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OPEN-TUBULAR MICROCAPILLARY LIQUID CHROMATOGRAPHY WITH ELECTRO-OSMOSIS FLOW USING A UV DETECTOR

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SUMMARY

The linear velocity of electro-osmosis in a capillary tube 30–90 cm × 30–200 μm I.D. was measured. The physical and geometrical parameters of electro-osmosis and its use are discussed. The *H* value for an unretained solute in disodium hydrogen orthophosphate–water is about one thirtieth of that obtained under laminar flow conditions. Chromatographic separations of aromatics using an octadecylsilane capillary column of I.D. 30 μm are demonstrated.

INTRODUCTION

Electro-osmosis has been used to pump solvents in both thin-layer and liquid chromatography[1–5]. Pretorius *et al.*[3] used electro-osmotic pumping for a packed column and an open tube (both 50 cm × 1 mm I.D.), and Jorgenson and Lukas[5] for a narrow packed column (68 cm × 0.17 mm I.D.). Electro-osmotic flow differs from laminar flow, and the former can have a sheared flow velocity only in the vicinity of the walls. In another words, the flow profile of electro-osmotic flow is much flatter than that of laminar flow[3], so the band broading of the solute in the former is much less than that in the latter.

Open-tubular capillary liquid chromatography has been in the development stage in recent years[6–9]. Optimization of capillary columns from theoretical considerations shows that a capillary column of I.D. between 2.5 and 10 μm might be suitable if a laminar flow is used. There is the possibility that inner diameters of open-tubular columns that slightly greater than this range might become optimal if electro-osmotic flow in open-tubular liquid chromatography is used.

EXPERIMENTAL

Straight glass tubes (30–90 cm × 30–200 μm I.D.) were drawn from Pyrex and soda-lime glass using a glass-drawing machine (GDM-1; Shimadzu, Kyoto, Japan). The inside surface of the Pyrex glass capillary was not modified and was used for the measurement of electro-osmotic flow. The inside surface of the soda-lime glass capil-

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lary was treated with 0.3 *N* sodium hydroxide solution for 2 days at room temperature or overnight at 50°C[6]. Then octadecylsilane (ODS) was chemically bonded on the surface as described earlier[6,10].

Detection and apparatus

A UV detector (mercury lamp, 254 nm, Type UVD-2; Shimadzu) was used. With the Pyrex glass column, on-column detection was used, the column passing through the UV cell port. The sensitivity was such that 10 ng of N-methyl-3-methylpyridinium iodide gave a peak height of 3 cm on the strip-chart recorder.

With the ODS capillary column, a quartz tube (0.1 mm I.D.) was coupled directly with the capillary column outlet using PTFE tubing[6]. The length of the PTFE tubing between the column outlet and the quartz cell (the "cell" is the section of the quartz tube on which UV light fell after passing through a slit) was 1 mm, and the volume of this section was 8 nl. The volume of the quartz cell, 1 mm long, was also 8 nl.

A high-voltage d.c. power supply (Shimadzu) was operated in both constant-voltage and constant-current modes and delivered up to 25 kV. Platinum–iridium tubing was used as the electrode.

Procedure

Tubes were filled with solvent by applying pressure or by dipping one end of the tube, which was kept 10 cm higher than the other end, in the solvent. After the capillary had been filled, both ends were dipped in small beakers. Sample introduction was carried out by using electro-osmotic flow[4,5] or by "downhill" flow in the same manner as for filling the capillary with solvent. The latter method could not used with the capillary column of 30 μm I.D.

In the disodium hydrogen orthophosphate–water solvent system, the charge on the electrode after cutting off the high-voltage input was negligible, but in the solvent system of lower conductivity, such as pure water and methanol, the charge on electrode was maintained for several minutes. For safety, most of the measurements of electro-osmotic flow were carried out in water containing an electrolyte as the solvent system.

RESULTS AND DISCUSSION

Electro-osmotic flow

The linear velocity, u, of a liquid under the influence of an applied electric field, E, is given by

$$u = \left(\frac{\varepsilon}{4\pi\eta}\right) E\zeta \tag{1}$$

where ε, η and ζ are the dielectric constant, the viscosity of the liquid and the zeta potential, respectively[3,11,12]. The zeta potential and the thickness of the diffusion double layer, δ, have the following relationship[11,13].

$$\zeta = \frac{4\pi\delta e}{\varepsilon} \tag{2}$$

$$\delta \approx [3 \cdot 10^7 \mid Z \mid (c^s)^{1/2}]^{-1} \text{ cm} \quad (3)$$

where e, Z and c^s are the amount of charge per unit surface area, the number of valence electrons and the concentration of the electrolyte in water, respectively. Eqn. 3 is valid for aqueous solutions at room temperature. Although eqn. 1 is strictly valid only if surface conduction is negligible larger than δ, these two restrictions might well be satisfied under the present experimental conditions. Although the models of eqns. 1–3 are different, they are assumed to be related to each other in the present paper.

From eqns. 1–3, the linear velocity of electro-osmosis is dependent on the physical parameters of the solvent and also on the concentration of the electrolyte present. The order of the velocities of acetonitrile, water and methanol is readily understood from the ratios of the dielectric constant to the viscosity of the solvent, 10, 7 and 6, respectively. In Table I lists the linear velocities of electro-osmosis of aqueous solutions. The effect of the concentration of disodium hydrogen orthophosphate in water on the flow velocity of electro-osmosis is also understandable from eqn. 3.

TABLE I

LINEAR FLOW VELOCITY OF ELECTRO-OSMOSIS AT 100 V/cm

Solvent	*Linear velocity (cm/sec)*	*Experimental conditions**
Distilled water	0.061	1
Methanol	0.034	1
Acetonitrile	0.14	1
0.05% Methanol–*n*-hexane	0.0006	1
0.01 *M* Na_2HPO_4–water	0.13	2
0.025 *M* Na_2HPO_4–water	0.093	2
0.05 *M* Na_2HPO_4–water	0.087	2
0.075 *M* Na_2HPO_4–water	0.077	2
0.1 *M* Na_2HPO_4–water	0.057	2
Methanol–benzene (1:9)	0.01	Ref. 2

* Experimental conditions: (1) capillary column, Pyrex, 57.6 cm × 132 μm I.D.; solute, benzene or pyridine; power supply, constant-voltage mode, 13 kV; (2) capillary column, Pyrex, 60 cm × 132 μm I.D.; solute, as above; power supply, constant-current mode, 200 μA (voltage per cm was varied from 302 to 96).

Although there is no geometrical parameter in eqns. 1–3, such as the inner diameter of the capillary tube, it is important to establish the effects of geometrical parameter in the chromatographic process. Fig. 1 shows the relationship between flow velocity and electric current for three different capillary tubes. The values of electric current for tubes 2 and 3 in Fig. 1 were normalized by using the ratio of their cross-sectional area to that of a 60 μm I.D. tube. Fig. 1 shows that flow velocity is proportional to the value of electric current per unit cross-sectional area, *i.e.*, current density and also electric field as the specific resistance is constant. There is no geometrical effect of the cross-sectional area of the capillary tube on the electro-osmotic flow under the experimental conditions used when the current density remains constant. E and u have a first-order relationship, as indicated by eqn. 1.

Capillary columns of 85 μm I.D. and lengths of 90, 60, 40 and 30 cm were tested. Under the same current conditions, the linear velocity of electro-osmotic flow

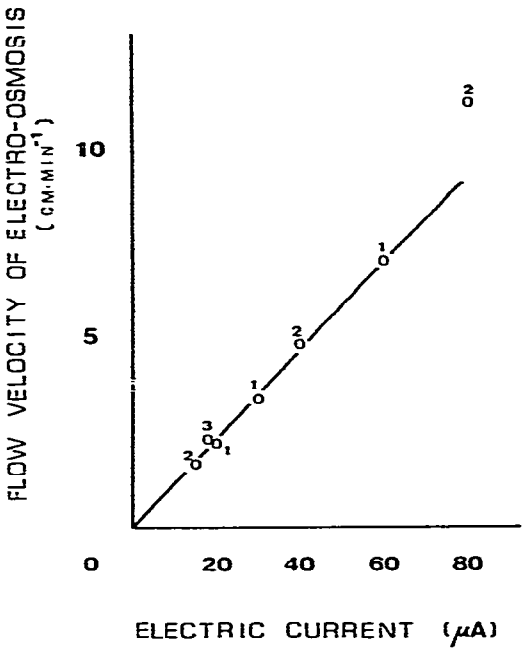

Fig. 1. Flow velocity of electro-osmosis *vs.* electric current. Pyrex capillary tube, 60 cm long and (1) 60, (2) 85 and (3) 200 μm I.D.; solvent, 0.05 *M* Na_2HPO_4–H_2O; solute, pyridine. The value of electric current of (2) and (3) means normalized value by using their current density.

and the height equivalent to a theoretical plate, *H*, for pyridine were constant in the system 0.05 *M* disodium hydrogen orthophosphate–water. It is suggested that the present injection procedure worked well without affecting *H*, and that the length of the capillary column did not effect the flow velocity.

Relationship between H and u

The relationship between *H* and *u* is shown in Fig. 2. As the total length of capillary tube in Fig. 2 is 75 cm (60 cm from the inlet to the detector and 15 cm from the detector to the outlet), a voltage input of 200 V/cm means that 15 kV is input at the end of the capillary.

The *H* values obtained are far less than the inner diameter of capillary column *ca.* 4.5%. In capillary chromatography the minimum value of *H* is given by $1/\sqrt{3}$ times the column radius[14]. The experimental value in Fig. 2 is about one sixth of the minimum value of *H* in which the flow profile in the tube is treated as laminar flow, although the linear flow velocity under experimental conditions is far greater than that giving the minimum *H* value.

If we calculate the value of *H* for pyridine from $r^2u/24D_m$, using $u = 0.09$ cm/sec, column radius $r = 61$ μm and diffusion coefficient of pyridine in pure water $D_m = 9.2 \cdot 10^{-6}$ cm²/sec, which was estimated by the method in ref. 15, the *H* value for pyridine is 152 μm. This value is about 29 times greater than the experimental value of 5.2 μm.

If we assume that the flow profile in the capillary tube is plug flow[3] and molecular diffusion alone is responsible for zone broadening[4,5], *i.e.*, axial diffusion

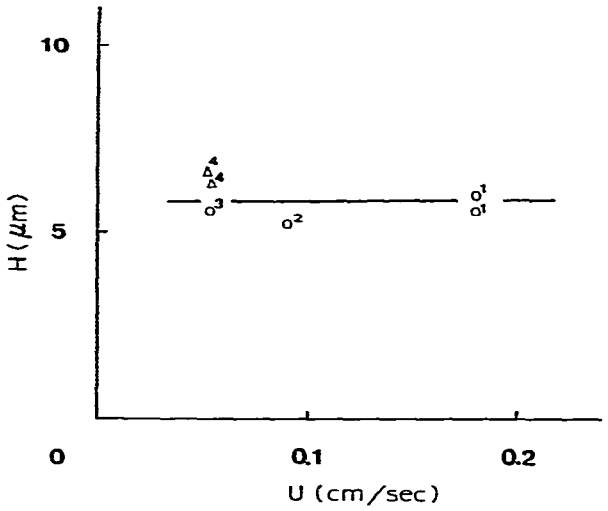

Fig. 2. Relationship between H and u. Pyrex capillary tube, 60 cm long and 132 μm I.D.; solvent, (○) 0.025 M Na_2HPO_4–H_2O and (△) 0.1 M Na_2HPO_4–H_2O; solute, pyridine; input voltage, (1) 193; (2) 136, (3) 96 and (4) 96 V/cm.

model, the calculated value of H for pyridine by using above parameters is 2 μm. The experimental value, 5.2 μm, is 2.6 times greater than this value, so the assumption does not hold for species that have no valence electron. In other words, the band broadening for an unretained solute is due not only to molecular diffusion but also to the flow profile of electro-osmosis in the capillary tube.

These results correspond well with Pretorius *et al.*'s results using column of 1 mm I.D. and 50 cm long[3]. They estimated that the H value was reduced 25-fold. Therefore, the band broadening in electro-osmotic flow is assumed to be in a capillary tube with a radius of about one fifth of the actual size, if we compare it with laminar flow. These results provide further evidence that the flow pattern due to electro-osmosis is flatter than that of laminar flow and less flat than that of plug flow.

In the range of linear flow velocities in Fig. 2, H seems to be independent of u and of the input voltage. Hence it is not essential to input a high voltage in order to obtain good separations provided that the analysis time is not important.

Chromatogram with ODS capillary column of 30 μm I.D.

As the ODS capillary column used had a very small inner diameter (30 μm), electro-osmotic flow as used for injection. As the effluent was kept in small beakers without covers, there was some possibility that the composition of the effluent might have changed with an increased water content, during the chromatographic run. Fig. 3 shows examples of typical chromatograms. The H values for benzene, pyrene and 1,3,5-triphenylbenzene are 40, 54 and 120 μm, respectively, which are up to twenty times higher than that for pyridine in Fig. 2. The mass transfer term might contribute considerably to the H values for aromatics. If we use better ODS capillary columns, the mass transfer term in H will decrease. It should be emphasized that chromatographic separation was achieved by using a column that is relatively short.

The potential for the use of electro-osmotic flow in capillary LC is great. A

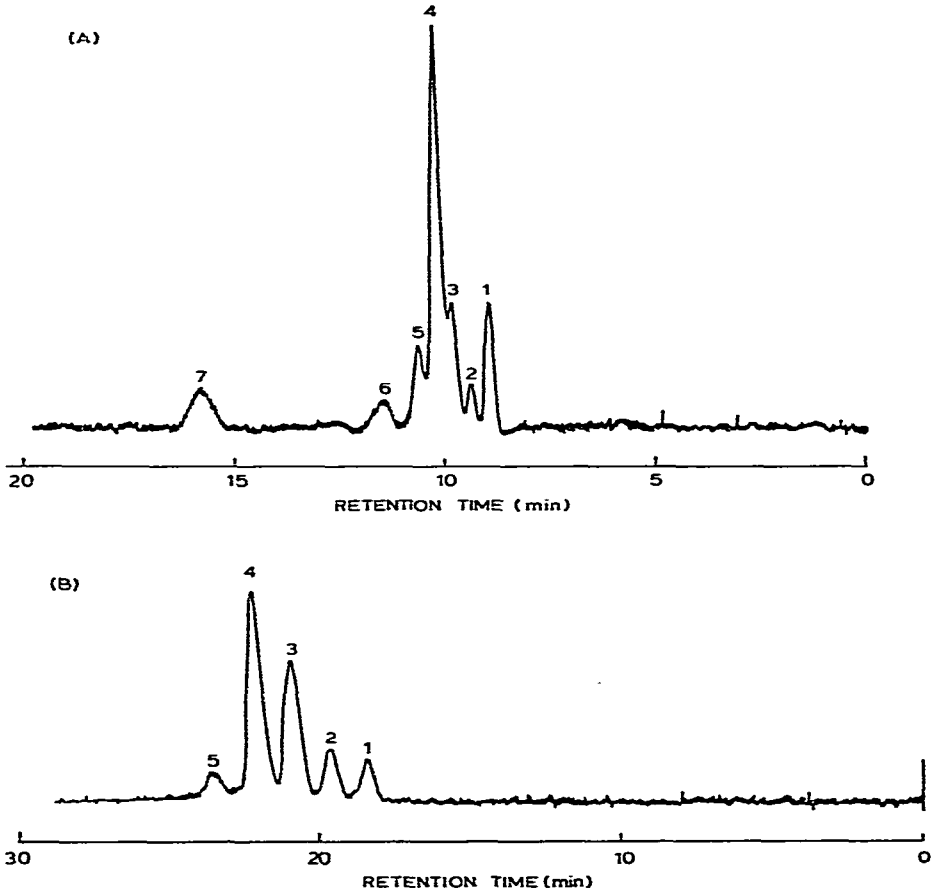

Fig. 3. Separation of aromatics on ODS capillary column (A) Column, 42 cm × 30 μm I.D.; effluent, acetonitrile–water (40:60); voltage, 13 kV. Sample: (1) = benzene; 2 = naphthalene; 3 = biphenyl; 4 = fluorene + anthracene; 5 = *p*-terphenyl; 6 = chrysene, 7 = 1,3,5-triphenylbenzene. (B) Effluent, acetonitrile–water (30:70); sample, as in (A) except 5 = pyrene. Other conditions as above.

good ODS capillary column of 10 μm I.D. under electro-osmotic flow would correspond to a column of 2 μm I.D. under laminar flow with respect to the estimation of *H* values due to the mobile phase for an unretained solute. In other words, we could achieve good separations without using a column with an extremely small inner diameter, such as 2 μm.

Attempts to make better ODS columns and narrower columns are in progress.

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