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Investigation of an enantioselective non-aqueous capillary electrochromatography system applied to the separation of chiral acids☆

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Abstract

A weak anion-exchange type chiral stationary phase (CSP) based on *tert*.-butylcarbamoylquinine as chiral selector and silica as chromatographic support was applied to non-aqueous capillary electrochromatography. The mobile phases used consisted of acetonitrile and methanol as organic solvents, and acetic acid and triethylamine were added as background electrolytes. The influence of several experimental parameters (electrolyte concentration, acetic acid–triethylamine ratio, acetonitrile–methanol ratio and temperature) was evaluated in order to obtain improved enantioselectivity and efficiency as well as short run times for the enantiomeric separation of negatively charged chiral analytes including benzyloxycarbonyl, *N*-(3,5-dinitrobenzyloxycarbonyl, 9-fluorenylmethoxycarbonyl, benzoyl, acetyl and *N*-(2,4-dinitrophenyl) derivatized amino acids and profens. Solvent composition of acetonitrile–methanol (80:20) and enhanced electrolyte concentrations up to 600 m*M* acetic acid at a constant acid–base ratio of 100:1 with high applied voltages of −25 kV proved to be optimum regarding short retention times and improved efficiencies. For example, the enantiomers of Fmoc-Leu could be separated in less than 10 min with a resolution factor of 6.9 and about 100 000 theoretical plates per meter.

Keywords: Non-aqueous electrochromatography; Enantiomer separation; Chiral stationary phases, electrochromatography; Amino acids; Profens

1. Introduction

Since the introduction of capillary electrochromatography (CEC) [1,2], this hybrid technique of capillary electrophoresis (CE) and capillary liquid chromatography (μLC) generated great interest in recent years [3–20].

The high efficiency values that can be achieved in CEC makes it an attractive technique also for the separation of enantiomers employing chiral stationary phases (CSPs). In this context, several enantioselective CEC applications utilizing capillary columns packed with chiral sorbents have been reported, dealing with protein type CSPs [21,22], cyclodextrin-based CSPs [23–25], ''Pirkle-concept'' type CSPs (e.g., naproxen derived CSP and Whelk-O1) [26], vancomycin [27,28] and teicoplanin [29] based CSPs, polymeric CSPs [30] based on silica particles covalently modified with poly-*N*-acryloyl-(*S*)-phenylalanine ethylester or using silica particles coated with cellulose tris-(3,5-dimethylphenyl-

☆Dedicated to Professor Csaba Horváth at the occasion of his 70th birthday

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carbamate), and chiral anion-exchange type CSPs [31,32]. All these mentioned CSPs are based on silica particles as chromatographic support material. Accordingly, the negatively charged residual silanol groups which are still present after chemical surface modification, will determine, contribute to or modulate the electroosmotic flow (EOF). Commonly these chirally modified sorbents are packed into capillaries and the chromatographic bed has to be stabilized by retaining frits. However, also monolithic type chiral stationary phases and continuous column packings have been developed for enantioselective CEC. In one approach, monolithic molecular imprinted polymer (MIP) CSPs have been prepared by in-situ polymerization of achiral monomers in presence of an enantiomeric template in a fused-silica (FS) capillary [17,33]. In another attempt, a monolithic CSP has been synthesized by in-situ copolymerization of a monomeric chiral selector, derived from (*S*)-valine and functionalized with methacrylic groups, and methacrylic type co-monomers and cross-linker in a FS capillary [34]. In these cases, the negatively chargeable acid groups stemming either from the (meth)acrylic acid or from other co-monomers, e.g., from 2-acrylamido-2-methylpropanesulfonic acid, will be responsible for the generation of a cathodic EOF.

With regards to the liquid phase used, the majority of CEC applications are performed with buffered aqueous–organic mobile phase systems similar to a reversed-phase mode. There are only a few reports dealing of CEC under non-aqueous conditions, both for achiral [35,36] and chiral separations [28,37], though better solubility of analytes and in particular lower occurring currents are convincing and powerful advantages of such non-aqueous separation conditions.

Recently, we reported on the use of silica based weak anion-exchange (WAX) type CSPs derived from quinine as chiral selector (SO) for the CEC enantioseparation of acidic chiral analytes (selectands, SAs) implementing reversed-phase conditions [31,32]. Thus, these WAX type CSPs have been operated with mobile phase conditions at which the basic quinuclidine residue of the quinine moiety is protonated thus being positively charged and acting as weak anion-exchanging group (see Fig. 1). In the protonated form the quinine carbamate type SO has for its CEC application a double function: (i) it represents the high-affinity chiral binding site for enantioselective SO–SA complexation and enantioselective molecular recognition driven by a Coulomb interaction resulting in an ion-exchange process. (ii) It may be the source for the generation of an anodic EOF.

Overall, however, the chirally modified silica surface of the CSP will have ''zwitterionic'' character due to the presence of negatively charged residual silanols and the positively charged amine function. Thus, the individual contribution of these groups on the actual EOF will strongly depend on the working pH of the mobile phase. As a result, the direction of the EOF depends on the pH yielding a cathodic EOF above the apparent isoelectric point (p*I*) of the

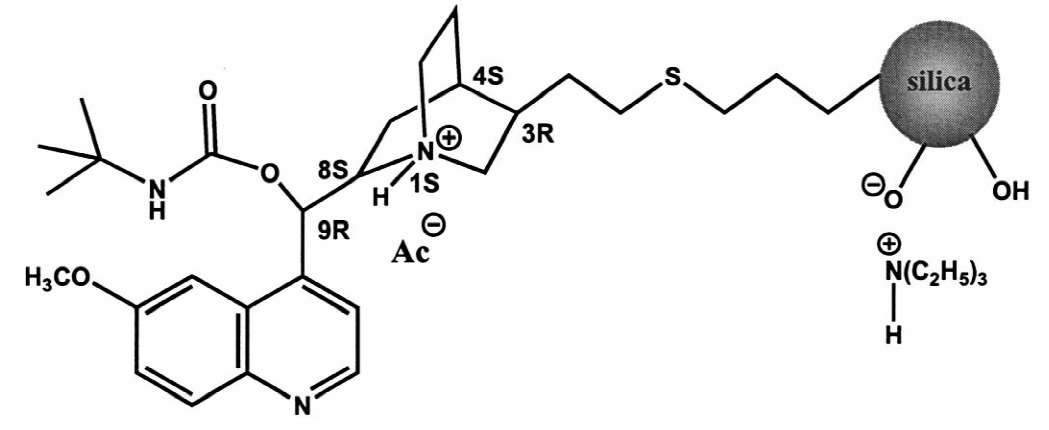

Fig. 1. Structure of the WAX type CSP investigated in this study.

surface modification and an anodic EOF below the apparent p*I* [31,32]. The latter conditions are preferred for the separation of acidic analytes. It is obvious that for such anion-exchange separation systems an anodically directed electrophoretic transport increment has to be considered in addition to transport with the EOF. This electrophoretic transport increment, which, of course, strongly depends on the pH of the mobile phase, can either be of minor influence to overall transport of the analytes (compared to electroosmotic transport), but under specific conditions it may become the dominating contribution to overall transport so that it may occur in case of an anodic EOF that the negatively charged analytes are eluted before the EOF marker.

Unfortunately, due to the rather low buffer concentrations that have to be used in aqueous CEC, in order to avoid Joule heating and bubble formation, and owing to strong ionic interactions between negatively charged SAs and positively charged SO rather long run times resulted under reversed-phase conditions [32]. For these aqueous–organic conditions it has been proven that it is rather difficult to balance the strong ionic interactions. In contrast, in the non-aqueous CEC mode (NA-CEC) much higher electrolyte concentrations can be used without significant heat generation resulting in higher elution strength (related to ion-exchange mechanism) and thus solving the problem of long run times.

Accordingly, in this study, the performance of the above cited weak anion-exchange type CSP, based on *tert*.-butylcarbamoylquinine covalently bonded to thiol-modified 3 μm porous silica particles (see Fig. 1), has been investigated under non-aqueous CEC conditions. Changing several experimental parameters which influence in a complex manner the involved processes (anion-exchange, electroosmotic mobility, electrophoretic mobility), the obtained results have been evaluated critically with regards to enantioselectivity, efficiency and speed.

2. Experimental

2.1. Materials

The synthesis of the CSP under study (see Fig. 1) has been described recently [32] and followed a standard protocol that is described in detail elsewhere [38].

Briefly, Hypersil 120, 3 μm (Hypersil, purchased from HPLC Service, Breitenfurt, Austria, Leonberg, Germany) was modified with 3-mercaptopropyltrimethoxysilane (elemental analysis of modified particles: 2.95% C, 0.61% H). Subsequently, the *tert*.-butylcarbamoylquinine (tBuCQN) SO [39,40] was covalently attached to this thiol-modified sorbent by radical addition reaction. Elemental analysis yielded the following results: 7.59% C, 1.13% H, 0.66% N, from which a mean SO coverage of 0.16 mmol SO/g CSP has been calculated. The chirally modified silica particles were slurry packed at 1000 bar into a fused-silica capillary of 100 μm I.D. with a chromatographic bed length of 25 cm and a total length of 33.5 cm. Retaining frits were fabricated by heat sintering of the CSP. A detection window was made by cutting off the polyimide coating close to the end frit (25 cm from inlet).

The analytes *N*-(3,5-dinitrobenzyloxycarbonyl) (DNZ) and *N*-(2,4-dinitrophenyl) (DNP) amino acids were prepared by derivatization of the respective amino acids with 3,5-dinitrobenzyl chloroformate (synthesized from phosgen and 3,5-dinitrobenzyl alcohol) and Sangers reagent (2,4-dinitrofluorobenzene; Aldrich), respectively, following standard derivatization protocols [41]. All other chiral analytes were purchased from Bachem (Bubendorf, Switzerland), Sigma or Aldrich.

Acetonitrile (ACN) and methanol (MeOH) were of HPLC grade and supplied by J.T. Baker (The Netherlands). Glacial acetic acid (AcOH) and triethylamine (NEt_3) were of analytical grade and from Fluka.

The mobile phases were filtered through a 0.2-μm nylon membrane filter and degassed by sonication prior to use.

2.2. Instrumentation

All experiments were carried out on a Hewlett-Packard HP ^{3D}CE system with external pressurization and HP ^{3D}CE ChemStation software. Throughout the study an external pressure of 8 bar was applied to both buffer vials.

3. Results and discussion

3.1. Influence of acid–base ratio

Clearly, also in the non-aqueous mode of operation of the currently investigated quinine carbamate based CSP ion-exchange mechanisms are the primary retention and selectivity principles. This, however, requires acidic mobile phase conditions so that the quinine moiety will be protonated and positively charged. Since the pH is not defined in a non-aqueous liquid phase we studied the influence of various acid–base ratios, i.e., glacial acetic acid to triethylamine of which the background electrolyte (BGE) is made up. Obviously, excess of acid has to be added to meet the aforementioned requirements. In this context, it should be emphasized that acid–base equilibria are shifted in organic solvent mixtures resulting in higher apparent pH values than expected for an aqueous mobile phase consisting of the same electrolyte system.

Advantageously, under such acidic conditions the net charge of the ''zwitterionic'' CSP is positive, yielding anodic flow and co-electrophoretic elution of the negatively charged analytes. From a practical point of view, the concentration of acetic acid in acetonitrile–methanol (80:20) mixture was kept constant at 200 m*M*, while the triethylamine concentration was varied as depicted in Fig. 2. Acetonitrile–methanol mixtures have been selected as solvent systems for the high EOF rates as well as reasonable solute and electrolyte solubility expected for such a polar solvent mixture. For the choice of the electrolyte it had to be considered that several salts (like phosphates, borates) are not very soluble in polar organic media, while much less solubility problems have been observed with triethylammonium acetate and acetic acid–triethylamine mixtures, respectively.

The actual retention and migration behavior, respectively, of the acidic analytes is a result of the three processes involved: adsorption to the anion exchanger that is determined by the actual capacity of the ion exchanger, movement with the EOF, and electrophoretic transport according to the effective electrophoretic mobility of the negatively charged species under the given conditions.

As can be seen from Fig. 2A, the observed mobilities (μ_{obs}) of 9-fluorenylmethyoxycarbonyl (Fmoc)-Leu enantiomers decrease with increasing triethylamine concentration, equivalent to an increase of the apparent pH of the liquid phase. This is due to lower electroosmotic mobility, μ_{eo} (negative sign

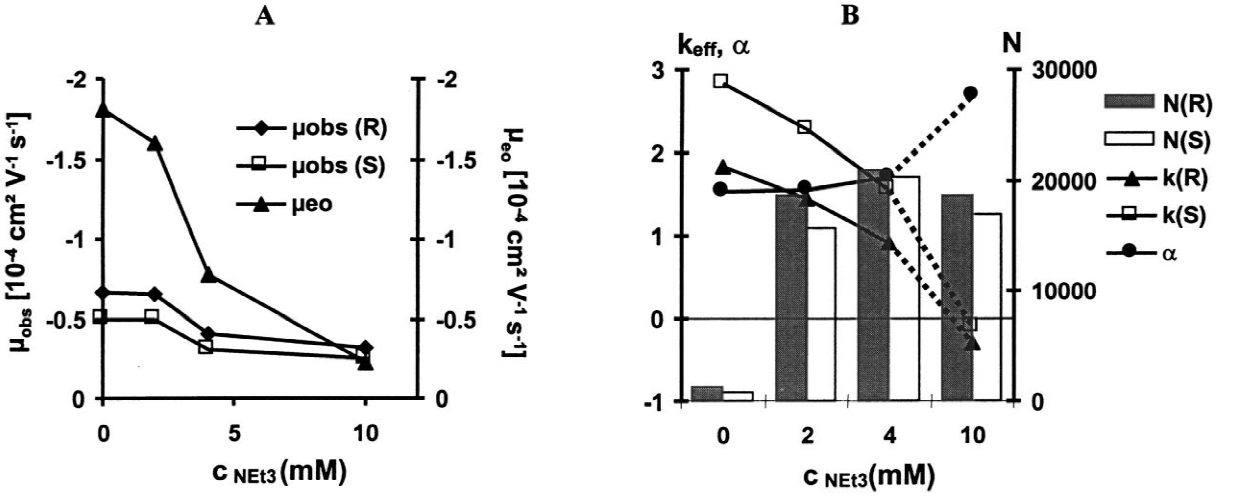

Fig. 2. Influence of various acid–base ratios at constant acetic acid concentration (200 m*M*) on (A) electroosmotic mobilities of the eluent (μ_{eo}) and observed mobilities of (*R*)- and (*S*)-Fmoc-Leu enantiomers (μ_{obs}), and (B) on retention factors (k_{eff}), enantioselectivity (α), and on theoretical plate numbers (*N*). Conditions: column: 335 mm (effective length 250 mm)×0.1 mm I.D.; mobile phase: ACN–MeOH (80:20)+200 m*M* acetic acid+various amounts of NEt_3; *T*: 20°C; voltage: −25 kV; injection: −10 kV/10 s; detection: UV at 254 nm; EOF marker: acetone.

indicates anodic direction), at higher triethylamine concentrations at which the actual potential of the anion exchanger (and thus EOF) is reduced. Simultaneously, the electrophoretic transport increment gains in significance with increasing triethylamine concentration, and as consequence, at 10 m*M* triethylamine this electrophoretic transport process contributes significantly to overall transport of the analytes indicated by elution of the Fmoc-Leu enantiomers before the EOF marker acetone.

For such a separation system, description of the separation by the chromatographic terminology is no longer appropriate, and consequently the electrophoretic terminology reporting observed mobilities as retention parameter is preferred (therefore, dotted lines are used in Fig. 2B for effective capacity values, k_{eff}, which give negative numbers for such separation systems, as well as the α value, which has in this case been calculated from the corresponding positive k_{eff} values). Fig. 3 shows the remarkable chromatogram obtained for Fmoc-Leu under the described conditions with 10 m*M* triethylamine showing the analytes eluting before the EOF marker.

Higher triethylamine concentrations than 10 m*M* have not been investigated owing to already high electric currents (higher than −10 μA) and also due

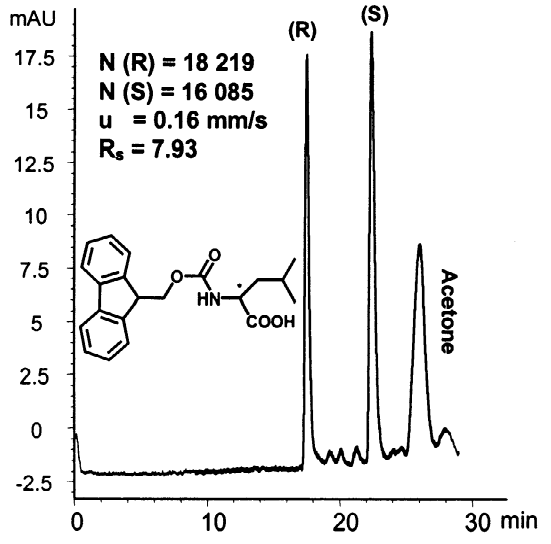

Fig. 3. Chromatogram of the enantioseparation of (*R*)- and (*S*)-Fmoc-Leu under conditions, at which the negatively chargeable SA enantiomers elute before the EOF marker. Electrolytes: 200 m*M* AcOH, 10 m*M* NEt_3. Other conditions as specified in Fig. 2.

to long run times (more than 30 min), although it seemed that slightly enhanced enantioselectivity values can be obtained at somewhat higher triethylamine concentrations. On the other side, in the investigated triethylamine range the highest efficiency was observed at 4 m*M* triethylamine with theoretical plate numbers of about 20 000. This was, unfortunately, accompanied by the doubling of retention times. Compromising high efficiencies and short analysis times at concentrations of 2 m*M* triethylamine, which corresponds to a molar ratio of AcOH–NEt_3 of 100:1, these conditions were selected for further optimization and considered to be adequate for practical applications of the given NA-CEC system. At this point we do not want further to interpret the experimental finding as this may be too speculative; as the current knowledge on the buffer and organic modifier effects observed in NA-CEC applied to charged solutes are complex and the deconvolution of the individual processes needs further investigation.

3.2. Influence of total electrolyte concentration

Besides the (apparent) pH of the liquid phase, the concentration of the total counterions is a key parameter to control retention in ion-exchange chromatography and CEC by balancing ionic interactions between oppositely charged solutes and sorbent. As already discussed, in NA-CEC the generated currents are considerably lower than under aqueous–organic conditions. As a consequence, the concentration of counterions and total buffer ions, respectively, in the mobile phase can be significantly higher. This clearly broadens the range of experimental conditions that can be used in CEC, and advantageously, greatly improves the flexibility as well as the versatility of CEC.

The acetate concentration studied ranged from 20 to 600 m*M* (at a constant acid–base ratio of 100:1) generating currents up to only −10 μA at an applied voltage of −25 kV. The EOF, unfortunately, is adversely affected by increasing the total BGE concentration as can be see from Fig. 4A. Obviously, double layer thickness and ζ-potential decrease with increasing BGE concentrations so that, as expected, the EOF velocity (anodic EOF) is reduced (see Fig. 4A). For example, a linear flow-rate of 1.52 mm/s

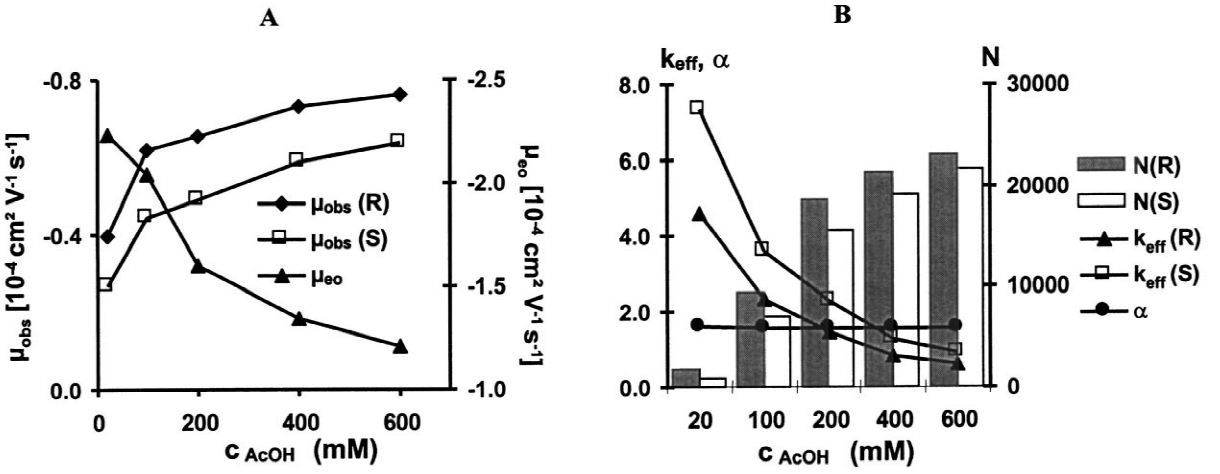

Fig. 4. Influence of increasing acetic acid (AcOH) concentration on (A) eluent mobility (μ_{eo}) and observed mobilities (μ_{obs}) of (*R*,*S*)-Fmoc-Leu, and (B) on retention factors (k_{eff}), enantioselectivity (α), and on theoretical plate numbers (N). AcOH–NEt_3 ratio=100:1. Other conditions as specified in Fig. 2.

was obtained at an acetate concentration of 100 m*M*, while at 600 m*M* acetate the linear flow-rate was only 0.9 mm/s (see also Fig. 5). The lower actual capacity of the chiral anion exchanger at higher counterion concentrations, however, leads to shorter retention times and higher observed mobilities (μ_{obs}) of the Fmoc-Leu enantiomers, or expressed in chromatographic terms to decreased retention factors (see Fig. 4A). Advantageously, α values remain more or less unaffected by the change of buffer concentrations, and in addition to shorter retention times, efficiency and symmetry of the peaks improved significantly at higher buffer and acetate concentrations. This observation is clearly demonstrated by Fig. 5, which shows the chromatograms of the enantioseparation of (*R*,*S*)-Fmoc-Leu at different acetic acid concentrations and constant acid–base ratio of 100:1.

3.3. Influence of organic solvent mixtures

The effect of various ACN–MeOH ratios (at a constant acid–base ratio of 200 m*M* acetic acid and 2 m*M* triethylamine) on electroosmotic characteristics and chromatographic behavior of (*R*,*S*)-Fmoc-Leu has also been investigated (see Fig. 6). In a recent study of non-aqueous CE systems, a maximum of ϵ/η ratio and thus of the EOF was found for an ACN–MeOH ratio of 80:20 [42]. However, as can be seen in Fig. 6A for the given NA-CEC system and the silica based WAX type CSP a maximum of EOF (u=1.59 mm/s) was obtained at an ACN–MeOH ratio of 60:40. In this context, it should be mentioned that acid–base equilibria are shifted at different extents in different solvent mixtures so that different apparent pH values are yielded with different ACN–MeOH mixtures. Commonly, ACN cause larger shifts than MeOH. Therefore, it can be supposed that the apparent pH value is higher for mobile phases with higher acetonitrile content.

Interestingly, the elution strength of the various ACN–MeOH mixtures (containing the same amount of BGE) is more or less directly related to the EOF velocity. Thus, highest observed mobilities and thus shortest retention times of (*R*)- and (*S*)-Fmoc-Leu enantiomers are obtained with eluents containing 40–60% methanol in acetonitrile. Although enantioselectivity steadily increases with increasing MeOH content (see Fig. 6B), better enantioseparations were obtained with MeOH contents in the range of 20–40%, as theoretical plate numbers declined dramatically at higher methanol contents (see Fig. 6B). These findings are clearly underlined by the chromatograms presented in Fig. 7, showing the enantioseparations of (*R*,*S*)-Fmoc-Leu achieved with different ACN–MeOH mixtures. With regards to optimum

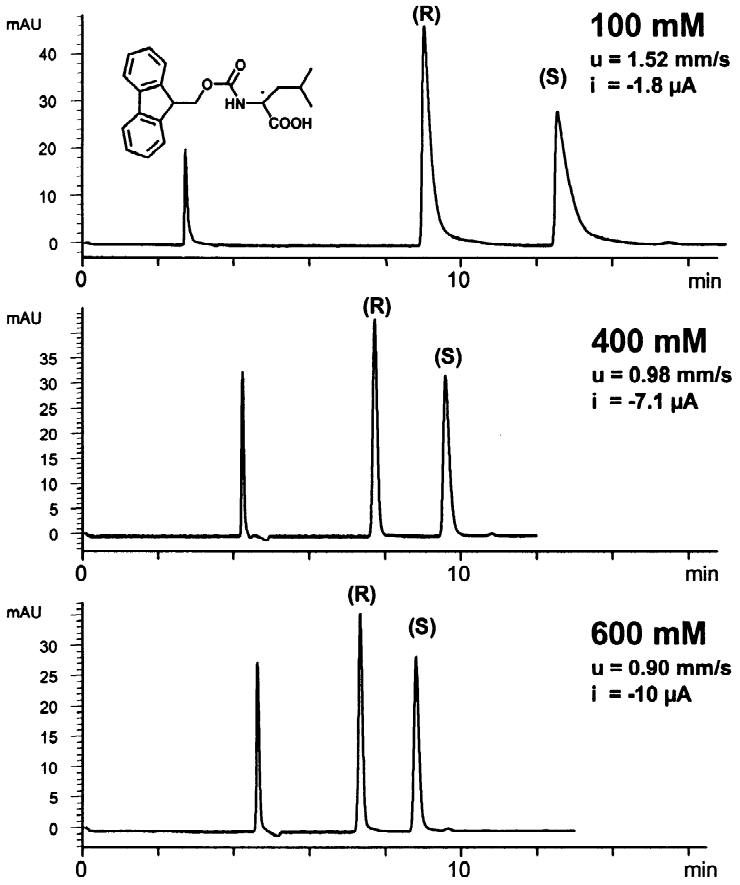

Fig. 5. Comparison of chromatograms of the enantioseparation of (*R,S*)-Fmoc-Leu obtained with different electrolyte concentrations. Conditions as specified in Fig. 4 and Fig. 2.

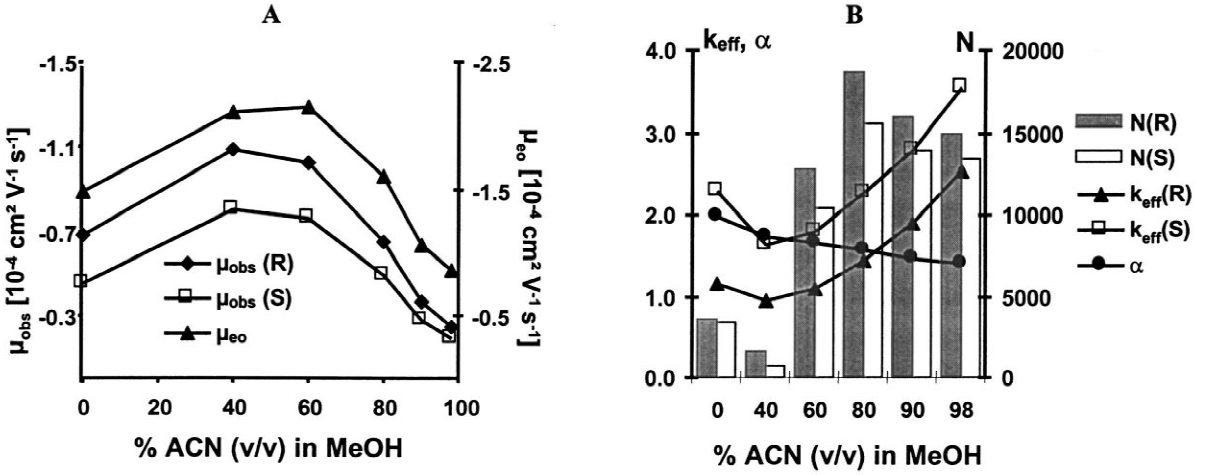

Fig. 6. Influence of varying acetonitrile–methanol ratios on (A) electroosmotic mobility (μ_{eo}) and observed mobilities (μ_{obs}) of (*R*)- and (*S*)-Fmoc-Leu, and (B) on retention factors (k_{eff}), enantioselectivity (α), and on theoretical plate numbers (*N*). Mobile phase: various ACN–MeOH mixtures+200 m*M* acetic acid+2 m*M* NEt_3; all other conditions as specified in Fig. 2.

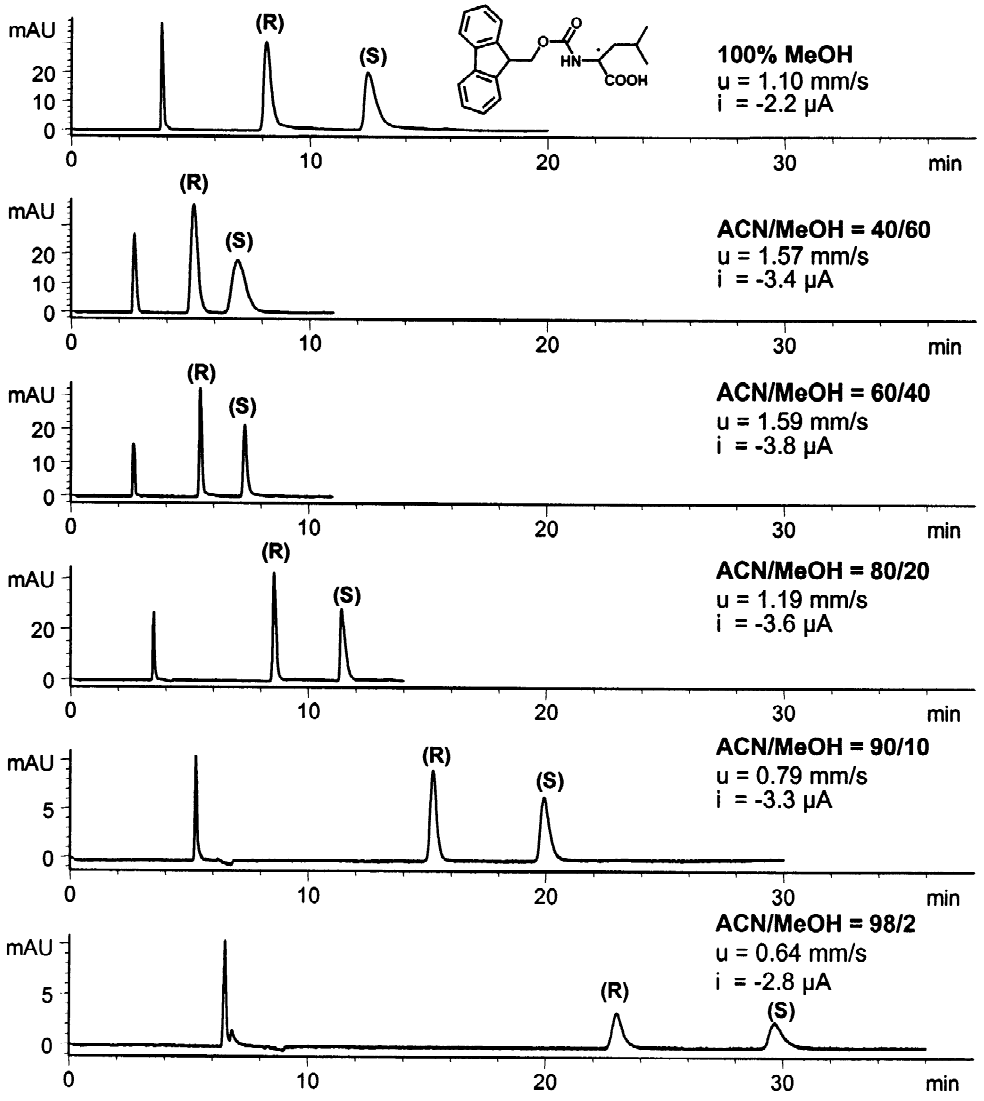

Fig. 7. Chromatograms of enantioseparation of (*R,S*)-Fmoc-Leu at various acetonitrile–methanol mixtures. Conditions as specified in Fig. 6 and Fig. 2.

efficiencies and short analysis time, conditions with an ACN–MeOH ratio of 80:20 were considered to be useful to continue further studies.

3.4. Influence of temperature

In fact, temperature has to be considered to exert a significant influence on chromatographic parameters related to thermodynamic figures of merit (like effective retention factors and enantioselectivity values) as well as to kinetic ones (like theoretical plate numbers and plate heights). In addition, one should bear in mind that in CEC temperature does also significantly effect flow-rates, since both ϵ/η ratio and ζ-potential, which primarily determine electroosmotic mobility, are dependent on temperature.

Accordingly, higher EOF rates are yielded at higher temperatures as demonstrated by Fig. 8A. For example, the linear flow velocity could be raised from $u=1.36$ mm/s at 15°C to $u=1.45$ mm/s at 50°C. The temperature dependence of effective retention factors (k_{eff}) and enantioselectivity (α) of

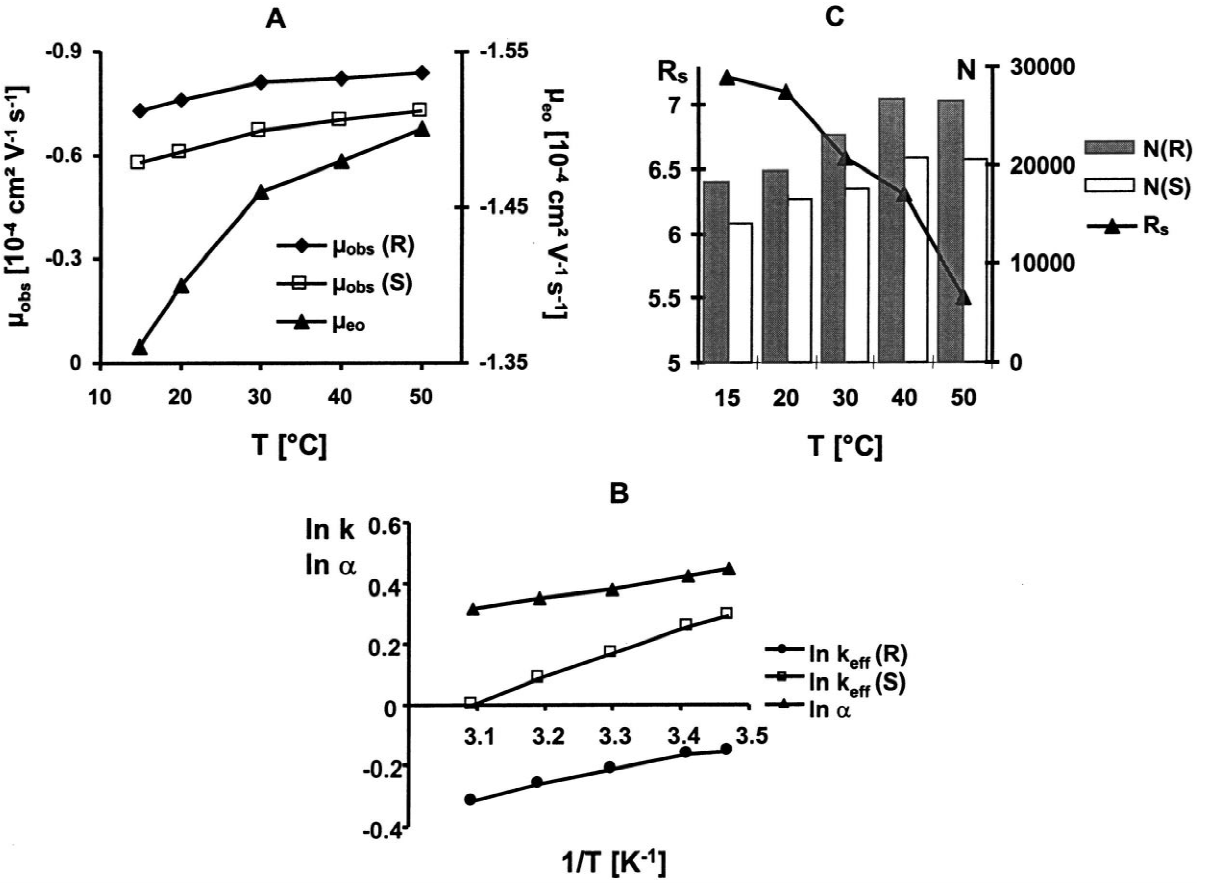

Fig. 8. Influence of column temperature on (A) electroosmotic mobility (μ_{eo}) and observed mobilities of (*R*)- and (*S*)-Fmoc-Leu (μ_{obs}), and (B) on retention factors (k_{eff}) and enantioselectivity (α) (demonstrated by Van 't Hoff plots), and (C) on resolution (R_s) and on theoretical plate numbers (N). Mobile phase: ACN–MeOH (80:20)+400 m*M* acetic acid+4 m*M* NEt_3; *T*: 15–50°C; other conditions as specified in Fig. 2.

(*R*,*S*)-Fmoc-Leu is presented in Fig. 8B in the form of van 't Hoff plots. As expected and in agreement with the corresponding HPLC data, at elevated temperatures retention factors as well as α values decreased indicating enthalpic control of the enantioseparation. Despite lower enantioselectivity, enhanced theoretical plate numbers at higher temperatures (up to 106 000 theoretical plates per meter) (see Fig. 8C) lead to greatly improved enantioseparations, also due to shorter run times; for Fmoc-Leu enantiomers optimum R_s of 7.22 has been observed at 15°C.

3.5. Enantioseparation of various acidic SAs

The above described optimized experimental conditions have been utilized to evaluate the enantioseparation capability of the quinine derived WAX type CSP under study for a larger set of different chiral acidic compounds under NA-CEC conditions. The results obtained are presented in Table 1. In contrast to CEC under hydro–organic conditions, the non-aqueous application enabled the use of high buffer concentrations, resulting in shorter retention times. Most of the injected analytes were eluted before 20 min and most of them showed in addition greatly improved peak symmetries. Overall, the enantioselectivity values obtained for the given experimental conditions were mostly somewhat smaller compared to optimized aqueous–organic mobile phase conditions. However, owing to the higher efficiencies achieved for all the investigated SAs (up to 22 000 theoretical plates per column,

Table 1
Data obtained for the CEC enantioseparation of various chiral acids on the quinine-derived WAX type CSP under study (see also Fig. 1) under optimized non-aqueous mobile phase conditions[a]

Racemic analyte	i (μA)[b]	t_0 (min)	t_1 (min)	t_2 (min)	k_{eff}[c] (1)	k_{eff}[c] (2)	α[d]	R_s	N (1)	N (2)	e.o.[e]
DNZ-Leu	−7.0	4.1	7.5	12.2	0.45	0.66	1.47	12.5	14 691	9452	*R*
DNZ-Phe	−7.2	4.2	10.4	15.7	0.60	0.74	1.22	10.8	14 231	10 017	*R*
DNZ-Pro	−7.2	4.2	7.6	8.0	0.45	0.47	1.06	1.6	16 810	14 494	*R*
Z-Leu	−7.3	4.3	7.1	7.6	0.40	0.44	1.09	2.5	20 658	20 107	*R*
Z-Phe	−7.7	4.4	9.8	10.4	0.55	0.58	1.05	1.8	18 848	10 744	*R*
Z-Tyr	−7.8	4.4	14.3	15.3	0.69	0.71	1.03	1.9	14 460	10 471	*R*
Bz-Leu	−8.0	4.5	8.0	11.3	0.44	0.60	1.37	10.6	20 340	13 174	*R*
Bz-Phe	−8.2	4.6	10.9	15.2	0.58	0.70	1.20	9.8	18 337	12 065	*R*
Ac-Phe	−8.8	4.9	9.7	11.1	0.50	0.56	1.13	4.2	15 854	15 639	*R*
Ac-Trp	−9.1	4.9	13.5	15.6	0.64	0.69	1.08	3.7	10 716	10 261	*R*
Fmoc-Ala	−7.8	4.4	8.9	10.4	0.50	0.57	1.15	4.9	14 948	15 388	*R*
Fmoc-Asn	−8.0	4.5	14.5	17.1	0.69	0.74	1.07	3.9	10 250	8021	*R*
Fmoc-Trp	−8.3	4.6	14.6	17.7	0.68	0.74	1.08	5.5	15 473	11 798	*R*
Fmoc-Arg	−8.6	4.7	14.6	18.1	0.68	0.74	1.09	6.0	14 003	12 076	*R*
DNP-Phe	−7.2	4.2	14.9	18.5	0.72	0.78	1.08	5.2	9302	8859	*S*
DNP-Lys	−7.8	4.4	18.8	23.4	0.76	0.81	1.06	4.8	10 336	6430	*S*
DNP-α-amino caprylic acid	−8.7	4.7	11.9	14.3	0.61	0.67	1.11	6.3	21 827	17 318	*S*
DNP-Phe	−9.2	4.9	13.7	16.7	0.64	0.71	1.10	7.0	20 245	17 956	*S*
DNP-Ser	−8.4	4.1	16.8	21.7	0.76	0.81	1.07	4.1	8532	6227	*S*
Dichlorprop	−6.9	3.9	11.6	12.5	0.66	0.69	1.04	2.4	18 027	15 896	n.d.
Suprofen	−7.1	4.1	6.3	6.5	0.34	0.36	1.05	0.7	12 041	10 779	n.d.
Flurbiprofen	−7.2	4.2	5.8	5.9	0.27	0.29	1.06	0.7	15 842	13 655	n.d.
Etodolac	−7.2	4.2	5.5	5.7	0.24	0.27	1.11	1.2	19 749	18 769	n.d.
Sulfinpyrazone	−7.9	4.5	9.2	9.8	0.51	0.54	1.06	1.9	15 149	16 633	n.d.

[a] Conditions: column: 335 mm (effective length 250 mm)×0.1 mm I.D.; mobile phase: ACN–MeOH (80:20)+400 m*M* acetic acid+4 m*M* NEt_3; *T*: 20°C; voltage: −25 kV; injection: −5 kV/5 s; detection: UV at 215 and 254 nm; EOF marker: acetone. DNZ=*N*-(3,5,-Dinitrobenzyloxycarbonyl; Z=benzyloxycarbonyl; Bz=benzoyl; Ac=acetyl; Fmoc=9-fluorenylmethoxycarbonyl; DNP=*N*-(2,4-dinitrophenyl).
[b] Fluctuations obtained because current slightly increased from run to run and analytes were not measured in this order
[c] $k_{eff}=(t_R-t_0)/t_0$.
[d] $\alpha=k_{eff}(2)/k_{eff}(1)$.
[e] Elution order, configuration of the first eluted enantiomer. n.d. Not determined.

corresponding to 88 000 theoretical plates per meter) and due to relatively short run times, reasonable and practically useful enantioseparations could be achieved (see also Fig. 9).

4. Conclusion

It could be shown that silica based chiral anion exchangers based on quinine carbamate, acting as chiral selector, can be successfully applied for CEC enantioseparation of chiral acids under non-aqueous mobile phase conditions. The low electric currents that are generated under purely organic mobile phase conditions allowed the use of high buffer concentrations (up to 600 m*M* acetic acid–6 m*M* triethylamine admixed to an acetonitrile–methanol mixture had been investigated without problems of Joule heating effects) and the application of high electric fields, thus overcoming the problem with long retention times due to the strong ionic interaction between positively charged chiral sorbent and negatively charged chiral analytes.

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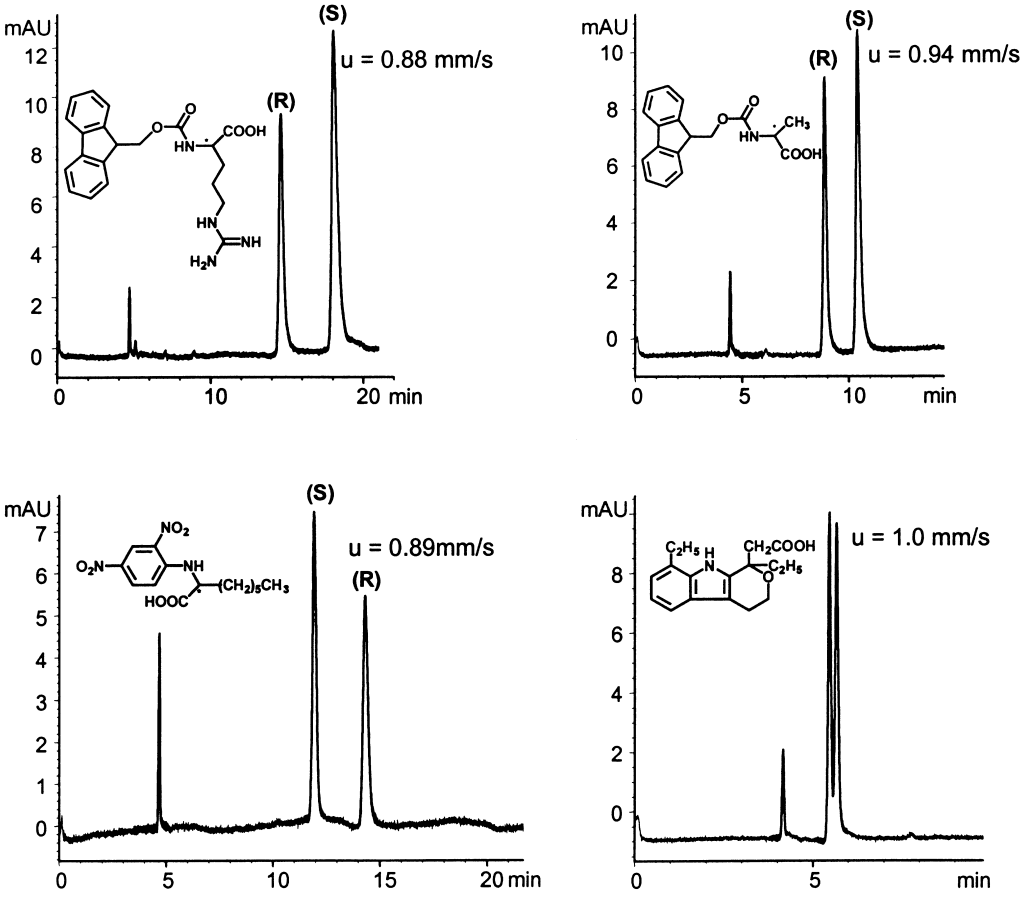

Fig. 9. Chromatograms of CEC enantioseparations of various chiral acids on a silica-based quinine-derived WAX type CSP under non-aqueous mobile phase conditions. Conditions as in Table 1.

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