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Electrokinetic reversed-phase chromatography with packed capillaries

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ABSTRACT

Possibilities and limitations for the applicability of electroendosmotic flow as an elution system in reversed-phase chromatography with packed capillaries were investigated. By using electroendosmotic elution, very high performance could be achieved. No loss of column efficiency was observed up to linear electroendosmotic flows of *ca.* 3 mm/s. With 50-μm I.D. fused-silica capillaries, packed with 3-μm ODS, reduced plate heights of 1.7–2.2 were obtained for test compounds ($k' = 0$–4.8) at a linear velocity of 2.6 mm/s. Electroendosmotic elution systems allowed the use of very small-particle packing materials. With 1.6-μm Monospher ODS, an extremely high chromatographic efficiency of up to 790 theoretical plates/s was obtained.

INTRODUCTION

Capillary liquid chromatography has been investigated by several groups because of its potential to increase the performance of chromatography for high-resolution analysis and for the analysis of extremely small samples [1]. Packed [2,3], drawn packed [4,5] and open-tubular [6,7] capillary columns have been employed and high efficiency in chromatographic performance has been demonstrated. Capillary electrokinetic separation techniques such as capillary zone electrophoresis [8–12], capillary micellar electrokinetic chromatography [13,14], capillary gel electrophoresis [15] and capillary isoelectric focusing [16] have stimulated further developments of capillary liquid chromatography. In these techniques (except capillary gel electrophoresis and capillary isoelectric focusing), the transport of solutes, both uncharged and charged, is based mainly on electroendosmotic flow induced by the electrical field. Electroendosmotic flow originates from the electrical double layer on the surface and this phenomenon has been fully described [17,18]. The flow profile of electroendosmotic flow is nearly flat compared with the parabolic flow profile of pressure pumping. This feature offers high plate efficiency in capillary electrokinetic separation techniques.

Electroendosmotic elution systems have been used in open-tubular [19–21] and drawn packed reversed-phase capillary chromatography [22] as an alternative to pressure elution systems. Jorgenson and Lukacs [23] discussed the applicability of electroendosmotic elution systems in packed capillary reversed-phase chromatography and reduced plate heights of 1.9 were obtained for a peak eluting at *ca.* 30 min using a 65 cm × 0.17 mm I.D. capillary packed with 10-μm ODS packing. However, they pointed out some difficulties in working with these systems.

For the analysis of very complex samples, it is necessary to increase the chromatographic performance. It is expected that electrokinetic reversed-phase chromatography with packed systems will allow the achievement of this goal and it also has the advantages of packed capillaries (higher capacity than open-tubular and drawn packed capillaries). In this paper, we describe the possibilities and limitations of applying electrokinetic elution systems in packed capillary reversed-phase chromatography.

EXPERIMENTAL

Preparation of packed capillaries

Fused-silica capillaries (50 μm I.D., 365 μm O.D.) (Polymicro Technologies, Phoenix, AZ, USA) were used as column materials. First, 4-μm spherical silica packings were packed into the capillaries in order to sinter the end-frit as follows. The end of the capillary was firmly tapped downwards into a tightly compacted pile of 4-μm spherical silica wetted with sodium silicate solution and deionized water. The packing was sintered at the end of the capillary by gently heating with a small microtorch flame for *ca.* 15 s. A slurry of 4-μm spherical silica packing (Superspher Si 60; Merck, Darmstadt, Germany) in acetonitrile (1:10, w/v) was prepared with ultrasonication (5 min) and pumped into the capillary at 5000 p.s.i. using a liquid chromatographic pump (Model 100 solvent metering system; Altex Scientific, Berkeley, CA, USA) and a stainless-steel tubing reservoir (350 mm × 2 mm I.D.). The base of the reservoir connected to the inlet of the capillary was placed in an ultrasonic bath during packing.

After packing, the mobile phase was replaced with distilled water and the capillary was equilibrated. The end-frit was sintered in *ca.* 19 cm from the outlet frit by very gentle heating. First the packing was dried and the polyimide coating was incinerated in the lower part of the flame, then the end-frit (3–5 mm) was sintered by heating in the middle of the flame for 20 s. During heating the capillary should be rotated slowly and the heating should be concentrated on the required position. The outlet frit was cut off and the capillary was emptied of the 4-μm spherical silica packings by pumping distilled water from each side. The polyimide coating was burned away to make a detection window. The capillary was flushed with acetonitrile and packed up to the inlet with a reversed-phase packing material (3-μm ODS-Hypersil, Shandon Southern Products, Runcorn, UK) as described above at 6000 p.s.i. In order to pack 1.6-μm Monospher ODS (Merck), the pressure was set at 9000 p.s.i. After the equilibration with distilled water, the length of the packed section and non-packed section was arranged by cutting the capillary ends. In electrokinetic reversed-phase chromatography, ODS silica packings are negatively charged, and the electro-

phoretic mobility is higher than the elektroendosmotic mobility. Therefore, it was necessary to sinter a frit at the inlet to prevent the packing from migrating out. The frit was sintered in the same way as the outlet frit.

Chromatographic system

Chromatographic runs were carried out with a laboratory-made apparatus similar to that used for capillary zone electrophoresis. The inlet of the capillary was connected to a stainless-steel six-port rotary valve including an injection port (Model 7010; Rheodyne, Cotati, CA, USA), in order to use high-pressure pumping to eliminate air bubbles from the capillaries. A carbon electrode from a positive polarity high-voltage power supply (Alpha MK II, Model 2907P, 0–60 kV; Brandenburg, Surrey, UK) and a stainless-steel tube from the valve were inserted in an anode chamber filled with the mobile phase. The outlet was inserted in a capped cathode vial. On-column detection was carried out with a Model 783A ultraviolet detector (Applied Biosystems, Foster City, CA, USA), which was modified to separate the detection unit from the aparatus by using optical fibres (600 μm I.D., 1 mm O.D.; Laaber Rüsselsheim, Germany). The samples were injected electrokinetically.

Reagents

Sodium tetraborate (Merck) was used to increase the pH of the mobile phase. Acetonitrile, benzyl alcohol, benzaldehyde, benzene and toluene were obtained from Merck, 1,2-dichlorobenzene, 1,2,3-trichlorobenzene, 1,2,3,4-tetrachlorobenzene, pentachlorobenzene and hexachlorobenzene from Aldrich-Chemie (Steinheim, Germany) and 1-naphthol and 2-naphthol from Fluka (Buchs, Switzerland). Distilled water was used to prepare the mobile phase. The mobile phase was filtered through a nylon 66 membrane (0.2-μm pore size). Isradipin [isopropyl methyl 4-(benzofurazanyl)-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylate] and its neutral by-products were obtained from Sandoz Pharma (Basle. Switzerland).

RESULTS AND DISCUSSION

Electroendosmotic flow in packed capillaries

The properties of electroendosmotic flow in

packed capillaries have been discussed [23,24] and are considered to be the same as in open-tubular capillaries, except that an electrical double layer exists on the surface of each silica-based particle in contact with the electrolyte. Several factors regulate electroendosmotic flow (F_{OS}). Field strength, pH and ionic strength of the mobile phase, organic modifiers and ionic modifiers have been investigated in open-tubular capillaries [9,19,25–27]. Therefore, these factors were also investigated in packed capillaries. The flow velocity was measured by monitoring the retention time of an unretained peak (thiourea). F_{OS} increased with decreasing molarity of sodium tetraborate. The highest F_{OS} was observed at concentrations of 2–4 mM. A curve similar to that in open-tubular systems was obtained for the pH of sodium tetraborate buffer adjusted with phosphoric acid vs. F_{OS}. F_{OS} increased between pH 6 and 8. No increase in F_{OS} was observed above pH 8–9. It was important to investigate the dependence on the concentration of organic modifiers, especially acetonitrile, in electrokinetic reversed-phase chromatography. As shown in Fig. 1, F_{OS} decreased on increasing the concentration of acetonitrile. The decrease in F_{OS} was only ca. 33% at 60% acetonitrile. This phenomenon was considered to be due to the decrease in dielectric constant and the magnitude of the zeta potential.
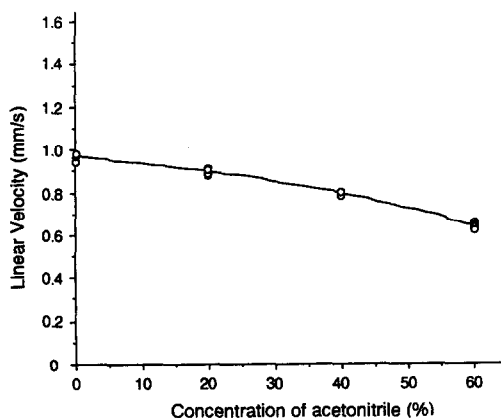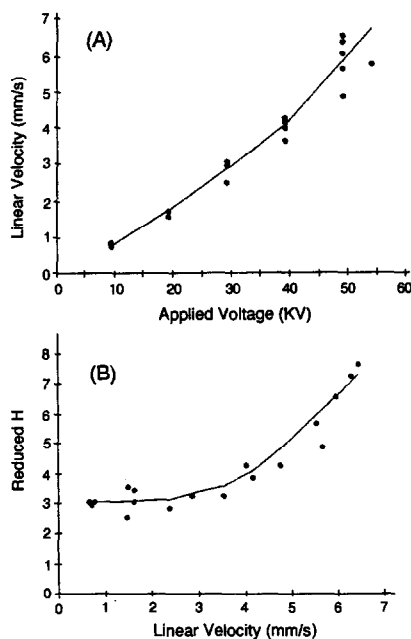

Fig. 2. (A) Dependence of electroendosmotic flow velocity on applied voltage; (B) reduced plate height vs. electroendosmotic flow velocity. Capillary, 143 mm; packing, Hypersil ODS (3 μm); mobile phase, 2 mM sodium tetraborate (pH 8.7)–80% acetonitrile; sampling, 1.5 kV for 4 s; sample, thiourea.

Fig. 1. Dependence of electroendosmotic flow linear velocity on the concentration of acetonitrile. Capillary, 640 mm × 50 μm I.D. × 365 μm O.D. packed with Monospher ODS (1.6 μm); applied voltage, 15 kV; mobile phase, 4 mM sodium tetraborate (pH 9.2)–acetonitrile.

Dependence of theoretical plate numbers on linear velocity

In order to evaluate the electroendosmotic elution system in packed capillary chromatography, the maximum applicable field strength and the dependence of plate numbers on linear flow velocity were investigated. For this purpose, a short capillary (143 mm) packed with Hypersil ODS (3 μm) was employed, so that it was possible to apply very high field strengths to the capillary. No formation of bubbles was observed up to 55 kV across the capillary (field strength ca. 2 kV/cm in the packed section) and a ca. 6 mm/s electroendosmotic flow was obtained. However, F_{OS} was unstable above 50 kV applied voltage and higher than the expected velocity, as shown by the curve of F_{OS} vs. applied voltage (Fig. 2A). This could be considered to be the result of heating effects. No decrease in plate efficiency was observed until F_{OS} = 3 mm/s (below ca. 30 kV, field strenght ca. 1.1 kV/cm) (Fig. 2B). This means that it is possible to perform rapid anal-
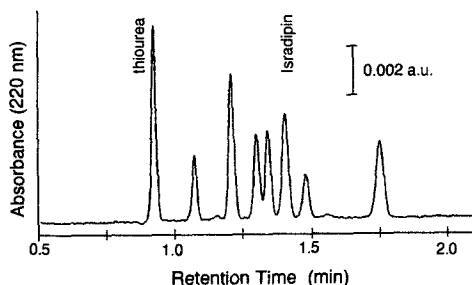
Fig. 3. Example of chromatograms obtained by electrokinetic reversed-phase chromatography with packed capillaries. A mixture of Isradipin, its by-products and thiourea was analysed. Applied voltage, 30 kV; other conditions as in Fig. 2. F_{OS} = 2.6 mm/s (current 1.8 μA).

Fig. 5. Example of rapid analysis. Linear velocity of electroendosmotic flow, 6.3 mm/s; applied voltage, 50 kV (current 2.9 μA). Peaks: left, thiourea (N = 6600, N/s = 290, h = 7.2); right, Isradipin (N = 8000, N/s = 230, h = 6.0). Other conditions as in Fig. 2.

yses without a decrease in plate numbers. An example of the chromatograms obtained with this capillary at F_{OS} = 2.6 mm/s is shown in Fig. 3. Isradipin and its six different by-products (k' = 0.17–0.90) were separated in 1.6 min. With longer capillaries (285 mm), better reduced plate heights were obtained, e.g., 1.8–2.2 for thiourea and benzene derivatives (k' = 0–4.8), as shown in Fig. 4. The decrease in the plate numbers at very high F_{OS} seems to be due to band broadening caused by the heating. This phenomenon has been observed in open-tubular electrokinetic chromatography [9,21] and discussed by Knox [24]. However, the peak shape was very
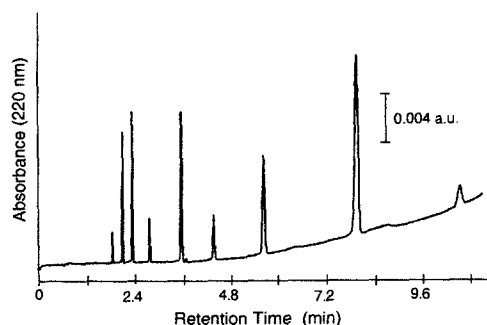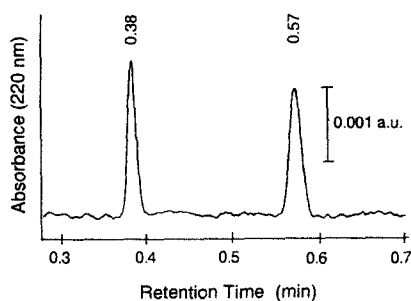

Fig. 4. High-resolution analysis of benzene derivatives. Peaks, from left to right: thiourea [N = 46 000, reduced plate height (h) = 2.0], benzyl alcohol (N = 54 000, h = 1.8), benzaldehyde (N = 56 000, h = 1.7), benzene (N = 47 000, h = 2.0), 1,2-dichlorobenzene (N = 54 000, h = 1.8), 1,2,3-trichlorobenzene (N = 52 000, h = 1.8), 1,2,3,4-tetrachlorobenzene (N = 49 000, h = 2.0), pentachlorobenzene (N = 43 000, h = 2.2) and hexachlorobenzene (N = 43 000, h = 2.2). Capillary, 285 mm; packing, Hypersil ODS (3 μm); mobile phase, 2 mM sodium tetraborate–80% acetonitrile; applied voltage, 45 kV (current 2.0 μA); sampling, 2.5 kV for 5 s. F_{OS} = 2.6 mm/s.

symmetrical even at ca. F_{OS} ≈ 6 mm/s, as shown in Fig. 5.

Curves of plate numbers vs. linear velocity for electrokinetic chromatography and pressure-driven chromatography were compared by using the same capillary (Table I). Pressure-driven chromatography was performed with the same procedure as electrokinetic chromatography and the sample solution was injected electrokinetically. With pressure elution, the highest plate number was obtained at ca. 0.9 mm/s and the value decreased subsequently with increasing linear velocity. With electroendosmotic elution, however, no changes in the plate numbers were observed from 0.8 to 2.6 mm/s, as shown in Table I and Fig. 2B, and improved plate numbers were observed at any velocity owing to the plug profile of electroendosmotic flow.

Stability and reproducibility of chromatographic performance

An expected problem with this technique was the stability of ODS silica packings at high pH. A high pH is necessary to obtain high electroendosmotic flow. In fact, it took some time to obtain stable conditions of F_{OS}, UV baseline and current with newly packed capillaries. Once stable conditions had been obtained, however, chromatography could be performed with some daily initial equilibration procedures as in conventional high-performance liquid chromatography. F_{OS} and the retention times of the test mixture containing Isradipin, its by-products and thiourea (k' = 0–0.9) were reproducible with relative standard deviation 1.6–2.2% in ten continuous runs at 40 kV with a capillary 285 mm × 50

TABLE I

COMPARISON OF PLATE EFFICIENCY BETWEEN ELECTROENDOSMOTIC ELUTION AND HYDROSTATIC PRESSURE ELUTION

Values of reduced plate heights for nine components ($k' = 0$–4.8) were averaged. The chromatogram obtained at an electroendosmotic flow of 2.6 mm/s is shown in Fig. 4.

Linear velocity (mm/s)	Reduced plate height	
	Electroendosmotic	Hydrostatic pressure
1.1 (0.9)[a]	2.0	3.0
2.6 (2.2)	1.9	3.6

[a] Value in parentheses: linear velocity of hydrostatic pressure flow.

μm I.D. $\times$ 365 μm O.D.) packed with Hypersil ODS (3 μm), and stable results were obtained for longer than 1 month.

The reproducibility of this technique between capillaries was also examined. Three capillaries of the same size packed with Hypersil ODS (3 μm) were prepared, and F_{OS} and k' of seven components were compared (Fig. 6). The deviation of k' values was about 9% in spite of good reproducibility of F_{OS} (3% deviation). This result may be due to differences in the packing conditions of the capillaries.
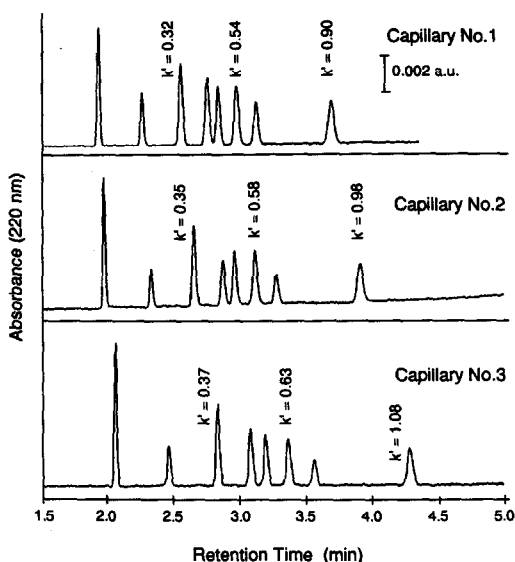

Fig. 6. Reproducibility of chromatographic performance between three capillaries. Three capillaries of the same size packed with Hypersil ODS were prepared and employed for electrokinetic reversed-phase chromatography. Applied voltage, 40 kV; other conditions as in Fig. 4. Sample as in Fig. 3.
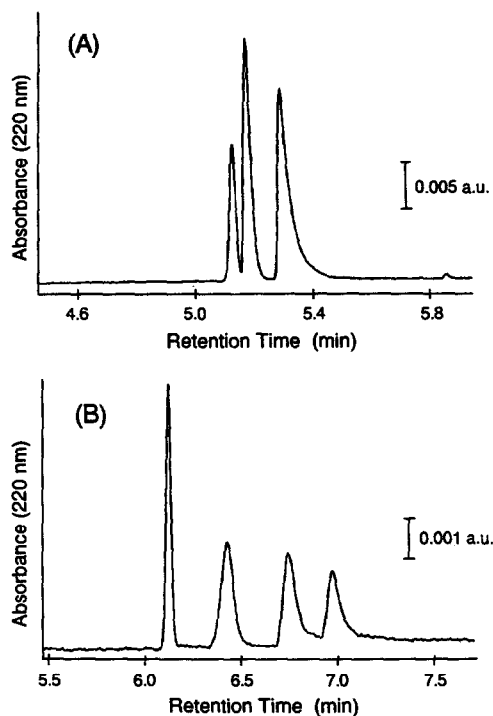

Fig. 7. Two examples of chromatograms obtained with 1.6-μm Monospher ODS. (A) Capillary, 680 mm $\times$ 50 μm I.D.; mobile phase, 4 mM sodium tetraborate (pH 9.2). $F_{OS} = 2.2$ mm/s. Peaks, from left to right: thiourea ($N = 243\,000$, $N/s = 790$), benzyl alcohol ($N = 220\,000$, $N/s = 710$), benzaldehyde ($N = 108\,000$, $N/s = 340$). (B) Capillary, 675 mm; mobile phase, 4 mM sodium tetraborate–20% acetonitrile. $F_{OS} = 1.8$ mm/s. Peaks, from left to right: thiourea ($N = 248\,000$, $N/s = 670$), toluene ($N = 47\,000$, $N/s = 120$), 2-naphthol ($N = 52\,000$, $N/s = 130$), 1-naphthol ($N = 62\,000$, $N/s = 150$). Applied voltage, 35 kV (current 1.3 α, 1.1 μA); sampling, 5 kV for 5 s.

Electrokinetic reversed-phase chromatography with small-particle packing material

It is of interest to use very small particles in capillary chromatography in order to increase the efficiency. Thus 1.6-μm Monospher ODS was used as the column packing. Two of the chromatograms are shown in Fig. 7. For the unretained compound (thiourea) more than 240 000 theoretical plates per 680 mm (the reduced plate height of 1.9) were obtained in 5.2 min (Fig. 7A). This leads to 790 theoretical plates/s, which is extremely high. From the curve of reduced plate height *vs.* linear velocity (Fig. 8), it can be expected to obtain an even higher efficiency than 800 theoretical plates/s when higher field strenghts are applied. With pressure elution, a *ca.* 0.24 mm/s linear velocity was obtained with the same capillaries at *ca.* 6000 p.s.i., and it would necessary to apply *ca.* 25 000 p.s.i. to the system to obtain even a 1 mm/s linear velocity. With 1.6-μm Monospher ODS, however, a significant decrease in plate numbers for retained compounds was observed. Toluene ($k' = 0.05$) showed gave about one fifth of the theoretical plates, as shown in Fig. 7B. The reason for this phenomenon is unclear, but similar results were observed with pressure elution. It might be due to the small capacity of the Monospher packing and the limitations of mass transfer.
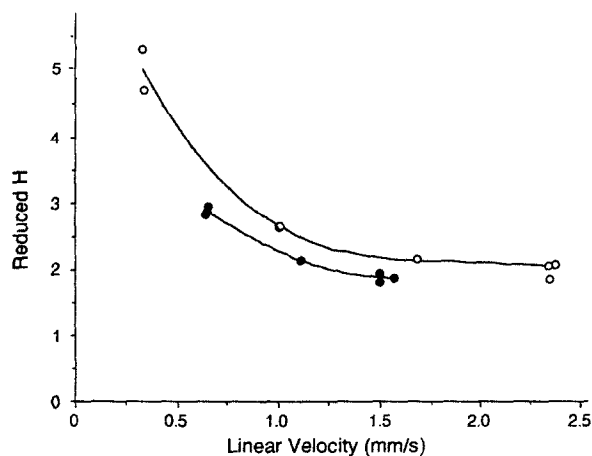
Wide-bore capillaries

This technique offers other possibilities. One is the use of capillaries of larger inside diameter without a decrease in plate efficiency and in order to improve the detection sensitivity. The sensitivity of detection was improved about threefold without any decrease in efficiency with 100 μm I.D. capillaries compared with 50 μm I.D. capillaries. Preliminary experiments indicated that the use of capillaries larger than 200 μm I.D. is possible.

CONCLUSIONS

The advantage of the application of electroendosmotic elution in liquid chromatography is the possibility of achieving high plate efficiencies (plates/s) without limitation of the particle seizes (specially for very small particles). This is due to the lack of pressure restrictions and the advantages of the plug profile of electroendosmotic flow. As shown here, electroendosmotic elution systems could be applied successfully in packed capillary reversed-phase chromatography. Very high performance (plates generated per unit time) could be achieved. There was no limitation to the use of a 1.6-μm packing, where 790 theoretical plates/s were obtained. This technique is very useful for rapid and high-resolution analyses and also in trace analysis because of the high capacity of packed capillaries.

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Fig. 8. Curves of reduced plate height *vs.* electroendosmotic flow velocity in a capillary packed with 1.6-μm Monospher ODS. Capillary, 620 mm; ($\bigcirc$) applied voltage 5–35 kV with 4 mM sodium tetraborate mobile phase; ($\bullet$) applied voltage 15–35 kV with 4 mM sodium tetraborate–60% acetonitrile mobile phase; sample, thiourea.

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