Journal of Chromatography, 632 (1993) 201-207
Elsevier Science Publishers B.V., Amsterdam

CHROMSYMP. 2612

Observation of flow profiles in electroosmosis in a rectangular capillary*

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ABSTRACT

The flow profile of electroosmosis in capillary electrophoresis was studied by using a dye and a rectangular capillary. The movement of the dye is observed with a microscope-video system, and then advances per unit time are measured from the recorded video tapes. The medium at the central portion moves like a plug flow, and the zone front at the edges are ahead of the central portion. The flow profile in a capillary column with a circular cross-section is proposed. The flow profiles of ionic solutes are also discussed.

INTRODUCTION

The flow profile in electroosmosis has been discussed under the conditions of electroosmotically driven open-tubular liquid chromatography [1–7], capillary zone electrophoresis (CZE) [8–13] and electroosmotically driven electrochromatography [14]. The flow profile in electroosmosis is different from the parabolic laminar velocity profile of pressurized flow. The former flow profile is much flatter than the latter. Thus very narrow peaks are obtained in electroosmotically driven liquid chromatography (ELC) and CZE. In early experiments, Pretorius *et al.* [1] and Tsuda *et al.* [3] obtained 10 and 30 times less band broadening in ELC than expected with pressurized flow. There have been several studies of flow profiles in electroosmosis [1,3,10,11]. Pretorius *et al.* [1] suggested that the profile is flat except in the region of a diffuse electric double layer near the column inner wall, where the flow profile is a quadratic velocity profile owing to the friction experienced by the viscous liquid flowing by the wall. Jorgenson and Lukas [8] assumed that the zone broadening in CZE is only generated by axial molecular diffusion of a solute and a medium. Tsuda *et al.* [3] found that it was difficult to explain the experimental results just from the axial molecular diffusion, and proposed that the flow profile might be a combined form of plug and Poiseuille flow. Guiochon and co-workers [10, 11] also assumed that the electroosmotic flow profile is an expression of a combination of plug and Poiseuille flow, proposed an equation and calculated the contribution of each to the real electroosmotic flow profile by using experimental data.

For the anaslysis of zone broadening under pressurized flow conditions, Taylor [15] used a method in which a coloured solution was introduced continuously into a narrow capillary tube at several different velocities. The concentration of the coloured

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* Part of this paper was presented at the ***2nd International Symposium on High Performance Capillary Electrophoresis, San Francisco, CA, January 24-26th 1990,*** and the ***15th International Meeting on Column Liquid Chromatography, Basle, June 3rd–7th, 1991.***

0021-9673/93/$06.00

solute at each axial point of the tube was measured after the zone front had travelled past the centre part of the capillary tube. He proposed equations for the zone broadening in which diffusion coefficients, flow velocity, column radius and time are included and also a general equation for band broadening in a capillary tube.

In this work, we used a similar method in which a fluorescent solution is continuously introduced into a capillary column, with the front profile being observed by through a microscope charge coupled device (CCD) camera-video cathode ray tube (CRT) system. A rectangular capillary tube [16], which has been used for CZE, is used because the flat sides cause fewer distortions in the observed zone front than do the more common round walls.

EXPERIMENTAL

Rectangular capillaries (1 mm × 50 μm) were purchased from Wilmad Glass (Buena, NJ, USA). A high-voltage power supply (O-30 kV with a reversible polarity output) is used (Hipotronics, Inc., Brewster, NY, USA). For the study of electroosmotic flow profiles, the applied voltage on a rectangular capillary (164 mm long) and electric current were 1.59 kV (97 V/cm) and 0.12 μA, respectively, for the experiments using pure methanol as a medium and Rhodamine 590–methanol solution as a sample zone. For the study of zone front of ionic molecules, 5 mM phosphate buffer (pH 6.8) as a medium and a rectangular capillary 300 mm long were used. The current through the capillary was monitored as a potential drop across a 64 kΩ resistor on the ground side of the circuit. A schematic diagram of the instrument used for the observation and recording of the zone front is shown in Fig. 1. The rectangular capillary was placed on XY stage- 1 under a Nikon (Tokyo, Japan) SMZ-2T stereoscope equipped with a Sony (Park Ridge, NJ, USA), DXC-101 color video CCD camera, VHS video recorder (National, Tokyo, Japan) and CRT (Sony). The zone front was kept under the microscope by adjusting the capillary position with XY stage- 1.

Syringes (A and B in Fig. 1) made of polyethylene were used as reservoirs, with electrodes set inside them. The medium or sample solution can be kept inside the syringe without closing the outlet part

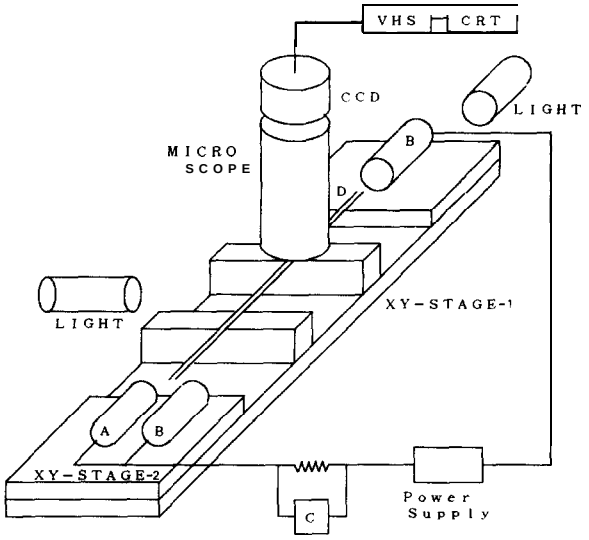

Fig. I. Schematic diagram of the instrument for visual observation of zone front. A and B are reservoirs of the medium and coloured sample solution, respectively. Each reservoir has its own platinum electrode. Current is measured at C. D is a rectangular capillary, 1 mm × 50 pm and 1.64 m long. Light is focused on the zone front, which is kept under a microscope by adjusting XY stage-1.

used for connection of a needle, which permits the rectangular capillary to be inserted through this orifice.

The operational procedure for the introduction of the sample solution is as follows. When one of the ends of the empty rectangular capillary is inserted into reservoir B, the medium in the reservoir is introduced into the capillary via capillary action. Then the other end is inserted into another reservoir B. Subsequently, the end of rectangular capillary is inserted into the outlet of the syringe containing sample solution (reservoir A in Fig. 1) with gentle and smooth motion of XY stage-2. This procedure is very important for producing a sharp zone front. Then a voltage is applied. Immediately after the application of the voltage, the coloured solution travels into the rectangular capillary and its zone front is followed under the microscope system by continuous or stepwise operation of XY stage- 1.

The sample solution was continuously introduced into the rectangular capillary in a similar fashion to frontal analysis in chromatography [17]. With the dye zone front illuminated using two lens-

es fed by two fibre-optic cables from a light source (150-W halogen lamp; Cole-Parmer Instrument, Chicago, IL, USA), the fluorescent image of the front was recorded.

Rhodamine 590, sulphorhodamine 640 and disodium fluorescein were purchased from Exciton Chemical (Dayton, OH, USA) and used as received.

RESULTS AND DISCUSSION

Electroosmotic flow in capillary tubing is generated under a potential gradient in various media, such as water, with electrolytes, pure methanol, acetonitrile, methanol-hexane [3], methanol-benzene [I] or mixtures of water and organic solvents [18]. The flow-rate of electroosmosis under unit potential gradient is dependent on the dielectric constant and viscosity of the medium, the amount of positive or negative surface charge on the inner wall and the diffuse double layer [3,9,13,19]. Linear flow velocities of electroosmosis under a unit potential gradient of methanol and acetonitrile are half and twice that of distilled water, respectively [3]. Electropherograms obtained using organic and aqueous organic media show almost same resolutions compared with those obtained using water or water with electrolyte [4,18]. Therefore, we consider that the use of methanol as a medium for the study of electroosmotic flow profile will gives a general answer. As methanol has high solubility for dyes, it is convenient for observation of zone front movements.

Rhodamine 590 in methanol is assumed to be neutral, hence the behaviour of the zone of Rhodamine 590 solution corresponds direclty to the behaviour of electroosmosis, *i.e.,* the zone front shows the flow profile of electroosmosis.

For the observation of the electroosmotic flow profile, we selected a rectangular capillary with height 50 μm, width 1 mm and length 164 mm, where these dimensions are defined as the Z, Y and X axes, respectively. The rectangular capillary is positioned so that the microscope images the XY surface of the capillary.

The use of a rectangular capillary for the observation of flow profiles in electroosmosis has several advantages. The flow velocity in electroosmosis is dependent on the amount of charge on the wall [13]. The medium in a rectangular capillary is surrounded by two sets of parallel plates, namely two XY plates and two XZ plates. At the centre part of the Y axis of the rectangular capillary the medium is affected mainly by the two parallel XY plates. The medium near the XZ plates is affected by both the two l-mm parallel plates (*XY*) and the 50-μm plate (*XZ*). Therefore, the geometric difference between the edge and centre of the l-mm plate makes the effect of the wall on the flow profile apparent. The difference in flow at the edge of the l-mm width (y axis) *versus* the centre would be caused by the effect of the 50-μm plate (*XZ*), because the effect of charges on the l-mm plate and its diffuse double layer would influence the flow profile equally at all parts of the XY plate.

When we inject continuously a dye-methanol solution into the rectangular capillary and force it with a pressurized flow by lifting up one of the ends of the rectangular capillary, we can observe a typical Poiseuille flow profile at its zone front. Under a potential gradient and keeping both ends of the capillary at the same level, we obtain completely different flow profiles from Poiseuille flow, as shown in Fig. 2.

The current is very stable during the run, *i.e.,* there is only a small difference in conductivity between pure methanol and 10^{-4} *M* Rhodamine-methanol solution. If there had been a considerable conductivity difference between them, a molecule entering the pure methanol zone from the zone front of Rhodamine-methanol would travel with higher velocity than those remaining in the sample zone, because the potential gradient in the pure methanol would be higher than that in Rhodamine-methanol zone. Diffusion of the zone front would result. A gradient of the concentration of Rhodamine the range of in the zone front along the X axis was not observed visually, and was not found in computerized images obtained from the recorded video tape.

Zone fronts of Rhodamine 590 in methanol solution are shown in Fig. 2. The period between the pictures is 1 s. The advance of the zone front between two pictures can be measured from photographs by matching the positions of the stationary marks on the rectangular capillary. The zones front shown in Fig. 2 is flat at the centre with small bends at both ends of the l-mm axis, namely near the 50-μm plates. The travelling of the zone front recorded on video tape was analysed by computer

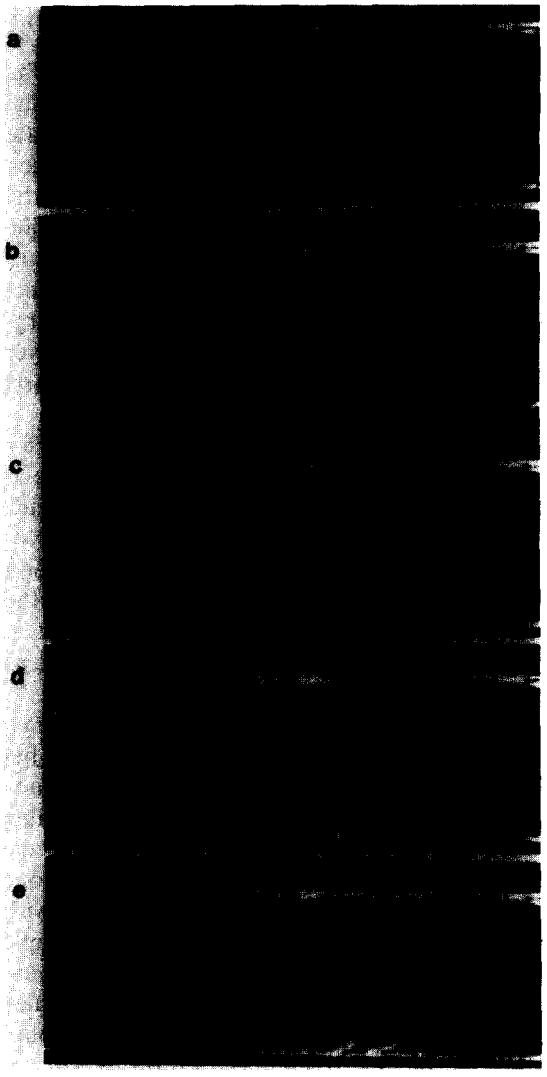

Fig. 2. Photographs of zone front of electroosmotic Row. Coloured sample solution: 0.1 m*M* Rhodamine 590 in methanol. Applied voltage and current, 1.59 kV and 0. I2 μA, respectively. The period between photographs is l s. The five photographs a-e are a series.

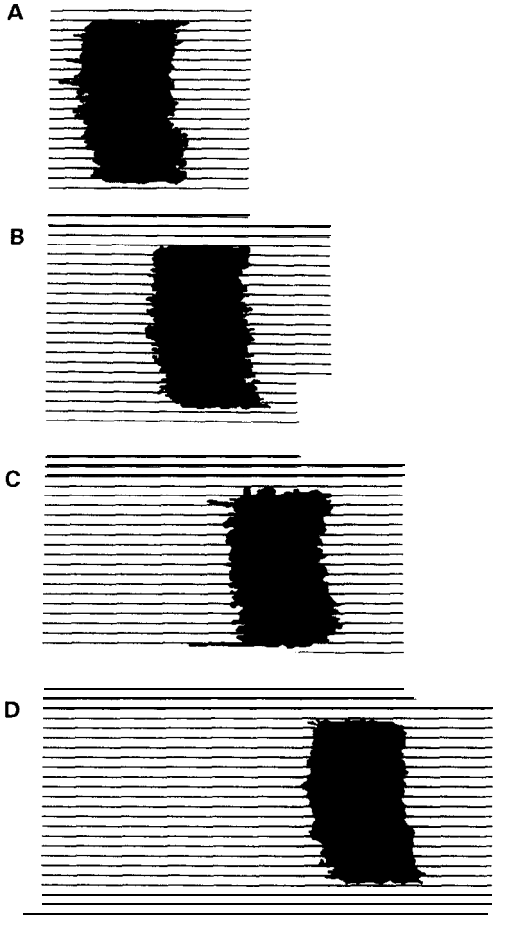

Fig. 3. Computed images of zone front on the *X* axis over a l-s span. The four images A-D are a series. Each image shows advances in the *X* axis over l s. The two ends of an image on the *X* axis correspond to zone fronts of l s apart.

software. The result obtained is the image of the advancing zone front, shown in Fig. 3. The four images of advances in Fig. 3 are over successive 4-s spans, and the l-mm axis is divided into seventeen sections. Both edges in the X-dimension of the image correspond to zone fronts.

The advances of the zone front along the *X* axis are summarized numerically in Table I. The average numbers at each Y section are similar. The flow profile is plug-like in the centre part of the capillary from sections 1 to 1.5. Unfortunately, the numerical values of the advances at sections 0 and 16 are not stable enough to estimate.

To examine the profile in the vicinity of the 50-μm plate, we focused the movement of the zone front with high magnification. One of the photographs obtained is shown in Fig. 4. The zone front at the near wall, namely near to 0 or 16 of the *Y*-axis, is further ahead (larger value of the X-axis) than the position of the zone front at the central portion.

It is concluded that at the central portion the ad-

TABLE 1

ADVANCES OF THE ZONE FRONT ALONG THE X AXIS OVER A l-s PERIOD AT Y SECTION

Mean of average value and its standard deviation are 7.69 and 0.12, respectively. The one unit of X-axis: 57.8 μm.

No. of Y section	Experiment No.				Average value
	1	2	3	4	
0	_	8.3	–	7.5	_
1	7.8	7.6	8.0	7.0	7.6
2	7.2	8.3	7.6	7.2	7.58
3	8.0	6.9	8.4	7.8	7.78
4	8.1	7.8	8.0	7.1	7.75
5	8.0	8.3	6.7	8.5	7.88
6	7.6	8.0	7.4	7.8	7.7
7	7.0	8.2	7.8	7.4	1.63
8	7.7	8.0	6.5	8.5	7.68
9	8.1	8.1	7.4	7.6	7.8
10	7.5	8.4	8.0	1.5	7.85
11	8.0	1.7	7.6	1.7	7.75
12	7.4	7.6	8.5	7.0	7.63
13	7.8	7.5	8.2	1.5	7.75
14	6.8	8.0	8.3	6.9	7.5
15	6.0	8.6	7.6	7.7	7.48
16	_	8.0	–	–	

vances are similar in Fig. 2 and 3 and Table I, and the zone fronts at the edges of the l-mm axis in Figs. 2 and 4 are ahead compared with the centre part. This phenomenon was observed in every experiment which we recorded.

We therefore suggest the following: the edge portion of the zone front advances at the very initial period, immediately after application of voltage on the rectangular capillary following the smooth operation of changing the reservoir from A to B. From then on the zone will maintain equal speeds at every point along the Y-axis. To our knowledge, this is the first time that the zone front pattern in electroosmosis has been directly observed. The centred part is behind edges. This finding is unique. The flow profile observed is very different from the suggestion of Pretorius *et* al. [1].

There is some possibility that capillary action of the dye-methanol solution into the rectangular capillary and/or adsorption of the solute on the inner wall during the operation of the introduction of the dye solution may occur, and they affect the zone front profile. The capillary action is very strong when a liquid is introduced into the empty rectangular capillary, but it might be negligible when a dye-methanol solution is introduced into the capillary filled with methanol and its concentration is low enough. Concerning the adsorption of the dye on the inner wall, it may be very weak or non-existent, because the washing out of the dye-methanol

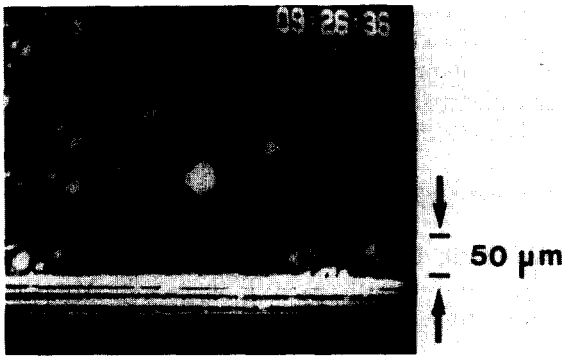

Fig. 4. Zone front near the 50-μm plate. Experimental conditions as in Fig. 2.

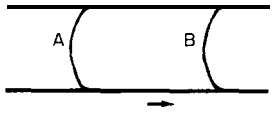

Fig. 5. Proposed zone front in a capillary column with circular cross-section of 100–50 μm I.D.

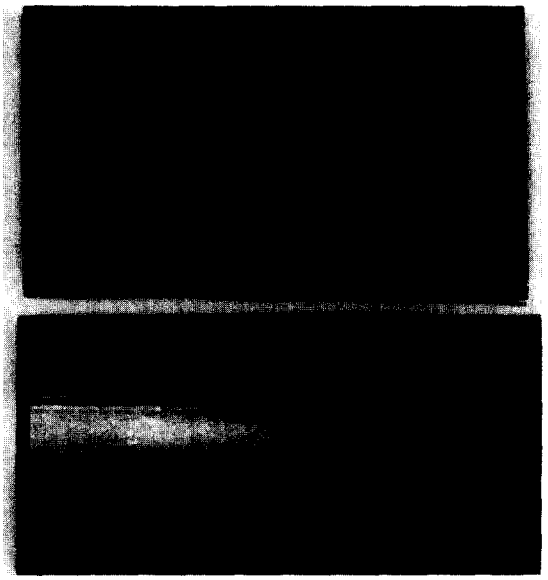

Fig. 6. Photographs of zone front of ionic solutes. (a) 0.05 m*M* Rhodamine 590 and (b) 0.1 m*M* disodium florescein in 5 m*M* phosphate buffer as ionic solutes. Applied voltage. 10 kV. A rectangular capillary (1 mm × 50 μm and 30 cm long) was **used.**

solution from the rectangular capillary with pure methanol was performed very easily. If there is some adsorption, the concentration at the region very near the *XZ* plate will decrease after the short period of development and it will result as the zone front at the edge will be behind to its central part. We did not observe this phenomenon. Also in the process of development the zone front at the edge is more advanced compared with the central part. Therefore, we conclude that there is not much effect of capillary action and adsorption during the operation of the introduction and the development of the zone.

The zone front in a capillary column with a circular cross-section of 100-50 μm I.D. will be different from the zone front shown in Fig. 2, because the geometrical dimension is different from the rectangular capillary used, especially the length of central portion. From the present experimental results, we propose the zone front profile in a 100-50 μm I.D. capillary column with circular cross-section as shown in Fig. 5. The central portion of the zone front is retarded compared with the edges, as with a rectangular capillary.

Flow profile of ionic solutes in aqueous solution

We consider that Rhodamine 590 in methanol is neutral, and its zone profile corresponds to that of electroosmosis. However, in 5 m*M* phosphate buffer (pH 6.8) with 5% ethylene glycol, Rhodamine 590 should have one positive charge and disodium fluorescein two negative charges. The zone front of these two ionic solutes in buffer solution is shown in Fig. 6. The negatively charged ionic solute has an electrostatic repulsion with the surface charge on the inner wall in its vicinity, and the positively charged ionic solute has an attraction with the wall. The zone fronts of both ionic solutes are different from those of neutral solutes. The difference is particularly pronounced at both ends of the l-mm axis (*Y*) of the rectangular capillary. The zone front at both ends of the Y axis moves forward or back owing to the positive or negative charge of the solute, respectively. Therefore, the zone front profile might be induced from electrostatic forces between ionic solutes and the charge on the surface of the inner wall under a potential gradient. The zone front of ionic solutes is not flat.

The advances per unit period at each Y axis are nearly equal as in electroosmosis, even though the zone front observed is not flat. More quantitative measurements of these advances at each Y section are in progress. The zone fronts of ionic dyes are dependent on their charges. The front zone profiles in electroosmosis and with ionic dyes each have a specific pattern. These findings are not explained by the general equation proposed for electroosmosis [9,19]. Although the diffuse double layer and the amount of surface charges on the inner wall are important factors, they might not have the power to affect molecules over a distance of several micrometres. The change in the nature of the medium under a potential gradient may be a key factor. Further experiments are planned to find the relationship between flow profiles and physical parameters.

ACKNOWLEDGEMENTS

We thank Pias Comp. (Tokyo, Japan) for the analysis of flow patterns. T.T. thanks the Ministry of Education, Japan, for the support of his leave. Beckman Instruments provided financial support for this research.

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