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Enantioseparations in non-aqueous capillary electrochromatography using polysaccharide type chiral stationary phases

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Abstract

Enantioseparations of chiral compounds with different structures were studied in non-aqueous capillary electrochromatography (NAQ CEC). Three different polysaccharide derivatives, cellulose tris(3,5-dimethylphenylcarbamate) (Chiralcel OD), amylose tris(3,5-dimethylphenylcarbamate) (Chiralpak AD) and cellulose tris(4-methylbenzoate) (Chiralcel OJ) were used as chiral stationary phases (CSPs). Methanolic or ethanolic ammonium acetate solutions served as a mobile phase. The effect of the type of the CSP, the loading of the chiral selector on wide-pore aminopropyl derivatized silica gel and operational parameters such as apparent pH, applied voltage, etc. on the EOF and chromatographic characteristics (α, N, R_S) were studied. NAQ CEC represents a valuable alternative and an extension to chiral separations by HPLC with common-size columns as well as to capillary LC and CEC in aqueous buffers.

Keywords: Enantiomer separations; Chiral stationary phases, CEC; Polysaccharide derivatives

1. Introduction

Capillary electrochromatography (CEC) separations have been essentially studied in aqueous–organic buffers at present [1–6]. Recently we found that non-aqueous CEC enantioseparations are feasible using a helically chiral polymethacrylate type chiral stationary phase (CSP) [7]. Lämmerhofer and Lindner have reported non-aqueous CEC enantioseparations using quinine derivatives as chiral mobile phase additives in combination with an achiral stationary phase (C_{18}) [8]. Although water as a component of a background electrolyte (BGE) offers certain advantages such as safety, non-volatility, low costs, etc., it is not always a desirable medium in capillary electrophoresis (CE) or CEC separations. This may be due to the limited solubility or stability of analytes in aqueous solvents. These problems can be solved by using non-aqueous solvents. In addition, non-aqueous background electrolytes (BGE) also generate lower currents and as a result lower Joule heat [8] and are better compatible with on-line coupling of a separation system to a mass spectrometer. However, the most important reason for the extension of CEC separations to non-aqueous solvents is that some stationary phases for high-performance liquid chromatography (HPLC) do not work effectively in the presence of water as a mobile phase. This relates to a certain extent to the most universal chiral stationary phases (CSPs) for HPLC-enantioseparations, i.e. polysaccharide derivatives. Although these materials possess significant chiral recognition ability in aqueous mobile phases [9] they were originally developed [10] and exhibit the widest

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applicability in non-aqueous mobile phases [11]. In our recent study it was shown that polysaccharide phenylcarbamates can be used for the enantioseparations in CEC in the presence of aqueous buffers [12].

In order to extend the application of polysaccharide type CSPs to enantioseparations in CEC and to gain more knowledge about the fundamentals of NAQ CEC the enantioseparations were studied by this technique using three widely used polysaccharide type chiral stationary phases, cellulose tris(3,5-dimethylphenylcarbamate) (Chiralcel OD), amylose tris(3,5-dimethylphenylcarbamate) (Chiralpak AD) and cellulose tris(4-methylbenzoate) (Chiralcel OJ).

2. Experimental

2.1. Chemicals and reagents

The racemic compounds (Fig. 1) were from different commercial sources and used without further purification. Microcrystalline cellulose (Avicel), wide-pore silica gel (LiChrospher 1000, 5 μm) and acetic acid 100% were from E. Merck (Darmstadt, Germany). Amylose B ($M_R \approx 16\,000$) was purchased from Nacalai Tesque (Kyoto, Japan). Ammonium acetate was purchased from Riedel-de Haën AG (Seelze-Hannover, Germany). Methanol and ethanol of HPLC quality, tetrahydrofurane, *n*-hexane, 2-propanol, pyridine and benzene were from J.T. Baker (Deventer, The Netherlands). 4-Methylbenzoylchloride and 3,5-dimethylphenylisocyanate were supplied from Aldrich (Daisenhofen, Germany), (3-aminopropyl)-triethoxysilane was purchased from Fluka (Buchs, Switzerland).

Cellulose tris(3,5-dimethylphenylcarbamate), amylose tris(3,5-dimethylphenylcarbamate) and cellulose tris(4-methylbenzoate) (Fig. 2) were prepared and isolated as methanol insoluble fractions as described [10,13–15]. Before coating with the polysaccharide derivatives wide-pore silica gel (LiChrospher 1000, 5 μm) was silanized using (3-aminopropyl)-triethoxysilane in benzene in the presence of a catalytic amount of dry pyridine at 80°C. The polysaccharide derivatives were dissolved in tetrahydrofurane and coated using different concentrations (4.8; 9.1; 13.0; 16.7 and 20.0% (w/w)) on previously aminopropylsilanized wide-pore silica gel by a static technique.

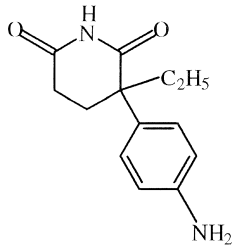
2.2. Common-size and capillary columns

Common-size columns were from Daicel Chem. Ind. (Daicel, Tokyo, Japan) under the commercial names Chiralcel OJ, Chiralcel OD and Chiralpak AD (4.6×250 mm). Capillary columns were packed as described previously [7,12]. Fused-silica capillaries of 100 μm I.D. from Polymicro Technologies (Phoenix, AZ, USA) were used. The inlet-end of the capillary was connected to a HPLC-precolumn (4.6×50 mm) which served as reservoir for the slurry of the packing material in *n*-hexane 2-propanol 90:10 (v/v). A commercially available HPLC column frit was connected to the outlet-end of the capillary in order to retain the packing material. The slurry of the packing material was ultrasonicated in a water-bath (15 min) and transferred into the reservoir. The system was closed tightly, pressure up to 400 bar was applied using a Knauer pneumatic pump (Knauer, Berlin, Germany) and maintained for 1 h. After complete reduction of the residual pressure (3–4 h), bidistilled water was pumped through the packed bed for 30 min. The outlet and inlet frits were sintered by local heating of the packed bed for approx. 10 s using a heating coil (700–800°C). The packed capillaries prepared according to this technique were used for capillary LC and CEC separations.

2.3. Equipments

2.3.1. HPLC enantioseparations in common-size columns

HPLC enantioseparations in common-size columns were performed using an E. Merck Hitachi L-6200A pump, an E. Merck Hitachi 655A UV-detector and an E. Merck Hitachi D-2500 recorder (E. Merck, Darmstadt, Germany). The flow-rates in the comparative HPLC separations were selected to match the retention and linear flow velocities observed in capillary LC and CEC. Other conditions (mobile phase, detection wavelengths, etc.) were identical in all separations.



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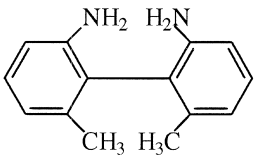

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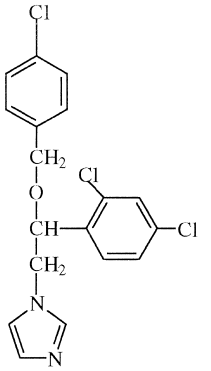

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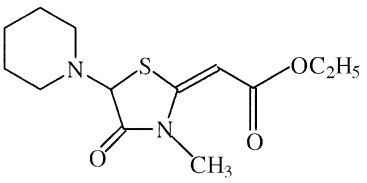

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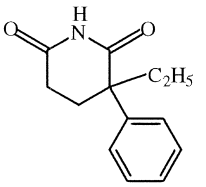

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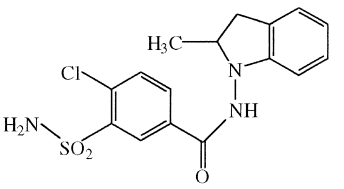

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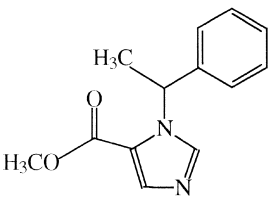

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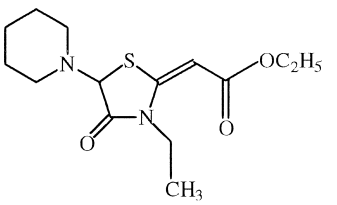

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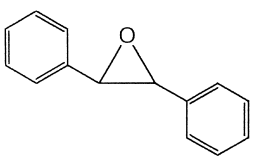

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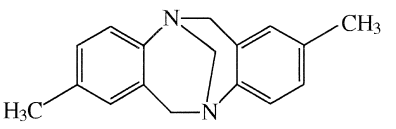

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Fig. 1. Structure of the chiral analytes: Aminoglutethimide (1), 2,2′-Diamino-6,6′-dimethylbiphenyl (2), Econazole (3), Etozolin (4), Glutethimide (5), Indapamide (6), Metomidate (7), Piprozolin (8), trans-Stilbene oxide (9), Troeger's base (10).

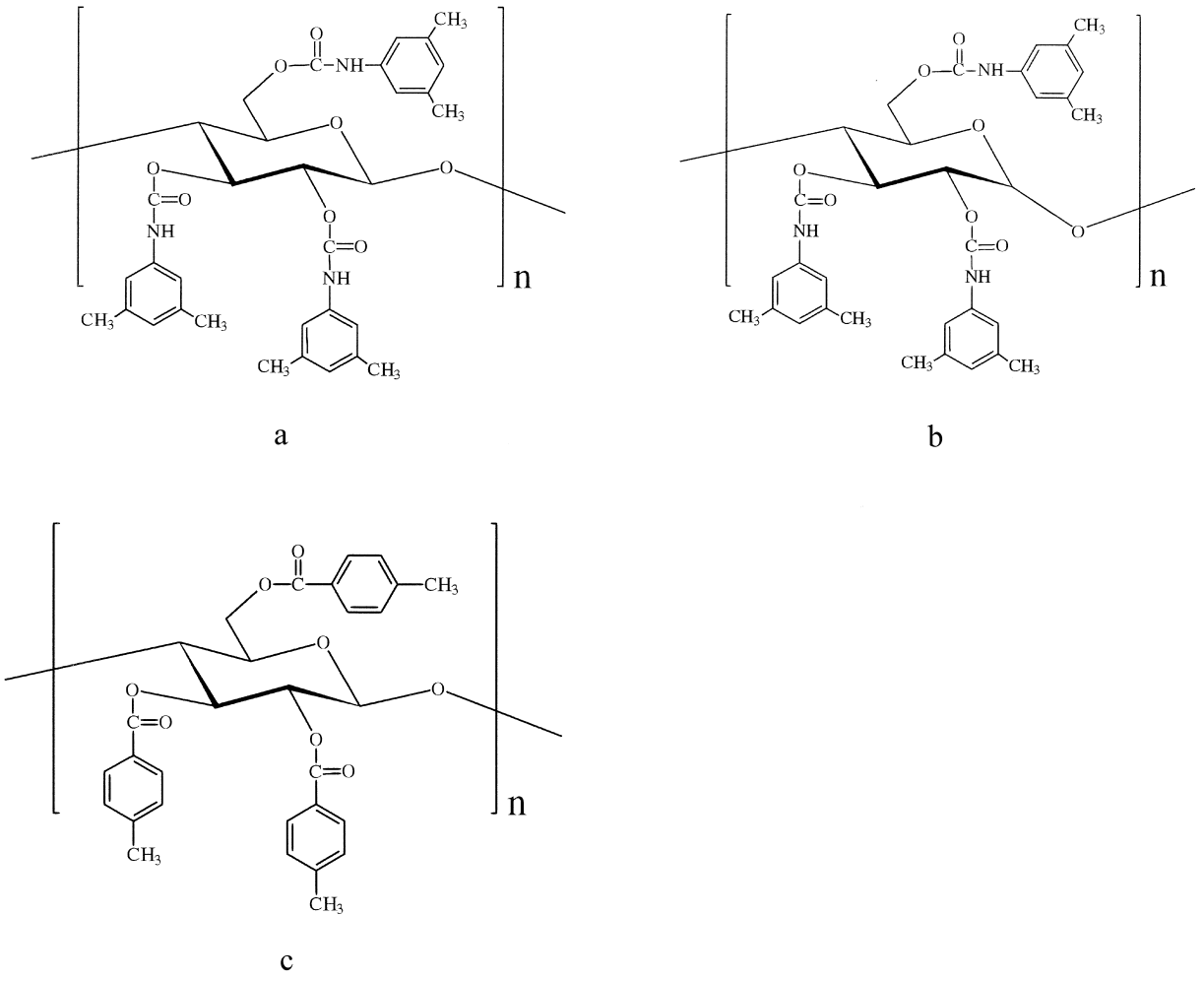

Fig. 2. Structure of the chiral stationary phases: (a) cellulose tris(3,5-dimethylphenylcarbamate) (Chiralcel OD); (b) amylose tris(3,5-dimethylphenylcarbamate) (Chiralpak AD) and (c) cellulose tris(4-methylbenzoate) (Chiralcel OJ).

2.3.2. Capillary LC and CEC

Capillary LC and CEC were performed with identical experimental set-up using a HP ^{3D}CE (Hewlett-Packard, Waldbronn, Germany) capillary electrophoresis instrument. For CEC separations the Beckmann P/ACE MDQ capillary electrophoresis equipment (Beckmann Instruments, Fullerton, CA, USA) was also used.

The apparent pH (pH*) of ammonium acetate solutions in alcohols was adjusted with acetic acid and measured with the pH-meter WTW pH 522 (Wissenschaftlich-Technische Werkstätten, Weilheim, Germany) without any corrections. The amount of glacial acetic acid required for adjustment of the apparent pH* was measured.

3. Results and discussion

3.1. Electroosmotic flow (EOF)

The electroosmotic flow (EOF) plays a decisive role for the migration of neutral analytes in CEC separations. The origin of the EOF in packed capillaries is not absolutely clear. In general, it is accepted that both, the silanol groups on the inner

surface of a fused-silica capillary and those on the surface of the packing material contribute to the EOF. However, as several previous studies indicated the packing material plays a major role in the generation of the EOF compared to the inner surface of a fused-silica capillary.

The packing material in this study is aminopropyl-silanized silica gel coated with neutral polysaccharide derivatives. Taking into account the relatively high loading (4.8–20.0% (w/w)) of neutral polysaccharide derivatives onto the silica gel one may assume that it will be difficult to generate an EOF in the capillaries packed with these materials. In contrast to this expectation, a relatively high anodic EOF was observed in the pH* range 3.0–7.8. The generation of the anodic and not the cathodic EOF definitely indicates that the surface of the aminopropylsilanized silica gel despite the coating seems to be the major contributor (at least in the pH* range below 5) to the EOF.

Once the significant contribution of the aminopropyl groups becomes evident in the effective EOF, the following questions arise: At first, not all silanol groups are derivatized with aminopropyl groups. The content of aminopropyl groups on silanized silica gel was ca. 0.75% (w/w) based on the content of nitrogen determined by elemental analysis. This means that in the appropriate pH* range the free silanol groups of the silica gel together with silanol groups on the inner capillary wall will also contribute to the overall EOF. In addition, based on the electric field inhomogenity along the packed and unpacked parts of the capillary [16–18] even the lengths of these parts will affect the observed EOF.

The anodic EOF was measured as a function of pH* of the methanolic ammonium acetate (10 m*M*) solution. The EOF increases with increasing pH* in the pH* range of 3.0–5.0 and then decreases (Fig. 3). The decrease of the EOF with increasing pH* in the pH* range 5.0–7.4 can be explained most likely as the result of the decreasing charge of the cationic aminopropyl groups and a concomitant increase of the charge of the anionic silanol groups, i. e. by increasing cathodic EOF which opposes the anodic

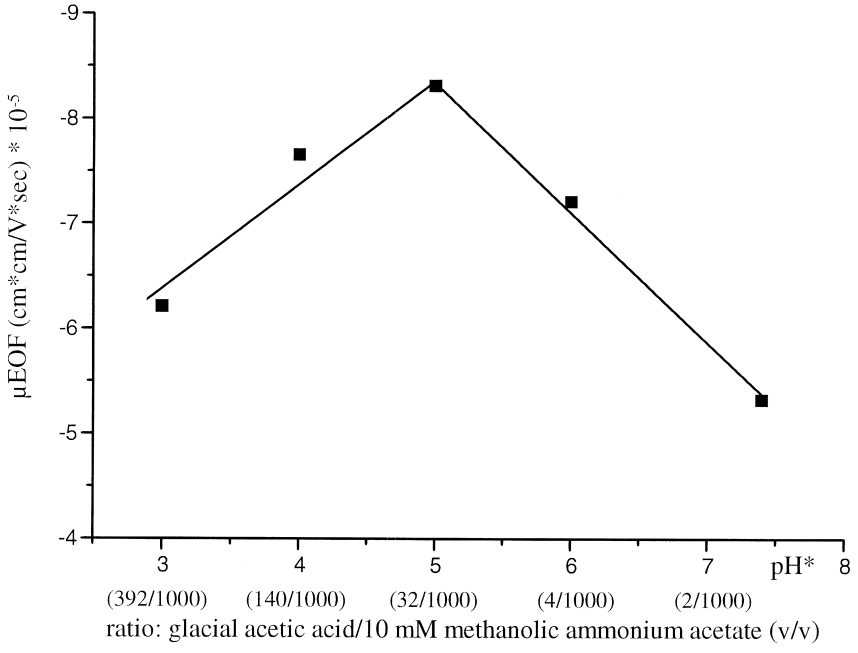

Fig. 3. pH* Dependence of EOF in the fused-silica capillary (100 μm×22/32 cm) packed with aminopropylsilanized silica gel coated with cellulose tris(dimethylphenylcarbamate) (20.0% (w/w)) in methanolic ammonium acetate solution (10 m*M*). Applied voltage: −10 kV, 6 bar on inlet and outlet vial. In the brackets the ratio of glacial acetic acid/methanolic ammonium acetate solution (10 m*M*) (v/v) required for pH* adjustment is indicated.

one. To our present knowledge it is difficult to explain the increase of the anodic EOF with increasing pH* in the range of 3.0–5.0. However, the significant amount of glacial acetic acid required for the pH* adjustment in this range and leading marked increase of the ionic strength of the buffer may responsible for this effect at least in part.

A selection of an appropriate marker for the EOF seems to be critical in CEC. Three compounds, tri-(*tert.*-butylbenzene), thiourea and acetone were examined as the EOF markers and exhibited similar retention and migration characteristics in the experimental conditions of this study.

Further experiments with thiourea as nonretained compound confirmed its suitability because no significant change of the chromatographic retention of this compound was observed with increasing loading of a polysaccharide onto the silica gel (Fig. 4a). A comparative study of the elution characteristics of thiourea in pure chromatographic and electrochromatographic runs allows to estimate the role of the polysaccharide loading for the anodic EOF. As shown in Fig. 4 with increasing polysaccharide loading the retention of thiourea increases only in the CEC runs (Fig. 4b). This means that only the decrease of the anodic EOF contributes to longer retention times. There is no effect of an increasing distribution of thiourea into increasing amounts of polysaccharides onto the silica gel.

As these studies showed the anodic EOF generated in ammonium acetate solutions in alcohols in fused-silica capillaries packed with aminopropylsilanized silica gel which is coated with neutral polysaccharide derivatives (4.8–20.0% (w/w)) is sufficient for per-

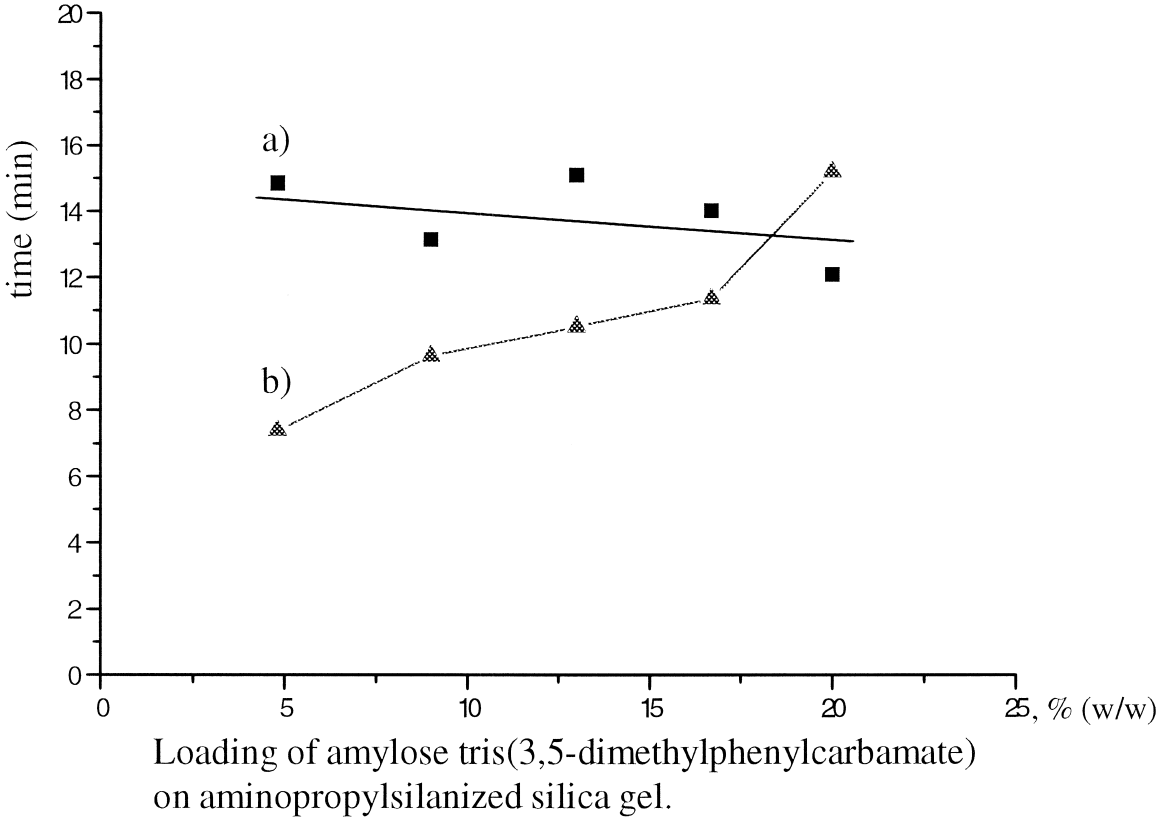

Fig. 4. Effect of the loading of amylose tris(3,5-dimethylphenylcarbamate) onto the aminopropylsilanized silica gel on the retention (a) and migration time (b) of thiourea in chromatographic (a) and CEC (b) runs. Separation conditions: Capillary: 100 μm×22/30.5 cm, buffer: methanolic ammonium acetate solution (10 m*M*, pH* 7.7 (without acetic acid)). (a) 12 bar on inlet vial. (b) −10 kV, 8 bar on inlet and outlet vial.

forming CEC separations. However, a better understanding of the EOF generation needs further studies which are in progress at present.

3.2. Chiral separations

3.2.1. Type of polysaccharide derivatives

CEC does not offer alternative chiral separation mechanisms for uncharged compounds compared to HPLC. Therefore, at the first glance it does not seem reasonable to compare 3 well studied polysaccharide derivatives for enantioseparations in HPLC, also in CEC. However, there are some questions which need to be answered before extending the experience with one polysaccharide derivative to other members of these chiral selectors. At first, chiral recognition ability, especially for polysaccharide phenylcarbamates, is less studied in pure alcohols [19]. In addition, some peculiarities from the viewpoint of the EOF generation are possible depending on the polymer coated onto the aminopropylsilica gel [20].

The data on enantioseparations of several racemic compounds in non-aqueous CEC in the capillaries packed with three different polysaccharide-type CSPs are summarized in Table 1. As shown in this table these CSPs possess some complementary chiral separation properties. In addition, in this study we found that the enantiomers of chiral compounds which were impossible to resolve with these CSPs in more convenient organic and aqueous mobile phases could be resolved in pure alcohols or ammonium acetate solutions in alcohols. The enantioseparation of (±)-metomidate is depicted in Fig. 5. Chiralcel OD does not exhibit a chiral resolving ability towards the enantiomers in methanolic ammonium acetate, whereas Chiralpak AD afforded a baseline enantioseparation. Thus, aminopropylsilica gel coated with neutral derivatives of polysaccharides may be used as CSPs for enantioseparations in non-aqueous CEC.

3.2.2. Effect of analyte loading on separation characteristics

As shown in previous study for similar capillary columns used in aqueous buffers sample loading may dramatically affect separations in capillary columns [12]. The same deteriorating effect of column overloading was confirmed also in non-aqueous buffers for several analytes. The example of the enantioseparation of trans-stilbene oxide (1 mg/ml solution in methanol) on Chiralpak AD material is shown in Fig. 6. Thus, as these data illustrate care must be taken not to overload capillary columns which contain a minute amounts of packing material.

Table 1
Enantioseparations in CEC using fused-silica capillaries (100 μm I.D.) packed with tris(3,5-dimethylphenylcarbamate) of cellulose and amylose and cellulose tris(4-methylbenzoate) coated in different amounts on aminopropylsilanized silica gel

CSP in % (w/w) of loading, length (cm)	Buffer	Racemate	Voltage (kV)	Pressure (bar)	k'_1	k'_2	α	R_s	N_1/m	N_2/m
OJ (20.0), 19/29	[a]	trans-Stilbene oxide	−5	6/6	0.80	1.08	1.35	2.36	23 667	21 659
OJ (20.0), 19/29	[a]	Econazole	−10	6/6	0.54	0.66	1.24	0.51	4159	2784
OJ (20.0), 19/29	[a]	2,2′-Diamino-6,6′-dimethylbiphenyl	−5	6/6	0.34	0.48	1.39	1.16	13 735	12 094
OJ (20.0), 19/29	[a]	Glutethimide	−10	6/6	0.29	0.40	1.38	0.80	9942	7274
OD (9.1), 24/34	[a]	Piprozolin	−10	6/6	0.10	0.21	2.01	1.55	23 609	15 618
OD (20.0), 22/32	[a]	Glutethimide	−15	6/6	0.26	0.38	1.48	1.33	14 982	13 286
OD (20.0), 22/32	[a]	Etozolin	−15	6/6	0.40	0.56	1.41	1.54	20 003	10 848
OD (20.0), 22/32	[a]	Troeger's base	−15	6/6	0.46	0.59	1.27	1.09	13 903	12 791
OD (20.0), 22/32	[a]	Indapamide	−15	6/6	0.12	0.27	2.13	1.59	14 144	12 187
OD (20.0), 22/32	[a]	Piprozolin	−15	6/6	0.31	0.59	1.89	2.86	18 687	14 384
AD (4.8), 22/30.5	[b]	Aminoglutethimide	−10	8/8	0.35	0.69	1.98	1.57	6577	2430
AD (4.8), 22/30.5	[b]	trans-Stilbene oxide	−10	8/8	0.03	0.08	3.23	1.36	51 496	41 989
AD (13.0), 22/30.5	[a]	Metomidate	−3	8/8	0.12	0.19	1.57	1.24	38 353	25 609
AD (20.0), 22/30.5	[a]	trans-Stilbene oxide	−5	8/8	0.56	0.81	1.45	2.76	31 150	21 537

[a] 10 m*M* Ammonium acetate in methanol (pH* 7.7 (without acetic acid)).
[b] 10 m*M* Ammonium acetate in ethanol (pH* 7.7 (without acetic acid)).

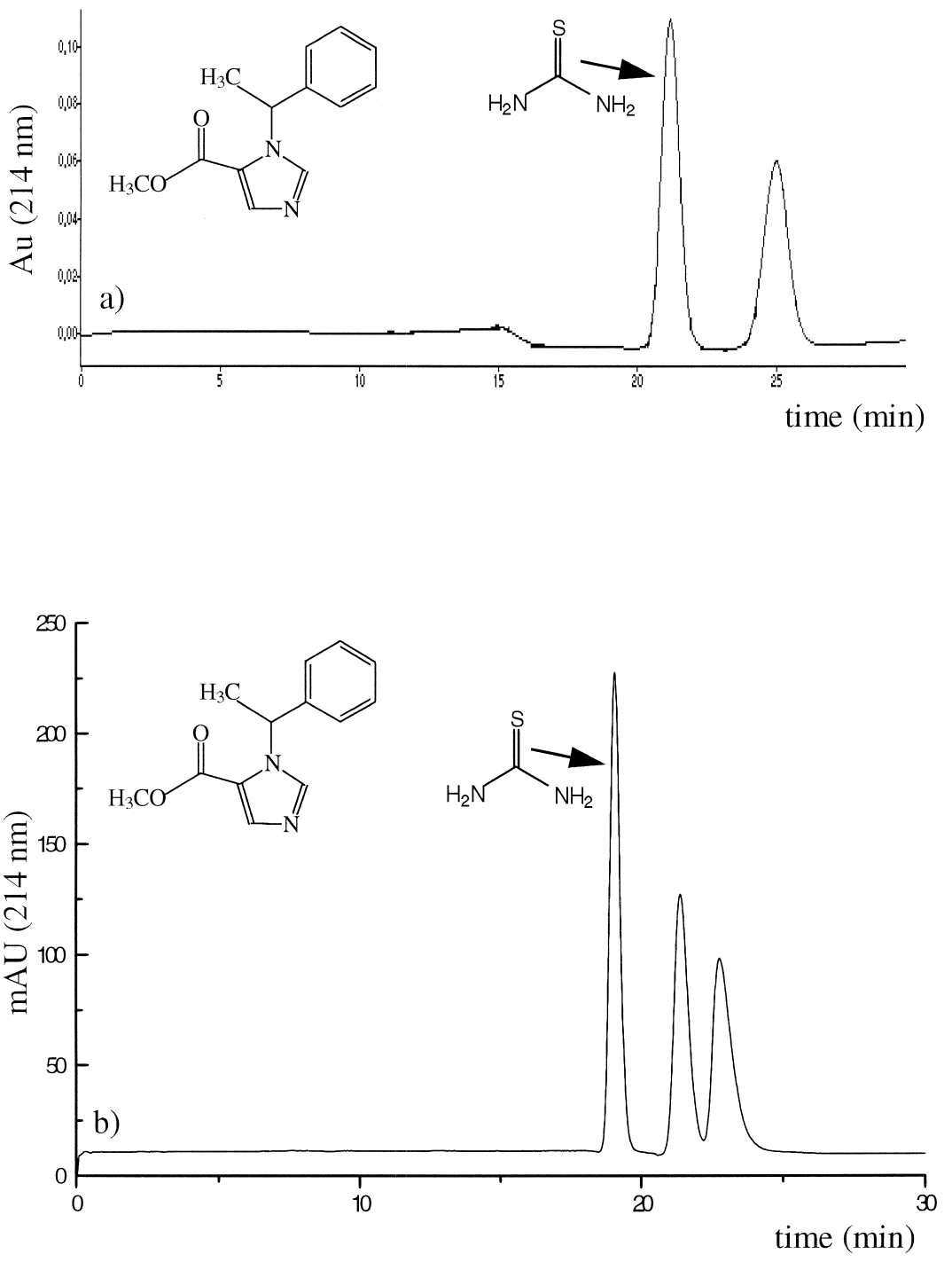

Fig. 5. Non-aqueous CEC enantioseparation of metomidate in the fused-silica capillaries (100 μm×22/30.5 cm) packed with Chiralcel OD (a) and Chiralpak AD (b) materials. Separation conditions: buffer: methanolic ammonium acetate solution (10 m*M*, pH* 7.7 (without acetic acid)). (a) Cellulose tris(3,5-dimethylphenylcarbamate) (20.0% (w/w)), −15 kV, 6 bar on inlet and outlet vial. (b) Amylose tris(3,5-dimethylphenylcarbamate) (13.0% (w/w)), −5 kV, 8 bar on inlet and outlet vial.

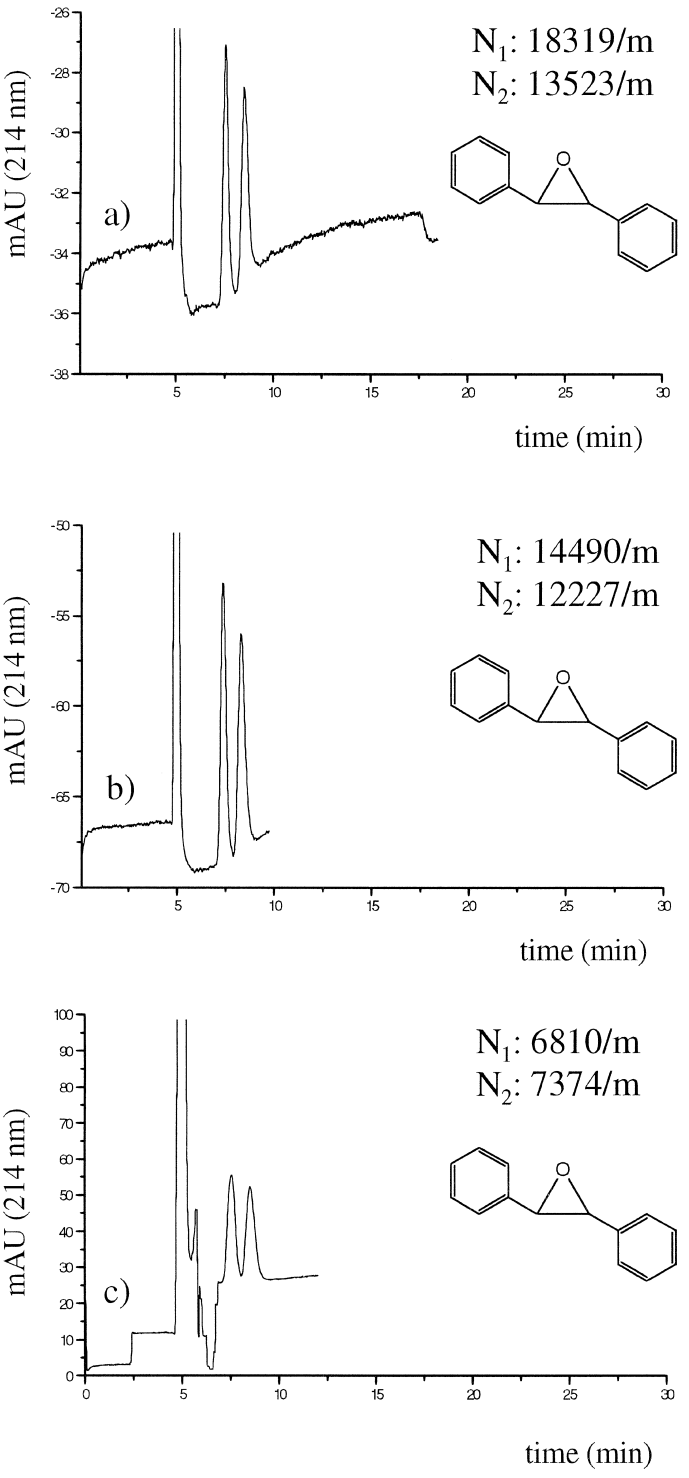

Fig. 6. Effect of sample loading on the enantioseparation of trans-stilbene oxide. Capillary: Amylose tris(3,5-dimethylphenylcarbamate) (20.0% (w/w)), (100 μm×22/30.5 cm), buffer: methanolic ammonium acetate solution (10 m*M*, pH* 7.7 (without acetic acid)). Injection with 12 bar for 12 s (a), 24 s (b) and 60 s (c). Voltage: −30 kV, 8 bar on inlet and outlet vial.

3.2.3. Effect of polysaccharide loading onto the silica gel on separation characteristics

As the experience shows [1–8,12,16–18] CEC offers certain advantages as a separation technique compared to HPLC in common-size columns. However, comparative studies between capillary LC and CEC in the same capillary are scarcely published [12]. Together with certain advantages such as the plug-like profile and the independence of the flow-rate on the particle size, in CEC some additional requirements apply to a separation medium such as electric conductivity, generation of the EOF, etc. Therefore, the potential advantages of CEC compared to capillary LC should be critically evaluated.

Switching from pressure-driven to electrokinetically-driven migration mechanism allows to flatten the parabolic flow profile caused by longitudinal diffusion. This means that parameter B will be mainly affected in the modified van Deemter equation for reduced plate heights [21]:

$$h = Av^{0.33} + B/v + Cv \quad (1)$$

where A is eddy diffusion and flow distribution component, B is the longitudinal diffusion component and C is the mass transfer component. Therefore, CEC will result maximal gain in the plate heights when this is controlled with a longitudinal diffusion and not with the mass transfer properties between a stationary and a mobile phase. However, it has been evidenced experimentally that mass transfer kinetics between a mobile phase and CSP may significantly affect a reduced plate height in chiral HPLC separations. This is especially valid for high-molecular weight CSPs [22,23].

In order to study the effect of the mass transfer kinetics on the plate height, van Deemter curves were constructed for CSPs with various loadings of chiral selectors. In addition, this was performed for analytes with different retention characteristics. Coated type CSPs offer certain advantages from this viewpoint compared to covalently bound ones because a continuous variation of a chiral selector loading onto the silica gel is possible.

There are two principal possibilities to affect the linear flow velocity in CEC: variation of the pH* of the separation medium and variation of the applied voltage. The former has a disadvantage that a pH* variation even in the wide range does not allow to cover a required range of a mobile phase linear velocities. In addition, variation of the pH* may also affect selector–selectand interactions. Therefore, the variation of applied voltage for an adjustment of a desired flow velocity seems to be favorable when CEC separation mechanisms do not overlap with pure CE separation mechanisms. In addition, most of the commercially available CE equipments allow to adjust the applied voltage with small increments over a wide range. Thus, van Deemter curves were constructed in this study using the applied voltage as variable.

The minimal plate heights observed for the thiourea peak were in the range of 13.4–14.0 μm (Fig. 7). This provides some information of diagnostic character about the quality of capillary preparation and in addition, it allows to optimize separation conditions.

In contrast to thiourea the plate numbers for retained chiral analytes strongly decreased with increasing loading of the polysaccharide derivative on the silica gel (Fig. 8). This effect was more pronounced for the more retained second enantiomers. Thus, the minimal plate height 21.5 μm for the first peak of piprozolin increased to 58.3 μm with increasing the loading of Chiralpak AD-polymer from 4.8% (w/w) to 13.0% (w/w). The related numbers for the second eluted enantiomer were 40.3 μm and 102.7 μm. These data indicate that the mass transfer kinetics between the stationary and mobile phases significantly affect peak dispersion of the piprozolin enantiomers.

Interestingly, no significant effect of the loading was observed for the enantiomers of trans-stilbene oxide in the range of 13.0–20.0% (w/w) (Fig. 9). This result agrees with expectations because the enantiomers of trans-stilbene oxide especially the less retained enantiomer possess a lower affinity to the Chiralpak AD compared to the enantiomers of piprozolin.

3.2.4. The effect of the pH* on enantioseparations in the CEC mode

The effect of the pH* can be multivariate in CEC separations. First, the EOF and consequently, the linear flow-rate of a mobile phase significantly changes depending on the pH*. Further, selector–

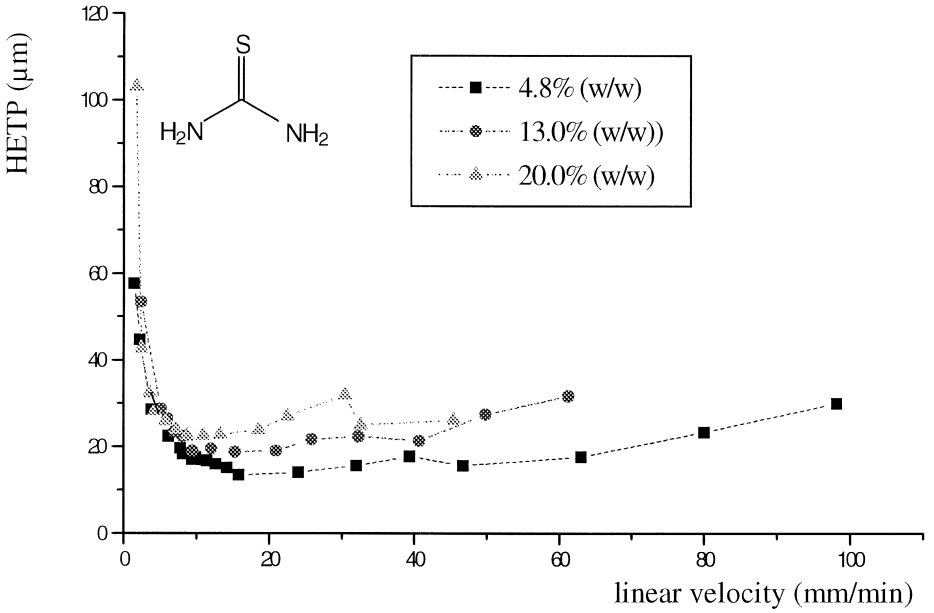

Fig. 7. Van Deemter curves for thiourea in the capillaries packed with aminopropylsilica gel (5 μm) containing 4.8%, 13.0% and 20.0% (w/w) amylose tris(3,5-dimethylphenylcarbamate). Separation conditions: Capillary: 100 μm×22/30.5 cm, buffer: methanolic ammonium acetate solution (10 m*M*, pH* 7.7 (without acetic acid)).

selectand interactions may vary depending on the pH*. The more significant pH*-depending effects may appear for those CSPs and analytes which change their effective charge in the pH* range studied.

The enantioseparation of indapamide in methanolic ammonium acetate solutions with different apparent pH* in capillaries packed with Chiralcel OD material is shown in Fig. 10. Both, the CSP and the analyte are neutral and most likely their effective charge does not change in this pH* range. This seems to be the reason for almost constant capacity factors for both enantiomers. The selectivity of enantioseparation decreases slightly in the pH* range 7.4–4.0 and drastically from pH* 4.0 to pH* 3.0. Thus, apparent pH* together with ionic strength can significantly affect the selector–selectand interactions.

3.2.5. The effect of the alcohol on CEC separations

In order to evaluate the potential of alcohols other than methanol as a separation medium and, in addition, to study the effect of the viscosity and diffusion characteristics on non-aqueous CEC enantioseparations, ethanol was also used. The anodic EOF decreased drastically when changing from methanol to ethanol. In Fig. 11 the enantioseparation of trans-stilbene oxide is shown in ethanol (b) and methanol (c) at the same applied voltage −10 kV. As expected, the retention times are shorter when using methanol. Slightly higher plate numbers for the enantiomers of trans-stilbene oxide were obtained in ethanolic solution and in combination with a significantly increased selectivity this resulted in better enantioseparation compared to methanolic solution. However, the analysis time was very long. In order to compare the enantioseparations in methanolic and ethanolic solutions at similar linear flow velocities, these separations were performed at different applied voltages (Fig. 11 a vs. b and c vs. d). At comparable linear flow velocities higher separation selectivities in ethanolic solution result in higher resolution factors (R_S). Thus, ethanol is a promising medium in non-aqueous CEC enantioseparations using polysaccharide derivatives.

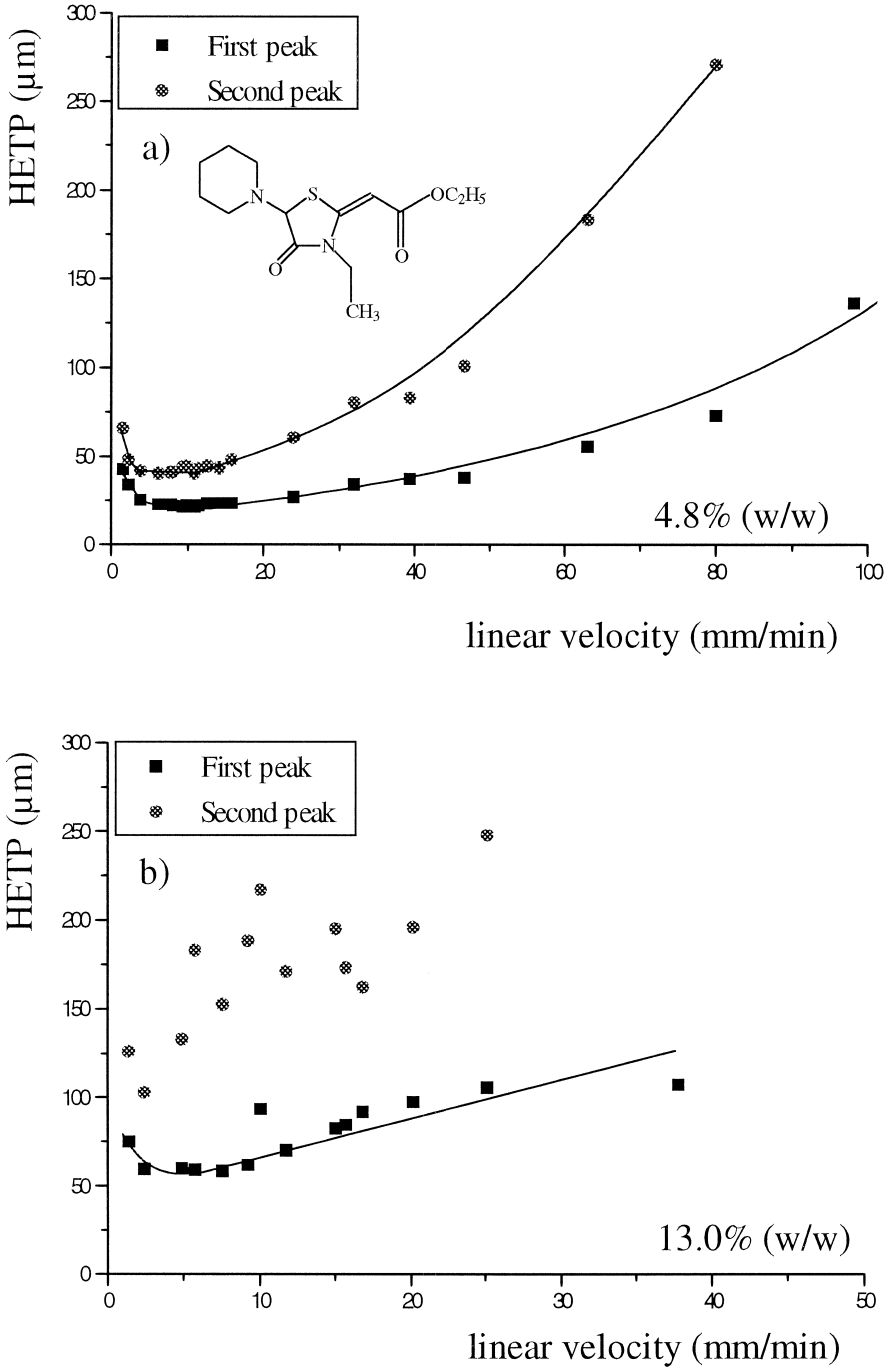

Fig. 8. Van Deemter curves for piprozolin in the capillaries packed with aminopropylsilica gel (5 μm) containing 4.8% (a) and 13.0% (b) (w/w) amylose tris(3,5-dimethylphenylcarbamate). Other separation conditions as in Fig. 7.

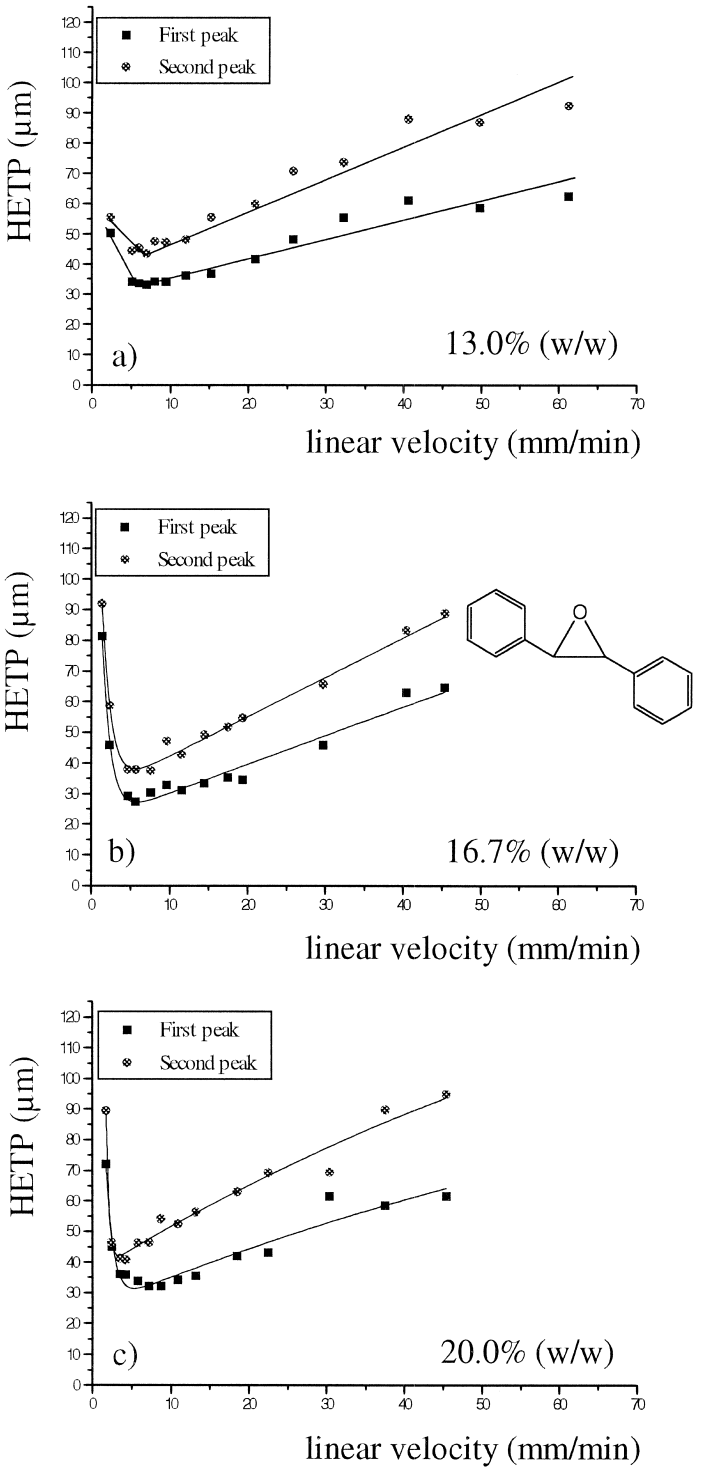

Fig. 9. Van Deemter curves for trans-stilbene oxide in the capillaries packed with aminopropylsilica gel containing 13.0% (a), 16.7% (b) and 20.0% (c) (w/w) amylose tris(3,5-dimethylphenylcarbamate). Other separation conditions as in Fig. 7.

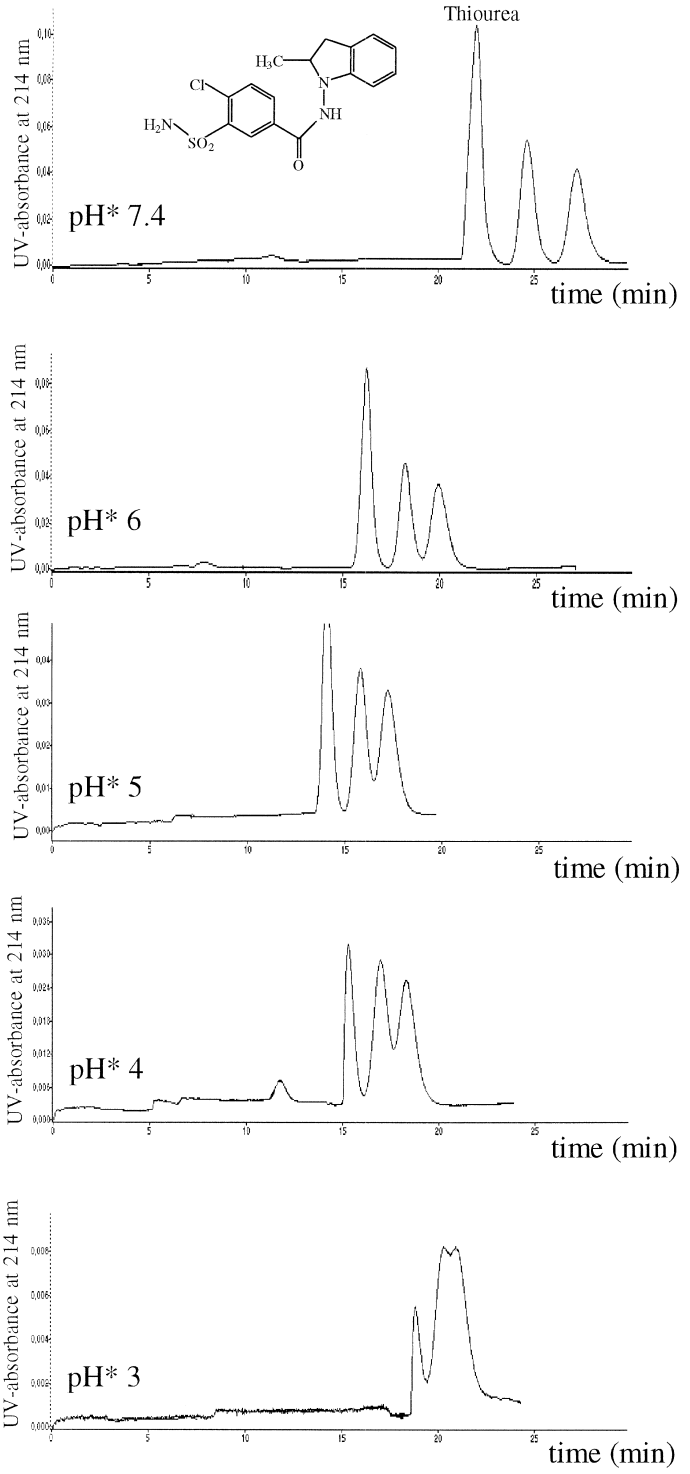

Fig. 10. Enantioseparation of indapamide at different apparent pH* of 10 m*M* methanolic ammonium acetate solutions. Separation conditions as in Fig. 3.

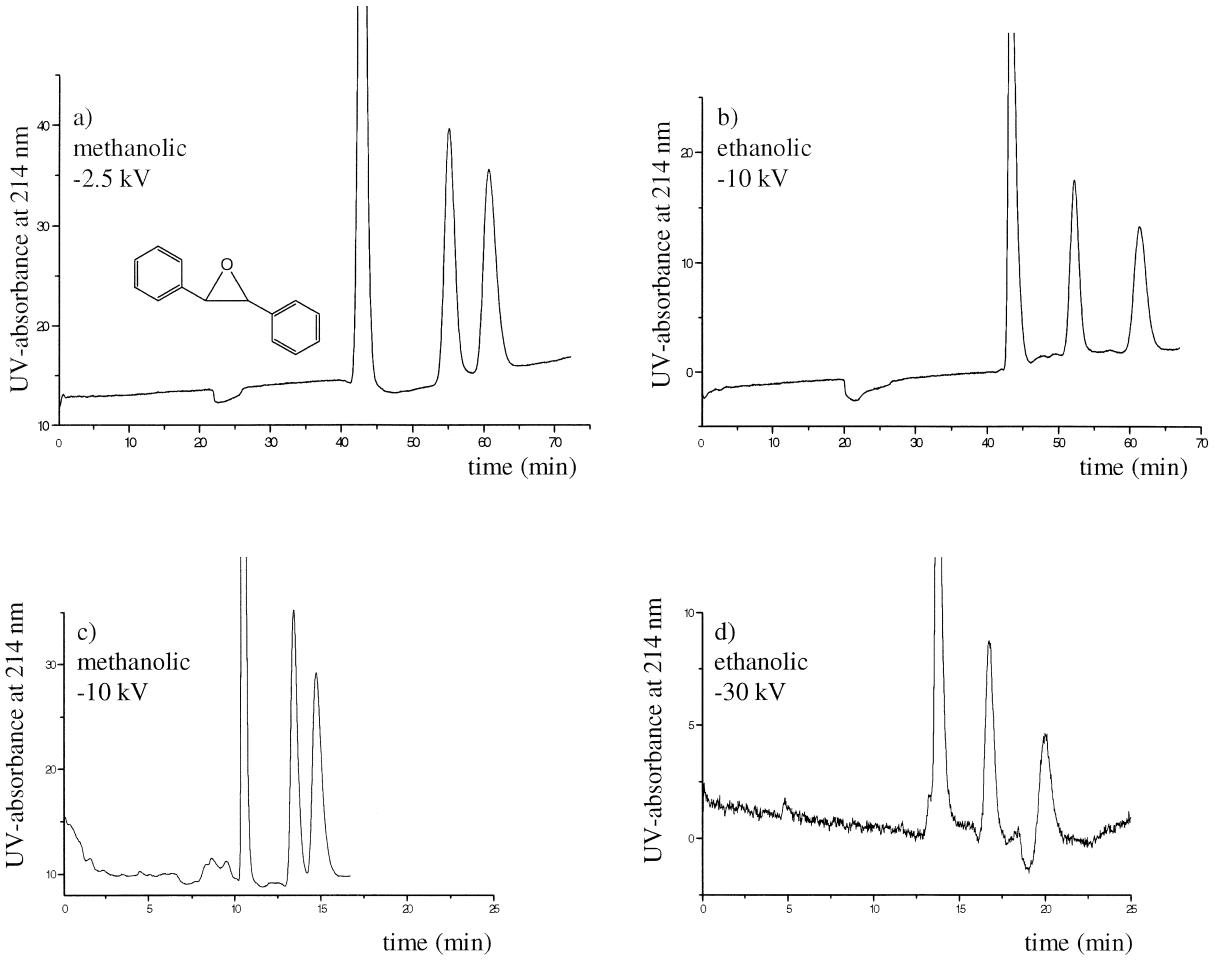

Fig. 11. Enantioseparation of trans-stilbene oxide in methanolic (a,c) and ethanolic (b,d) ammonium acetate solutions at the same applied voltage −10 kV (b,c) and comparable linear flow velocities (a vs. b and c vs. d). Capillary: Amylose tris(3,5-dimethylphenylcarbamate) (13.0% (w/w)), (100 μm×22/30.5 cm), buffer: (a) and (c): methanolic ammonium acetate solution (10 m*M*, pH* 7.7 (without acetic acid)), (b) and (d): ethanolic ammonium acetate solution (10 m*M*, pH* 7.7 (without acetic acid)).

3.2.6. HPLC enantioseparations in common-size columns vs. capillary LC and CEC

The advantages of miniaturized separation system such as low costs, less environmental problems, etc. are obvious. However, the question is if capillary LC offers at least adequate or better separations compared to LC in common-size columns and further, if it is possible to obtain a significant gain in peak efficiency by changing from a pressure-driven to an electrokinetically-driven flow.

In Fig. 12 the enantioseparations of metomidate are shown using commercially available common-size HPLC column Chiralpak AD (Fig. 12a) and a 100 μm×22.0/30.5 cm fused-silica capillary packed with a similar material. It seems that the higher amount of coated chiral selector (25% (w/w)) in the commercial column leads to higher capacity factors of the analyte as well as to higher selectivities of the enantioseparations. Considering different particle sizes of the packing material (7.5 μm and 5.0 μm in the common-size and capillary columns, respectively) reduced plate heights were compared. These were somewhat lower especially for the less retained first peak in capillary LC (Fig. 12b) compared to HPLC

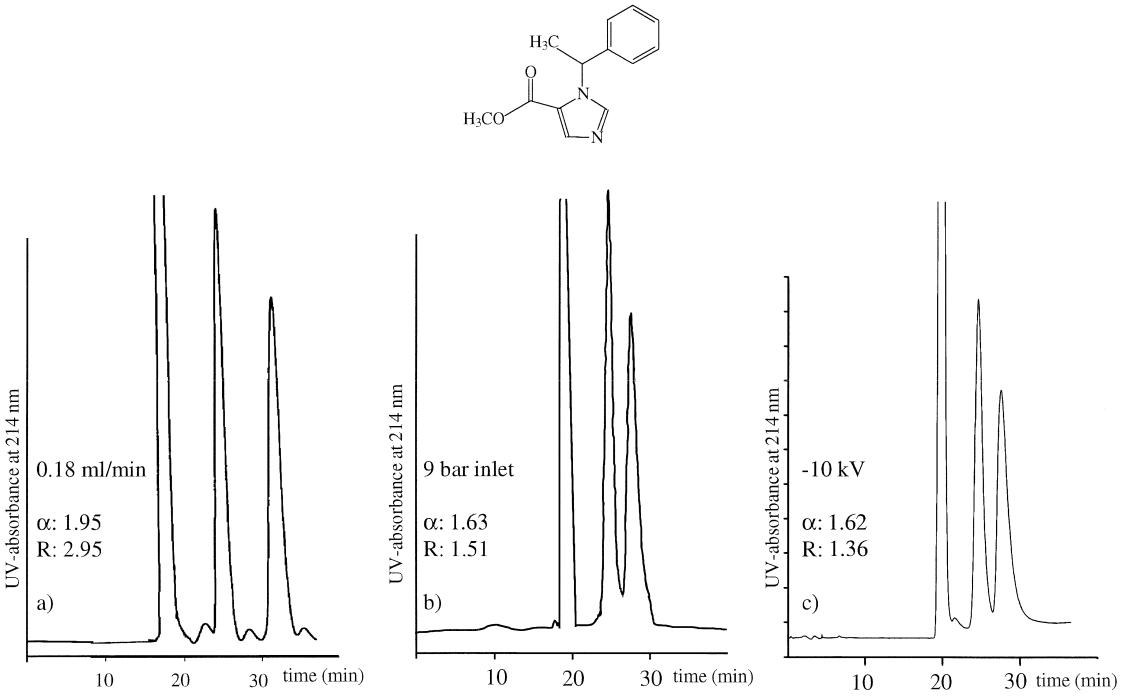

Fig. 12. Enantioseparation of metomidate using commercially available Chiralpak AD column (4.6×250 mm) (a), in capillary LC (b) and CEC (c) (100 μm×22/30.5 cm) for (b) and (c). Background electrolyte: 10 m*M* ammonium acetate in methanol pH* 7.7 (without acetic acid).

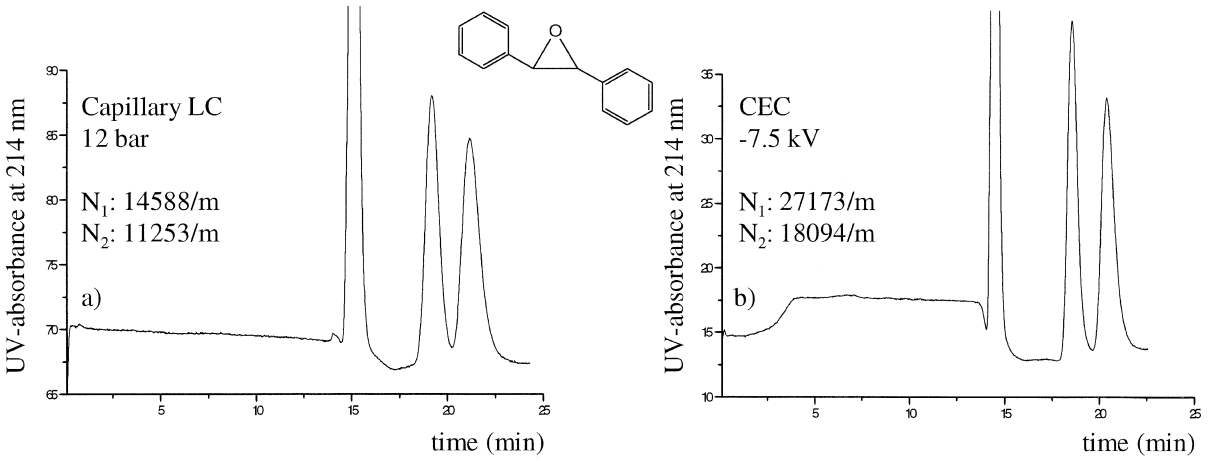

Fig. 13. Enantioseparation of trans-stilbene oxide in capillary LC (a) and non-aqueous CEC (b). Capillary: Amylose tris(3,5-dimethylphenylcarbamate) (13.0% (w/w)) (100 μm×22/30.5 cm). Mobile phase: 10 m*M* ammonium acetate in methanol (pH* 7.7 (without acetic acid)). Pressure in (a) 12 bar. Voltage in (b) −7.5 kV, 8 bar on inlet and outlet vial.

separations in common-size columns (Fig. 12a). Under similar linear flow-rate conditions plate heights in capillary LC and CEC were comparable in this particular case. (Fig. 12c) However, for most separations in this study moderately higher (approx. 15–75%) peak efficiencies were observed in CEC compared to capillary LC (Fig. 13). This moderate but reliable improvement allows to look for further possibilities of efficiency improvement in CEC.

4. Conclusions

The study illustrates for the first time the feasibility of non-aqueous CEC separations using polysaccharide-type chiral stationary phases. It is shown that despite high loadings of the neutral polysaccharide derivatives on aminopropylsilanized silica gel a significant anodic electroosmotic flow can be generated in methanolic and ethanolic ammonium acetate solutions in fused-silica capillaries packed with these materials. A wide variety of chiral compounds can be resolved in the CEC mode.

CEC offers significant advantages compared to HPLC in common-size columns due to the miniaturization. In addition, moderate but reliable improvements of the peak efficiency was observed in the CEC mode compared to capillary LC in the same capillaries. In general, CEC in non-aqueous solvents seems to be a promising new technique for analytical enantioseparations.

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