Direct Observation on Electroosmotic Flow Profile and Pressurized Flow Profile of the Fluorinated-Bonded Silica Packed Rectangular Capillary

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ABSTRACT

Electroosmotic flow (EOF) profiles and pressurized flow profiles in fluorinated-bonded silica packed rectangular capillary were investigated. A microscope CCD-video system was used to observe the flow profiles of 0.03 mM Rhodamine-6G in cyclohexanol, containing 0.1mM sodium dodecyl sulfate. The 2.3 cm homemade slurry-packed column was used. The fluorescence property of Rhodamine-6G in the solution after excitation with white light made it possible to directly observe the profiles through the microscope CCD-video system. The rectangular capillary was transparent and caused less distortion of images, enabling clearer images to be observed. Pressurized flow was generated by lifting one end of the capillary. The fluorescent samples moved towards the other end of the capillary. The images were captured, recorded and analysed. The pressurized flow profiles were parabolic. The parabolic shapes were different in skewness and peakedness along different sections of the capillary. High voltage 3 kV was applied to the capillary. EOF was then generated and moved towards the cathode. Flow velocities were then calculated from the moving distance of fluorescent samples against time. It was found that EOF velocities were not different along the capillary, indicating the homogeneity of surface charges along the column. The mean of average EOF velocity and its percent relative standard deviation (%RSD) were 2.22 ?m/sec and 0.68 respectively. The EOF profiles were flat, compared to the previous controversial EOF profiles in open tubular column. It might be due to the negative charges, occurring on this stationary phase during the application of the electric field as discovered in previous reports. This provided more evidence to indicate the benefits of using fluorinated-bonded silica packed column. EOF profile of this phase was much flatter than pressurized flow profile. It implied higher efficiency of separation, using Capillary Electrochromatography (CEC) rather than using High Pressure Liquid Capillary Chromatography (HPLCC).

Key words: Electroosmotic flow, Pressurized flow, Fluorinated-bonded silica, Capillary electrochromatography, Capillary chromatography, Rectangular capillary

INTRODUCTION

Capillary chromatography is one of the main tools for separating mixtures and it is widely accepted as the most recent advance in separation techniques. There are two ways to drive a liquid through a column (open and packed column), either by application of a

hydrostatic pressure difference or by a potential difference (Tsuda et al., 1993a, 1995). Capillary electrophoresis and electrochromatography utilize the potential difference as a driving force which is known as electroosmotic flow (EOF) (Tsuda et al., 1993a, 1995).

EOF is generated at the solid-liquid interface of the support material which in most cases is silica, the main component of the capillary column. Nowadays, several other packing materials are also used as the electroosmotic-generating support materials such as octadecylsilane and cationic exchange resin. A fluorinated-bonded silica packed column was developed in previous studies (Chaiyasut et al., 1999a, 1999b, 1999c, 2000).

Pressurized flow is the driving force used in High Pressure Liquid Chromatography (HPLC) and High Pressure Liquid Capillary Chromatography (HPLCC). In pressure difference systems with open tubular columns (OTCs) or packed capillary columns (PCCs), there is laminar (Poiseuille) flow which has a parabolic velocity profile as a result of distribution of shear stress in a viscous fluid whereas in potential difference systems, the flow does not have a parabolic velocity profile. Flow profiles have been studied by different methods, both simulation (Patankar et al., 1998; Osuga et al., 1999) and observation (Tsuda et al., 1993a, 1993b, 1997). The observed and the proposed flow profile patterns differ in these previous reports. The flow profile patterns are different and depend on the type of the packing materials, that is whether it is an open or packed column.

In this report, observations on the EOF profile and the pressurized flow profile in a fluorinated-bonded silica packed rectangular capillary were made. A microscope CCD-camera was used to observe directly the flow profiles. The images were captured and recorded. The flow profile patterns were then analyzed.

MATERIALS AND METHODS

Apparatus

The apparatus for the observation of the flow profile pattern consisted of a rectangular capillary column (0.05 mm x 0.5 mm, 2.3 cm long, 500 nL) (Wilmad Glass, Buena, NJ, USA), two polyethylene reservoirs (approximate volume of each: 250 µl) on a glass slide (Matsunami, Osaka, Type S-1214), two platinum-wire electrodes inserted to the two reservoirs, a voltage power supply (Kikusui Electronics Corp., Kawasaki, Type CCD-X2), a video recorder-CRT monitor (Aiwa, Type VX-T14G3) and a video timer (minimum time resolution 10 ms, ForA, Tokyo, Type VTG33). The configuration of the apparatus is shown in Figure 1.

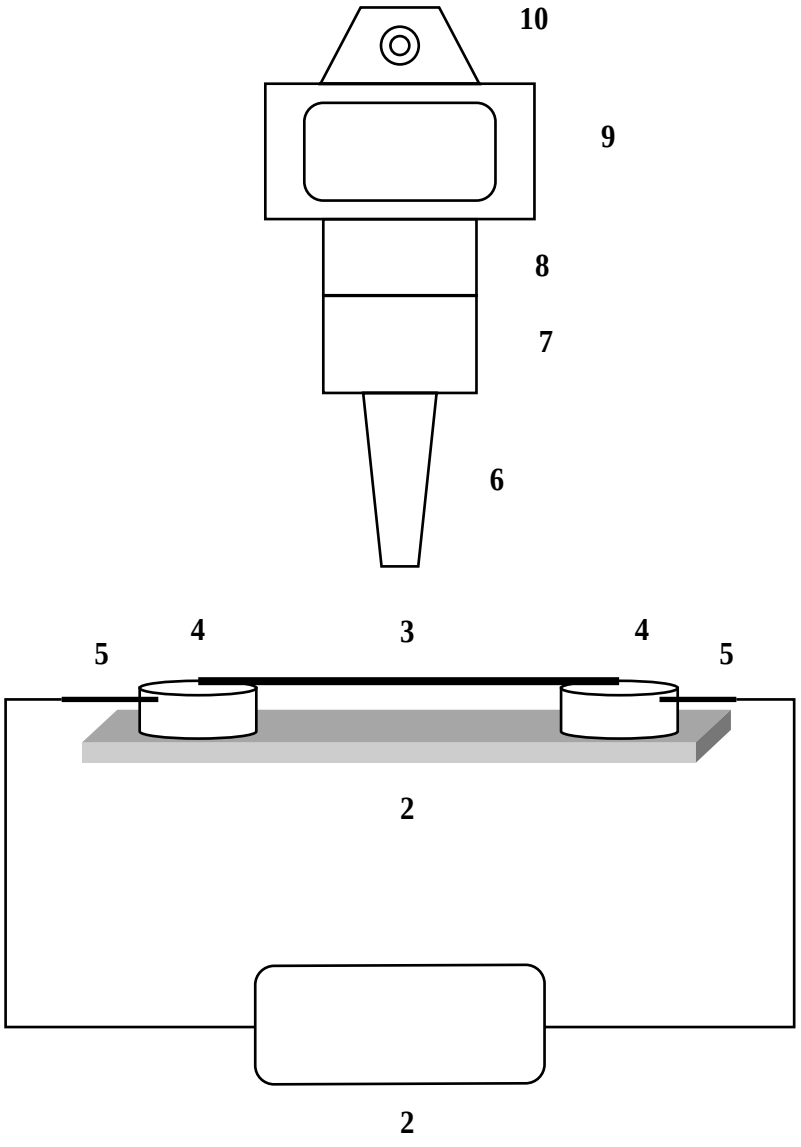

Figure 1. Schematic diagram of apparatus for observation of flow profiles (1) dc power supply (2) slide glass (3) short rectangular capillary (0.05mm x 0.5mm, 2.3cm long) (4) small polyethylene reservoirs (volume, 250 μl) (5) platinum wire elec trode (6) microscope (7) CCD camera (8) CRT monitor (9) video recorder (10) video timer.

Column Preparation

A homemade slurry-packed capillary column was used. A rectangular capillary was packed with fluorinated-bonded silica (Fluofix120E, 80421, particle diameter 5 μm, pore size 120 A˚, surface area 300 m^2/g). The structure of the fluorinated bonded phase was – $OSi(CH_3)_2(CH_2)_3C(CH_3)_2(CF_2)_2CF_3$. The packing procedure was as follow: An inner end frit 0.5 mm in length was prepared and fluorinated-bonded silica was packed under pressure using 0.1% cyclohexanol in carbon tetrachloride as the slurry solvent. After the column was fully packed, an outer end frit was prepared. The column was then flushed with cyclohexanol. The length of the column was 2.3 cm.

Materials

All solvents and reagents used were obtained from Merck and Wako Pure Chemical Co., Ltd. All reagents were guaranteed reagent grade. The solvents and buffer solutions were degassed, using an ultrasonic bath 30 minutes prior to use.

Methods

Cyclohexanol containing 0.1 mM sodium dodecyl sulfate (SDS) was used as the mobile phase of a packed capillary column. The SDS was added to increase the conductivity of the mobile phase. A cyclohexanol solution containing 0.03 mM Rhodamine-6G and 0.1 mM SDS was used as the sample solution for the observation of the pressurized flow and EOF in a rectangular capillary.

The rectangular capillary was fully filled with cyclohexanol. One reservior was filled with 0.1 mM SDS in cyclohexanol and the other with 0.03 mM Rhodamine-6G and 0.1 mM SDS in cyclohexanol. The levels in both reservoirs were kept at an equal height to avoid the generation of a pressurized flow and the capillary was kept horizontal. To draw the Rhodamine-6G from the capillary, a positive high voltage was applied first to the reservoir filled with cyclohexanol and then to the reservoir filled with Rhodamine-6G solution. The applied high voltage of 3 kV generated the EOF, and the Rhodamine-6G cyclohexanol solution was introduced into the capillary. The EOF profile of the Rhodamine-6G cyclohexanol solution was observed, using a microscope-CCD camera system and recorded on videotape. Pressurized flow profile of the same solution in this packed capillary column was then observed by lifting one end of the capillary reservoir 5 mm higher than the other end. Flow velocity (μm/sec) was calculated from the image captured through the CCD camera.

RESULTS AND DISCUSSION

A rectangular capillary was used because the flat sides would cause less distortion of the observed zone front than the more common round walls of a cylindrical capillary. The transparency property of the rectangular capillary enabled clearer observation of the particles inside the capillary column.

Rhodamine-6G in the solution can undergo the process of generating fluorescence under the experimental conditions. White light was used for excitation of the fluorescent dye at two directions having a 90 degree angle difference. In this way, the flow profile pattern of the Rhodamine-6G in solution could be observed as a progression of the liquid along the packed capillary column.

Cyclohexanol was the best solution from among several different trial solvents in this experiment. With this solvent, clearer images of the flow profile could be observed.

The homemade Miniaturization Total Analysis System (μTAS) was used to observe the flow profile in this study. With this μTAS apparatus, EOF profiles and pressurized flow profiles were easily generated without any difficulty. It was also adapted in a current research on single-cell studies. With the help of the positive applied voltage, the diffused Rhodamine-6G solution in the packed column, once inserted into the column between the reservoirs, was easily withdrawn back to the reservoir. This enabled the flow profile phenomenon to be observed from the beginning of the column.

The CCD camera with a timer recorder also enabled the image to be recorded with time and the flow velocity to be analyzed manually and directly. The flow velocity was calculated from the moving distance of Rhodamine-6G solution with time. The results are shown in Table 1. The interval between pictures was in second unit. The advance of the zone front, taking place between two pictures, can be measured from photographs by matching the positions of the stationary marks on the rectangular capillary. The EOF velocities of different section are shown in Table 1. The mean of average EOF velocity is 2.22 μm/sec. High precision of EOF velocity observations is obtained. The %RSD, five replicated observations, of EOF velocities of each section is less than 3.94. The %RSD of mean of average EOF velocities is 0.68. There are no differences among the average EOF velocity values of all sectors using the one-way analysis of variance at the 95 percent confidence interval ($F = 0.162$). This indicated that the EOF profiles of the observed solution were harmonized along the column.

Table 1. EOF velocity (μm/sec) of the advances of the zone front of EOF along the different section of capillary.

Number of section along the capillary	EOF velocity (μm/sec) of five replicated observations					Average EOF velocity (μm/sec)	% RSD of EOF velocity
	1	2	3	4	5		
1	2.12	2.23	2.28	2.15	2.31	2.22	3.68
2	2.34	2.18	2.25	2.15	2.30	2.25	3.63
3	2.09	2.15	2.18	2.25	2.30	2.19	3.77
4	2.11	2.16	2.28	2.23	2.32	2.22	3.86
5	2.24	2.13	2.33	2.26	2.15	2.22	3.70
6	2.24	2.14	2.12	2.32	2.21	2.21	2.65
7	2.25	2.31	2.18	2.13	2.21	2.22	3.09
8	2.23	2.25	2.16	2.07	2.29	2.20	3.94
9	2.19	2.12	2.31	2.25	2.29	2.23	3.48
10	2.18	2.23	2.11	2.31	2.14	2.19	3.60
11	2.22	2.29	2.11	2.31	2.16	2.22	2.82
12	2.25	2.17	2.32	2.09	2.22	2.21	3.91
13	2.29	2.20	2.33	2.12	2.24	2.24	3.64
14	2.31	2.17	2.09	2.24	2.21	2.20	3.71
15	2.18	2.13	2.16	2.24	2.34	2.21	3.76

Mean of average EOF velocity and its %RSD are 2.22 μm/sec and 0.68, respectively.

Pressurized flow profiles of the fluorinated-bonded silica capillary column are shown in Figure 2. The flow profile was parabolic. The parabolic shapes of this pressurized flow profile are different in skewness and peakedness along the section of capillary. Therefore, it was very difficult to select any representative point in order to calculate the pressurized flow velocity along the column. From rough calculation, it indicated that the flow velocity at the wall was zero and in the center approximately being twice the mean flow rate.

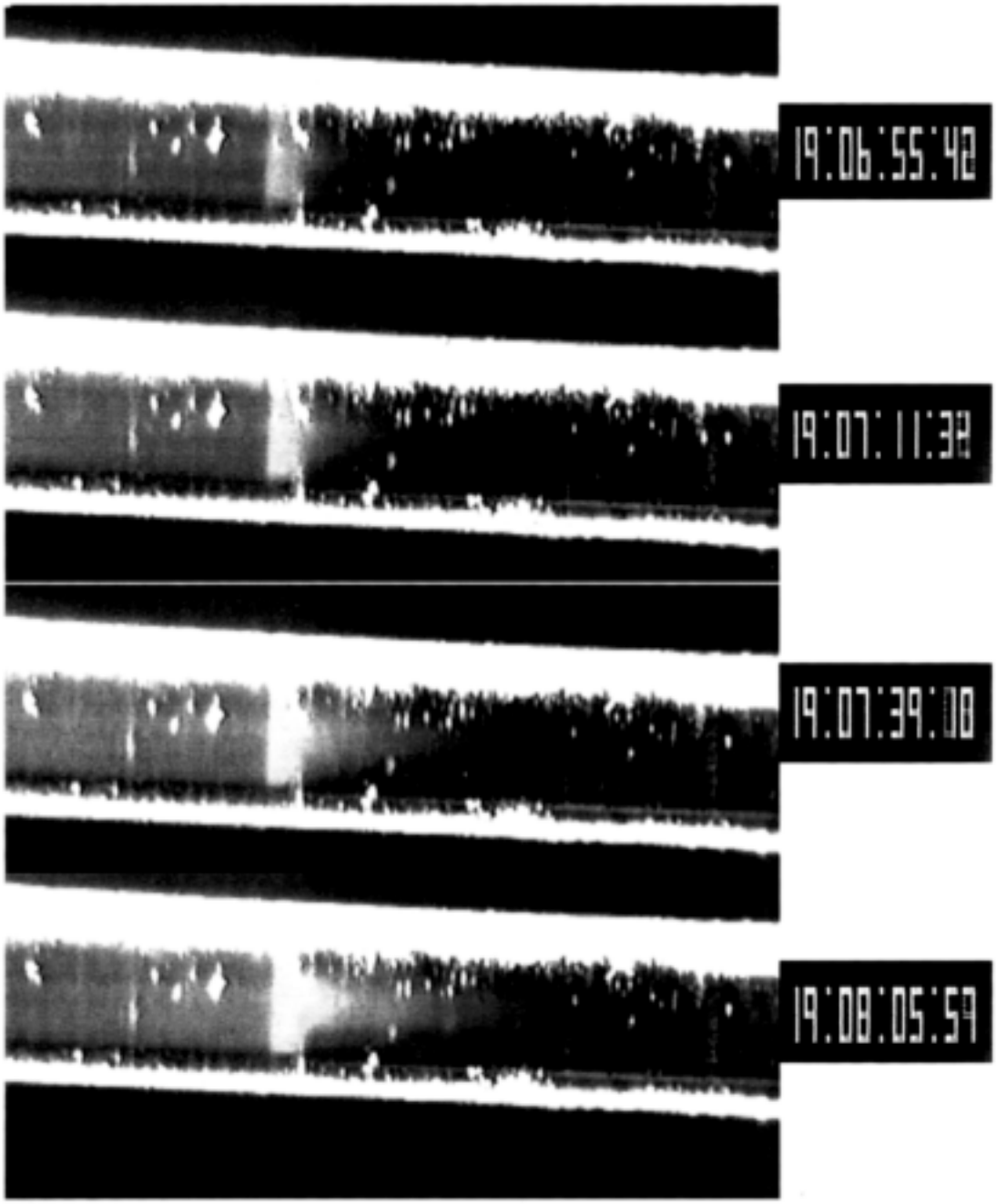

Figure 2. Photographs of the pressurized flow profiles in fluorinated-bonded silica packed capillary columns. The photograph time recorder is shown in the right corner.

EOF profiles are also shown in Figure 3. The flow profile is flat. EOF profiles differ from laminar flow profiles and the former can have a shear flow velocity only in the vicinity of the wall. In other words, the flow profile of electroosmotic flow is much flatter than that of laminar flow. This produces the very narrow peaks obtained by electrostatic driven force compared with the pressure driven force.

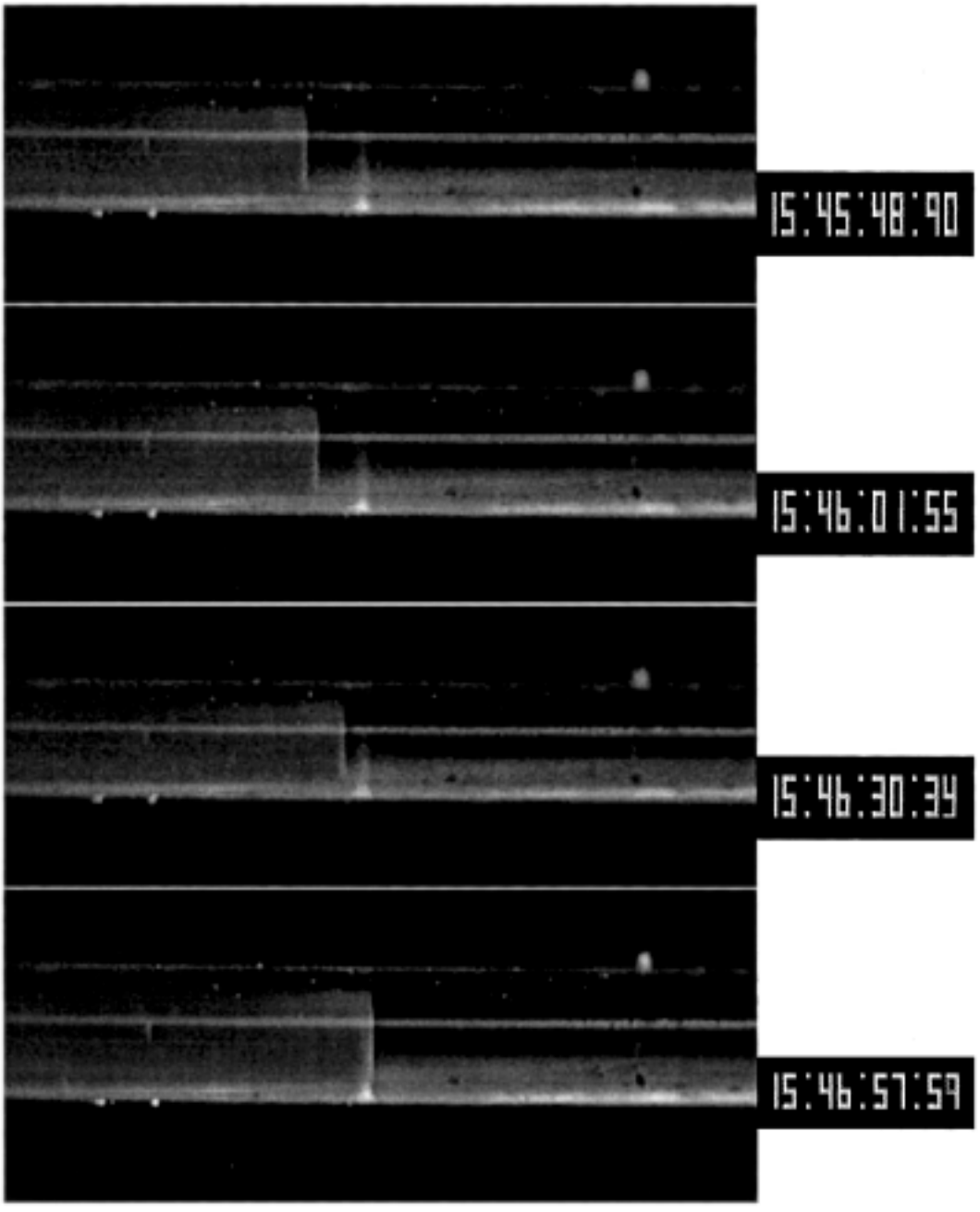

Figure 3. Photographs of the electroosmotic flow profiles (EOF) in fluorinated-bonded silica packed capillary columns. The photograph time recorder is shown in the right corner.

Pretorius et al., (1974) suggested that the EOF profile was flat except in the region of the diffused double layer near the column inner wall in an open tubular column. Tsuda et al., (1983) proposed that the EOF flow profile might be a combination of plug and Poiseuille flow as observed in the open tubular column. The EOF flow profile observed in Tsudaís experiment (Tsuda et al., 1993a, 1993b, 1995) was the reversed parabolic shape. The center part lagged behind the edges. He explained that it might be due to the pumping power requirement, enabling the solution flow to exit at the outlet of the capillary. The other explanation was the electrocharge balance requirement. The positive charges coagulated mostly at the double layer near the capillary wall. Therefore, the negative ions concentrated at the center of capillary and caused the reversed parabolic flow pattern.

In this experiment, the EOF profile in the fluorinated-bonded silica packed capillary column was different from the above-proposed EOF profile of open tubular column. Hence, EOF profile of this study was a flat flow profile. The negative charge on the liquid-solid interface of the support material are well-known mechanisms of generating EOF. It causes the flat flow profile compared with the pressurized flow generating mechanism.

From previous reports (Chaiyasut et al., 1999a), the fluorinated structure on the silica surface of the fluorinated-bonded silica has a dipole that comes from fluorine atoms. The negative charges of the fluorinated structure on the silica surface of the fluorinated-bonded silica phase occurred during applying the electric field (Chaiyasut et al., 1999c, 2000). These phenomena might have a crucial role on generating the EOF. If the negative charge occurred homogenously on the surface of the packing material in the packed column, the EOF should be simultaneously generated at nearly all regions in the capillary column. This might be an explanation of the flat flow profile phenomenon in this experiment and provided more evidence to indicate the benefits of using a fluorinated-bonded silica packed column. This can generate the flat EOF profile compared to the controversial EOF profile in the open tubular column.

The flat flow profile of EOF has the main effect in producing the narrow peak. Less band broadening results in higher efficiency in CEC and HPLCC packed with fluorinated-bonded silica. From previous studies (Chaiyasut et al., 1999a, 1999b, 1999c, 2000), fluorinated-bonded silica has the unique characteristic in chromatographic phenomena such as higher negative charges on surfaces and interaction of fluorine atoms with the analytes, resulting in unique separation parameters. The flat flow profile of this stationary phase is another beneficial characteristic of this phase.

This phase might be the stationary phase of choice in CEC technique. More research is being performed, using the fluorinated-bonded silica stationary phase with CEC and microcolumn high pressure liquid chromatography techniques.

CONCLUSIONS

The mini-electrophoresis apparatus can be used to direct observe EOF and pressurized flow profile of the fluorinated-bonded silica packed capillary column. By using rectangular capillary, the clear progression zone fronts were observed. This apparatus are being further utilized to study the electrovoltage phenomena on single cell level analysis as well as the

physiochemical properties of packed phase. In this report, observed pressurized flow profile were parabolic. It corresponded very well with previous reports. However, EOF profile in this study is flat compared to the reverse parabolic shape previously reported. The mean of average EOF velocity is 2.22 μm/sec. EOF profiles of the solution were consistence along the column with %RSD of mean average EOF velocity of 0.68. The amount of the negative charges occurred on the fluorinated-bonded silica stationary phase might contribute to the harmonization of EOF flow velocity and profile. This technique, direct measurement of the EOF was achieved. Therefore, the medium used in the EOF measurement could be quite completely the same as that used in the separation condition. EOF velocities calculated by this method would be more realistic than other indirect methods. It is the first time to direct observe the EOF profiles and pressurized flow profiles of fluorinated-bonded silica gel as a stationary phase in CEC and HPLCC. The flat flow profile of EOF is the benefit of using this phase with CEC technique.

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