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ELECTRO-OSMOSIS

A NEW CONCEPT FOR HIGH-SPEED LIQUID CHROMATOGRAPHY

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SUMMARY

Electro-osmosis has been used to pump solvents in both thin-layer and high-speed liquid chromatography. The advantages of this technique over conventional methods of driving solvent are discussed.

INTRODUCTION

Possibly the two most successful descendants of classical column liquid chromatography are thin-layer chromatography (TLC) and what has come to be known as high-speed liquid chromatography (HSLC). Both have been extensively described (see, e.g., refs. 1 and 2). For our purpose, it suffices to draw attention to only those aspects where, as is described below, electro-osmosis may be usefully employed.

HSLC requires, for its successful operation, columns packed with small (1-20 μm) support particles, covered with a thin layer (pellicular) of chromatographic material, through which solvent is driven at relative high linear flow-rates (ca. 0.1-1 $\text{cm}\cdot\text{sec}^{-1}$). Most commonly, the solvent is driven through the column by applying sufficient pressure (up to 10,000 p.s.i.) and this introduces several disadvantages:

- (i) special pumps and inlet devices must be used;
- (ii) columns must be constructed from mechanically strong materials; glass columns, which are useful for handling many biological samples, are not easily constructed,
- (iii) columns are, perforce, narrow-bore and preparative chromatography becomes technically formidable;
- (iv) detector dead volumes must be kept extremely small (several microlitres) in order to avoid additional band spreading.

Less commonly, the solvent is driven by placing the entire column in a strong centrifugal field³. Among the disadvantages of this technique are:

- (i) columns are short (ca. 10 cm);
- (ii) detection must be carried out, after separation has taken place, by extruding the packing from the column;
- (iv) the entire available column length can seldom be effectively used.

Attempts to further improve the performance of HSLC along these lines would certainly involve formidable technical problems.

In TLC, the solvent is driven by capillary action. This introduces the disadvantages of centrifugal HSLC and, more seriously, the fact that flow-rates are low and decrease as the solvent front progresses.

THEORY OF ELECTRO-OSMOSIS

Electro-osmosis is the flow of liquid, in contact with a solid surface, under the influence of a tangentially applied electric field. Detailed descriptions of the phenomena may be found in many standard texts (see, *e.g.*, refs. 4 and 5). For the present purpose, it suffices to say that electro-osmosis is essentially attributable to the formation of an electric double layer, at the solid-liquid interface, due to the preferential adsorption of ions. The double layer is visualized as consisting of a relatively immobile layer of ions on the solid surface and a diffuse layer extending into the liquid. Under the influence of a tangential electric field, a portion of the diffuse layer moves because of the electric forces acting on the excess ionic charge in it, and a shear plane is set up at some distance from the solid surface. A constant flow-rate is reached when the force exerted on the counter ions (and thus on the liquid as a whole) is balanced by the frictional forces arising from the viscosity of the liquid. The potential at the shear plane is called the zeta (ζ) potential. The "thickness" of the double layer, δ , is, somewhat arbitrarily, defined as the distance from the immobile layer to a point at which the potential is equal to 0.37 times the potential at the interface between the immobile and diffuse layers.

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

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

where ϵ and η are the dielectric constant and viscosity of the liquid, respectively. Eqn. 1 is strictly valid only if surface conduction is negligible and if the channels through which the fluid flows are considerably larger than δ . The first effect is relatively unimportant⁴; the second is dealt with below.

Zeta potentials depend on the nature of the solid surface and the ionic state of the liquid. Polar solvents, *e.g.*, water, give rise to zeta potentials of as much as 100 mV in contact with either polar, *e.g.*, glass, or non-polar, *e.g.*, graphite, surfaces⁴. Non-polar, non-conducting solvents, *e.g.*, heptane, do not normally exhibit a zeta potential when in contact with either polar or non-polar surfaces. However, by adding small concentrations ($10^{-5} M$) of substances such as calcium diisopropyl salicylate, zeta potentials similar to those quoted above can be obtained⁶. It can be accepted, therefore, that adequate zeta potentials can be attained in any chromatographic system by using such additives.

From eqn. 1, we can estimate that useful linear velocities ($0.1-1 \text{ cm} \cdot \text{sec}^{-1}$) can be generated in chromatographic systems using realistic voltages (up to $1500 \text{ V} \cdot \text{cm}^{-1}$). Because of the low concentrations involved, heating is seldom a problem.

Eqn. 1 is independent of the channel (and thus particle) size. This means that

solvent flow should proceed at the same rate in a column packed with fine as with coarse particles. Furthermore, the flow should be substantially unaffected by inhomogeneities in the packing.

Electro-osmotic flow differs from laminar flow (whether caused by pressure, centrifugation or capillary forces) in another important respect. As shown schematically in Fig. 1, laminar flow causes a parabolic velocity profile which, in chromatography, increases resistance to mass transfer in the mobile phase and thus also the plate height. In electro-osmotic flow, the flow-profile is much flatter, and resistance to mass transfer occurs only in the region of laminar flow, *i.e.*, in the region δ . The numerical value of δ depends essentially on the conductance of the liquid and it may be as low as several micrometers in reasonably conducting solvents such as those mentioned above⁷. Band spreading in open tubes (*e.g.*, UV detectors) may thus be considerably reduced.

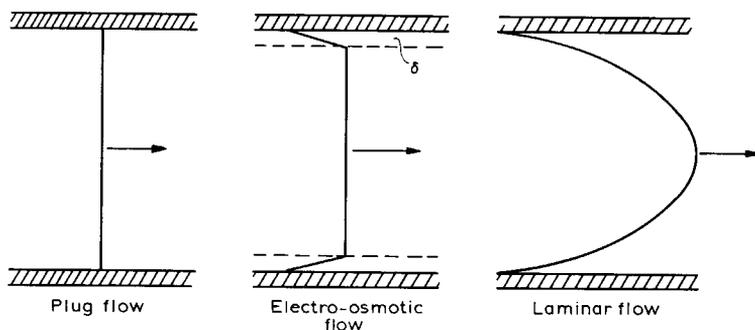


Fig. 1. Flow profiles under various types of flow.

Finally, it may be pointed out that electro-osmotic flow does not generate any hydrostatic pressure in a tube or column provided that the ends of the tube are open. Flow by electro-osmosis is thus possible in TLC. Furthermore, wide-diameter columns for preparative chromatography can be contained in thin-walled material.

EXPERIMENTAL AND RESULTS

The experiments described here are presented merely to confirm some of the conclusions arrived at in the preceding discussion; there has been no attempt, at this stage, to fully exploit the possibilities in a chromatographic sense.

High-speed TLC

Samples were run on TLC plates (Kieselgel 60-F-254, Merck, Darmstadt, G.F.R.) (which had been silanized by dipping the plates in a 5% solution of dichlorodimethylsilane in toluene for 15 min, then in pure methanol for the same period and finally oven dried) under electro-osmotic flow and under capillary flow using the apparatus shown in Fig. 2. In the case of the electro-osmotic TLC, the plate was first wetted with solvent and an electric field of $2000 \text{ V} \cdot \text{cm}^{-1}$ was applied. Solvent accumulating at the top of the plate was simply removed by suction.

The results are shown in Fig. 3. The improvement in analysis time is evident.

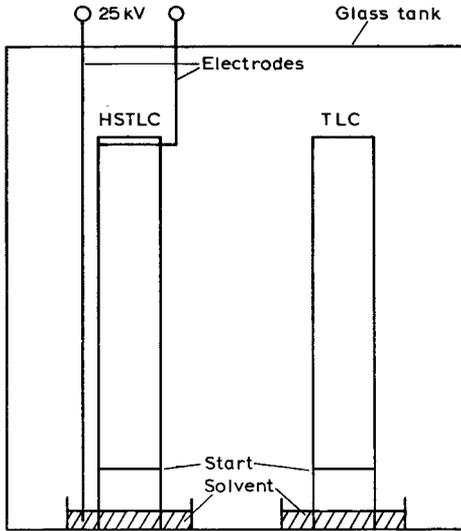


Fig. 2. Apparatus for high-speed thin-layer chromatography (HSTLC).

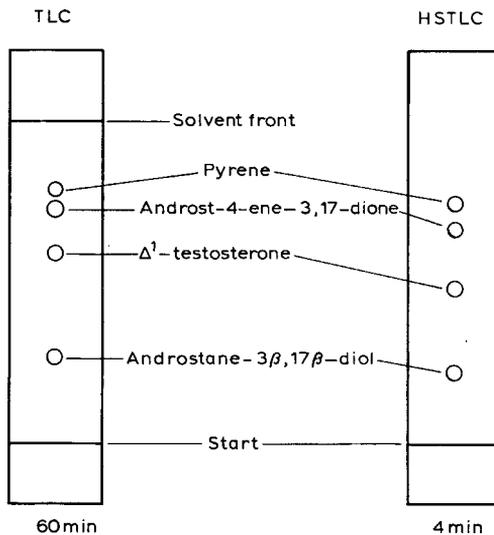


Fig. 3. Comparison of separation by TLC and HSTLC using electro-osmosis.

Flow in open tubes

Band spreading under electro-osmotic flow in an open tube was measured using the apparatus shown in Fig. 4. The wide-bore return path was necessary to prevent laminar back-flow of liquid. Tubes (50 cm long, 1 mm I.D.) were made of quartz so that bands could be detected by means of UV absorption (Uviscan III, Buchler, Fort Lee, N.J., U.S.A.). Samples of 0.1 μ l of benzene were injected as shown; the solvent was methanol. Voltages of approximately 5 kV were applied

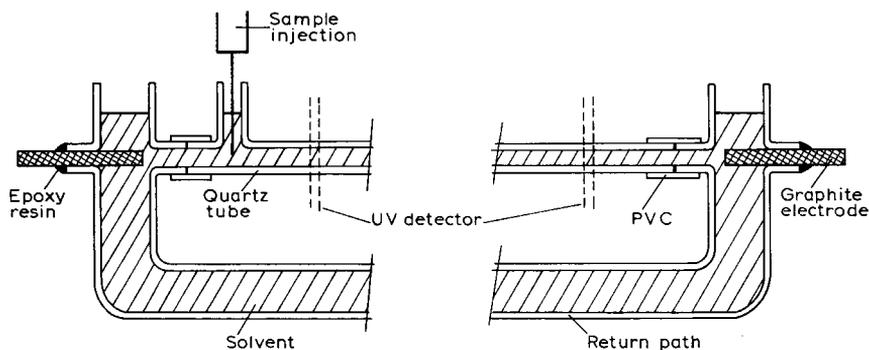


Fig. 4. Apparatus for studying electro-osmotic flow in open tubes.

across the tubes through graphite electrodes. Solute band widths were measured at two points along the column and the plate height was calculated from these data. Measurements were made at various flow-rates, which were obtained by varying the applied voltage.

Similar experiments were carried out to measure band spreading under laminar flow. For this purpose, one end of the quartz tube was connected to a solvent reservoir and the other to a needle valve.

The results of these experiments are shown in Fig. 5 and agree well with values calculated from the Golay equation⁸:

$$H = \frac{2D}{u} + \frac{r^2}{24D} \cdot u \quad (2)$$

using D (diffusion coefficient) = $10^{-5} \text{ cm}^2 \cdot \text{sec}^{-1}$ and r (radius) = 0.05 cm.

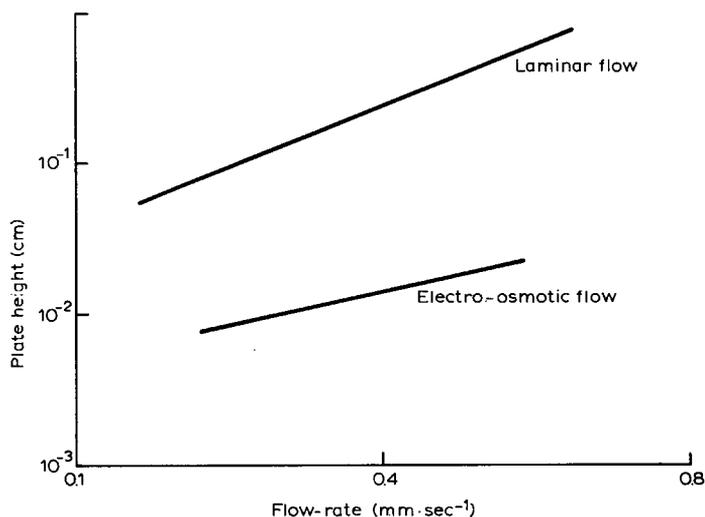


Fig. 5. Plate height *versus* flow velocity in open tubes for laminar and electro-osmotic flow.

Band spreading under electro-osmotic flow is considerably less than that observed under laminar flow. By using the data in Fig. 5 and assuming $r = \delta$ in eqn. 2, the value of δ can be estimated as 0.01 cm, which is not unreasonable.

Flow in microparticulate columns

Glass columns (5 cm long, 1 mm I.D.) were packed with microparticulate silica (Silanox, Cabot Corp., Boston, Mass., U.S.A.) by centrifugation in the manner outlined by Piel⁹. According to Piel, a hydrostatic pressure of *ca.* 4000 p.s.i. is needed in order to force solvent through such fine packings.

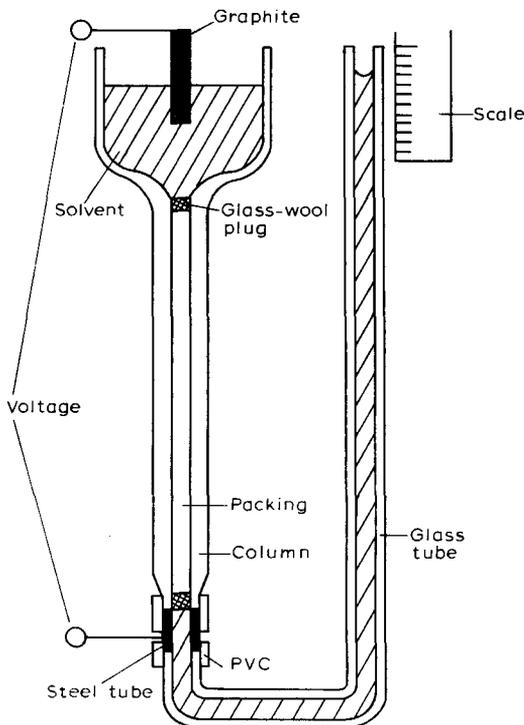


Fig. 6. Apparatus for measuring flow through microparticulate packing.

Electrodes were fitted to the column as shown in Fig. 6 and flow could be measured by observing the surface of liquid in the open tube connected to the column. Using methanol-benzene (1:9) as solvent, linear flow-rates (in the packing) of $0.2 \text{ mm} \cdot \text{sec}^{-1}$ were obtained at field strengths of $2000 \text{ V} \cdot \text{cm}^{-1}$.

Longer columns (*ca.* 50 cm in length), which cannot be packed by centrifugation, were successfully packed by filling the columns with a slurry of the microparticulate silica and reversing the field so that the particles were sedimented by electrophoresis. Packing densities comparable with those obtained by centrifugation could be attained in this way. Further exploitation of this system awaits the design of a suitable inlet system and detector.

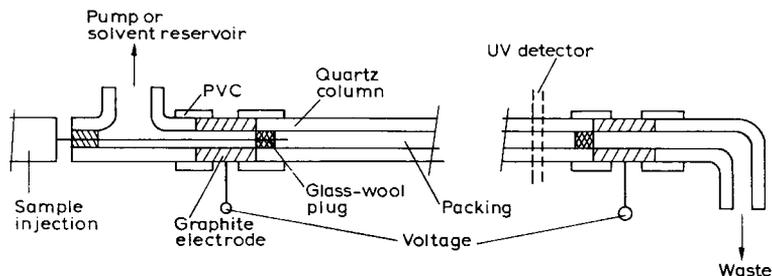


Fig. 7. Apparatus for measuring plate heights for laminar and electro-osmotic flow through packed columns.

Plate-height measurements and flow in packed columns

Plate heights in packed columns (50 cm long, 1 mm I.D.) using electro-osmotic flow were measured, using the apparatus shown in Fig. 7. Columns were packed with Porasil (75–125 μm) coated with *n*-octane in the normal way² and the solvent used was methanol–water (3:2). Samples of 1 μl of acetone, which was not retained, were injected into the packing and driven through the column by electro-osmosis. Plate heights and solvent velocities were determined as described above.

Similar experiments using laminar flow were carried out by pumping solvent through the apparatus shown in Fig. 7. Plate heights were determined by the same means as used above for electro-osmotic flow.

The results of these experiments are shown in Fig. 8. The plate heights under electro-osmotic flow are lower than those resulting from laminar flow. These experiments do not justify a convincing explanation of this difference in plate height. The slightly smaller slope of the electro-osmotic curve suggests smaller resistance to mass transfer in the mobile phase, possibly due to a flatter flow profile. Extrapolation of the curves to zero velocity suggests that eddy diffusion is smaller under electro-osmotic

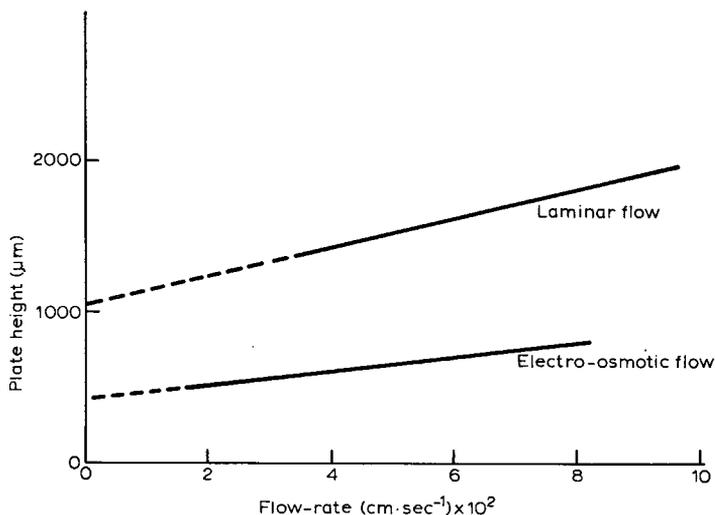


Fig. 8. Plate height versus flow-rate in packed columns using laminar and electro-osmotic flow.

than under linear flow, which implies that the former flow is less affected than the latter by irregularities in the packing.

CONCLUSIONS

Electro-osmotic flow can be induced in a variety of systems of interest in liquid chromatography; the fact that trace amounts of ion-producing substances must be added to the solvent in certain cases does not appear to introduce any insurmountable problems. Adequate flow-rates can be attained by using acceptable electric field strengths. The flow-rate is substantially independent of the size of the channels; this introduces a new approach to further improving column efficiency.

The fact that no hydrostatic pressure is generated simplifies inlet systems and opens the way for preparative HSLC using wide-diameter columns. The relatively flat flow profile reduces band spreading arising from resistance to mass transfer in the mobile phase; this can be expected to be of more importance in reducing band spreading in open tubes, and thus in detector design, than in improving the performance of columns packed with very fine particles.

Electrophoresis and electro-osmosis can be made to take place in opposite directions; the exploitation of this as a means of effecting continuous chromatography are obvious.

NOTE BY THE EDITOR OF THE *JOURNAL OF CHROMATOGRAPHY*

Electro-osmosis as mechanism for development has already been used by D. L. Mould and R. L. M. Syngé [*Analyst (London)*, 77 (1952) 964 and *Biochem. J.*, 58 (1954) 571] using collodion membranes as the support in a fore-runner to gel filtration in the separation of substances according to their molecular weights. The technique is called "Electrokinetic ultrafiltration".

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