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Electrically driven open-tubular liquid chromatography

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ABSTRACT

Electrically driven (ED), as opposed to pressure-driven (PD), open-tubular liquid chromatography (OTLC) was evaluated for different types of open-tubular columns, with inner diameters in the range 5–25 μm. The efficiency of ED-OTLC was found to be better than that of PD-OTLC by a factor of *ca.* 2, in agreement with theory. Injection of the sample in ED-OTLC by electromigration appears to be much simpler than that with the split-injection procedures in PD-OTLC. Electroosmotic mobility was found to depend little on the application of an ODS coating. With the mobile phase used at 60 000 V/m, the maximum linear velocity was 1.4 mm/s. The potential of ED-OTLC in 10–25 μm I.D. capillaries is demonstrated.

INTRODUCTION

Open-tubular liquid chromatography (OTLC) has been extensively investigated both theoretically and experimentally[1–16] in the last 20 years. Efforts have been made in column preparation[3–9] and in developing injection and detection systems[8–16]. Although the performance of OT columns has proved to be much better than that of packed columns, at present OTLC is used only in university laboratories and research institutes. Commercial OTLC instruments are still not available, which can be attributed to the experimental difficulties in efficiently operating OTLC, owing to the extremely small dimensions of the system. It has been found that the inner diameter of OTLC columns should be smaller than 10 μm[2,3] and that the injection and detection volumes should be about 40 and 100 pl, respectively. These small volumes imply the need for a very specialized type of detector, *e.g.*, on-column laser-based optical detectors, electrochemical detectors and mass spectrometric devices[10–16]. Although such detection systems have been tried, the insufficient sensitivity and the low mass loadability of OT columns[9,17] still inhibit the interest of instrument manufacturers in developing an OTLC instrument.

In contrast to the lack of commercial development of OTLC, that of capillary electroseparation techniques has expanded since the introduction of capillary zone electrophoresis around 1980 by Mikkers *et al.*[18] and Jorgenson and Lukacs[19]. Interest in these techniques is growing rapidly, as demonstrated by the enormous increase in

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the number of publications and the introduction of several commercial instruments.

In capillary electroseparation techniques, a high electric potential is applied across a capillary, filled with a buffer, and separations of charged species, based on a difference in charge/mass ratio can be carried out by an electrophoretic process. In addition to charged species, uncharged species can also be transported by the electroosmotic flow, which is induced by the electric field. Currently five capillary electroseparation techniques have been demonstrated experimentally[18–28]:

(i) (free solution) capillary zone electrophoresis (CZE);
(ii) capillary gel electrophoresis;
(iii) capillary micellar electrokinetic chromatography;
(iv) isoelectric focusing; and
(v) capillary electroosmotic chromatography.

These techniques are normally carried out in 10–100 μm I.D. fused-silica capillaries and in most instances on-column detection is used[18–28]. As with OTLC, the detection is one of the major problems. However, considering the great interest in capillary electroseparation techniques, renewed research efforts[27–30] in micro detection will certainly lead in the near future to commercially available ultramicro detectors having better characteristics with respect to sensitivity.

In pressure-driven (PD) OTLC, sample introduction is usually effected by split injection, which requires a relatively large sample volume. When the sample volume is extremely small, the split technique cannot be applied and another means of introducing the sample is necessary, *e.g.*, by disconnecting the column and injecting the sample into the capillary with a microsyringe, as demonstrated by Kennedy and Jorgenson[31,32]. However, this complicates the techniques. In capillary electroseparation techniques the sample introduction is performed by means of electromigration[33,34] or by the vacuum suction method[35]. Both techniques lend themselves to automation. However, the vacuum suction technique may fail with the small I.D. capillaries used in OTLC. Therefore, the electromigration injection is the method of choice for electrically driven (ED) OTLC. The method is very simple, applicable to neutral species and allows manipulations of small sample volumes. Moreover, the method is also applicable to charged species, but the amount of injected solute is affected by the electrophoretic mobility of the solute.

Capillary electroosmotic chromatography in open-tubular columns will henceforth be termed electrically driven OTLC (ED-OTLC). It was first demonstrated in 30–200 μm I.D. columns by Tsuda *et al.*[24] in 1982. They found larger plate heights than expected theoretically and showed some separations of polycyclic aromatics in a 30 μm I.D. capillary. Capillary electroosmotic chromatography in packed fused-silica capillaries was demonstrated by Knox and Grant[36] in 1987. They observed an improvement in plate height by a factor of 2 when comparing ED and PD chromatography in the same column. They also argued that an ED system allows the use of much smaller particles for column packings.

In OTLC, plug flow, as opposed to Poisseuille flow, leads to a smaller contribution from slow equilibration in the mobile phase, the C_m term. We therefore considered it worthwhile to assess the possibilities of using electroosmotic flow as the driving force for OTLC in 10–25 μm I.D. capillaries, and to explore the use of electromigration injection in combination with ED-OTLC.

THEORY

Electroosmosis

The driving force for an ED system is electroosmosis, which results from the existence of the electric double layer. If a surface is in contact with an electrolyte, there will be an excess of charge near that surface. With fused silica, the excess charge is normally positive. Because of the presence of the excess of charge, the application of an electric field will bring about movement of the liquid, which is called the electroosmotic flow. This phenomenon has been treated in more detail by Rice and Whitehead[37] and Van de Goor *et al.*[38]. The electroosmotic flow has a linear velocity v_{eo}, which is given by

$$v_{eo} = E\mu_{eo} = E\varepsilon_o\varepsilon_r\,\zeta/\eta \tag{1}$$

where μ_{eo} is the electroosmotic mobility, E is the applied electric field, η is the viscosity, $\varepsilon_o\varepsilon_r$ is the dielectric constant of the electrolyte and ζ is the zeta potential near the wall[37,38]. The electroosmotic mobility depends on the viscosity, pH and ionic strength of the electrolyte and on the character (charge density) of the surface of the capillary. The flow profile is virtually rectangular. A deviation from the rectangular shape occurs in the wall region, the size of which is of the order of the size of the diffuse electric double layer, 1–100 nm[39]. Because of the finite size of the double layer, in extremely narrow ($\leqslant 1$ μm) channels and at low ionic strengths, the plug flow and eqn. 1 may not be valid (double-layer overlap)[37,39]. However, such conditions did not occur in our work.

Comparison of plate height between pressure- and electrically driven systems

Axial diffusion and slow equilibration in the mobile and stationary phase are the main processes contributing to band broadening in OTLC. This is expressed in the theoretical plate height equation derived by Golay[40] for a PD system, and by Martin and Guiochon[41] and Giddings[42] for an ED system. These two equations are very similar and deviate only in the function of k', $f(k')_m$. This is attributed to the difference in the flow profiles between the two systems, *viz.*, a plug flow profile in an ED system and a parabolic flow profile in a PD system. The overall plate height equation is valid in both PD and ED systems:

$$H = \frac{2D_m}{u} + f(k')_m \cdot \frac{d_c^2\,u}{D_m} + f(k')_s \cdot \frac{d_f^2\,u}{D_s} \tag{2}$$

where H is the plate height, u is the linear velocity, k' is the capacity factor, D_m and D_s are the diffusion coefficients in the mobile and stationary phase, respectively, d_c is the inner diameter of the column, d_f is the thickness of the stationary phase layer and $f(k')_m$ and $f(k')_s$ are functions of k'.

$f(k')_m$ for the PD system is

$$f(k')_m^{PD} = \frac{(1 + 6k' + 11k'^2)}{96\,(1 + k')^2} \tag{3}$$

and for an ED system, assuming a perfectly flat flow profile,

$$f(k')_m^{ED} = \frac{k'^2}{16\,(1 + k')^2} \quad (4)$$

The main difference between an ED and a PD system is, as mentioned before, the flow profile. This is expressed in different plate height curves, calculated with eqns. 2–4, as shown in Fig. 1A and B for a 25 and a 10 μm I.D. capillary, respectively, for three k' values. As can be seen, for a 25 μm I.D. capillary the gain in efficiency by using an ED system instead of a PD system is by a factor of *ca.* 3. Knox and Grant[36] pointed out that with capillary electroosmotic chromatography in wider bore capillaries, the C_m term, as in OTLC, increases rapidly when retention occurs. For a 10 μm I.D. capillary the same phenomenon occurs but, owing to the smaller inside diameter, the slow mass transfer term is much smaller and less significant. If a 25 μm I.D. capillary in an ED system is compared with a 10 μm I.D. capillary in a PD system, the loss in plate height is by only a factor of 3. This phenomenon opens up the possibility of using larger diameters in ED-OTLC, which has the advantage that there are fewer practical problems with regard to detection, loadability and column preparation.

In Fig. 2, $f(k')_m^{ED}$ and $f(k')_m^{PD}$ are plotted *versus* k'. It is clear that both functions increase with increasing retention. Also, it is clear that $f(k')_m^{ED}$ is always smaller than $f(k')_m^{PD}$. The improvement is very large at low k' values, but is still greater by a factor of 11/6 for k' up to infinity. The lower $f(k')_m^{ED}$ results in a lower plate height for an ED system compared with a PD system.

EXPERIMENTAL

Apparatus

In Fig. 3 an ED-OTLC system is shown, which in principle is similar to a CZE system. It consists of a 0–60 kV d.c. high-voltage power supply (Model R603/05P; Wallis, Worthing, U.K.). Platinum electrodes are used for the connection of the supply with the buffer reservoirs, located at each end of the capillary. The total set-up was placed in a laboratory-built polycarbonate box, which was thermostated in the following way: a radiator, mounted in one of the side walls of the box, was connected to a circulating liquid thermostat (Model TL 1620; Haake, Berlin, F.R.G.), and an air fan was placed in front of the radiator to ensure an air flow of constant temperature around the capillary. On-column detection was carried out at the cathodic side, using laser-induced fluorescence detection as described previously[9]. Sample injection was performed by the electromigration technique[33]. Acetonitrile–phosphate buffer (0.05 or 0.1 *M*, pH 7.0) (2:3, v/v) and methanol–phosphate buffer (0.05 *M*, pH 7.0) (1:1, v/v) were used as mobile phases.

Materials and chemicals

The solvents used were analytical-reagent grade methanol and toluene (Merck, Darmstadt, F.R.G.), acetonitrile (Rathburn, Walkerburn, U.K.) and distilled water, freshly deionized by passage through a PSC filter assembly (Barnstead, Boston, MA, U.S.A.). Prior to use all solvents were filtered by vacuum suction through 0.5-μm

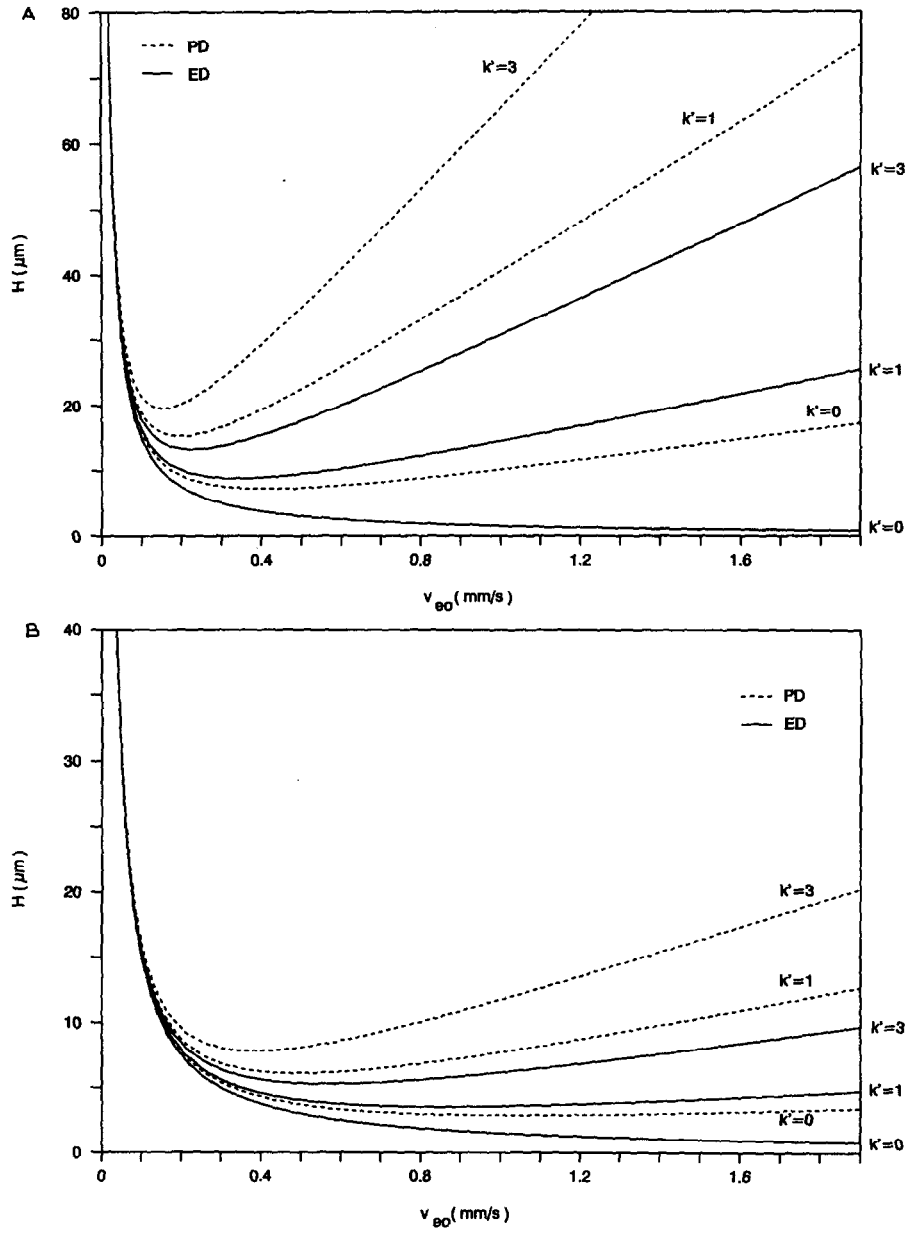

Fig. 1. Theoretical plate height curves for an electrically driven (ED) system (solid lines) and a pressure driven (PD) system (dashed lines) with (A) a 25-μm I.D. capillary and (B) a 10-μm I.D. capillary for three different k' values.

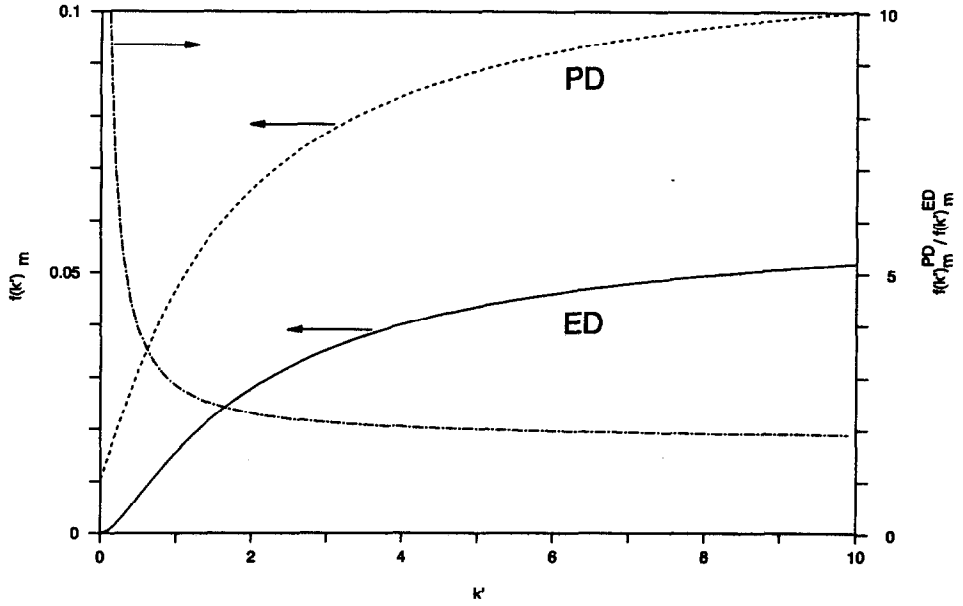

Fig. 2. $f(k')_m$ functions for a pressure-driven system, $f(k')_m^{PD}$ (dashed line), and for an electrically driven system, $f(k')_m^{ED}$ (solid line), plotted *versus* k'. The dot-dashed line represents the $f(k')_m^{PD}/f(k')_m^{ED}$ ratio.

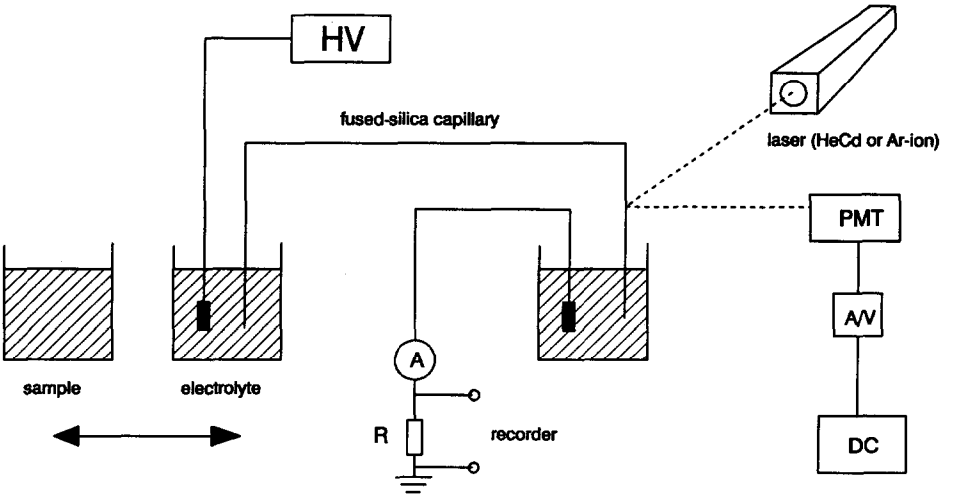

Fig. 3. Experimental set-up. HV = high-voltage power supply; PMT = photomultiplier tube; A/V = current/voltage converter with amplifier; DC = data collection; A = current monitor; R = resistance (= 10 kΩ).

filters (Model FH; Millipore, Bedford, MA, U.S.A.). The polycyclic aromatics used as test compounds were obtained from Janssen (Beerse, Belgium) and Aldrich (Brussels, Belgium). Stock solutions of the test compounds were prepared in the mobile phase. The 10, 25 and 50 μm I.D. fused-silica capillaries were obtained from Polymicro Technology (Phoenix, AZ, U.S.A.). Monochlorodimethyloctadecylsilane (ODS) was obtained from Aldrich (Beerse, Belgium).

Preparation of the capillary

The etched and the porous silica layered (PSL) fused-silica capillaries were prepared according to Tock and co-workers[8,9]. The capillaries were dried at 200°C for at least 2 h while being purged with helium.

Chemical modification

The dried capillary was filled with a 5% (w/v) solution of ODS in toluene. Both ends were sealed in a flame and the capillary was heated at 140°C for 6 h. Finally, the capillary was rinsed with toluene and acetonitrile or methanol before use.

RESULTS AND DISCUSSION

Electroosmotic mobility measurements

One of the most important parameters in ED-OTLC is the electroosmotic mobility in chemically modified capillaries under reversed-phase conditions. These values can be used to predict the accessible linear velocity in ED-OTLC. The electroosmotic flow measurements were carried out according to the method of Huang *et al.*[43]. Briefly, this method is as follows: the used electrolyte is replaced with an electrolyte with a lower conductivity, which results in a gradual decrease in the current when a high voltage is applied until the capillary is completely filled with the new electrolyte. The time, t_m, necessary to reach the new current level can be used to calculate the electroosmotic mobility, μ_{eo}, according to

$$\mu_{eo} = \frac{L}{t_m E} \tag{5}$$

where L is the total length of the capillary. It should be noted that changes in the concentration of the electrolyte must be small (not more than 10%) to avoid changes in the zeta potential during the μ_{eo} measurements, as shown by Zare *et al.*[43]. Table I summarizes the electroosmotic flow measurements in 5–50 μm I.D. capillaries. These measurements were carried out at a sufficiently weak applied electric field to be sure that thermal effects were excluded. It can be seen that μ_{eo} is independent of the inner diameter of the capillary. It can also be observed that μ_{eo} is virtually the same for ODS-treated and bare surfaces, and is not influenced by the presence of porous silica with the mobile phase used. The difference in μ_{eo} observed when methanol and acetonitrile are used as the organic modifier in the electrolyte can be explained by differences in the viscosity and dielectric constant of the electrolytes.

Band broadening due to injection

In order to rule out the possibility of extra-column contributions to band

TABLE I

ELECTROOSMOTIC MOBILITY, μ_{eo}, MEASURED IN VARIOUS CAPILLARIES

Values are averages of at least duplicate measurements.

Capillary		μ_{eo} $(cm^2/V \cdot s) \cdot 10^4$	
Type	d_c *(μm)*	*Acetonitrile–buffer*[a] *(2:3)*	*Methanol–buffer*[a] *(1:1)*
ODS	5	2.40	–
ODS	10	2.38	1.16
PSL-ODS[b]	10	2.18	–
ODS	25	2.49	–
ODS	50	2.43	–
Etched	50	2.37	1.13

[a] 0.1 *M* phosphate buffer (pH 7).
[b] PSL = capillary with a porous silica layer[8,9].

broadening, some conditions governing the injection procedure are discussed.

As shown in Fig. 1A and B, theoretical plate heights for $k' = 0$ as low as 1.5 μm can be obtained. The distance variance, $\sigma^2_{z,col}$ $(= LH)$, is 0.75 mm^2 for a 50-cm capillary. When a 5% increase in peak width by external broadening effects is accepted, the following should hold:

$$\sigma^2_{z,tot} \leqslant (1.05\ \sigma_{z,col})^2 \tag{6}$$

or

$$\sigma^2_{z,tot} \leqslant 1.103\ \sigma^2_{z,col} \tag{7}$$

The extra band broadening due to on-column laser-induced fluorescence detection can be neglected[10], and hence $\sigma^2_{z,tot} = \sigma^2_{z,col} + \sigma^2_{z,inj}$, where $\sigma^2_{z,inj}$ is the extra variance caused by the injection zone length. To preserve efficiency, $\sigma^2_{z,inj}$ is not allowed to exceed 0.077 mm^2. The variance $\sigma^2_{z,inj}$ can be expressed as

$$\sigma^2_{z,inj} = \frac{l^2_{inj}}{K^2} \tag{8}$$

where l_{inj} is the length of the injection plug and K^2 is the injection profile factor[44]. This means that the length of the injection plug should be less than 0.96 mm (the injection volume for an unretained solute would be 75 pl in a 10 μm I.D. capillary) when injection of a rectangular plug is assumed with $K^2 = 12$. The restrictions on the injection zone length become even more critical when the injection profile approaches an exponential profile. In this instance one will find a lower K^2 value and thus a smaller maximum acceptable injection zone length.

Using the electromigration technique as described in detail by Rose and Jorgenson[33], it is possible to inject very small sample volumes. For capillary zone electrophoresis, capillary micellar electrokinetic chromatography and capillary gel electrophoresis, discrimination between the sample components appears because charged compounds migrate with a different velocity into the column as a result of

their different electrophoretic mobilities. This implies that the amount of sample components injected depends on their electrophoretic mobility. However, in ED-OTLC electroosmotic chromatography preferably neutral compounds are separated and they all migrate with the same electroosmotic velocity into the capillary. It should be noted that retained components will focus during the sample introduction by a factor of $1 + k'$. Hence for these components larger volumes can be tolerated.

In Fig. 4 the square of the injection volume, V_{inj}^2, is plotted *versus* the column variance, $\sigma_{v,tot}^2$, for a 10 μm I.D. capillary and a pair of solutes with small retention. The column variance is calculated with the measured time standard deviation at 0.6 of the total peak height multiplied by the electroosmotic flow. V_{inj} is varied between 19 and 280 pl by increasing the injection time from 2 to 30 s at a constant injection voltage of 2.4 kV for anthracene ($k' = 0.13$) and 9-anthracenecarbonitrile ($k' = 0.09$). Values for the injection volume were calculated by means of eqn. 1. The electroosmotic flow during the separation was 95 pl/s ($v_{eo} = 1.08$ mm/s). The slope of the plotted lines in the graph of $\sigma_{v,tot}^2$ *versus* V_{inj}^2 equals the reciprocal of K^2 and an average value of $K^2 = 8.0$ was found in this experiment. Various phenomena might be responsible for the deviation in K^2 value from the value for a block, 12. In the first place, the rise and fall times in the voltage[28] may have influenced the amount injected. We also found that the angle at which the capillary is cut is critical: with obliquely cut capillaries K^2 values as low as 3 were found. Finally, Grushka *et al.*[45] found that spontaneous, not electrically induced sample introduction takes place. However, for the present experiments we considered the observed value of 8 to be satisfactory.

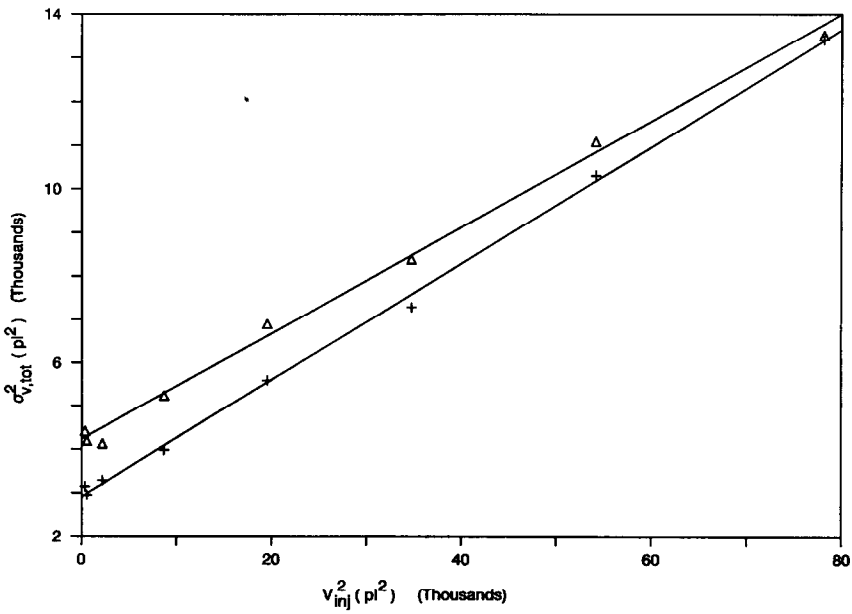

Fig. 4. Total volume variance, $\sigma_{v,tot}^2$, *versus* the square of the injection volume for a 50.0 cm × 10 μm I.D. PSL-ODS capillary. Injection voltage = 2.4 kV; $l_{inj\text{-}det}$ = 25.0 cm; separation voltage = 24.6 kV; current = 2.2 μA. Compounds: +, 9-anthracenecarbonitrile ($k' = 0.09$); △, anthracene ($k' = 0.13$). The lines were drawn from calculations by the linear square root method.

Band broadening by thermal effects

The effects of self-heating on efficiency in an ED system can be expressed as

$$H_{col} = H_{ED} + H_{TH} \tag{9}$$

where H_{ED} is the plate height for an ED system as shown in eqn. 2 and H_{th} is the plate height caused by thermal effects. The latter can be a significant source of band broadening in capillary electroseparation techniques. The magnitude of the temperature gradient from the centre of the capillary to the inner wall, ΔT, can be calculated from[39,46]

$$\Delta T = Q\, d_c^2/16\kappa \tag{10}$$

where κ is the thermal conductivity of the electrolyte, d_c is the inner diameter of the capillary and Q is the power density[39,47]:

$$Q = 4EI/\pi d_c^2 \tag{11}$$

where I is the current. Combination of eqns. 10 and 11 yields a general relationship between the power per unit length (EI) and the temperature gradient:

$$\Delta T = EI/4\pi\kappa \tag{12}$$

Jones and Grushka[46] assumed that a ΔT of less than 1 K across the bore of the capillary will not seriously decrease the efficiency. For $\Delta T = 1$ K and $\kappa = 0.4$ W/(m · K), $EI = 5.02$ W/m. In ED-OTLC, EI is much smaller because of the low current in 10 μm I.D. capillaries. Applying a field of 68.4 kV/m across a 10 μm I.D. capillary filled with, *e.g.*, phosphate buffer (0.1 *M*)–acetonitrile results in a current of 3.42 μA and EI in this instance is 0.23 W/m. Hence it can be said that very small thermal gradients can be expected in 10 μm I.D. capillaries.

Knox[39] derived an expression for H_{TH}:

$$H_{TH} = 10^{-8} \cdot \frac{\varepsilon_o \varepsilon_r \zeta}{D_m \eta \kappa^2} \cdot E^5 d_c^6 \lambda^2 c^2 \tag{13}$$

where λ is the equivalent conductivity and c is the concentration of the electrolyte. Using some typical parameters (some of the values are rough estimates) from Table II shows that thermal band broadening caused by heating effects can be neglected for diameters smaller than 100 μm.

Apart from the thermal non-uniformity of the liquid, problems may also arise as a result of the mean temperature rise of the liquid, due to slow heat transfer through the tube material and cooling bath medium. Such temperature increases may affect the reproducibility and stability of components. In contrast to the internal thermal profiles, these effects can be diminished by applying cooling, as shown by various workers[39,47]. The demands on temperature control become more severe when larger inner diameters are used, but even with 25 μm I.D. capillaries in ED-OTLC the entire capillary may heat up by several degrees, as will be shown below.

TABLE II

BAND BROADENING CAUSED BY THERMAL EFFECTS, H_{TH}, AND THE TEMPERATURE EXCESS, θ, OF THE TUBE WALL RELATIVE TO THE SURROUNDING AIR AS A FUNCTION OF THE CAPILLARY DIAMETER FOR ACETONITRILE–BUFFER (2:3)

E = 50 000 V/m, λ = 0.00735 m^2/Ω · mol, c = 60 mol/m^3, η = 1.1 · 10^{-3} Pa s, $\varepsilon_o\varepsilon_r$ = 7.08 · 10^{-10} C^2/N · m^2, ζ = 40 · 10^{-3} V, D_m = 1 · 10^{-9} m^2/s, κ = 0.4 W/m · K, Q = 1102 W/cm^3 (calculated with $Q = E^2 \lambda c$), k' = 0 and u = 1.5 mm/s.

I.D. (μm)	*O.D.* (μm)	H_{ED} (μm)	H_{TH} (μm)	θ (K)
10	175	1.33	9.7 · 10^{-8}	1.5
25	325	1.33	2.3 · 10^{-5}	7.7
50	350	1.33	1.5 · 10^{-3}	29.9
100	300	1.33	0.098	125.6
200	500	1.33	6.2	431.0

The temperature increase of the tube relative to the surrounding air, θ, can be calculated from an expression derived by Knox[39] but slightly modified for the fact that formation of heat takes place only in the part of the tube filled with electrolyte:

$$\log \theta = 1.70 \log d_o\ (\mu m) + \log Q[(d_i/d_o)^2]\ (W/cm^3) - 4.20 \qquad (14)$$

where d_i and d_o are the inner and outer diameter of the tube, respectively. It can be concluded from Table II that for a 10 μm I.D. capillary there will be only a very small temperature increase (1.5 K) of the capillary as a whole. This is calculated for

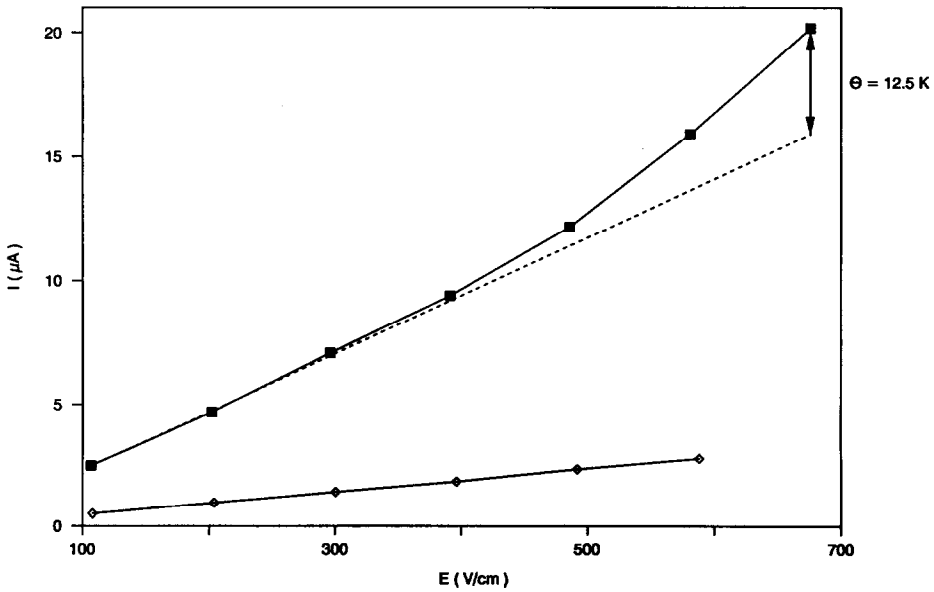

Fig. 5. Plot of current *versus* electric field for natural convection on a 10 μm I.D. × 175 μm O.D. (◇, L = 50.0 cm) and a 25 μm I.D. × 325 μm O.D. (■, L = 50.6 cm) capillary in still air.

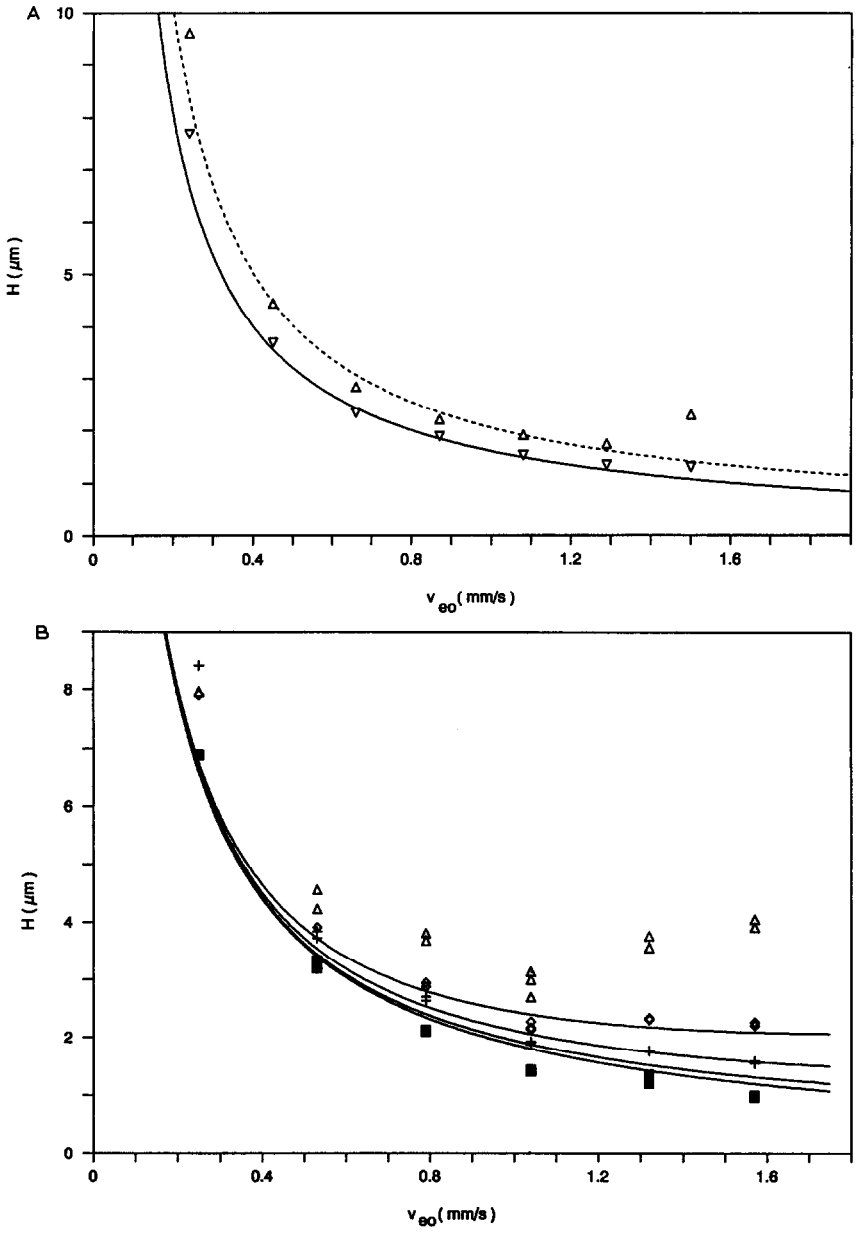

Fig. 6. *H versus* v_{eo} curves for (A) a 10-μm I.D. PSL-ODS capillary and (B) an etched ODS 10-μm I.D. capillary. Mobile phase: phosphate buffer (pH 7.0)–acetonitrile (3:2). V_{inj} = 50 pl; T = 21.0 ± 0.2°C. (A) L = 50.0 cm, $l_{inj\text{-}det}$ = 25.0 cm. ▽, 9-Anthracenecarbonitrile (k' = 0.09); △, anthracene (k' = 0.13); the lines are theoretical lines. (B) L = 45.0 cm, $l_{inj\text{-}det}$ = 22.0 cm. ■, 9-Anthracenemethanol (k' = 0); +, 9-methylanthracene (k' = 0.12); ◇, 1,2-benzanthracene (k' = 0.24); △, 9-phenylanthracene (k' = 0.42).

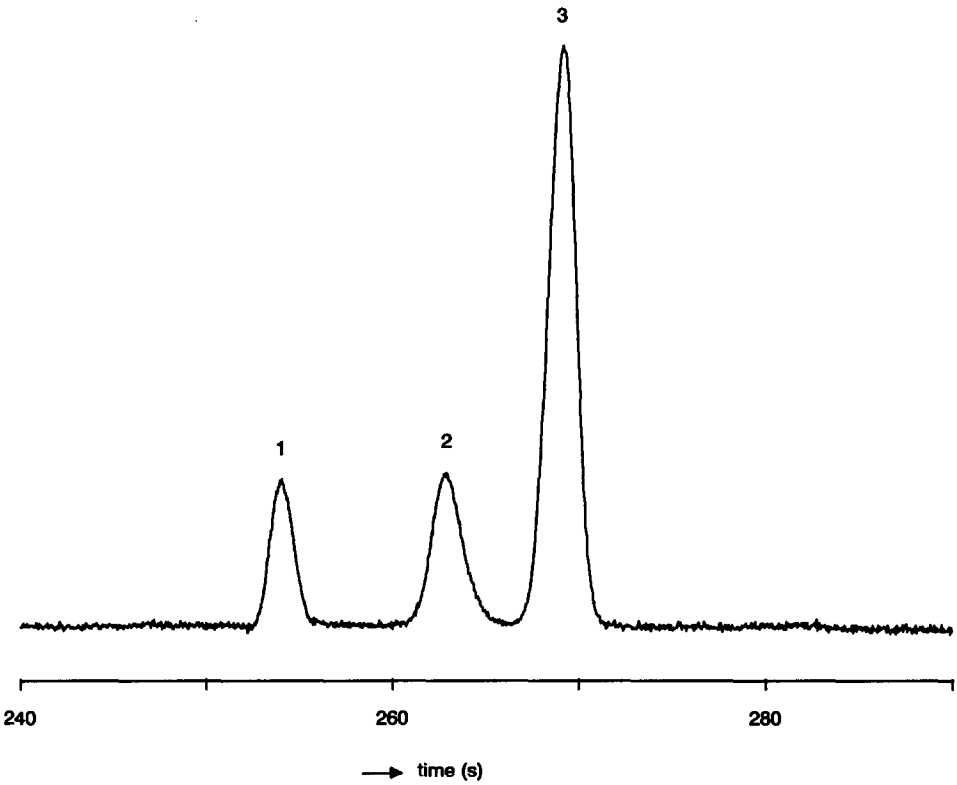

Fig. 7. Chromatogram of three polycyclic aromatics obtained with a 10-μm I.D. ODS-capillary. Applied voltage = 17.5 kV; current = 0.9 μA; L = 45.9 cm, $l_{inj\text{-}det}$ = 22.0 cm. Peaks: 1 = 9-anthracenemethanol (k' = 0); 2 = anthracene (k' = 0.03); 3 = 9-methylanthracene (k' = 0.06). Other details as in Fig. 6.

a situation without a cooling device in the experimental set-up. As shown by Nelson *et al.*[47], the current will increase faster than in proportion to the increase in applied voltage, as a result of poor heat dissipation in still air in 50 μm I.D. and larger capillaries. In Fig. 5 the current, I, is plotted against the applied electric field, E, for 25 and 10 μm I.D. capillaries. It was found that with the 25 μm I.D. capillary there is a small tendency to deviate from linearity, whereas with the 10 μm I.D. capillary a linear relationship holds. Applying a field of 67.6 kV/m ($Q = 2.01 \cdot 10^3$ W/cm^3) in a 25 μm I.D. × 325 μm O.D. capillary gives a calculated value for the temperature increase of θ = 14 K. Fig. 5 shows that a 25% positive deviation from linearity in the current occurs at this electric field strength. It is known that the conductivity changes by 2% per degree[47]. Hence we find an experimental estimate for θ of 12.5 K.

Chromatography

In Fig. 6A a H *versus* v_{eo} curve is shown for a 10-μm I.D. PSL-ODS capillary with an injection volume of about 20 pl. It can be seen that the minimum observed plate heights were 1.4 μm for 9-anthracenecarbonitrile and 1.7 μm for anthracene, and also that good agreement with theory is obtained. Results with another 10 μm I.D. ODS

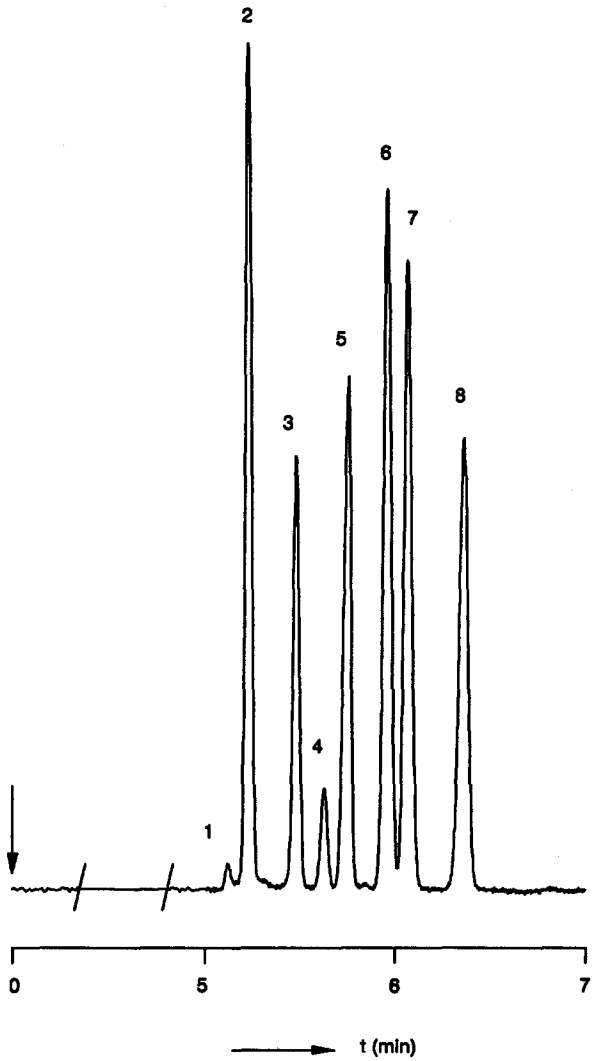

Fig. 8. Chromatogram of eight polycyclic aromatics obtained with a 10-μm I.D. PSL-ODS capillary. Applied voltage = 20 kV; L = 49.0 cm; $l_{inj\text{-}det}$ = 26.5 cm; current = 0.7 μA; T = 25°C; V_{inj} = 9 pl. Mobile phase: 0.05 M phosphate buffer (pH 7.0)–methanol (1:1). Peaks: 1 = naphthoquinone (k' = 0); 2 = 9-anthracenemethanol (k' = 0.03); 3 = 9-anthracenecarbonitrile (k' = 0.06); 4 = anthracene (k' = 0.09); 5 = 7,8-benzoflavone (k' = 0.12); 6 = fluoranthene (k' = 0.16); 7 = pyrene (k' = 0.19); 8 = 9-vinylanthracene (k' = 0.24).

capillary are shown in Fig. 6B. Again, plate heights as low as 1.2 μm could be observed for the unretained 9-anthracenemethanol and 2.3 μm for 1,2-benzanthracene (k' = 0.24). These values correspond to plate numbers of 183 000 and 94 000, respectively. The data in Fig. 6B show good agreement with theory for solutes of low retention. However, at high velocity and retention, a deviation of increasing magnitude occurs, amounting to a factor of nearly 2 in the worst case. This deviation is as yet unexplained.

Some examples of the high performance of ED-OTLC can be seen in Fig. 7, where a sample of three polycyclic aromatics was injected. The peaks show a Gaussian shape and are baseline resolved, despite the small differences in k' values. Another example of a separation is shown in Fig. 8, where eight polycyclic aromatics are separated in less than 400 s, while the group of peaks appears baseline resolved in a time interval of only 90 s.

CONCLUSIONS

It has been shown for the first time that it is possible to perform ED-OTLC in chemically modified 10–25 μm I.D. capillaries near the optimum of the H *versus* v_{eo} curve. An advantage of ED-OTLC is that injection is easier than in a PD-OTLC system, where dynamic or static split injection devices are necessary in order to obtain a sufficiently small injection volume. It is found that very small volumes of uncharged components can be introduced with the electromigration injection technique. It gives the opportunity to analyse very small volumes of sample, *e.g.*, sampling directly from tissue or individual cells. The use of larger I.D. capillaries in ED-OTLC without losing to much efficiency gives better prospects for loadability, optical detection systems and preparation of stationary phases.

The electroosmotic mobility has been found to be independent of the inner diameter of the capillary. Its value in ODS-treated tubes is hardly different from that in untreated tubes and allows adequate mobile phase velocities when typical reversed-phase mobile phases are used.

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