concentrations. The use of neutral (polyacrylamide) and positively charged (aminopropyl) covalently coated capillaries to control the EOF in chiral separation has also been demonstrated with high repeatability in NACE [10]. Unfortunately, the efficiency was lower than for uncoated capillaries, which counteracted the benefits of a low EOF.

In the present study, dichloromethane, 1,2-dichloroethane and 2-PrOH have been added to the methanolic BGE in different amounts (Fig. 4). The solvents all decreased the EOF, but the BGE containing dichloromethane gave a bad repeatability. This was due to its high volatility, which gave a continuous decrease in the dichloromethane content in the BGE vials. The use of 1,2-dichloroethane, with a higher boiling point, enhanced the repeatability in the migration time (RSD = 2.2%, $n = 10$). This made it possible to use the same BGE for a long time and it was not replaced in the vials during the whole analysis time (5 h). The lower volatility would also permit the use of higher currents, which has advantages when the objective is a fast ''high throughput'' separation on a short capillary with high electric field strengths. The lower EOF was probably due to the lower dielectric constant to viscosity (ϵ/η) ratio of the mixtures, compared to MeOH (Table 1). This

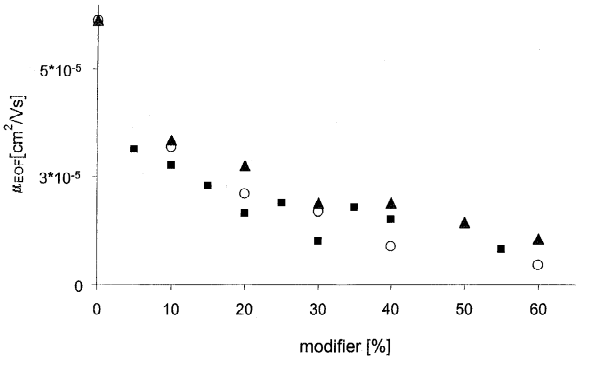

Fig. 4. Electroosmosis in non-aqueous solvents. Solute: mesityl oxide. BGE: 125 m*M* *L*-ZGP and 50 m*M* NH_4Ac with different amounts of 2-PrOH, dichloromethane or 1,2-dichloroethane in MeOH. For other conditions, see Section 2.3. ■, Dichloromethane; ○, 1,2-dichloroethane; ▲, 2-PrOH.

ratio has proven to be an important factor for determining the EOF [21].

(*S*)-Propranolol was used as a model compound to calculate the efficiency (number of plates) at different amounts of 1,2-dichloroethane in the BGE (Fig. 5). The efficiency decreased gradually up to an amount of 40% (v/v) 1,2-dichloroethane. However, a steep decrease to ~2000 plates was observed above 50% addition. This was probably due to the increased migration times, which increase the diffusion time of the solute in the capillary. The change in solvent properties, with improved $\Delta\mu$ and decreased EOF, often counteracted the lower efficiency, with, in many cases, a higher enantioresolution as a result (Table 2). This was an advantage compared to covalently coated capillaries, which can only reduce the EOF [10].

3.3. Solute structure and enantioseparation

In the present study, 21 different chiral amines (Fig. 1b) were used as test solutes. All the test solutes, except labetalol, pronethalol, bambuterol, pindolol and remoxipride, have previously been separated using *L*-ZGP as CMPA in HPLC [22–24]. A partial or complete enantioseparation was obtained for 13 of those solutes (Table 2). Tendencies (Kf> 0) for enantioseparation at higher concentrations of dichlorometane or 1,2-dichloroethane were observed also for alprenolol, atenolol, salbutamol, terbutaline and remoxipride (data not shown). Thus, enantioselective formation of diasteromeric ion-pairs was obtained with *L*-ZGP for all the solutes, except propranolol, sotalol and isoprenaline (data not shown). However, it should be stressed that no further optimization of the separation conditions for these substances was performed. Unfortunately, it was not possible to use exactly the same BGE solvent (e.g. 100% dichloromethane) that previously had been used as mobile phase solvent in HPLC, due to very long migration times.

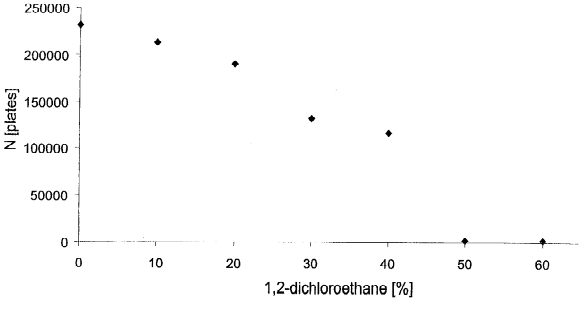

Fig. 5. Efficiency and 1,2-dichloroethane concentration. Solute: *S*-propranolol. BGE: 125 m*M* *L*-ZGP and 50 m*M* NH_4Ac with different amounts of 1,2-dichloroethane in methanol. For other conditions, see Section 2.3.

In CE the enantioseparation can only be based on a difference in the formation constants of the diastereomeric ion-pairs, i.e. ''three point interaction'', as no stationary phase is available, unless the complexes are electrically charged [19]. Charged diastereomeric complexes may have different mobilities [22]. In HPLC, on the other hand, it is possible to use any selector without a ''three point interaction'' with the solute, provided the established diastereomeric complexes differ in their affinity to the stationary phase.

Some statements have earlier been made about the relations between the structure of the solute and the enantioselectivity in HPLC for the chiral selector *L*-ZGP [23–26]. One is that the solutes that give the highest observed stereoselectivity have an amino function and a second polar function or an aromatic ring attached directly to the asymmetrical centre or at a distance of one methylene group from it [27]. However, the relationship between the structure and enantioselectivity is complex, and cannot easily be predicted from the present study. Isoprenaline fulfils the statement above, but has not been enantioseparated either in HPLC [24] or in the current work. This might be due to non-stereoselective hydrogen bondings between the hydoxy groups attached to the aromatic ring and the chiral selector instead of interactions near the asymmetrical centre.

In Fig. 6, an electropherogram of the racemic drugs bupivacaine, mepivacaine and prilocaine, is presented. The results show that the enantioseparation for prilocaine was lower than for mepivacaine and bupivacaine. This might be caused by the side-chain of prilocaine, which is not ring-closed and probably has a more flexible conformation. The higher enantioresolution for mepivacaine compared to bupivacaine might be due to less steric hindrance in the complexation with *L*-ZGP, as mepivacaine has a shorter alkyl substituent on the ring nitrogen.

Table 2
Enantioresolution and observed mobility in different solvents

Substance	BGE: 100% MeOH			BGE: 20% 2-PrOH			BGE: 20% dichloromethane			BGE: 20% 1,2-dichloroethane		
	μ_{obs_1}, $\times 10^4$	$\Delta\mu$, $\times 10^6$	f/g	μ_{obs_1}, $\times 10^4$	$\Delta\mu$, $\times 10^6$	f/g	μ_{obs_1}, $\times 10^4$	$\Delta\mu$, $\times 10^6$	f/g	μ_{obs_1}, $\times 10^4$	$\Delta\mu$, $\times 10^6$	f/g
Mepivacaine	1.83	3.9	1.0[f]	1.16	3.8	1.0[g]	1.38	3.6	1.0[h]	0.85	1.7	0.5
Bupivacaine	1.75	1.0	0.9	1.22	1.8	0.5	1.20	1.6	0.8	1.54	1.7	0.9
Tocainide	1.82	1.4	0.5	1.16	1.2	0.7	1.34	1.9	0.8	1.36	1.3	0.9
Labethalol	1.18	1.1	0.2	0.73	0.4	>0	0.78	0.3	>0	0.84	0.9	0.3
Prilocaine	1.74	0.9	0.2	1.07	0.8	0.4	1.24	0.5	>0	0.68	0.4	0.1
Pindolol	1.72	0.5	>0	1.09	0.6	0.2	1.20	1.1	0.6	1.36	1.0	0.5
Oxprenolol	0.65	0	0	1.30	0.7	0.2	1.45	1.1	0.6	1.48	0.9	0.5
Metoprolol	1.70	0	0	1.11	0	0	1.22	0.6	0.1	1.27	0.6	0.1
Terodiline	NA			1.32	1.7	1.0[i]	1.35	2.1	1.0[j]	0.85	1.1	0.5
ψ-Ephedrine[a]	NA			NA			1.66	1.79	0.8	NA		
Bambuterol[b]	1.87	0	0	1.07	0	0	1.33	0	0	1.35	0	0
Pronethalol[c]	1.87	0	0	1.23	0	0	1.35	0	0	1.39	0	>0
p-Hydroxyalprenolol[d]	1.58	0	0	1.02	0	0	1.13	0	0	1.28	0	>0
EOF[e]	5.20			0.30			0.17			0.20		

BGE: 125 m*M* *L*-ZGP and 50 m*M* NH_4Ac in MeOH or methanol with 20% addition of 2-PrOH, dichloromethane or 1,2-dichloroethane. NA, not analyzed. For other conditions, see Section 2.3

[a] Kf in 55% CH_2Cl_2 = 0.9.
[b] Kf in 55% CH_2Cl_2 = 0.9.
[c] Kf in 55% CH_2Cl_2 = 0.8.
[d] Kf in 55% CH_2Cl_2 = 0.6.
[e] Mesityl oxide.
[f] R_s = 1.8.
[g] R_s = 4.4.
[h] R_s = 2.2.
[i] R_s = 1.7.
[j] R_s = 1.50.

The substances were affected differently by the solvents (Table 2), and there was no clear trend in the changes in Kf. This was in agreement with previous findings using *L*-ZGP as a chiral selector in HPLC when 2-PrOH or ACN was added to a methanolic mobile phase [24]. A comparison of $\Delta\mu$ shows that the majority of the solutes had the highest $\Delta\mu$ in the BGE containing dichloromethane or 1,2-dichloroethane. This might be the result of a better environment for ion-pair formation. However, mepivacaine, bupivacaine, prilocaine and labetalol had a higher value of $\Delta\mu$ in MeOH without solvent addition. The fact that some substances had a higher $\Delta\mu$ in MeOH might be due to a lower degree of charge of the protolytes in the other solvents.

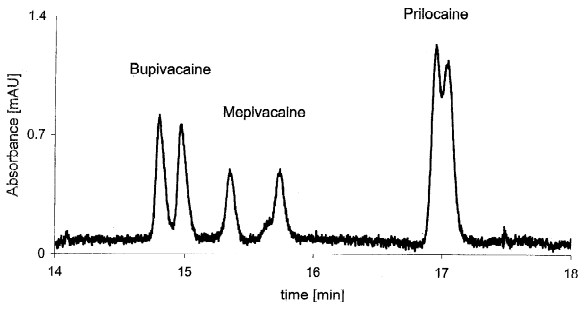

Fig. 6. Chiral separation of bupivacaine, mepivacaine and prilocaine. BGE: 125 m*M* *L*-ZGP and 50 m*M* NH_4Ac in MeOH. For other conditions see Section 2.3.

The decrease in the EOF also enhanced the chiral resolution (Kf) for some of the solutes when dichloromethane and 1,2-dichloroethane were added (Figs. 4 and 7, Table 2). The addition of 2-PrOH decreased the EOF, but gave a lower enantioresolu-

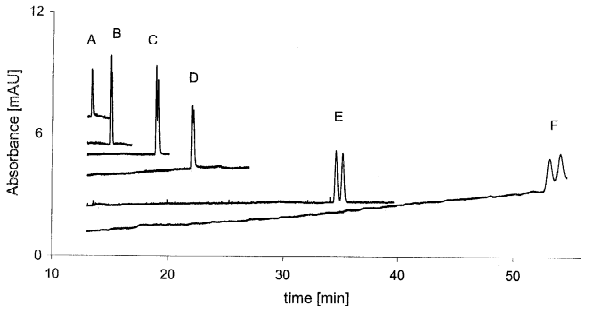

Fig. 7. Chiral separation of pindolol in different solvents. BGE: 125 m*M* *L*-ZGP and 50 m*M* NH_4Ac with or without addition of 2-PrOH, dichloromethane or 1,2-dicloroethane in MeOH. For other conditions, see Section 2.3. Electropherogram A: *L*-ZGP and NH_4Ac in MeOH; B: 20% 1,2-dichloroethane; C: 20% dichloromethane; D: 20% 2-PrOH; E: 55% 1,2-dichloroethane; and F: 55% dichloromethane.

tion than the chlorinated solvent mixtures. The low EOF and effective mobility in 2-PrOH was probably due to the higher viscosity in this solvent. However, the degree of ion-pair formation may be lower in 2-PrOH, due to a higher dielectric constant than in, for example, 1,2-dichloroethane, which can explain the lower enantioresolution observed in this solvent.

The amount of 1,2-dichloroethane in the BGE had a great influence on the enantioresolution for pindolol (Figs. 7 and 8). The higher enantioresolution (Kf) depends on the combined effect of a decreased EOF (Fig. 4) and an increased $\Delta\mu$ (Fig. 8). It is apparent that the lower efficiency (Fig. 5) observed at higher concentrations of 1,2-dichloroethane did not have a major influence on the enantioresolution in this case. Amounts of 1,2-dichloroethane higher than 60% gave no observable peaks within reasonable time ($\mu_{obs} < 3.8\times10^{-5}$ cm^2 V^{-1} s^{-1}). This was a combination of the low charge of the enantiomers and the low ($\mu_{EOF} < 4\times10^{-6}$ cm^2 V^{-1} s^{-1}) EOF. Bupivacaine on the other hand, reached a maximum for $\Delta\mu$ at ~20% of 1,2-dichloroethane (Fig. 8). However, Kf for bupivacaine decreased when adding 1,2-dichloroethane even though the EOF was lowered. A decrease of the number of theoretical plates contributed to this effect.

3.4. Short-end injection

Nowadays, the accelerating use of high throughput screening in preclinical drug research, with large number of samples, causes a bottleneck in analysis.

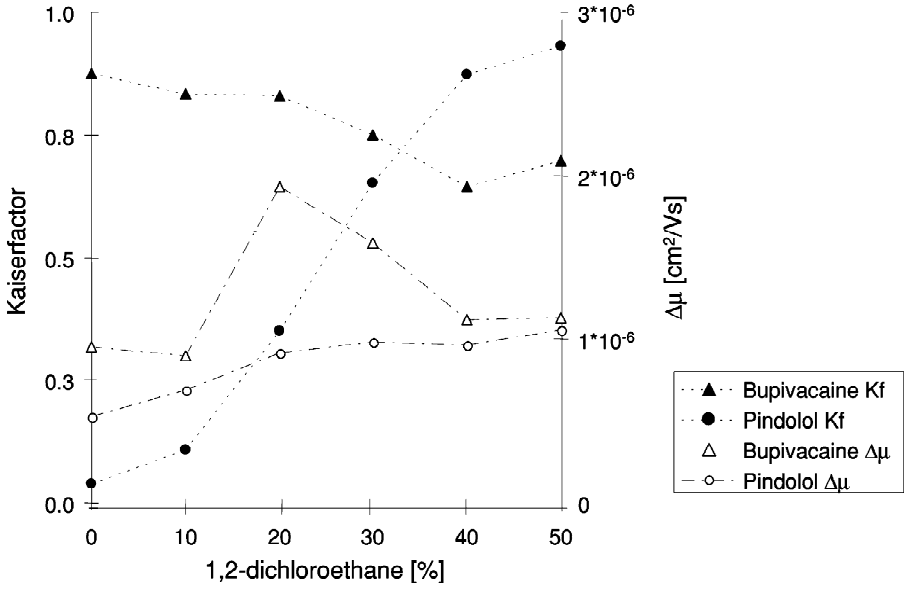

Fig. 8. Influence of 1,2-dichloroethane on enantioresolution and $\Delta\mu$ of pindolol and bupivacaine. △ = $\Delta\mu$ Bupivacaine; ○ = $\Delta\mu$ pindolol; ▲ = kf bupivacaine; ● = kf pindolol. BGE: 125 m*M* *L*-ZGP and 50 m*M* NH_4Ac in MeOH with addition of 1,2-dichloroethane added to the BGE. For other conditions, see experimental section 2.3.

Furthermore, high throughput analysis will save time also in ordinary method development. Jansson and Roeraade [28] separated propranolol from felodipine within 35 s (19.5-cm effective capillary length) using pure *N*-methylformamide as BGE. Altria et al. have shown an enantioseparation of picumeterol and clenbuterol within 2 min with dimethyl-β-cyclodextrin as the chiral selector and using short-end injection (8.5-cm effective capillary length) [17]. Also Bergholdt and Lehmann have shown enantioseparation of ormeloxifene within 40 s using sulphated β-cyclodextrin as chiral additive, and with ''extended'' short-end injection the separation time was decreased to 8.4 s [29]. The analysis also included a 60-s introduction of a selector plug after the sample zone.

In the present study, enantioseparation of the local anaesthetic drug mepivacaine was performed within 72 s by short-end injection on the cathodic side (Fig. 9). The background electrolyte contained 175 m*M* *L*-ZGP and 50 m*M* NH_4Ac with 20% 2-PrOH in MeOH, and the effective length of the capillary was 8.5 cm. This demonstrates the potential of NACE for chiral high throughput analysis.

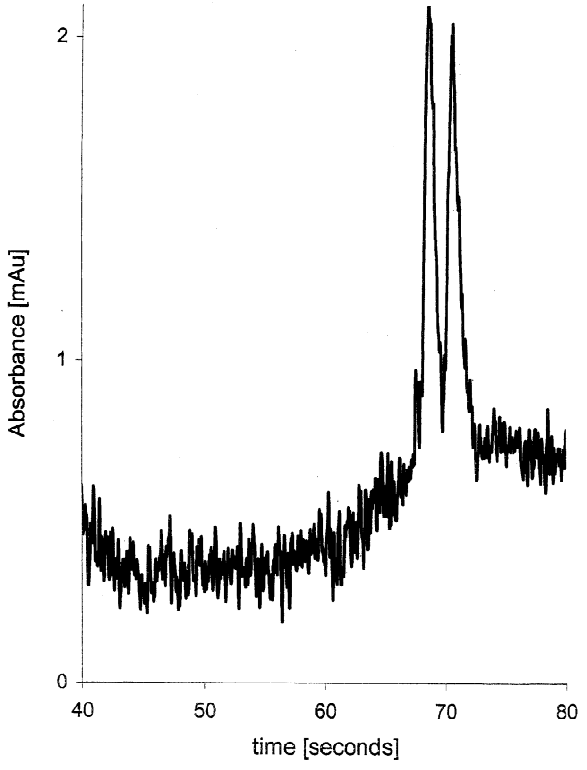

Fig. 9. Fast separation by short-end injection of mepivacaine. BGE: 175 m*M* *L*-ZGP and 50 m*M* NH_4Ac with 20% 2-PrOH in MeOH. Total length 30 cm, length to detector, 8.5 cm. Voltage: 20 kV. For other conditions, see Section 2.3.

4. Conclusions

N-Benzoxycarbonylglycyl-*L*-proline (*L*-ZGP) has been introduced as a chiral counter-ion for separation of enantiomeric amines in non-aqueous capillary electrophoresis. Separation of different types of pharmacologically active compounds (e.g. mepivacaine, pindolol) was performed. The majority of solutes previously enantioseparated in HPLC was also enantioseparated in NACE, which proves that enantioselective complexation with *L*-ZGP prevails.

In order to overcome the problem with high EOF in MeOH, addition of 2-PrOH, dichloromethane and 1,2-dichloroethane was evaluated. A distinct decrease in the EOF was observed with enhanced enantioresolution for a majority of the substances as a result. The system with 20% 1,2-dicloroethane gave good repeatability (RSD 2.2% for the migration time, $n=10$).

Enantioseparation of mepivacaine was performed within 72 s by short-end injection on the cathodic side.

Acknowledgements

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