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Capillary electrochromatography: normal-phase mode using silica gel and cellulose-based packing materials[1]

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Abstract

Cellulose-based packings and native silica gel have been employed as stationary phases in normal-phase capillary electrochromatography. The electroosmotic flow produced with non-aqueous mobile phases (acetonitrile, methanol, hexane–ethanol–methanol) is sufficiently high (mobility up to $\mu_{eo}=0.152$ cm^2 s^{-1} kV^{-1}) and stable for chromatographic separations. Only electroosmotic flow has been used for the propulsion of the mobile phase through the chromatographic packings. The elution order obtained with a mixture of test solutes corresponds to the elution order expected for normal-phase chromatography. © 1997 Elsevier Science B.V.

Keywords: Electroosmotic flow; Stationary phases, electrochromatography; Electrochromatography; Silica gel; Cellulose

1. Introduction

Capillary electrochromatography (CEC) is the liquid chromatographic technique, involving principles of electrokinetic analysis. CEC is conventionally performed with stationary phases typical for liquid chromatography [1,2]. Packed [1–6] or open tubular [7–12] capillaries are used in CEC. In both cases, aqueous/organic, buffered mobile phases are preferred. In CEC the system of mobile and stationary phases should result in an electroosmotic flow in the capillary under the application of an electric field. Classical understanding of the electroosmotic flow predicts the necessity to employ aqueous/organic buffered mobile phases. The aqueous media so far used as mobile phases in CEC are similar to mobile phases used in reversed-phase HPLC. This similarity was utilised in order to transfer mobile and stationary phases typical for reversed-phase HPLC into CEC and it resulted in a rapid growth of the number of applications employing silica gel based reversed-phase packing materials and aqueous mobile phases in CEC [3–13]. Therefore, CEC is sometimes identified as a reversed-phase liquid chromatography with an electric field applied across the length of the column [13]. Recently the use of non-aqueous media in capillary electrophoresis has been reported [14–18]. The application of non-aqueous mobile phases in combination with polar stationary phases in CEC, to our knowledge, has not been discussed in the literature to date. CEC separation of polycyclic

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aromatic hydrocarbons and fullerenes using octadecyl silica and non-aqueous mobile phases (acetonitrile modified with methylene chloride or tetrahydrofuran) was recently reported by Whitaker and Sepaniak [19]. The non-aqueous system provided greater solubility for the hydrophobic solutes and small currents.

In this paper we present experimental results obtained with non-aqueous mobile phases in CEC and experience gained in normal-phase CEC separations.

2. Experimental

2.1. Apparatus and operation

CEC was performed with an in-house made apparatus, described in Ref. [10]. The high-voltage source was a Model CZE 1000 R (Spellman, Planeview, USA). A UV-MII ultraviolet detector (Pharmacia, Uppsala, Sweden) modified by the installation of ball lenses was utilised for in-column detection. Detection was performed at 254 nm. A L-6210 HPLC pump (Merck Hitachi, Darmstadt, Germany) was used for conditioning of packed capillary. Chromatograms were recorded with a 740 Data module (Waters Millipore, Milford, USA).

Fig. 1 shows the configuration of the CEC apparatus employed in this study. The inlet of packed capillary was immersed into a reservoir filled with mobile phase, equipped with a platinum electrode. The outlet of the capillary was connected tightly by a modified fitting and rubber seal to a conventional T-piece used in HPLC. The T-piece was grounded and it served as the second electrode. The second and third ports of the T-piece were connected to a 2-way valve and a capillary filled with conditioning solvent. The second end of the conditioning solvent capillary (65 cm×0.5 mm I.D. PTFE capillary) was coupled HPLC pump. PTFE capillaries were employed for the connections-manifold of the CEC system.

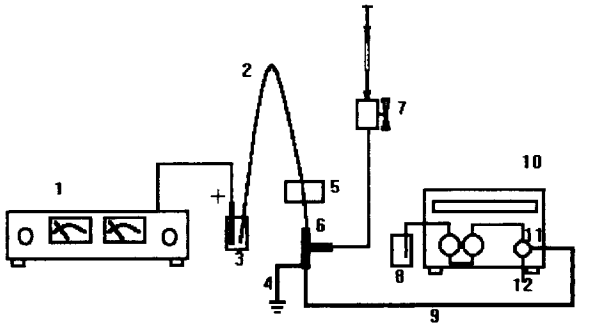

Fig. 1. Configuration of the CEC apparatus employed: 1, high voltage source; 2, capillary; 3, elution solvent reservoir with electrode; 4, grounding; 5, detection unit; 6, T-piece; 7, 2-way valve with injection port; 8, pumping liquid; 9, conditioning solvent reservoir; 10, HPLC pump; 11, pump valve; 12, waste.

2.1.1. Conditioning of the capillary column

The conditioning solvent was injected through the injection port of the 2-way valve into the conditioning solvent reservoir (injection valve and valve of HPLC pump were open). Conditioning was performed at 30 bar pressure pumping conditioning solvent with HPLC pump through the capillary column, after having closed the 2-way valve and the HPLC pump valve. Packed capillaries were conditioned for 30–45 min. After the conditioning procedure, pressure was gradually decreased by gentle opening of the HPLC pump valve.

2.1.2. Capillary electrochromatography

In the CEC operation mode, the HPLC pump valve was kept open. Electroosmotic flow was used for the propulsion of the mobile phase. Injection of analytes was performed electrokinetically (7 s at 5 kV).

2.1.3. Measurement of the electroosmotic flow

A 10-μl syringe (Hamilton, Reno, Nevada, USA) without the piston was mounted into the injection port of the 2-way valve and used in order to measure the flow-rate of the electroosmotic flow (EOF). For excluding a leakage through the HPLC pump valves and changes of the extracolumn liquid volume, occurring because of thermal fluctuation, the branch of the manifold, including conditioning liquid reservoir and HPLC pump, was disconnected from the T-piece.

2.2. Columns

Fused-silica capillaries of 360 μm O.D. and 150

μm I.D. (CeramOptec, Bonn, Germany) were packed by the slurry method. A DSTV-122 (Haskel, USA) pneumatic pump was used to pack the capillaries. The inlet frits were sintered after the tapping the capillary ends into wetted bare Silica 100 (~5 μm fraction, Macherey-Nagel, Düren, Germany). A slurry of the packing material in methanol (20% w/v) was packed into the capillary at pressures up to 200 bar. During the packing procedure, the slurry in the packing reservoir was stirred and the capillary was immersed into an ultrasonic bath. The outlet frits were made in the same manner as the inlet frits.

A 1.0-cm length detection window was made by removing polyimide layer of the capillary with hot sulphuric acid at 3.5–4.5 cm from the capillary outlet.

The following columns were employed in this study: column (i) packed with Polygosil 100-10, d_p=10 μm, mean pore diameter=10 nm (Macherey-Nagel) at 100 bar pressure, 341(301) mm×150 μm; column (ii) packed with octadecylated cellulose C_{18}-Granocel-14Sh (d_p=7 μm) at 200 bar pressure, 283(243) mm×150 μm; column (iii) packed with neutral cellulose matrix Granocel-14Sh (d_p=7 μm) at 50 bar pressure, 283(243) mm×150 μm. C_{18}-Granocel-14Sh and Granocel-14Sh were prepared in-house [20,21].

2.3. Chemicals

Analytes used for the chromatographic experiments were purchased from various companies. Acetonitrile (Riedel-de Haën, Hannover, Germany) was distilled before use. Methanol and ethanol were HPLC grade (Merck). Hexane was from the Department of Chemistry, University of Marburg (Germany).

3. Results and discussion

3.1. Cellulose-based packing materials

Rigid cellulose-based packing materials were obtained by physical–chemical modification of the conventional cellulose gel Granocel-14 [20] used for column liquid chromatography of biopreparations. Rigidity of cellulose particles was increased by shrinkage of the cellulose hydrogel Granocel-14 beads (fraction 10–20 μm) during the drying with a Mini Spray Dryer (Büchi, Switzerland) [21]. The cellulose xerogel Granocel-14Sh obtained was irreversibly shrunken (size of particles d_p=7 μm) and demonstrated a high mechanical stability.

Octadecylated cellulose was obtained by the reaction of epoxy-activated cellulose matrix Granocel-14Sh with octadecanol [21]. An electron micrograph of synthesised octadecylated cellulose C_{18}-Granocel-14Sh microparticles is shown in Fig. 2.

3.2. Selection of mobile phases

Several mobile phases (acetonitrile, methanol, methanol–ethanol–hexane) were tested in this study. Pure methanol or acetonitrile were selected as monocomponent relatively polar mobile phases for normal-phase CEC. Hexane was added to methanol in order to obtain less polar mobile phases. Because of the poor solubility of pure hexane in methanol, a mixture of hexane–ethanol (85:15, v/v) was used instead of pure hexane to form ternary mobile phases with methanol. Due to its high viscosity, pure ethanol or ethanol–hexane mixtures were not used in these studies.

3.3. Electroosmotic flow

The electroosmotic flow in CEC is the crucial factor that makes electrochromatographic technique

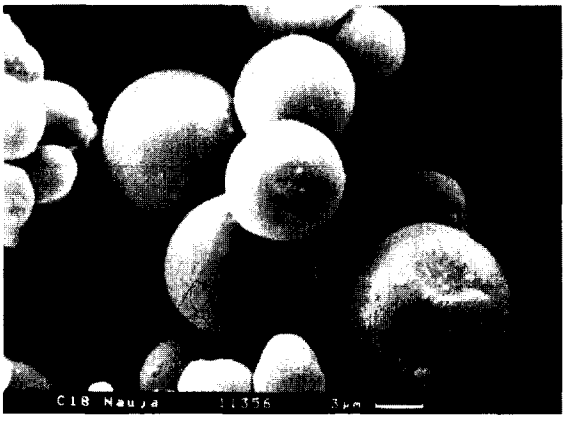

Fig. 2. Electron micrograph of octadecylated cellulose particles C_{18}-Granocel-14Sh.

superior to pressure-driven chromatography due to the different flow profiles generated [3,22]. Higher efficiency for open-tubular CEC compared to conventional open-tubular liquid chromatography can be obtained only at elevated EOF velocities. At very low linear velocities (less than 0.05–0.1 mm/s), calculations have shown no difference in efficiency for flat and parabolic flow profiles [23].

The volume fraction of the mobile phase ϕ_{mob} was calculated using Eq. (1):

$$\phi_{mob} = \frac{4t_M u}{L_d \pi d_c} \quad (1)$$

where t_M is the retention time of methanol in seconds, u is the flow rate measured with a syringe in μl/s, L_d is the length of capillary from the inlet to detector in mm, d_c is the I.D. of capillary in mm.

Electroosmotic flow velocity was calculated using Eq. (2):

$$u_{eo} = \frac{4u}{\varphi_{mob} \pi d_c^2} \quad (2)$$

Results of the evaluation of the electroosmotic mobility, μ_{eo} ($u_{eo} = \mu_{eo} U/L_t$, where U is voltage in kV, L_t is total length of capillary in cm) in non-aqueous CEC with bare silica gel and cellulose-based packing materials as stationary phases, are presented in Table 1. As is seen in Table 1 non-polar hexane in the mobile phase reduced the electroosmotic flow considerably. Currents generated in CEC with non-aqueous mobile phase were considerably lower than those obtained with aqueous mobile phase. For instance, current, measured with C_{18}-Granocel-14Sh packed column (ii), was 0.5 μA at 20 kV voltage, mobile phase methanol–ethanol–hexane (50:7.5:42.5, v/v) and 64 μA with aqueous mobile phase, acetonitrile–20 mmol sodium tetraborate buffer in water (4:6), at 20 kV voltage. Due to low current and smaller heat generation during a normal-phase electrochromatographic run, the electroosmotic flow could be increased by applying a higher voltage. The EOF demonstrated a linear dependence on the voltage applied. Fig. 3 shows u_{eo} obtained with Polygosil 100-10 packed column (i) and methanol as mobile phase. No significant changes or pulsation of the EOF were observed within several days operation

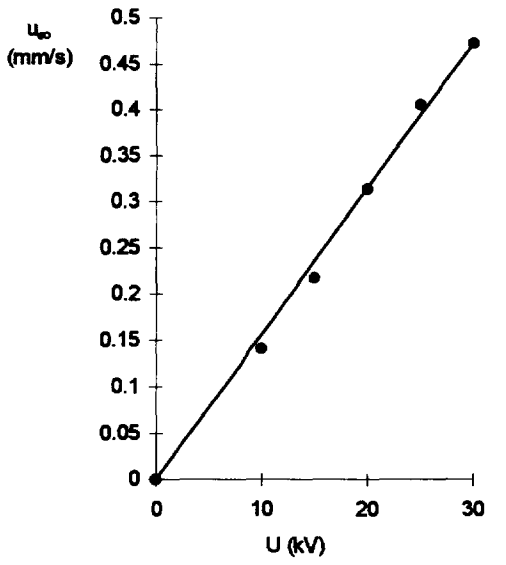

Fig. 3. Dependence of the electroosmotic flow velocity u_{eo} on the voltage applied. Column (i) 341(301) mm×150 μm; stationary phase Polygosil 100-10; mobile phase methanol–ethanol–hexane (50:7.5:42.5, v/v).

Table 1
Electroosmotic mobility, μ_{eo}, in normal-phase capillary electrochromatography with different mobile phases

Column	Packing material	φ_{mob}	μ_{eo} ($cm^2 s^{-1} kV^{-1}$)			
			Acetonitrile	Methanol–ethanol–hexane (v/v)		
				100:0:0	75:3.75:21.25	50:7.5:42.5
(i)	Bare silica Polygosil 100-10	0.650	–[a]	0.107	0.083	0.053
(ii)	Octadecylated cellulose C_{18}-Granocel-14Sh	0.631	0.152	0.146	–[a]	0.037
(iii)	Neutral cellulose Granocel-14Sh	0.734	–[a]	0.08.6	0.052	0.027

Measurements were carried out at 20 kV.
[a] Not determined.

of this column in the normal-phase mode. Alterations in μ_{eo} were caused mostly by reconditioning or rinsing of the packed capillary by means of a HPLC pump. Therefore special care was taken to preserve the packing structure and capillary end-frits from pressure shocks.

Both, the silica gel Polygosil 100-10 and the cellulose-based stationary phases C_{18}-Granocel-14Sh and Granocel-14Sh demonstrated relatively stable EOF velocities and easy handling in the normal-phase mode. The relative standard deviation of u_{eo} with a mixed stationary phase [Polygosil 100-10–C_{18}-Granocel-14Sh 1:1 (v/v)] and methanol as the mobile phase was 3.1% [20]. Due to low heat generation, the formation of bubbles during the chromatographic run did not constitute a problem in normal-phase CEC. Comparing μ_{eo} obtained at different modes, a decrease of about 2 was observed with the Polygosil 100-10 packed column (i) changing from an aqueous mobile phase [$\mu_{eo}=0.212$ $cm^2\,s^{-1}\,kV^{-1}$ with mobile phase acetonitrile–2 *M* aqueous solution of $Na_2B_4O_7$ (7:3, v/v) at 20 kV voltage] to a non-aqueous mobile phase [$\mu_{eo}=0.107$ $cm^2\,s^{-1}\,kV^{-1}$ with pure methanol at 20 kV voltage (Table 1)]. In case of a cellulose-based packing material C_{18}-Granocel-14Sh [column (ii)] a remarkable increase of μ_{eo} was observed in the normal-phase mode [$\mu_{eo}=0.146$ $cm^2\,s^{-1}\,kV^{-1}$ with pure methanol at 20 kV voltage (Table 1)] compared with that obtained in the reversed-phase mode [$\mu_{eo}=0.100$ $cm^2\,s^{-1}\,kV^{-1}$ with mobile phase acetonitrile–2 *M* aqueous solution of $Na_2B_4O_7$ (7:3, v/v) at 20 kV voltage]. The latter phenomenon can be explained by an increase of the hydrodynamic resistance of the cellulose-based packing in aqueous media, which occurred because of the swelling of the packed particles.

3.4. Normal-phase CEC

Fig. 4 shows the separations of test solutes obtained with bare silica Polygosil 100-10 packed column (i). The elution order of the solutes follows that expected for normal-phase liquid chromatography. With a low-polar mobile phase, methanol–ethanol–hexane (50:7.5:42.5 v/v) the least polar benzene eluted before the more polar acidic compounds phenol and resorcinol, the most polar compounds 2-aminophenol and 1,2-diaminobenzene were most strongly retarded and eluted in the last position (Fig. 4a). The chromatographic selectivity, observed in case of higher polarity mobile phase (Fig. 4b), was poor and insufficient to separate all the components of the test-mixture.

Comparison of the stationary phases investigated showed that the neutral cellulose Granocel-14Sh packed column (iii) had the best selectivity in normal-phase CEC separations. Complete separation of the test-solutes was obtained even with pure methanol, as a relatively polar mobile phase. Retention factors calculated for solutes eluted with mobile phases of different polarity are summarised in Table 2. The increase of the volume fraction of non-polar hexane in the mobile phase caused higher retention factors and better resolution.

The octadecylated cellulose packing material C_{18}-Granocel-14Sh demonstrated interesting behaviour. This packing material was originally synthesised and used for reversed-phase partition chromatography [21]. A C_{18}-Granocel-14Sh packed column (ii) in combination with aqueous mobile phases was used for reversed-phase CEC [24]. However, it has also demonstrated normal-phase CEC separations in non-aqueous media. The amphiphilicity observed with this packing material can be explained by the chemical structure of the octadecylated cellulose. Besides lipophilic properties, originating in the octadecyl ligands, it has also hydrophilic properties because of the polar hydroxyl groups included in the structure of the adsorbent. All the hydroxyl groups of the octadecylated cellulose consist of unconverted hydroxyl groups of the cellulose matrix and hydroxyl groups, that are produced by the reaction of the octadecanol anchoring to the epoxy-activated cellulose. Fig. 5 shows chromatograms of the test solutes obtained with the column (ii). Octadecylated cellulose in combination with methanol as relatively polar non-aqueous mobile phase (Fig. 5a) has demonstrated lower selectivity. The less polar compounds benzene, phenol and resorcinol were not separated under these conditions. The addition of non-polar hexane to the mobile phase improved the resolution so that all compounds of the test-mixture were separated.

The application of amphiphilic stationary phase packed capillary for the NP CEC was very valuable because it provided the opportunity to compare the

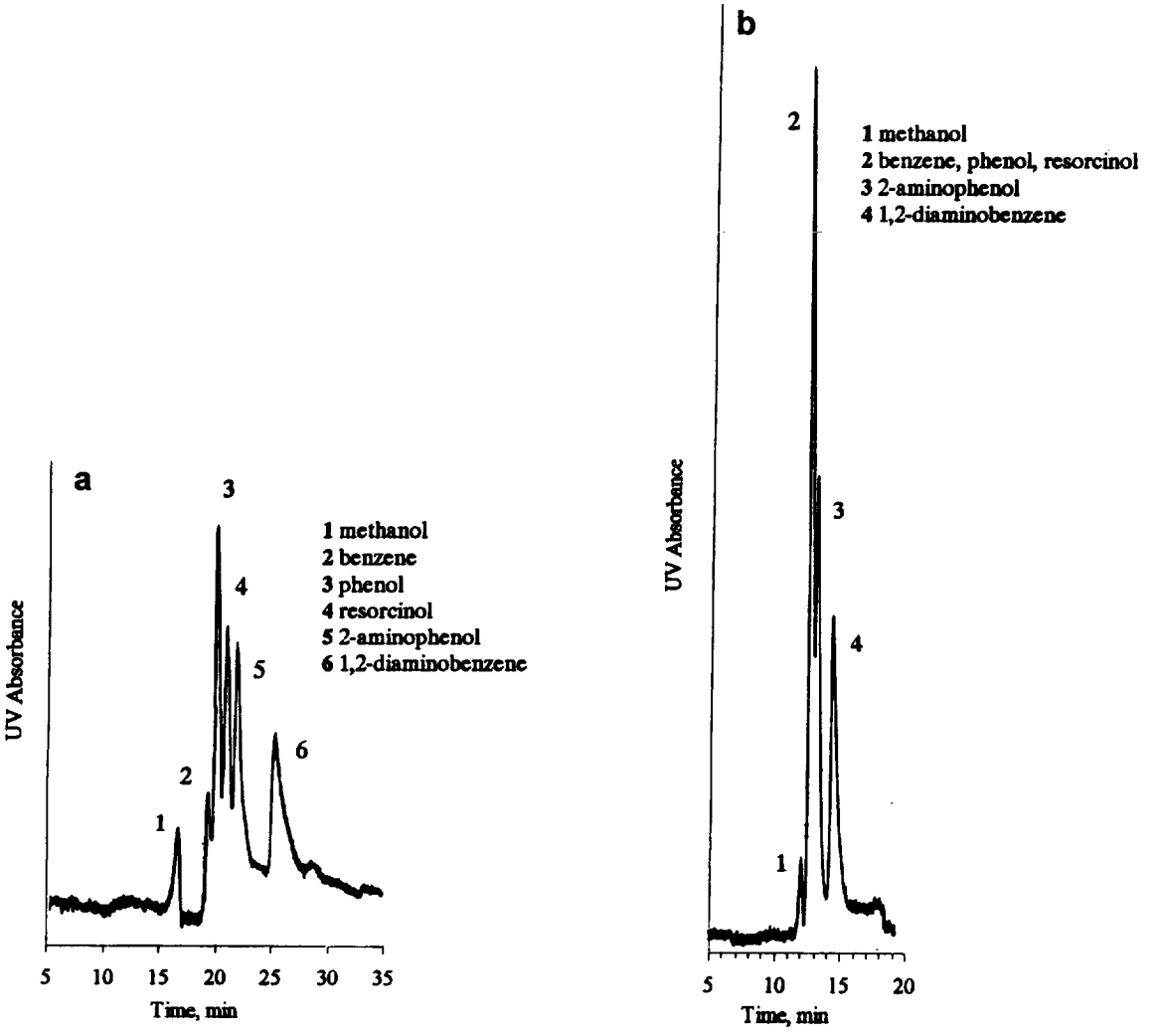

Fig. 4. Normal-phase CEC separations obtained with bare silica Polygosil 100-10 packed column (i) 341(301) mm×150 μm and methanol–ethanol–hexane mobile phases of different composition: (a) 50:7.5:42.5 (v/v) and (b) 75:3.7:21.25 (v/v) at 20 kV voltage; detection: UV, 254 nm; injection: electrokinetic, 7 s, 5 kV.

behaviour of the same capillary in both, reversed and straight modes of CEC. This case demonstrated stability of EOF and easier operation in normal-phase mode CEC.

The stability of the packing material is of key importance if an in-column detection (detection in the packing material) is performed. Due to semi-rigidity of the cellulose-based stationary phases, a remarkable drift of the baseline was observed (Fig. 5). Considerable stabilisation of the baseline was obtained with mixed packing material, Polygosil 100-10–C_{18}-Granocel-14Sh, (1:1, v/v) [24].

The efficiency of CEC separations performed in this study was relatively low. In no case did the number of theoretical plates exceed 21 000 m^{-1}. A similar efficiency was observed with octadecylated cellulose C_{18}-Granocel-14Sh in the reversed-phase mode [24]. Using the same electrochromatographic system with conventional ODS silica gel (d_p = 3 μm and 5 μm) packed capillaries the plate number (N)

Table 2
Influence of the mobile phase on the retention factor (k)

Analyte	k with mobile phase methanol–ethanol–hexane (v/v)		
	100:0:0	75:3.75:21.25	50:7.5:42.5
Benzene	0	0.16	0.30
Phenol	0.06	0.29	0.44
Resorcinol	0.15	0.55	1.01
2-Aminophenol	0.32	0.83	1.35
1,2-Diaminobenzene	0.86	1.26	–[a]

Data obtained with column (iii), stationary phase: Granocel-14Sh, other conditions see Fig. 4.
[a] Not determined.

ranged between 29 000 and 44 000 m^{-1} [10]. Efficiency of separation in part is limited by sensitivity of UV detector used in these studies and the necessity to overload the capillary columns for ease of detection. In order to obtain higher efficiency, further optimisation of the packing procedure and packing materials for normal phase CEC is needed.

4. Conclusions

This study has shown the possibility of performing

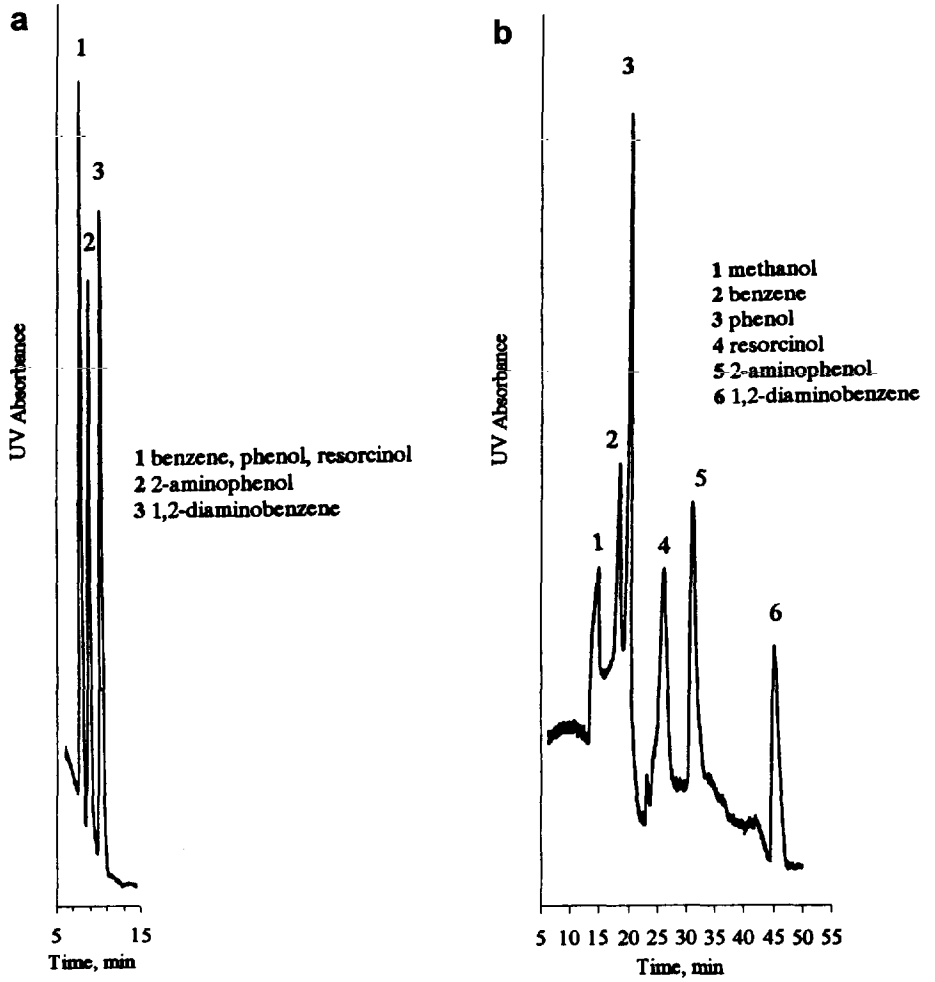

Fig. 5. Normal-phase CEC separations obtained with octadecylated cellulose C_{18}-Granocel-14Sh packed column (ii) 283(243) mm×150 μm with (a) methanol and (b) methanol–ethanol–hexane (50:7.5:42.5, v/v), as mobile phases at 20 kV, other conditions see Fig. 4.

CEC in the normal-phase mode. The electroosmotic flow was stable and sufficient to perform CEC with non-aqueous mobile phases (acetonitrile, methanol, methanol–ethanol–hexane) and silica gel or cellulose-based adsorbents as stationary phases. The increase of the volume fraction of the non-polar component hexane in the mobile phase results in higher retention factors and better resolution, however, it also decreased the electroosmotic mobility.

Normal-phase CEC offers completely different selectivities in comparison with that obtained with conventional octadecylated silica gel packing materials in reversed-phase CEC. Normal-phase mode extends the applicability of the CEC for analytes with very limited solubility in aqueous media.

Acknowledgments

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