Journal of Chromatography B, 875 (2008) 296–303

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Enantioseparations with cellulose tris(3-chloro-4-methylphenylcarbamate) in nano-liquid chromatography and capillary electrochromatography☆

Salvatore Fanali[a,*], Giovanni D'Orazio[a], Ketevan Lomsadze[b], Bezhan Chankvetadze[b]

[a] Institute of Chemical Methodologies, Consiglio Nazionale delle Ricerche, Area della Ricerca di Roma I, Via Salaria Km 29,300, 00016 Monterotondo Scalo, Roma, Italy
[b] Department of Physical and Analytical Chemistry and Molecular Recognition and Separation Science Laboratory, School of Exact and Natural Sciences, Tbilisi State University, 380028 Tbilisi, Georgia

ARTICLE INFO

Article history:
Received 14 May 2008
Accepted 6 July 2008
Available online 25 July 2008

Keywords:
Enantiomers
Capillary electrochromatography
Nano-liquid chromatography
Polysaccharide-based chiral stationary phases

ABSTRACT

Cellulose tris(3-chloro-4-methylphenylcarbamate) was coated onto native and aminopropylsilanized silica in order to prepare chiral stationary phases (CSPs) for enantioseparations using nano-liquid chromatography (nano-LC) and capillary electrochromatography (CEC). The effect of the chiral selector loading onto silica, mobile phase composition and pH, as well as separation variables on separation of enantiomers was studied. It was found that CSPs based on cellulose tris(3-chloro-4-methylphenylcarbamate) can be used for preparation of very stable capillary columns useful for enantioseparations in nano-LC and CEC in combination with polar organic mobile phases.

© 2008 Elsevier B.V. All rights reserved.

1. Introduction

In the last years there has been an increasing interest in the development of methods for separation of enantiomeric mixtures. These methods have been applied with success in different areas such as pharmaceutical, agrochemical, biomedical, etc. analyses. Since the separation of enantiomers is based on interactions between analytes and a chiral stationary phase (CSP), the attention has been focused on development of new CSPs for efficient enantioseparations. A wide number of chiral stationary phases have been successfully used in HPLC including brush type [1], proteins [2,3] cyclodextrins [4,5], chiral synthetic polymers [6,7], polysaccharide derivatives [8–10], chiral ion-exchangers [11,12], chiral ligand-exchange material [13], macrocyclic antibiotics [14,15], etc. Polysaccharide phenylcarbamate derivatives are most widely used CSPs for enantioseparations in HPLC able to resolve more than 80% of commercially available chiral compounds. Polysaccharide phenylcarbamate derivatives are obtained by the reaction of polysaccharide with corresponding phenylisocyanate derivatives. Stable CSPs are prepared by coating of polysaccharide phenylcarbamates onto the surface of silica particles [8–10,16].

The use of polysaccharide derivates as CSP may offer several advantages such as availability of starting polysaccharides as natural sources and the easy derivatization of the hydroxyl groups.

The presence of modified phenylcarbamates in the polysaccharide structure may produce CSPs with different enantiorecognition capability depending on the type and position of the substituents on the phenyl group [8,9,16].

A series of phenylcarbamate derivatives having both electron-donating and electron-withdrawing groups on the phenyl moiety exhibit interesting enantiomer resolving ability [9,16,17–19].

The applicability of cellulose tris(3,5-dimethylphenylcarbamate) (CDMPC) in open tubular capillary chromatography and capillary electrochromatography was demonstrated by Francotte and Jung in 1996 [19]. Open-tubular capillary columns for nano-LC and CEC applications suffer from low sample loading capacity, low enantiomer resolving capability and low stability.

Later on silica particles modified with CDMPC [20–24], cellulose tris(3,5-dichlorophenylcarbamate) [25,26], and amylose tris(3,5-dimethylphenylcarbamate) [27] were used for enantioseparations in nano-LC and CEC.

To our best knowledge cellulose tris(3-chloro-4-methylphenylcarbamate) has not yet been used for enantioseparations by CEC and nano-LC. Therefore, in this study the chiral selector was coated onto the native or aminopropylsilanized silica and used as packed CSP for separation of enantiomers of selected neutral chiral compounds by means of nano-LC and CEC.

☆ This paper is part of the Special Issue 'Enantioseparations', dedicated to W. Lindner, edited by B. Chankvetadze and E. Francotte.

* Corresponding author. Tel.: +39 0690672256; fax: +39 0690672269.
E-mail address: salvatore.fanali@imc.cnr.it (S. Fanali).

1570-0232/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2008.07.017

Six neutral selected racemic analytes, namely etozoline, lorazepam, oxazepam, temazepam, thalidomide and *trans*-stilbene oxide were tested in order to verify the recognition capability of the polysaccharide derivative. The effect of experimental parameters such as mobile phase type and composition, loading of the coated chiral selector on retention, separation factor and peak efficiency was investigated. The results obtained in CEC were compared with those achieved in nano-LC.

2. Experimental

2.1. Chemicals and samples

All chemicals were of analytical reagent grade and used as received.

Methanol (MeOH), acetonitrile (MeCN), sodium hydroxide (NaOH), formic and acetic acids were purchased from Carlo Erba (Milan, Italy). Ammonium hydroxide solution (30%) was from Riedel-de Haen (Seelze, Germany). Racemic lorazepam, oxazepam, temazepam and *trans*-stilbene oxide were obtained from Sigma (St. Louis, MO, USA), while etozoline and thalidomide were kindly provided by the Institute of Pharmaceutical and Medicinal Chemistry, University of Münster, Germany.

Cellulose tris(3-chloro-4-methylphenylcarbamate) (Fig. 1) was synthesized and characterized by elemental analysis, FT-IR and H NMR spectra as described previously [9,16].

Deionized water was obtained by using the Milli-Q system (Millipore, MA, USA).

Buffer solutions were prepared every week and stored at +4 °C. Mobile phase were daily prepared by mixing the suitable volumes of organic solvents, water and buffer solutions.

Stock standard solutions of each studied compound were prepared by dissolving the appropriate amount of sample in methanol with the exception of etozoline and thalidomide that were dissolved in acetonitrile. The final concentration of stock standard solutions was 1 mg/mL that were daily diluted with MeOH (0.1 mg/mL). All solutions were kept at 4 °C.

2.2. Instrumentation

A microp H 2001 pH Meter (Crison, Barcelona, Spain) was used for pH measurements. An ultrasonic bath model FS 100b Decon (Hove, UK) was employed to sonicate solutions.

The fused silica capillaries (100 μm I.D. × 375 μm O.D.) were purchased from Composite Metal Services (Hallow, Worcestershire, UK). These capillaries were packed by using a PerkinElmer Series 10 HPLC pump (Palo Alto, CA, USA).

2.2.1. CEC

An Agilent Technologies ^{3D}CE system (Waldbronn, Germany) equipped with UV–vis diode array detector (DAD) was used in this study for CEC experiments. Detection was performed at 214 nm.

The temperature of capillary column was controlled by an air thermostating system while the vial carousel was at room temperature.

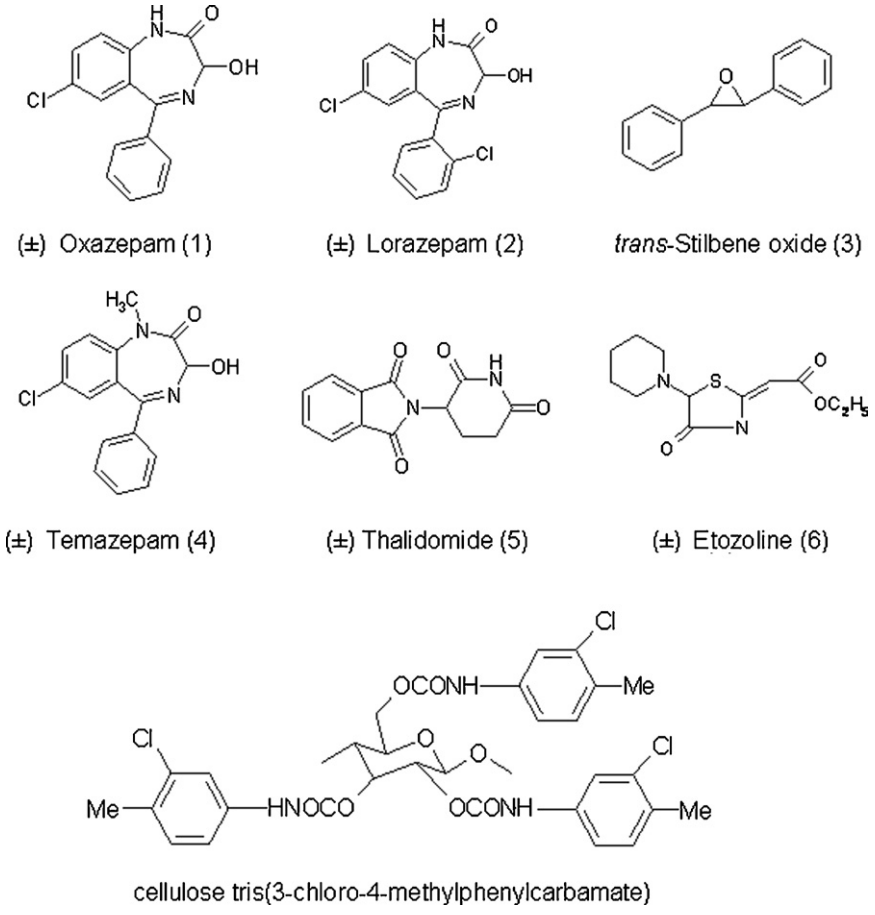

Fig. 1. Structure of chiral analytes and cellulose tris(3-chloro-4-methylphenylcarbamate).

[3D]CE Chemstation software (rev. A.09.01, Agilent Technologies) was used for data collection and processing.

2.2.2. Nano-liquid chromatography

Nano-liquid chromatography experiments were carried out by using a laboratory assembled instrumentation.

The separation system was constructed based on a conventional gradient HPLC pump Spectra System P2000 (Thermo Separation Products, TSP, San Jose, CA, USA), a modified injector valve (Separserve GmbH, Münster, Germany) with a 50-μL loop as a reservoir for the mobile phase, a 60-nL injection valve from Vici (Valco, Houston, TX, USA). Additionally, a packed capillary column and a on-column Spectra Focus PC1000 UV-Vis detector (TSP, San Jose, CA, USA) were used.

The flow rate of the mobile phase was reduced from μL/min to nL/min by using a laboratory made split system. For this purpose a stainless steel T piece (Vici Valco, Houston, TX, USA) was connected at one end to the pump through a 50 cm × 130 μm I.D. PEEK capillary. The other two ends were connected with the reservoir valve and to the waste by using 5 cm × 500 μm I.D. and 70 cm × 50 μm I.D. PEEK capillaries, respectively.

Nano-injector and mobile phase reservoir were coupled by using a 50 cm × 20 μm I.D. PEEK capillary. The capillary column was directly connected to the nano-injector valve and inserted into an on-column detector cell for on-line UV measurements. The set-up of the laboratory-assembled instrument used in this study is shown in Fig. 2.

The micro-pump was operating in isocratic mode delivering methanol (20–240 μL/min) to the split and then to the modified valve containing the selected mobile phase. The split system provided the nano-flow in the range of 80–1000 nL/min (split ratio about 1/240).

The flow rate into the capillary was estimated measuring the mobile phase volume after connecting the outlet-column to a micro-syringe (HAMILTON, Reno, NV, USA) through a Teflon tube (TF-350; LC Packing, CA, USA) for 5–10 min.

The UV detector was linked to a Compaq pro-line computer by means of a RS 232 serial port. The system was controlled by Spectra System PC1000 Software for OS2/WARP-IBM version 3.0 (Eremont, CA, USA). The detection was performed at 214 nm.

2.3. Preparation of stationary phase

Cellulose tris(3-chloro-4-methylphenylcarbamate) was dissolved in tetrahydrofurane (THF) and coated onto silica particles of different chemistry as described in Refs. [20,21,25–27] in order to prepare CSPs used for packing the capillary columns used in this study. The following CSP were prepared: (i) native silica particles (5 μm) coated with 6 or 12 or 25% (w/w) polysaccharide derivative and (ii) aminopropylsilanized silica (5 μm) coated with the chiral selector at a concentration of 25% (w/w). The data obtained with capillaries packed with the described CSPs were compared with those achieved using a native silica (SP-1000; 5 μm) without the chiral selector.

2.4. Preparation of capillary columns

The capillary columns were prepared by using the slurry packing method as described previously [25–28]. Briefly, one end of the fused silica capillary (100 μm I.D. × 375 μm O.D.) was connected to a stainless steel HPLC pre-column (50 mm × 4.1 mm I.D., Valco, Houston, TX, USA) containing the slurry of the packing material. The opposite side of the capillary was connected to a HPLC mechanical frit (Valco, Houston, TX, USA) to retain the packing material. The stationary phase (50 mg) was suspended in 1 mL of 80/20 (v/v) MeOH/H_2O, sonicated for 15 min and transferred into the reservoir.

The capillary was packed at 35 MPa (350 bar, 5000 psi) for a length of about 30 cm. After removing the slurry mixture, the packed material was flushed with water for about 30 min and the inlet and outlet frits were prepared by using a heating coil (about 700 °C for 5–6 s). The excess of stationary phase was removed by flushing the capillary with the mobile phase also providing a fast equilibration of the material. A detection window was prepared 0.5 cm after the outlet frit by removing the polyimide layer with a razor. 25.0 and 34.0 cm were the packed and total length of the capillary. The same capillary was employed for all experiments firstly in CEC and thereafter in nano-LC.

3. Results and discussion

3.1. Electroosmotic flow (EOF)

The presence of EOF is essential for CEC separations, especially when analyzing uncharged compounds. In fact, a high EOF may allow fast analysis with high efficiency. The magnitude of the EOF is influenced by several experimental parameters such as applied voltage, composition of both, stationary phase and background electrolyte, pH of the latter, etc.

In this study the EOF was evaluated by measuring the retention time of the negative perturbation obtained after injection of MeOH analyzed in capillaries containing different stationary phases. Experiments were carried out using native silica gel particles alone or modified with aminopropyl groups also coated with 25% (w/w) polysaccharide derivative. The influence of the apparent pH of the polar organic mobile phase pH on EOF was investigated in the range of 2.5–8.5.

As can be seen in Fig. 3 the increase of background electrolyte's pH caused a raising of EOF mobility when the capillary was packed with native silica-based CSP. The EOF generated in the capillary column packed with CSP based on the aminopropyl silica was anodic. EOF mobility in this case, in contrary to above mentioned, decreased by increasing the apparent pH of the background electrolyte.

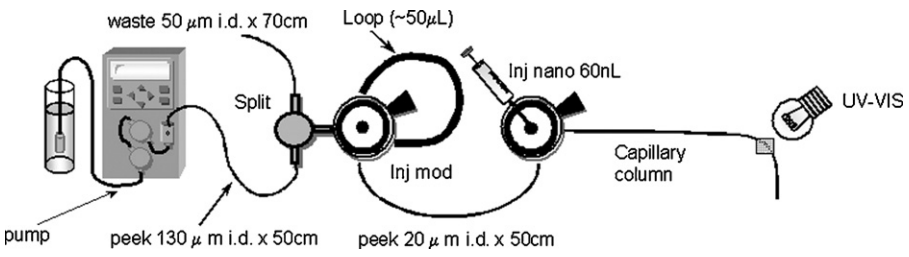

Fig. 2. Scheme of nano-LC system.

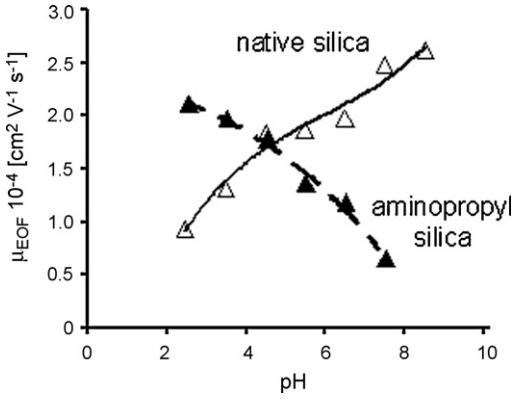

Fig. 3. Effect of the pH of the background electrolyte on the electroosmotic flow. Experimental conditions were as follows: capillary column: 100 μm I.D. × 375 μm O.D.—L_{packed} = 25.0 cm, L_{eff} = 25.5 cm; stationary phase: native silica (5 μm) or aminopropyl silica (5 μm) coated with 25% (w/w) cellulose tris(3-chloro-4-methylphenylcarbamate). Mobile phase: 1/49/50 (v/v/v) 500 mM buffer solution with different apparent pH/MeOH/ACN; ammonium formate (pH 2.5–3.5), ammonium acetate (pH 4.5–6.5), sodium borate (pH 7.5–8.5); injection: 10 bar × 0.2 min and plug, 10 bar × 0.2 min, applied voltage, +15 and −15 kV for native and aminopropyl silica, respectively; temperature: 20 °C; pressure: 10 bar on both vials.

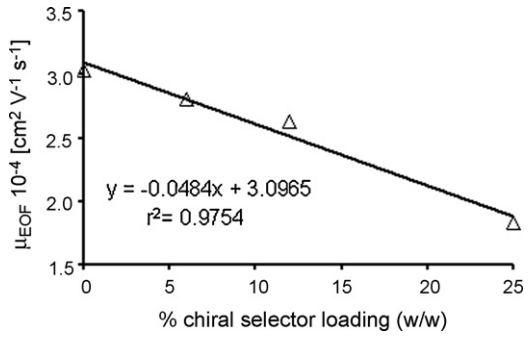

Fig. 4. The effect of chiral selector loading onto the silica on the EOF mobility. Stationary phase: native silica (5 μm) coated with 0, 6, 12, 25% (w/w) cellulose tris(3-chloro-4-methylphenylcarbamate). Applied voltage, +15 kV. For other experimental conditions see Fig. 3.

Considering the data reported above, for further experiments with native silica-based CSPs a mobile phase with the buffer at pH 6.5 was selected.

Capillaries packed with native and aminopropyl silica coated with 25% (w/w) chiral selector were also tested in order to study the effect of applied voltage on EOF.

By increasing the voltage from 10 to 25 kV (absolute value) a linear increase of EOF mobility was observed as expected. Although a relatively high current was recorded (10 and −7.9 μA at 25 and −25 kV, respectively), good correlation factors r^2 = 0.9956 and 0.9917 were observed for native and aminopropyl silica, respectively. Therefore, the negative effect of Joule heating on peak dispersion can be neglected.

Increasing the amount of coated polysaccharide derivative onto the silica in the range up to 25% the EOF decreased (Fig. 4). The observed decrease of the EOF is due to increasing shielding of silanol groups responsible for the EOF generation and is in a good agreement with previous results [25–27].

3.2. Selection of the optimum mobile phase for the enantiomeric separations by CEC and nano-LC

Although polysaccharide phenylcarbamate derivatives are commonly used for enantioseparation with normal-phase eluents [8–10,16–18,29–31], since late 1980s reversed-phase [32,33] and more recently pure polar organic eluents have been also used [25–27,34].

Preliminary nano-LC experiments were carried out using the native silica CSP containing 25% (w/w) chiral selector and analyzing three model samples, namely oxazepam, lorazepam and *trans*-stilbene oxide. Using only methanol or acetonitrile as mobile phases, the three studied racemic compounds showed enantioselective interaction with the CSP and good separation of enantiomers were observed. (The resolution factors were for oxazepam and *trans*-stilbene oxide in MeOH *Rs* = 2.31 and 1.86, respectively, and for lorazepam in ACN 1.1).

In order to find mobile phases exhibiting similar enantioresolution capabilities employing the same CSP in both techniques the same polar organic solvents as used in nano-LC were tested next in CEC experiments. Therefore, buffer solutions were added to the methanol/acetonitrile mixtures at different concentration ratios in order to have the appropriate conductivity. Experiments carried out by using mobile phases with 50–100% methanol in mixture with acetonitrile and 5 mM of ammonium formate (pH 2.5 and 3.5) or ammonium acetate (pH 4.5, 5.5 and 6.5) were not successful due to unstable current during the analysis.

Stable currents were observed by using acetonitrile at concentrations higher than 50% (v/v). Consequently, the mobile phase composition was modified considering various ACN/MeOH and MeOH/water ratios thus affecting the polarity and other physical properties of the solvent used. The example of the effect of the mobile phase composition on the retention and separation of enantiomers is shown in Fig. 5.

With increasing the polarity of the mobile phase, the retention factor of the three studied compounds raised (Fig. 5a). This shows that hydrophobic forces governing dipole–dipole and π–π interac-

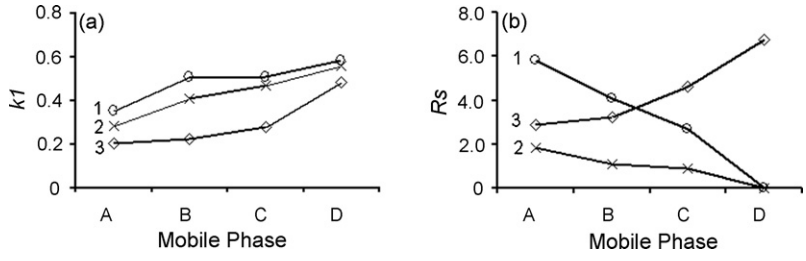

Fig. 5. Effect of mobile phase composition on the retention and enantioseparation factors in CEC experiments. Stationary phase: native silica coated with 25% (w/w) cellulose tris(3-chloro-4-methylphenylcarbamate). Sample 50 μg/mL in methanol of (1) oxazepam, (2) lorazepam, (3) *trans*-stilbene oxide. Mobile phase: (A) 1/49/50 (v/v/v) 500 mM ammonium acetate pH 6.5/MeOH/ACN, (B) 1/19/80 (v/v/v) 500 mM ammonium acetate pH 6.5/MeOH/ACN, (C) 1/10/9/80 (v/v/v/v) 500 mM ammonium acetate pH 6.5/H_2O/MeOH/ACN, (D) 1/19/80 (v/v/v) 500 mM ammonium acetate pH 6.5/H_2O/ACN; injection: 10 bar × 0.2 min and 10 bar × 0.2 min plug. Applied voltage, +15 kV; temperature, 20 °C; 10 bar on both vials. For other conditions see text.

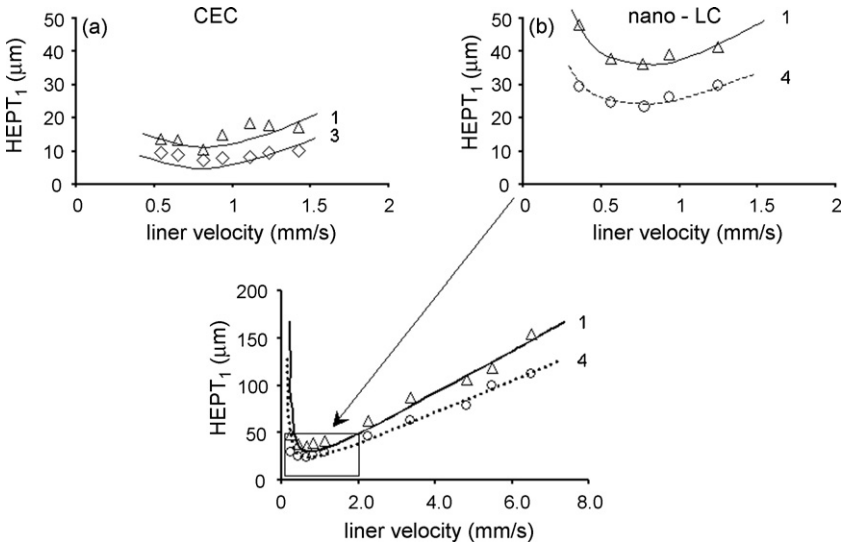

Fig. 6. The dependence of plate heights on linear flow velocity of the mobile phase in (a) CEC and (b) nano-LC. Experimental conditions: (a) applied voltage, 10–27 kV and 10 bar both on vials during runs. Mobile phase: 1/49/50 (v/v/v) 500 mM ammonium acetate with apparent pH 6.5/MeOH/ACN. Sample 50 μg/mL in methanol of (1) oxazepam, (3) *trans*-stilbene oxide and (4) temazepam. For other conditions see Fig. 5 and text.

tions between aromatic moieties of analytes and phenyl groups on the CSP are involved in the separation process.

In agreement with numerous previous observations, higher affinity for the stationary phase did not result in higher chiral recognition. In the case of chiral diazepines (lorazepam and oxazepam) (Fig. 5b) the enantiomers most likely interacted with the polar carbamate moiety via hydrogen bonding with the NH and carbonyl groups while additional dipole–dipole interactions occurred between the analyte and carbonyl groups of the chiral selector. The increase of the water's and methanol's amount in the mobile phase decreased the enantioresolution for both of above-mentioned analytes. Thus, water and methanol present in the mobile phase appeared to be strong competitors for hydrogen bonding with enantiomers for chiral site on phenylcarbamate derivative of cellulose.

3.3. Comparative enantioseparation in CEC and nano-LC

Experiments were carried out in order to study the effect of the linear flow rate of the mobile phase on the chromatographic performance of the modified polysaccharide stationary phases by using both CEC and nano-LC. For this purpose, the native silica-based CSP with 25% (w/w) chiral selector was used for the enantiomeric separation of some racemic analytes. Namely, temazepam and oxazepam were separated in nano-LC, *trans*-stilbene oxide and oxazepam in CEC.

The effect of the linear flow rate on plate height in CEC and nano-LC is shown in Fig. 6a and b. As it can be seen from these figures, the minimum plate heights (20 and 5 μm) were observed at the linear flow rates 0.70 and 0.81 mm/s in nano-LC and CEC, respectively. Based on the data one can conclude that CEC is a more efficient technique for enantiomer separations compared to nano-LC. The shape of the van Deemter curve observed in CEC was also flatter especially in the range of higher linear flow rates. Indeed, in CEC the significant intraparticle flow of the analytes might be responsible for the improved mass-transfer. This means a lower C term in the van Deemter equation [25–27,35].

A plug-like profile of the electrokinetically driven flow and low contribution of longitudinal diffusion were also responsible for the lower peak broadening in CEC versus nano-LC.

The above-mentioned linear flow rate allowing the highest efficiency were applied for the enantiomeric resolution of six pairs of enantiomers by using CEC and nano-LC. All analyzed compounds were baseline resolved in less than 15 min.

The number of theoretical plates observed in CEC (58,000–145,000 m^{-1}), were approximately 2.5 times higher than those

Table 1
CEC and nano-LC enantioseparations of standard racemic compounds by using capillary column packed with native silica coated with 25% (w/w) cellulose tris(3-chloro-4-methylphenylcarbamate)

	Sample	t_0 (min)	k_1	α	Plates$_1$/m	Plates$_2$/m	*Rs*
Nano-LC	Etozoline	6.40[a]	0.38	1.52	59,876	58,348	4.05
	Lorazepam		0.23	1.16	15,943	15,122	<1
	Oxazepam		0.29	1.73	28,092	33,080	3.28
	Temazepam		0.56	2.21	43,376	40,216	9.08
	Thalidomide		0.32	1.21	61,998	59,266	1.39
	trans-Stilbene oxide		0.17	1.34	57,254	55,146	1.31
CEC	Etozoline	5.56[a]	0.38	1.65	122,747	84,283	6.50
	Lorazepam		0.21	1.21	83,128	77,718	1.27
	Oxazepam		0.29	1.77	98,694	75,597	5.77
	Temazepam		0.62	2.32	79,420	58,558	12.90
	Thalidomide		0.34	1.21	113,806	75,857	2.03
	trans-Stilbene oxide		0.19	1.38	145,593	130,767	2.70

For experimental conditions see Figs. 5 and 6.
[a] Mean value.

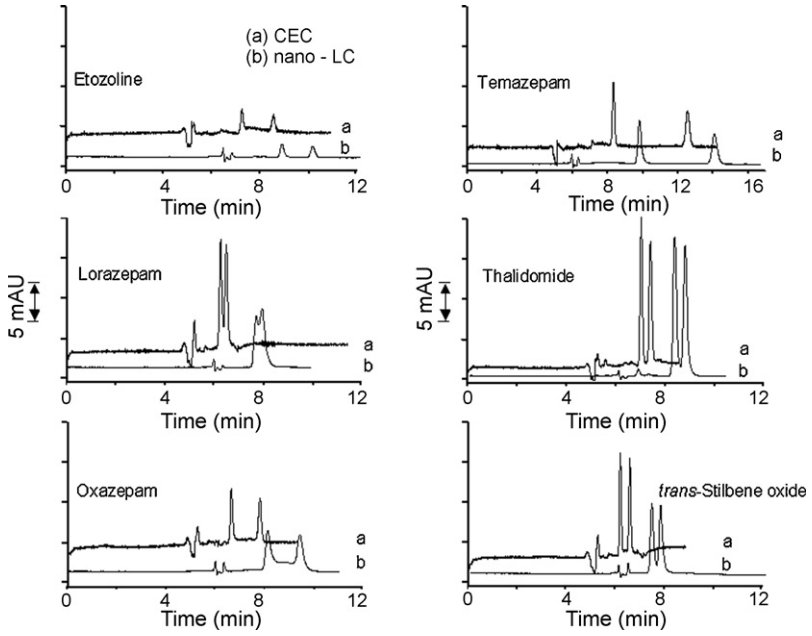

Fig. 7. CEC and nano-LC separation of studied enantiomers. Experimental conditions: (a) injection, 10 bar × 0.2 min and 10 bar × 0.2 min plug; +15 kV-10 bar on both vials, 20 °C (b) 250 nL/min; 60 nL injection. For other conditions, see Figs. 5 and 6.

recorded in nano-LC (15,000–62,000 m^{-1}) (Table 1). Quite similar retention factors were observed for a given chiral compound in both separation techniques (Table 1). Based on this, it can be concluded that the analytes being neutral compounds migrated towards the capillary outlet by EOF and only chromatographic mechanism was involved in separation under CEC conditions. The separation factors (α) were in the range of 1.16–2.32. As expected, comparable α values were observed in nano-LC and CEC while higher enantiomer resolution (*Rs*) was obtained when using electrodriven technique (Fig. 7). The plateau observed between resolved peaks of oxazepam indicates that this analyte undergoes enantiomerization under separation conditions in both nano-LC and CEC experiments.

3.4. Effect of polysaccharide loading onto the native silica gel on enantioseparations in nano-LC and CEC

Coated-type polysaccharide-based CSPs are less stable in some mobile phases compared to CSPs containing covalently attached chiral selector. On the other hand, coated-type CSPs may offer

Table 2
Effect of the chiral selector loading onto native silica on separation parameters measured in CEC experiments

% (w/w)	Sample	t_{eof} (min)	k_1	α	Plates$_1$/m	Plates$_2$/m	*Rs*
% chiral selector loaded							
0	Etozoline	3.17[a]	–	–	–	–	–
	Lorazepam		–	–	–	–	–
	Oxazepam		–	–	–	–	–
	Temazepam		–	–	–	–	–
	Thalidomide		–	–	–	–	–
	trans-Stilbene oxide		–	–	–	–	–
6	Etozoline	3.43[a]	0.03	2.34	279,468	283,695	2.17
	Lorazepam		–	–	–	–	–
	Oxazepam		0.04	1.91	132,709	143,289	1.58
	Temazepam		0.08	2.48	185,529	176,014	5.54
	Thalidomide		0.04	1.30	232,359	241,406	0.63
	trans-Stilbene oxide		0.01	1.00	278,811	231,647	0.49
12	Etozoline	3.66[a]	0.13	1.65	171,724	154,800	3.56
	Lorazepam		0.08	1.20	115,486	99,233	0.62
	Oxazepam		0.11	1.84	120,634	115,424	3.35
	Temazepam		0.22	2.31	127,573	89,990	8.53
	Thalidomide		0.11	1.25	185,305	175,082	1.30
	trans-Stilbene oxide		0.06	1.43	189,680	176,709	1.22
25	Etozoline	5.26[a]	0.38	1.65	122,747	84,283	6.50
	Lorazepam		0.21	1.21	83,128	77,718	1.27
	Oxazepam		0.29	1.77	98,694	75,597	5.77
	Temazepam		0.62	2.32	79,420	58,558	12.90
	Thalidomide		0.34	1.21	113,806	75,857	2.03
	trans-Stilbene oxide		0.19	1.38	145,593	130,767	2.70

For experimental conditions see Fig. 7. –: data not reported because analytes were not resolved in their enantiomers and were eluted together with the EOF.
[a] Mean value.

Table 3
Comparison of native and aminopropyl silica support by using nano-LC and CEC

Silica support	Sample	t_0 (min)	t_{eof} (min)	k_1		α		Rs	
		Nano-LC	CEC	Nano-LC	CEC	Nano-LC	CEC	Nano-LC	CEC
Sepapak-2, 25% 5 μm									
Native silica	Etozoline	6.40[a]	5.26[a]	0.38	0.38	1.52	1.65	4.05	6.50
	Lorazepam			0.23	0.21	1.16	1.21	<1	1.27
	Oxazepam			0.29	0.29	1.73	1.77	3.28	5.77
	Temazepam			0.56	0.62	2.21	2.32	9.08	12.90
	Thalidomide			0.32	0.34	1.21	1.21	1.39	2.03
	trans-Stilbene oxide			0.17	0.19	1.34	1.38	1.31	2.70
Aminopropyl silica	Etozoline	6.32[a]	7.49[a]	0.31	0.37	1.63	1.64	3.21	5.51
	Lorazepam			0.26	0.28	1.04	1.18	<1	1.03
	Oxazepam			0.29	0.37	1.70	1.69	2.73	5.15
	Temazepam			0.54	0.63	2.21	2.30	7.66	12.20
	Thalidomide			0.27	0.32	1.23	1.23	1.20	2.08
	trans-Stilbene oxide			0.14	0.16	1.40	1.40	1.33	2.15

Experimental conditions: 25% (w/w) chiral selector. Applied voltage, +15 and −15 kV for native and aminopropyl silica support, respectively. For other experimental conditions see Fig. 7.
[a] Mean value.

the advantage of easy loading of a silica with variable amounts of polysaccharide derivative [25–27,34–36].

In order to investigate the effect of a chiral selector loading on the chromatographic performance, the studies were performed with capillaries packed with native silica particles coated with cellulose tris(3-chloro-4-methylphenylcarbamate) at concentration of 6, 12 and 25% (w/w). The experiments were performed in both CEC and nano-LC mode.

The effect of chiral selector concentration on the EOF, analyte retention, separation factor of enantiomers, peak efficiency and resolution is shown in Table 2. Increasing the polysaccharide derivative concentration from 0 to 25% lead to a reduction of the EOF from 3.03×10^{-4} cm^2 V^{-1} s^{-1} down to 1.83×10^{-4} cm^2 V^{-1} s^{-1}. This effect is the result of increasing shielding of surface silanol groups due to the increased amount of the chiral selector [36].

As expected the retention and enantioseparation factor increased but the peak efficiency decreased with increasing loading of the chiral selector onto the surface of silica [27,37]. The lower efficiency observed at higher chiral selector concentration is most likely due to the lower mass-transfer [23,27,37].

The effect of chiral selector concentration on enantiomeric resolution in nano-LC was similar to that observed in CEC (results not shown).

3.5. Comparison of native and aminopropyl silica as support for chiral selector

Experiments were performed by using the same mobile phase and the same chiral selector adsorbed onto native and aminopropyl silica. Separation of enantiomers with these materials was performed in both nano-LC and CEC.

When using CSP based on aminopropyl silica in CEC the polarity was reversed because an anodic EOF was observed.

The separation data (k, α and Rs) for the six standard racemic analytes observed in nano-LC and CEC using the two different silica supports are shown in Table 3.

As these data indicate (see also Fig. 3) a slightly lower EOF was observed in CEC experiments with the capillary column prepared based on aminopropyl silica while the retention factors of most of analytes were comparable in both cases. The same trend was observed for the α values, too.

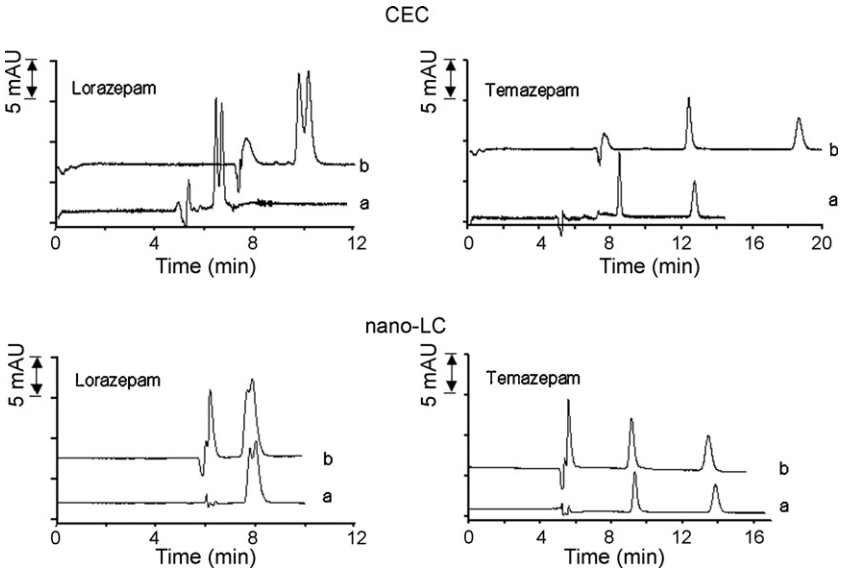

Fig. 8. CEC and nano-LC separation of selected analytes using CSPs based on (a) native and (b) aminopropyl silica supports coated with 25% (w/w) cellulose tris(3-chloro-4-methylphenylcarbamate). Applied voltage, +15 and −15 kV for CEC experiments in case (a) and (b), respectively. For other experimental conditions see Fig. 7.

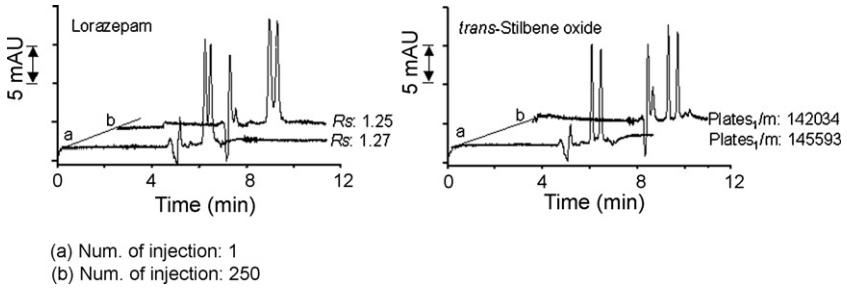

Fig. 9. Electrochromatograms of the enantiomeric separation of lorazepam and *trans*-stilbene oxide obtained using the same column in different days. Stationary phase, native silica support coated with 25% (w/w) cellulose tris(3-chloro-4-methylphenylcarbamate). For other experimental conditions see Fig. 7.

Comparing the data reported in Table 3 concerning the nano-LC experiments carried out with capillary columns based on native and aminopropyl silica, it can be observed that the *k* values were generally lower when the aminopropyl-based CSP was employed. Similar enantioseparation factors were obtained using the two stationary phases. Observing the *Rs* it can be concluded that the CSP based on native silica allowed to achieve higher resolution with the exception of lorazepam that was poorly resolved on both phases.

A comparison of the enantiomeric separation by using nano-LC and CEC with above-mentioned two capillary columns is shown in Fig. 8.

3.6. Column stability

In order to assess columns stability, experiments were carried out by CEC. Two polar organic solvents (MeOH and ACN) with apparent pH 2.0, 5.0 and 7.5 were used. Lorazepam and *trans*-stilbene oxide enantiomers were analyzed. The chiral stationary phases were stable at least for 300 runs from the viewpoint of the EOF, analyte retention, enantioseparation factors and peak efficiency.

As an example of column stability the electropherograms obtained by CEC analyzing lorazepam and *trans*-stilbene oxide enantiomers are shown in Fig. 9.

The above described results clearly indicate that the capillary columns packed with silica modified by cellulose tris(3-chloro-4-methylphenylcarbamate) are stable and can be used with polar organic solvents also for routine analysis.

4. Conclusions

From the results of this study it can be concluded that capillary columns packed with silica coated with cellulose tris(3-chloro-4-methylphenylcarbamate) can be used for miniaturized pressure- and electro-driven separation of enantiomers. The CSPs exhibited a high recognition capability towards almost all studied compounds in both CEC and nano-LC. The separation characteristic was affected by the amount of coated polysaccharide derivative. Higher efficiencies and enantioresolutions were observed when working with CEC. The prepared columns were tested for long time showing a good stability. No principal difference was observed for studied neutral analytes when using as a support native and aminopropyl silica in nano-LC. On the contrary, in CEC somewhat higher peak efficiency and enantioresolution were achieved for neutral analytes under this study by employing native silica.

Acknowledgement

B.C. thanks Georgian National Scientific Foundation (GNSF) for providing financial support for the part of this study (Project No. GNSF/ST 06/-071).

References

[1] W.H. Pirkle, J.M. Finn, J.L. Schreiner, B.C. Hamper, J. Am. Chem. Soc. 103 (1981) 3964.
[2] S. Allenmark, B. Bomgren, H. Boren, J. Chromatogr. 264 (1983) 63.
[3] J. Hermansson, J. Chromatogr. 269 (1983) 71.
[4] T. Ward, D.W. Armstrong, in: M. Zief, L.J. Crane (Eds.), Chromatographic Chiral Separations, Chromatographic Science Series, vol. 40, Marcel Dekker, New York, 1988, p. 131.
[5] I. Kartozia, G. D'Orazio, B. Chankvetadze, S. Fanali, J. Cap. Elec. Microchip Technol. 9 (2005) 31.
[6] Y. Okamoto, S. Honda, I. Okamoto, H. Yuki, J. Am. Chem. Soc 103 (1981) 6971.
[7] G. Blaschke, B. Brocker, W. Fraenkel, Angew. Chem. Int. Ed. Engl. 25 (1986) 830.
[8] Y. Okamoto, M. Kawashima, K. Hatada, J. Am. Chem. Soc. 106 (1984) 5357.
[9] B. Chankvetadze, E. Yashima, Y. Okamoto, Chem. Lett. (1993) 617.
[10] Y. Okamoto, Y. Kaida, J. Chromatogr. A 666 (1994) 403.
[11] M. Lämmerhofer, W. Lindner, J. Chromatogr. A 741 (1996) 33.
[12] R. Sardela, M. Lämmerhofer, B. Natalini, W. Lindner, J. Sep. Sci. 31 (2008) 1072.
[13] P. Rumeliotis, K.K. Unger, A.A. Kurganov, V. Davankov, J. Chromatogr. 255 (1983) 51.
[14] A. Berthod, Y. Liu, C. Bagwill, D.W. Armstrong, J. Chromatogr. A 731 (1996) 123.
[15] G. D'Orazio, Z. Aturki, M. Cristalli, M.G. Quaglia, S. Fanali, J. Chromatogr. A 1081 (2005) 105.
[16] B. Chankvetadze, E. Yashima, Y. Okamoto, J. Chromatogr. A 670 (1994) 39.
[17] B. Chankvetadze, L. Chankvetadze, S.H. Sidamonidze, E. Yashima, Y. Okamoto, J. Pharm. Biomed. Anal. 14 (1996) 1295.
[18] B. Chankvetadze, L. Chankvetadze, S.H. Sidamonidze, E. Yashima, Y. Okamoto, J. Pharm. Biomed. Anal. 13 (1995) 695.
[19] E. Francotte, M. Jung, Chromatographia 42 (1996) 521.
[20] K. Krause, M. Girod, B. Chankvetadze, G. Blaschke, J. Chromatogr. A 837 (1999) 51.
[21] M. Meyring, D. Strickmann, B. Chankvetadze, G. Blaschke, C. Desiderio, S. Fanali, J. Chromatogr. B 723 (1999) 255.
[22] K. Otsuka, C. Mikami, S. Terabe, J. Chromatogr. A 887 (2000) 457.
[23] D. Mangelingis, M. Maftouh, Y.V. Heyden, J. Sep. Sci. 28 (2005) 691.
[24] F. Qin, Y. Liu, X. Chen, L. Kong, H. Zou, Electrophoresis 26 (2005) 3921.
[25] B. Chankvetadze, I. Kartozia, J. Breitkreutz, Y. Okamoto, G. Blaschke, Electrophoresis 22 (2001) 3327.
[26] B. Chankvetadze, I. Kartozia, Y. Okamoto, G. Blaschke, J. Sep. Sci 24 (2001) 635.
[27] L. Chankvetadze, I. Kartozia, C. Yamamoto, B. Chankvetadze, G. Blaschke, Y. Okamoto, J. Sep. Sci. 25 (2002) 653.
[28] S. Fanali, E. Camera, B. Chankvetadze, G. D'Orazio, M.G. Quaglia, J. Pharm. Biomed. Anal. 35 (2004) 331.
[29] Y. Okamoto, M. Kawashima, K. Hatada, J. Chromatogr. 363 (1986) 173.
[30] B. Chankvetadze, L. Chankvetadze, Sh. Sidamonize, E. Yashima, Y. Okamoto, J. Pharm. Biomed. Anal. 14 (1996) 1295.
[31] R. Cirilli, R. Ferretti, B. Gallinella, F. La Torre, G. La Regina, R. Silvestri, J. Sep. Sci. 28 (2005) 627.
[32] K. Ikeda, T. Hamasaki, H. Kohno, T. Ogawa, Chem. Lett. (1989) 1089.
[33] D. Mangelings, J. Discry, M. Maftouh, D.L. Massart, Y.V. Heyden, Electrophoresis 26 (2005) 3930.
[34] B. Chankvetadze, I. Kartozia, C. Yamamoto, Y. Okamoto, J. Pharm. Biomed. Anal. 27 (2002) 467.
[35] B. Chankvetadze, I. Kartozia, J. Breitkreutz, M. Girod, M. Knobloch, Y. Okamoto, G. Blaschke, J. Sep. Sci. 24 (2001) 251.
[36] M. Girod, B. Chankvetadze, G. Blaschke, J. Chromatogr. A 887 (2000) 439.
[37] M. Girod, B. Chankvetadze, Y. Okamoto, G. Blaschke, J. Sep. Sci. 24 (2001) 27.