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Sensitivity enhancement effect in off-column detection for capillary electrophoresis

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Abstract

The sensitivity enhancement effect in off-column detection for capillary electrophoresis was investigated. A capillary is divided into a separation and coupling capillaries by an on-column fracture coated with cellulose acetate porous polymer. The electroosmotic flow produced in the separation capillary acts as a pump and makes the buffer fluid flow through the coupling capillary. In the separation capillary, sample ions are induced to proceed not only by the buffer fluid flow but also by electrophoretic migration. On the other hand, in the coupling capillary, ions move only by the buffer fluid flow. The difference in the migration velocity of the sample ions between the separation and coupling capillaries results in concentration (for a cationic sample) or dilution (for an anionic sample) of the sample zone after passing through the fracture. Two cation dyes, rhodamine B and safranine O, were used as model samples. An off-column absorbance detection method with a 542.8-nm He–Ne laser as light source was used for demonstration of the sensitivity enhancement effect. The maximum concentration factor observed in the experiments was about 4.7 for Safranine O. The factors affecting the enhancement factor in off-column detection with a practical conductive joint are discussed.

Keywords: Capillary electrophoresis; Sensitivity enhancement; Detectors, electrophoresis; Rhodamine B; Safranine O; Dyes

1. Introduction

Detection methods developed for capillary electrophoresis (CE) can be mainly divided into two types, on-column and off-column detection [1]. In on-column detection methods, such as UV absorbance and laser-induced fluorescence detection, part of separation capillary is used directly as a detection cell, and thus the detector has no influence on the separation efficiency of CE. However, the high separation electric field used in CE usually interferes with the detection in some other detection modes such as electrochemical methods [2–4]. A mass spectrometric method for CE is impossible with the on-column mode [5–7]. Therefore, these detection methods are usually performed with an off-column configuration. In off-column detection in CE, a coupling capillary is usually connected to the separation capillary by a conductive joint so as to

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isolate the detector from the high electric field. Analytes are first separated in the separation capillary, then transported through the coupling capillary towards the detector. Also for post-columnn derivatization, to improve the detectability of the separated compounds, an off-column system is used.

Most studies on off-column detection for CE have concentrated on the development of an effective conductive joint, improvement of the separation efficiency and its application to various samples. Many conductive joints, such as on-column fracture [8,9], porous joints [10,11] and solid-state field decouplers [12], have been developed and applied to the analysis of real samples. The decrease in separation efficiency with off-column detection systems and methods for its improvement [3,10] have also been investigated. In this paper, we report on the sensitivity enhancement effect in off-column detection for CE. The study was carried out using an off-column UV absorbance detector, but the conclusions are also valid for other off-column detectors.

2. Experimental

Fig. 1 illustrates the experimental set-up. A high-voltage power supply (Glassman High Voltage) provided the separation voltage. A 25-cm fused-silica capillary (100 μm I.D. $\times$ 360 μm O.D) with an on-column fracture coated with cellulose acetate porous polymer was used. The

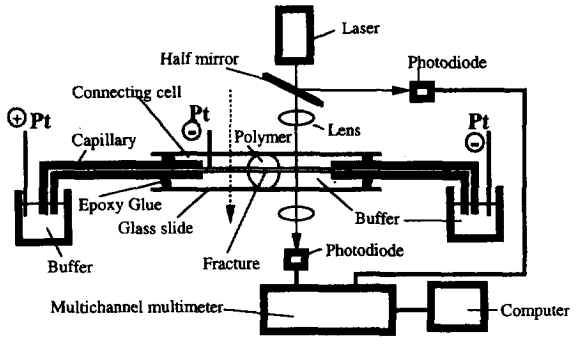

Fig. 1. Illustration of the experimental set up.

left-hand part of the capillary in Fig. 1 (between the +-electrode and the fracture) was used as the separation capillary; its length was 15 cm. The right-hand part of the capillary was 10 cm in length and was used as the coupling capillary, where no electric field existed in the experiments. The cellulose acetate porous polymer-coated fracture was made according to the method described by Whang and Chen [8]. Two glass microscope slides were used for sandwiching the fracture, and were fixed with an epoxy glue so as to form a connecting cell. The connecting cell was mounted on an X–Y–Z stage to adjust the location at which laser light was passed through. The connecting cell was also filled with buffer solution. The electrodes immersed in the connecting cell and vial 2 were connected to the ground end of the power supply. The polyimide coating around the fracture was burned off so that light could pass through the capillary.

A He–Ne laser (power 0.5 mW, wavelength 542.8 nm) was used as a light source. The laser light was divided into signal and reference beams by a half-mirror. The intensity of the reference beam was monitored by a photodiode. The signal beam was focused to the capillary by a lens with a focal length of 10 mm. After passing through the capillary, the signal beam was focused on another photodiode. The signals from the two photodiodes were input into a multi-channel digital multimeter (Iwatsu) and converted into the absorbance signal. The absorbance data were recorded by a personal computer.

The buffer solution was 2 mM sodium acetate–acetic acid (pH 4.4). To the buffer solution, the cationic surfactant cetyltrimetylammonium bromide (CTAB) with a concentration from 0 to $3 \cdot 10^{-5}$ M was added to suppress the electroosmotic flow [13]. The model samples rhodamine B and safranine O, which absorb the laser light, were dissolved in the buffer solution. Their concentration were $1 \cdot 10^{-4}$ and $5 \cdot 10^{-5}$ M, respectively. The model samples were injected into the capillary by a gravity flow injection method (height 10 cm, injection time 10 s).

The signal beam was first passed through the separation capillary about 0.5 cm from the fracture, as shown by the dotted arrow in Fig. 1. The

electropherograms of the model samples were obtained by applying a separation voltage of 15 kV. Second, the signal beam was passed through the coupling capillary, about 0.5 cm from the fracture. Electropherograms of the model samples were obtained under the same conditions as for the separation capillary, and were compared.

In the experiments on the determination of electroosmotic flow and electrophoretic migration velocities, a UV detector was used as reported previously [14]. Acetone was used as a model sample for the determination of the electroosmotic flow velocity.

3. Results and discussion

In the off-column detection mode for CE, while electroosmotic flow is produced in the separation capillary, no electroosmotic flow exists in the coupling capillary since no electric field exists in it. The electroosmotic flow in the separation capillary acts as a pump and causes transport of buffer fluid through the coupling capillary. The flow velocity v of the buffer fluid depends on the electroosmotic flow and length ratio of the separation capillary to coupling capillary [3]:

$$v = v_{eo}[l/(l + l')] \quad (1)$$

where v_{eo} is the electroosmotic flow produced in the separation capillary, and l and l' are the length of the separation and coupling capillaries, respectively.

In the separation capillary, a sample zone moves with a velocity $v \pm v_{ep}$ (v_{ep} is electrophoretic migration velocity of the sample, + is for a cationic sample and − is for an anionic sample; the meanings of + and − below are the same). When the sample zone passes through the fracture, its migration velocity becomes v, since no electric field exists and thus v_{ep} of the sample in the coupling capillary is zero.

For an ideal conductive joint through which buffer ions could pass while sample ions could not, this difference in the migration velocities between the separation and coupling capillaries results in concentration (for a cationic sample) or dilution (for an anionic sample) of the sample zone after passing through the fracture. This means that the detection sensitivity for a cationic sample will be enhanced and that for an anionic sample decreased in the coupling capillary, compared with that in the separation capillary.

If the length of the sample zone in the separation capillary is L, the time required to pass through the fracture for the whole sample zone is

$$t = L/(v \pm v_{ep}) \quad (2)$$

During this period, the front of the sample zone will move to a distance L' in the coupling capillary:

$$L' = tv = L[v/(v \pm v_{ep})] \quad (3)$$

L' is the length of the sample zone after passing through the fracture. Eq. 3 shows that the sample zone is stacked by a factor of $v/(v \pm v_{ep})$ in the coupling capillary. This means that the concentration of the sample zone is increased by a factor of $(v \pm v_{ep})/v$, i.e., the detection sensitivity in the coupling capillary is enhanced by a factor of $(v \pm v_{ep})/v$ compared with that in the separation capillary. Here, we refer to this factor as the concentration factor or enhancement factor α:

$$\alpha = (v \pm v_{ep})/v = 1 \pm (1 + l'/l)(v_{ep}/v_{eo}) \quad (4)$$

However, for a practical conductive joint, the loss of sample ions on passing through the joint must be considered since not only buffer ions but also sample ions permeate through the conductive joint. The permeabilities of sample ions through the joint depend on the properties of the joint, the composition and concentration of the buffer solution, the electric current in the electrophoresis process and other factors. Another loss of sample ions occurs via adsorption on the joint. Therefore, the practical enhancement factor α_p is given as

$$\alpha_p = 1 \pm (1 + l'/l)(v_{ep}/v_{eo}) - \beta \quad (5)$$

where β is a parameter characterized by per-

meability and adsorption of sample ions in the joint. Eq. 5 shows that the practical enhancement factor for off-column detection depends greatly on β, i.e., permeability and adsorption of sample ions in the practical joint. Eq. 5 suggests that selective detection might be possible by designing a selective joint, for which some samples are permeable while others are not.

For the cellulose acetate porous polymer-coated fracture, which was used as a joint in these experiments, an accurate value of β is unknown at present because of the lack of the fundamental data on permeabilities and adsorption of samples. However, this joint has been reported to show little permeation for samples such as thiamine [8]. In a preliminary experiment, the loss due to the permeation and adsorption in the joint was also found not to be great for the model samples rhodamine B and safranine O. Therefore, we use it for demonstration of the sensitivity enhancement in off-column detection. Fig. 2A and B show the electropherograms obtained in the separation and coupling capillaries, respectively. Obviously, the absorbance of safranine O is enhanced when detected in the coupling capillary. On the other hand, rhodamine B is hardly enhanced. This is because v_{ep} of rhodamine B is much smaller than v_{eo}, as the concentration factor α is nearly 1 according to Eq. 4. Therefore, the sensitivity enhancement effect in off-column detection is particularly large for ions with large electrophoretic mobilities.

Although the sample zones of safranine O and rhodamine B are stacked by a factor of $v/(v + v_{ep})$ in the coupling capillary, the obtained peak width and interval between the peaks in the coupling capillary do not change compared with those in the separation capillary. This is because the migration velocity of the sample zone decreases by a factor of $v/(v + v_{ep})$ in the coupling capillary. The broad peaks and lower theoretical plate number in Fig. 2 are mainly due to the long coupling capillary used in the experiment, which results in a laminar flow and broadening of the peak [3,10]. Another reason is adsorption of the cationic dye sample on the capillary surface. Despite the lower theoretical plate numbers from the electropherograms, the demonstration of the sensitivity enhancement effect, which is the objective of these experiments, is still successful. The theoretical plate numbers can be improved by decreasing the length of the coupling capillary [3,10]. Recently, a pressure compensation technique has also been proposed to improve the decrease in theoretical plate number and separation efficiency of off-column detection for CE [3].

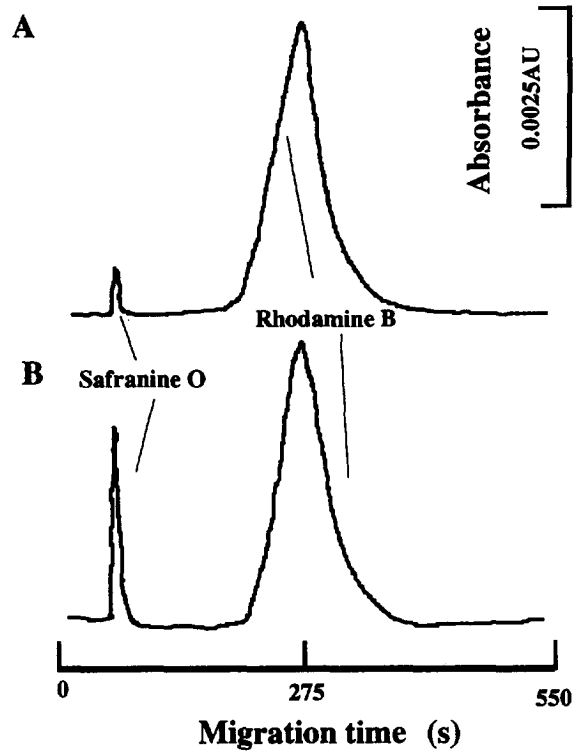

Fig. 2. Electropherograms for the model samples obtained in (A) the separation and (B) the coupling capillaries.

Fig. 3 shows the relationships between the enhancement factor and the velocity of electroosmotic flow, which is adjusted by changing the concentration of cationic surfactant CTAB added to the buffer solution [13]. The theoretical results are calculated from Eq. 4 by using v_{eo} and v_{ep}, which are determined by another experiment with the use of the UV detector. Experimental results for α are calculated from either the peak area or peak height. It is clear that the enhance-

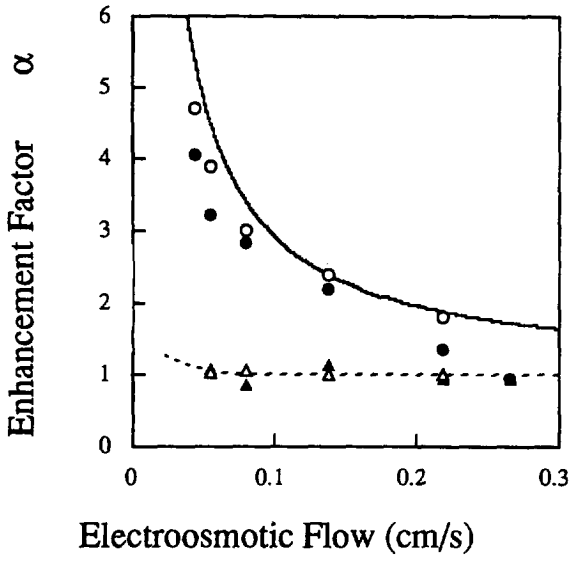

Fig. 3. Relationship between enhancement factor and electroosmotic flow velocity. The theoretical results are calculated according to Eq. 4. Electrophoretic migration velocities v_{ep} are 0.19 and 0.01 cm/s for safranine O and rhodamine B, respectively. Solid and dotted lines are calculated results for safranine O and rhodamine B, respectively. Experimental results obtained from peak height for (●) safranine O and (▲) rhodamine B and those obtained from peak area for (○) safranine O and (△) rhodamine B.

ment factor for cationic samples increases with decrease in the electroosmotic flow velocity.

The difference in the theoretical and the experimental values of α obtained from the peak area in Fig. 3 might correspond to β in Eq. 5, i.e., loss due to the permeation and adsorption of the model samples in the joint. This suggests that it could be used to evaluate the permeation and adsorption of a sample in a joint, about which little has been reported. Fig. 3 also shows that the enhancement factors obtained from peak height are smaller than those from peak area. Adsorption of samples on the joint, and diffusion of the sample zone along the axis direction of the capillary might contribute to the difference. The diffusion of the sample zone leads to a decrease in peak height, but the peak area should not change. The smaller the volume injected into the capillary, the smaller are the effect of diffusion and the broadening estimated. In the demonstration experiments, a large volume of sample was injected so that good reproducibility could be obtained. However, in real experiments, a small sample volume should be injected.

For the anionic samples, as indicated in Eqs. 3 and 4, the length of the sample zone will be increased in the coupling capillary and the enhancement factor is <1. Therefore, the sensitivity will be decreased for anionic samples with off-column detection. For the case where the electroosmotic flow was reversed (from the − to the +-electrode) by adding some reagents [14], the sensitivity enhancement would be observed for anionic rather than cationic samples. However, it is impossible to enhance the sensitivity for cationic and anionic samples simultaneously in off-column detection of CE.

It should be noted that although the concentration effect occurs in the coupling capillary for off-column detection, the sensitivity enhancement only exists with concentration-sensitive detectors such as a UV absorbance detector. For mass-sensitive detectors such as a mass spectrometer, the sensitivity enhancement does not exist. Also, the zone concentration in off-column detection is compared with that in on-column detection, and thus the sensitivity enhancement in off-column detection is compared with that in on-column detection. This is different from those selective sample preconcentration techniques [15–21] using discontinuous buffer systems in the sample preconcentration and the electrophoresis processes, where the concentration is compared with the sample solution.

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