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Enantiomeric separation by capillary electrophoresis with an electroosmotic flow-controlled capillary

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Abstract

Perfect control of electroosmotic flow (EOF) was achieved by dovetailing successive multiple ionic–polymer layer (SMIL) coated capillaries. The direction and magnitude of the EOF was perfectly controllable over the pH range 2–13. Zone diffusion was not observed, even if the inner wall of the dovetailed capillary was discontinuous, or if the sample zone passed through the connected part of the capillary because the RSDs of migration time, theoretical plates, symmetry factor and S/N of the marker were almost the same when seamless capillary and dovetailed capillary were compared. The dovetailed capillary was applied to cyclodextrin modified capillary zone electrophoresis. The control of the EOF enabled us to control both the resolution and the migration order of the enantiomers. The migration time was also controllable and, therefore, the best condition between separation and migration time could be determined by controlling the EOF. Partial filling affinity electrokinetic chromatography with a protein used as a chiral selector was also studied. The migration of the pseudo-stationary phase was controllable by EOF, and detection of the solute at 214 nm was possible. Therefore, the EOF-controlled dovetailed capillary has great potential to expand the application of the separation technique.

Keywords: Enantiomer separation; Electroosmotic flow; Dovetailed capillaries; Successive multiple ionic–polymer layer coated capillaries; Coated capillaries; Benzoin

1. Introduction

Capillary electrophoresis (CE) has been widely used as a separation tool because of its high separation efficiency [1]. The basic theory of the resolution (R_s) of capillary zone electrophoresis (CZE) can be explained by the following equation: [2]

$$R_s = \frac{1}{4} \cdot \sqrt{\frac{V}{D}} \cdot \sqrt{\frac{l}{L}} \cdot \frac{\Delta\mu_{ep}}{\sqrt{|(\mu_{ave} + \mu_{EOF})|}} \quad (1)$$

where V is the applied voltage, l is the effective length of the capillary, L is the total length of the capillary, D is the diffusion coefficient, $\Delta\mu_{ep}$ is the difference in electrophoretic mobilities of the two solutes, μ_{ave} is the average electrophoretic mobility of the two solutes, and μ_{EOF} is the mobility of the electroosmotic flow (EOF). The inherent EOF was positive in the direction of anode to cathode, and it was reversed to minus in the direction of cathode to anode. Eq. (1) was especially applicable for the optimization of conditions for the enantiomeric separation and control of the migration order of the enantiomers.

One way to enhance the R_s was to increase the voltage, *V*. Although a special instrument such as the ultra-high-voltage CZE system could apply voltage

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up to 120 kV [3], the voltage limitation of the commercially available CE system was about 30 kV. Hence, there was a limitation to the increase in V available in order to control the R_s. Another way to increase the R_s is by control of the EOF. The maximum R_s could be obtained when $(\mu_{ave} + \mu_{EOF}) = 0$. However, the migration time of the enantiomer would then be infinite. Therefore, if the EOF could be perfectly controlled independently of V, separation could be performed at the best balance between R_s and migration time.

The control of EOF had been investigated by such techniques as applying a radial electric potential gradient across the capillary wall [4–8], by suppressing or reversing the EOF using a permanent coating [9–11], and by suppressing or reversing the EOF by applying a dynamic coating [12,13]. However, the magnitude and the direction of the EOF over a wide pH range had not been successfully controlled. Another approach to control the EOF is to connect capillaries which have a different zeta potential. The EOF of the connected capillary could be altered by the length of each segment and the intrinsic EOF of each capillary. Although this concept was first developed by Nashabeh and El Rassi [14,15], the controllable range of the EOF was narrow because the capillaries they connected were an uncoated capillary and a polyether-coated capillary. The controllable pH range and the magnitude of the EOF were dependent on the EOF of the uncoated capillary.

In this study, we set out to achieve perfect control of the EOF by connecting the coated capillary which was produced by a procedure of successive multiple ionic–polymer layers (SMIL), developed by our group [16–18] and named by us a ''dovetailed capillary''. The SMIL capillary had a pH-independent EOF over the pH range 2–13 from anode to cathode when an anionic dextran sulfate modified capillary (SMIL-DS) was produced, and from cathode to anode when a cationic polybrene modified capillary (SMIL-PB) was produced. The coating on the inner wall of the capillary was very durable, and it was tolerant of either acidic or alkaline solution. Therefore, the direction and the magnitude of the EOF would be perfectly controllable if the SMIL capillary was dovetailed.

The EOF-controllable dovetailed capillary was then applied to enantiomeric separation. Although there were many applications of enantiomeric separation by CE [19–22], perfect control of resolution had not been achieved. Reversal of the EOF could perform the reversal of the migration order [23]. It is important to control the migration order because the accuracy and limit of quantitation (LOQ) of the minor enantiomer were improved when it was detected before the major enantiomer. This was realized by changing from separation mode cyclodextrin-modified capillary zone electrophoresis (CD–CZE) to cyclodextrin-modified micellar electrokinetic chromatography (CD–MEKC) in our study [24]. Another way of reversing the migration order is by charged CD [25,26] and other methods which were classified in our previous paper [24].

The perfect control of the EOF made possible by dovetailing capillaries has the potential to control the resolution, migration time and the migration order of the enantiomer because the SMIL capillary could generate the EOF over a wide pH range. In this study, we will demonstrate the feasibility of controlling the EOF in this way.

2. Experimental

2.1. Reagents and materials

Polybrene (PB) (Aldrich, Milwaukee, WI, USA) was used as a cationic coating reagent, and dextran sulfate (Sigma, St. Louis, MO, USA) was used as an anionic coating reagent. An EOF marker, formamide, was obtained from Pharmacia Biotech (Uppsala, Sweden). The cationic compound chlorprenaline was synthesized at Eisai (Ibaraki, Japan). 2,6-Di-*O*-methyl-β-CD (DMCD) (Nacalai, Kyoto, Japan) was used as a chiral selector for the enantiomeric separation of chlorprenaline. Benzoin was obtained from Tokyo Kasei (Tokyo, Japan). Flavoprotein was purified from chicken eggs [27]. Other reagents were analytical grade.

2.2. Apparatus

A Beckman P/ACE 2100 (Fullerton, CA, USA) was used for the CE system. An uncoated fused-silica capillary of 50 μm I.D.×365 μm O.D. was

obtained from Polymicro Technology (Phoenix, AZ, USA). A neutral capillary, μSIL DB-WAX, was obtained from J&W Scientific (Folsom, CA, USA). The SMIL-PB and SMIL-DS capillaries were prepared by the method of successive multiple ionic–polymer layer coating [16–18]. The SMIL coating was the permanent coating in which the mechanism was based on the physical adsorption. The opposite charge of ionic polymers was successively adsorbed on the inner wall of the capillary. The dovetailed capillary was jointed using a PTFE sleeve obtained from LC Packings (San Francisco, CA, USA).

2.3. Procedure

Before each analysis, all the buffer solutions were passed through a 0.45 μm membrane filter. The capillaries were washed with a buffer solution for 3 min before each run. The samples were injected under pressure. The EOF was controlled using a dovetailed capillary. The total length of the capillary was fixed, and the ratio of each segment was varied. The electric field was fixed to 300 V/cm.

3. Results and discussion

3.1. The control of EOF by dovetailing capillaries

When each capillary segment that had a different magnitude and direction of EOF was dovetailed, the EOF of the united capillary could be described as follows: [14]

$$\mu_{\mathrm{EOF}} = \mu_{\mathrm{EOF}-d} \cdot \frac{L_d}{L_d + L_c} + \mu_{\mathrm{EOF}-c} \cdot \frac{L_c}{L_d + L_c} \qquad (2)$$

where L_d is the length of the detection capillary which had a detection window, L_c is the length of the EOF control capillary which had no detection window, $\mu_{\mathrm{EOF}-d}$ is the EOF of the detection capillary, and $\mu_{\mathrm{EOF}-c}$ is the EOF of the EOF control capillary. In this study, $L_d/(L_d+L_c)\cdot 100$ (%) was defined as the dovetail ratio, and the EOF of the capillaries that had various dovetail ratios was studied.

The EOF of the capillaries we prepared to produce the dovetailed capillary is shown in Fig. 1a. The SMIL-DS capillary and the uncoated capillary had the EOF direction from anode to cathode. For a pH range above 4.5, the EOF of the uncoated capillary was faster than for the SMIL-DS capillary, while the SMIL-DS capillary had faster EOF below pH 4.5. On the other hand, when the SMIL-PB capillary was employed, the EOF was pH-independent from cathode to anode. Within the range of the EOF of these three capillaries, the EOF could be controlled without changing the electric field when the dovetail technique was used. The control of the EOF at pH 2.7 from anode to cathode and cathode to anode is shown in Fig. 1b and c, respectively, as an example. This time, a neutral capillary was used as the EOF control capillary. The EOF of the neutral capillary was assumed to be 0 ($m^2\ V^{-1}\ s^{-1}$), and hence, the predicted value of the EOF could be obtained by multiplying the dovetail ratio by $\mu_{\mathrm{EOF}-d}$. The observed values of EOF had good linearity ($r=0.999$). In addition, the observed values agreed well with the predicted values. Those results demonstrated that the direction and the magnitude of the EOF was controllable over a wide pH range without changing the electric field when the uncoated, the SMIL-DS and the SMIL-PB capillaries were dovetailed.

3.2. Effect of dovetailing the capillary to sample zone

The effect of dovetailing capillaries to the sample zone should be discussed. An EOF marker, formamide, was used for the evaluation of the zone diffusion. The dovetail ratio was adjusted to 65% by dovetailing uncoated and neutral capillaries, where the migration time of EOF was almost the same as that of the seamless SMIL-DS capillary at pH 7. The result are shown in Table 1. Good reproducibility of migration time from both capillaries was obtained. The theoretical plates, symmetry factor and S/N of the dovetailed capillary were almost the same when compared with the seamless capillary. Although the simulation of the non-uniform zeta potential showed significant zone dispersion [28], the sample zone of the actual experiment was not affected by the discontinuous inner wall of the capillary.

3.3. Control of the resolution and migration order in CD–CZE

The control of EOF by the dovetailed capillary

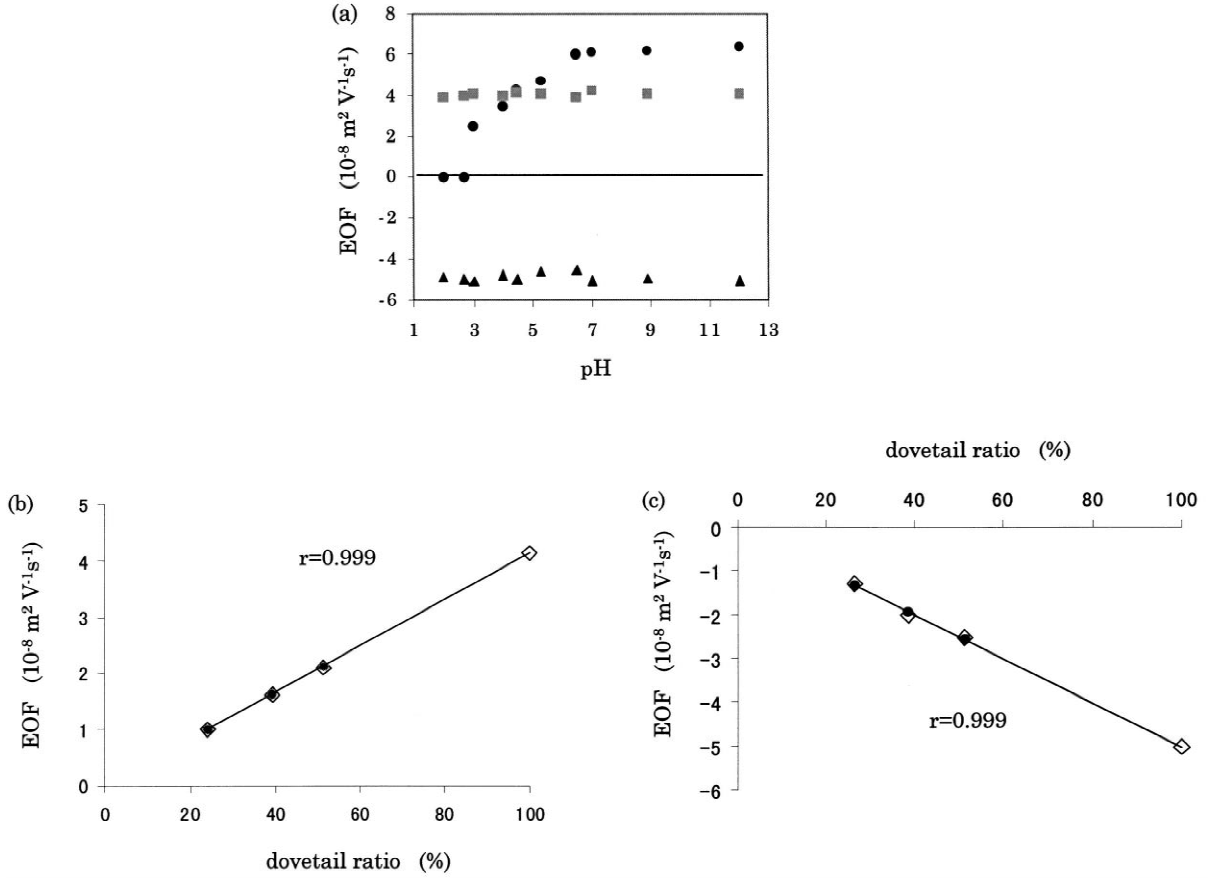

Fig. 1. Control of EOF. (a) EOF of uncoated (●), SMIL-DS (■) and SMIL-PB capillaries (▲). (b) EOF control from anode to cathode at pH 2.7 (detection capillary: SMIL-DS, EOF control capillary: Neutral). (◊) Observed value, (●) predicted value. (c) EOF control from cathode to anode at pH 2.7 (detection capillary: SMIL-PB, EOF control capillary: Neutral). (◊) Observed value, (●) predicted value. Conditions: Detection, 214 nm; electric field, 300 V/cm; buffers, phosphate buffer at pH 2–3 ($I=0.05$), acetate buffer at pH 4–5 ($I=0.05$), phosphate buffer at pH 6–7 ($I=0.05$) and borate buffer at pH 8–13 ($I=0.05$); capillary 30 cm (23.5 cm effective length)×50 μm I.D., the sample was injected from EOF control capillary.

Table 1
Effect of dovetailing capillaries on sample zone at pH 7 ($n=5$)

	Dovetail ratio[a] (%)	RSD of t_{EOF}[b] (%)	Theoretical plates[c] (m^{-1})	Symmetry factor[d]	S/N
Seamless capillary (SMIL-DS)	100	0.6	129 000	1.18	13
Dovetailed capillary (uncoated/neutral)	65	0.7	122 000	1.12	12

[a] Dovetail ratio (%)$=L_d/(L_d+L_c)\times 100$.
[b] Migration time of the EOF.
[c] Theoretical plates$=5.54\ (t/W_{0.5})\ t_{EOF}$, migration time. $W_{0.5}$, width of the peak at the half peak height.
[d] Symmetry factor$=W_{0.05}/(2f)$. $W_{0.05}$, width of the peak at 1/20 of the peak height. f, distance between the perpendicular from the peak height.

was applied to CD–CZE analyses. The ($\mu_{EOF} + \mu_{ave}$) was especially important for the control of the resolution and the migration order when the neutral CD was used as the chiral selector. This means that when μ_{EOF} was reversed and ($\mu_{ave} + \mu_{EOF}$)>0 became ($\mu_{ave} + \mu_{EOF}$)<0, the migration order of the enantiomers was reversed. If the enantiomer was partially separated, an R_s value greater than 1.5 could be absolutely achieved by adjusting the ($\mu_{ave} + \mu_{EOF}$) value close to 0. Therefore, perfect control of the EOF by dovetailed capillary can govern both the resolution and the migration order of the enantiomers by controlling the ($\mu_{ave} + \mu_{EOF}$) value.

The cationic compound chlorprenaline was selected as a test sample in order to demonstrate the above theory. First, 100% neutral capillary (dovetail ratio 100%) was used in order to perform ($\mu_{EOF} + \mu_{ave}$)> 0 separation. The result is shown in Fig. 2a. The sample was injected from the anode, and the (−)-enantiomer migrated faster than the (+)-enantiomer. The baseline separation was achieved, and the tailing was suppressed because the inner wall of the capillary was coated by the neutral polymer.

Next, the migration order of the enantiomers should be discussed. The separation was performed by cationically coated SMIL-PB capillary (dovetail ratio 100%) in order to reverse EOF and avoid the interaction between the cationic solutes and the capillary wall. The polarity was reversed, and the sample was injected from the cathode. This separation was classified as the ($\mu_{EOF} + \mu_{ave}$)<0 separation. The result is shown in Fig. 2b. The (+)-enantiomer migrated faster than the (−)-enantiomer, and the migration order of the enantiomers was reversed from Fig. 2a. However, baseline separation was not observed. When the electric field was applied up to 500 V/cm, baseline separation was still not achieved (data not shown). Therefore, we controlled the EOF to 50% (dovetail ratio 50%) using the dovetailed capillary without changing the electric field, in order to achieve baseline separation in the shortest time. The result is shown in Fig. 2c. The resolution was improved and the baseline separation was achieved. Therefore, efficient separation could be performed under both modes, and the best conditions of migration time and resolution could be determined by controlling EOF.

These data showed the feasibility of controlling the separation and migration order using a perfectly EOF-controlled dovetailed capillary.

3.4. Application of the dovetailed capillary to enantiomeric separation by partial filling affinity electrokinetic chromatography (EKC)

Proteins, antibiotics and many other chiral micelles are known to be useful as chiral selectors for enantiomeric separation, but the drawback of affinity EKC is the high UV background of the pseudo-stationary phase. For example, when the ovomucoid was used as a chiral selector by Ishihama et al. [29], the concentration of the chiral selector had to be compromised to a lower concentration level than the concentration that could achieve best separation because of the UV absorption of the chiral selector. In order to overcome this problem, the partial filling technique was developed. It could avoid the effect of the UV background by adjusting the plug of the pseudo-stationary phase before the detection window [30,31]. However, the problem still exists because migration time and the resolution are not controllable, because in most cases, the neutral capillary was used in order to avoid the adsorption of the pseudo-stationary phase to the capillary wall.

In this study, the dovetailed capillary was applied to partial filling affinity EKC for the separation of the neutral compound, benzoin. Flavoprotein was used as a chiral selector. Flavoprotein (M_r 32 000–36 000, isolectric point, pI 3.9–4.1) is the protein which was first used as a chiral selector by Mano et al. [27] in CE. A phosphate buffer of pH 7 (Ionic strength, I=0.01) was selected as the buffer condition. At this pH, the pseudo-stationary phase migrated from cathode to anode. If conventional affinity EKC or partial filling affinity EKC was performed in this condition with the neutral capillary, the effect of the UV background could not be avoided. On the other hand, if an adequate magnitude of EOF could be generated to the opposite direction of the electrophoretic mobility of the pseudo-stationary phase, partial filling affinity EKC without disturbance of the background would be possible. Therefore, we intended to move the sample zone in the opposite direction of the electrophoretic mobility of the pseudo-stationary phase by controlled EOF by neutral and SMIL-DS dovetailed capillary,

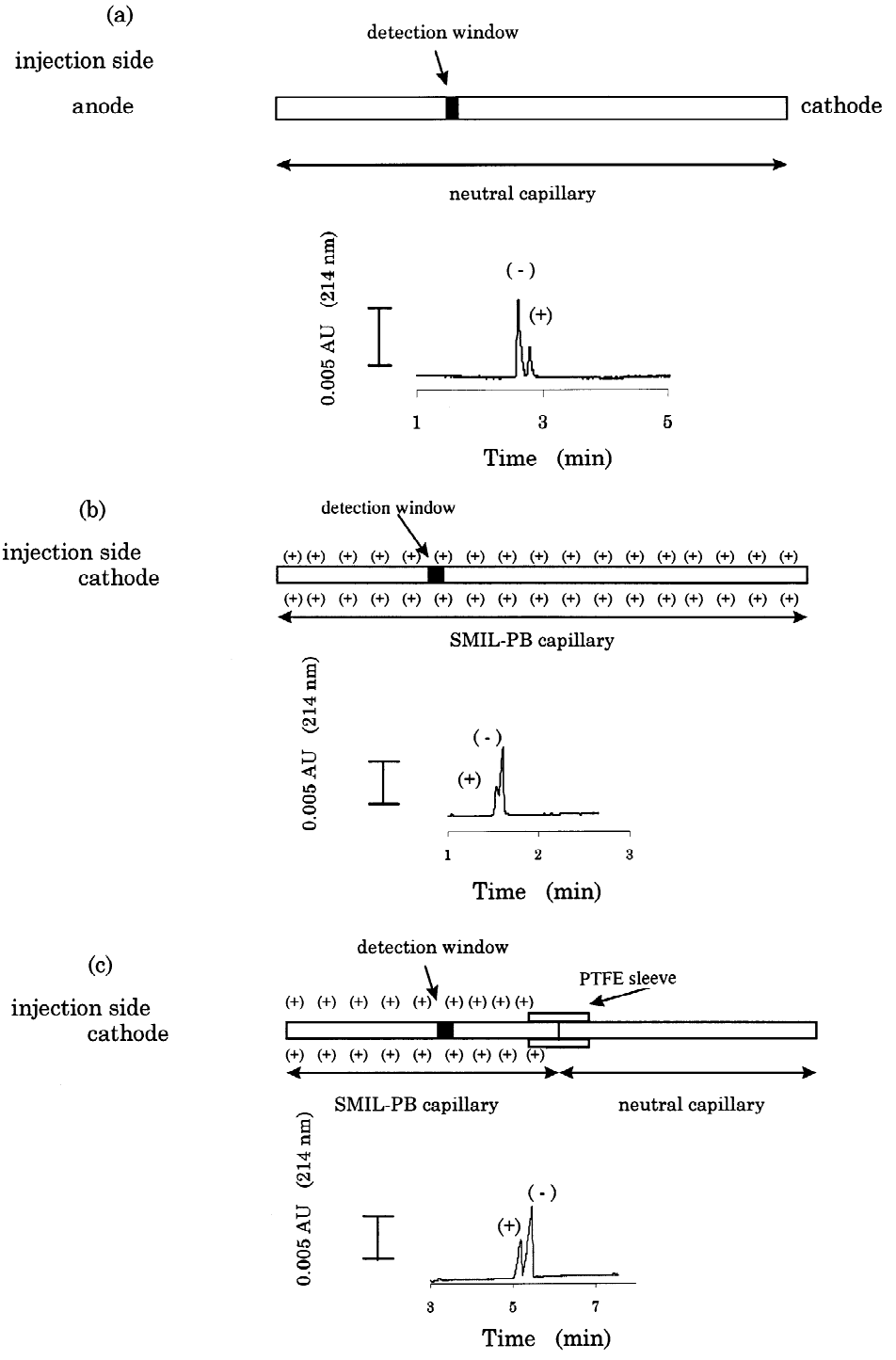

Fig. 2. Control of the resolution and the migration order of the enantiomers. (a) $(\mu_{EOF} + \mu_{ave}) > 0$ separation, dovetail ratio 100%. (b) $(\mu_{EOF} + \mu_{ave}) < 0$ separation, dovetail ratio 100%. (c) $(\mu_{EOF} + \mu_{ave}) < 0$ separation, dovetail ratio 50%. Conditions: Detection, 214 nm; electric field, 300 V/cm; buffers, phosphate buffer at pH 2.7 ($I = 0.05$); capillary 30 cm (23.5 cm effective length)×50 μm I.D., the sample was injected from the detection capillary.

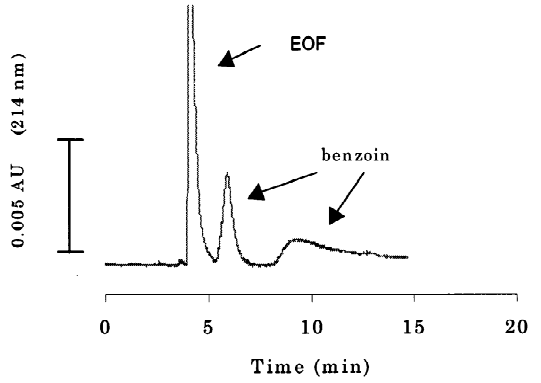

Fig. 3. Partial filling affinity EKC of benzoin by flavoprotein used as a pseudo-stationary phase. Conditions: Detection, 214 nm; electric field, 300 V/cm; buffers, phosphate buffer at pH 7.0 ($I=0.01$), capillary 35 cm (28 cm effective length)×50 μm I.D., (detection capillary: Neutral 25 cm, EOF control capillary: SMIL-DS 10 cm), the sample was injected from the EOF control capillary. Injection procedure: Flush phosphate buffer pH 7.0 ($I=0.01$) for 3 min, and next, inject 200 μ*M* of flavoprotein dissolved in phosphate buffer pH 7.0 ($I=0.01$) for 3 s prior to the 2 s injection of benzoin dissolved in water–methanol–dimethyl sulfoxide (4:4:2).

in order to achieve efficient separation in a reasonable time, avoid the influence of the background and avoid the absorption of the protein to the capillary wall.

The dovetailed capillary was prepared from the SMIL-DS and the neutral capillary. The result is shown in Fig. 3. Benzoin was detected at 214 nm without the interference of the UV background of the pseudo-stationary phase, and enantiomeric separation was achieved.

These data showed the effectiveness of controlling EOF for partial filling affinity EKC as well as CD–CZE.

4. Conclusions

Perfect EOF control was achieved using the dovetailed capillary without changing the electrical field. Zone diffusion could not be observed even if the inner wall of the capillary was discontinuous, and the EOF could be adjusted by changing the length of the different SMIL coated capillaries.

This dovetailed capillary was applied to CD–CZE. The resolution was controllable by adjusting the EOF using a dovetailed capillary. In addition, the migration order of the enantiomer could be reversed when $(\mu_{ave}+\mu_{EOF})>0$ was turned to $(\mu_{ave}+\mu_{EOF})<0$.

The partial filling affinity EKC was performed by controlling EOF toward the opposite direction to the pseudo-stationary phase in order to separate the racemic mixture of benzoin. Detection was possible at 214 nm, and good separation was obtained in a reasonable time. This technique would also be applicable to capillary electrophoresis–mass spectrometry (CE–MS) because the control of pseudo-stationary phase migration by EOF would avoid the introduction of chiral selectors to MS, which can be the cause of signal suppression. These results and potential applications show the usefulness of the EOF-controlled dovetailed capillary.

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