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Short communication

On-line sample preconcentration of cationic analytes by dynamic pH junction in capillary electrophoresis

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Abstract

To improve detection sensitivity of cationic analytes, a dynamic pH junction technique was examined. Dynamic pH junction is an on-line focusing method in capillary electrophoresis (CE) based on the difference in the analyte's mobility between the background electrolyte (BGE) and sample matrix. The effects of pH values and concentrations of the BGE and the sample matrix on dynamic pH junction were examined. Optimization of analyte focusing resulted in enhanced detection responses of about 100–160-fold in terms of peak heights for some anilines in comparison to conventional injections. In particular, the concentration limits of detection (LOD) (S/N = 3) for the test anilines obtained with dynamic pH junction were from 1.9 to 3.7 ppb with UV detection without any pretreatment procedure.

Keywords: Dynamic pH junction; Sample handling; Anilines

1. Introduction

In recent years, capillary electrophoresis (CE) has been developed as a powerful separation and analysis technique for complex mixtures. Its advantages include high efficiency, small sample requirements, short analysis time, and wide application range. One of the disadvantages of UV detection in CE is the low concentration sensitivity resulting from the inherently small dimensions of the capillary and the small sample volume injected. Currently, overcoming this low concentration sensitivity has been the objective of many investigations. On-line sample preconcentration is known to be an effective approach for enhancement of concentration sensitivity, since the preconcentration step is performed within the same capillary used for separation. Four of the most widely accepted on-line sample preconcentration techniques are sample stacking [1–8], sweeping [9–15], transient isotachophoresis (t-ITP) [16–20], and dynamic pH junction [21–28].

Sample stacking is one of the simplest of these techniques in capillary zone electrophoresis (CZE) [1,2]. It has been widely used for the analysis of anions or cations alone or in mixtures [2–6]. Sweeping, which was originally developed for micellar electrokinetic chromatography (MEKC) [9], has been demonstrated to be a useful method to improve detection sensitivity of CE [10–13]. This technique has been extended to CZE separation of neutral solutes involving complexation with borate [14,15]. t-ITP is based on the use of

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at least two electrolytes of different mobilities, such as a leading electrolyte and a terminating electrolyte, to focus the analytes that possess intermediate mobilities [16–20].

Dynamic pH junction is a focusing method based on the difference in the analyte's ionization and mobility in a multi-section electrolyte system [21–28]. Dynamic pH junction of several weakly acidic and zwitterionic analytes has been shown to enhance concentration sensitivity approaching limit of detection at nanomolar levels even when using UV detection [21,22]. The influence of buffer pH, buffer co-ion and ionic strength (conductivity) in sample solution relative to background electrolyte (BGE) on analytes focusing were extensively examined. Recently, a combination of dynamic pH junction and sweeping, dynamic pH junction-sweeping, has been reported to permit the analysis of picomolar flavin metabolites in biological samples using laser-induced fluorescence detection [27,28].

In this paper, to improve detection sensitivity of some cationic analytes, a dynamic pH junction is examined. The effects of pH and concentration of BGE and sample matrix are discussed.

2. Dynamic pH junction model

Fig. 1 illustrates a dynamic pH junction model based on the results of experiments. In step A, the capillary is conditioned with a BGE (pH 4.5) that contains cetyltrimethylammonium chloride (CTAC) and a long plug of the test analytes prepared in the sample matrix (pH 2.0) is injected. The analytes are positively charged under this condition. In step B, when a negative separation voltage is applied, the interface between the BGE and sample zones move at the same mobility of the electroosmotic flow (EOF) toward the anode, but positively charged analytes migrate in the same direction at slower velocity than EOF due to their high electrophoretic mobility toward the cathode. The pH of the sample zone increases at the rear interface between the BGE and sample zones because acetate ions enter the sample matrix from the BGE. The positively charged analytes change to neutral by deprotonation at the rear interface due to the change in pH. Therefore, the focusing the analytes occurs at the dynamic pH junction. Finally the neutralized and focused analyte zones migrate as independent zones through BGE (step C). It should be noted the analytes must be partially charged to be separated each other by CZE.

3. Experimental

3.1. Apparatus

All electrophoresis experiments were performed using an Agilent CE capillary electrophoresis

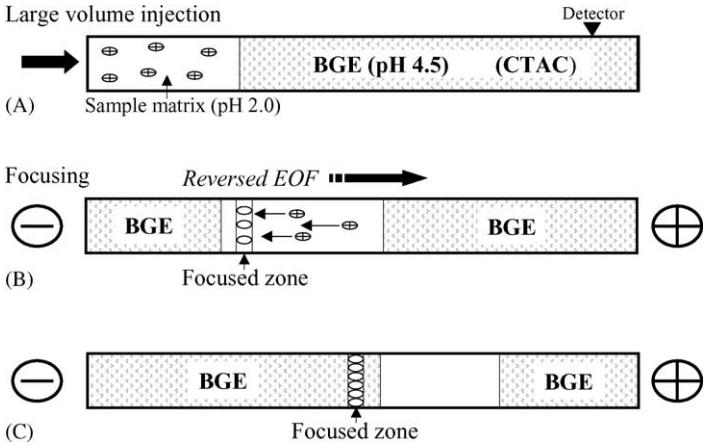

Fig. 1. Schematic diagrams of a dynamic pH junction model: (A) capillary is conditioned with a BGE (pH 4.5), then the analyte prepared in sample matrix (pH 2.0) is injected by pressure for a much longer time compare to normal injection; (B) focusing of the analyte occurs because of its mobility changes in the two zones (BGE and sample zones); (C) focusing analyte zone migrates independently of the sample matrix.

system (Agilent Technologies, Waldbronn, Germany) equipped with a diode-array detection (DAD) system. Separations and focusing were carried out with fused-silica capillaries, 60 cm (51.5 cm effective length) × 50 μm i.d. (Polymicro Technologies, Phoenix, AZ). Detection wavelength was set at 200 nm and the capillary temperature was thermostated at 25 °C. Samples were introduced by pressure (50 mbar, 1 mbar = 100 Pa) injection. The separation voltage was set at negative or positive 17 kV. Deionized water was prepared with a Milli-Q system (Millipore, Bedford, MA, USA). The pH of solutions was measured and adjusted with the aid of a Beckman Φ34 pH meter (Fullerton, CA, USA). Conductivities of sample and separation solutions were measured using a Horiba ES-12 conductivity meter (Kyoto, Japan).

3.2. Reagents and samples

Acetic acid, sodium dihydrogenphosphate, phosphoric acid, sodium hydroxide (NaOH), aniline, *m*-anisidine, *p*-bromoaniline were purchased from Wako (Osaka, Japan). Sodium acetate and methanol were obtained from Nacalai Tesque (Kyoto, Japan). CTAC was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). All reagents were of analytical-reagent grade and used without further purification. BGE solutions were prepared from stock solution of 500 mM sodium acetate and acetic acid. Sample matrices were prepared from stock solution of 500 mM phosphoric acid and sodium dihydrogenphosphate. Stock solutions of three anilines containing 100 μg/ml each of aniline, *m*-anisidine, *p*-bromoaniline were prepared in 50% aqueous methanol. All solutions were sonicated and filtered through 0.45 μm filters prior to CE experiments.

3.3. CE procedure

A new capillary was conditioned prior to use with 1.0 M NaOH solution for 20 min, followed by methanol for 20 min, 0.1 M NaOH for 20 min, purified water for 20 min, and finally the BGE for 20 min. Before each injection, the capillary was rinsed successively for 3 min with 1.0 M NaOH, methanol, deionized water and the BGE to ensure adequate run-to-run reproducibility. Sample injections were done by pressure (50 mbar). The measurements were run at least in triplicate to ensure reproducibility. The velocity of a liquid in the capillary at 50 mbar pressures was determined by using a neutral marker to approximate the length of the zones injected at different intervals. Other experimental conditions are described in the text or figures.

4. Results and discussion

4.1. Effect of the pH and concentration of the BGE on separation and focusing

In uncoated capillary, the positively charged samples tend to be adsorbed on the capillary wall, resulting broad and tailing peaks. In this report, to prevent peak tailing, we added the cationic surfactant, CTAC. Generally, the addition of cationic surfactants to the BGE causes anodic EOF owing to the capillary wall being positively charged by the adsorption of cationic surfactants [29,30]. Under this condition, negative polarity was applied at the inlet electrode to detect the analytes.

To begin with, the effect of the pH of BGE on separation was examined. The separation of three anilines was achieved using a BGE containing 250 mM acetate (pH 4.5) with and without CTAC. At pH 4.5 without CTAC, the migration order, aniline ($pK_a = 4.63$) > *m*-anisidine ($pK_a = 4.23$) > *p*-bromoaniline ($pK_a = 3.86$), is directly related to their pK_a values. On the other hand, when 2 mM CTAC was added to BGE, the migration order was *m*-anisidine, *p*-bromoaniline, aniline. Since CTAC is present at a concentration higher than its critical micelle concentration (ca. 1 mM), micelles must be formed. The migration order different from expected can be explained in terms of partial interaction between the analytes and micelles, i.e. *p*-bromoaniline is incorporated by the micelle than *m*-anisidine. During separation optimization, injection was performed hydrodynamically to produce a plug length of 0.74 mm (1 s injection).

The pH of BGE was kept constant and the effect of the concentration of BGE on separation was examined in the range of 150–300 mM. The separation of peaks is improved by increasing the concentration of BGE. The optimum concentration of BGE was found to be 250 mM in terms of separation and sensitivity enhancement.

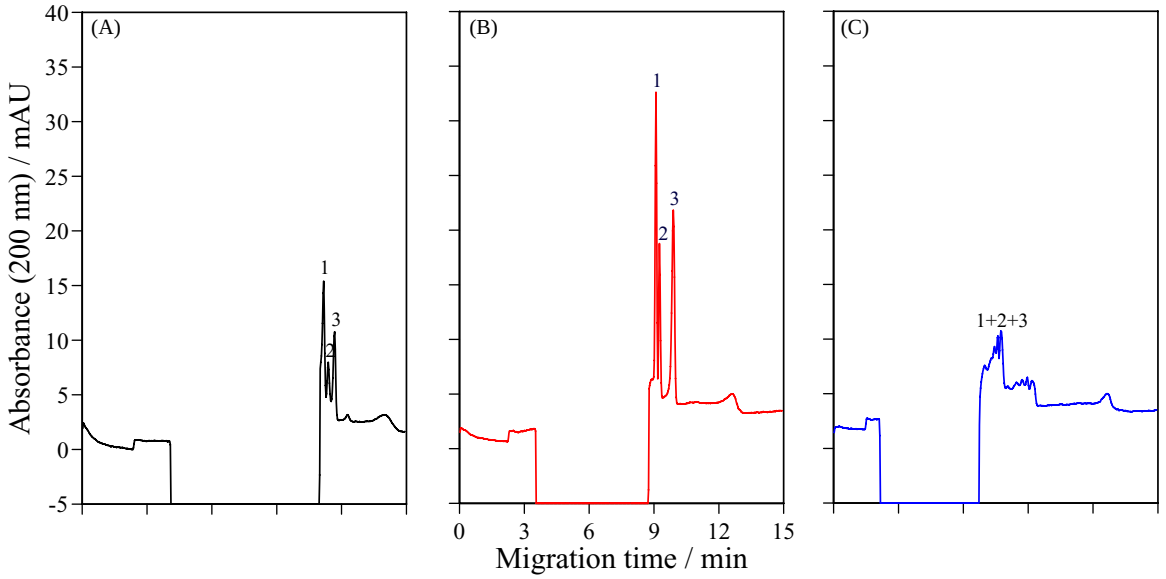

Fig. 2. Effect of sample matrix pH on separation and focusing. BGE, 250 mM acetate and 2 mM CTAC (pH 4.5); pH of sample matrix: (A) 1.6; (B) 2.0; (C) 2.5; sample matrix, 100 mM phosphate; concentration of samples, *m*-anisidine (peak 1, 1 ppm), *p*-bromoaniline (peak 2, 1 ppm), aniline (peak 3, 1 ppm); injection length, 26 cm; applied voltage, −17 kV; capillary, 60 cm total (51.5 cm to detector). Other conditions are as described in Section 3.

4.2. Effect of the sample matrix pH on separation and focusing

The sample matrix pH and ionic strength are the two most important factors, which influence the focusing of weakly ionic analytes in dynamic pH junction [21–23]. The effects of sample matrix pH on the separation and focusing of the test analytes were examined in the pH range of 1.6–2.5 (Fig. 2). The injection plug length was fixed at 26 cm (350 s injection). As shown in Fig. 2A, when the pH of the sample matrix was 1.6 the analytes was not focused very well. When the acidity of the sample matrix was too high, the acetate ions emanating from the rear interface between the sample zone and BGE were not able to able to form a sharp boundary in pH. Here, the positive charges of the analytes were not neutralized sufficiently, and no marked change in electrophoretic mobility. In Fig. 2C (pH 2.5), separation and focusing were not successful because the pH difference between BGE and sample matrix was too small. On the other hand, under pH 2.0 three anilines were well separated and focused as shown in Fig. 2B. When the analytes were prepared in the BGE (pH 4.5) and then injected for 26 cm, only broad peaks were observed without improvement of sensitivity (figure is not shown).

4.3. Effect of the sample matrix concentration on separation and focusing

Fig. 3 shows the effect of the sample matrix concentration on separation and focusing. The sample matrix pH and injection plug length was kept at 2.0 and 26 cm, respectively. In Fig. 3B (150 mM), *m*-anisidine and *p*-bromoaniline co-migrated, while aniline showed very broad peak. In Fig. 3A (50 mM), the separation of three anilines was incomplete and sensitivity enhancements were lower than in Fig. 2B. As can be seen in Fig. 2B, the optimum concentration of sample matrix was found to be 100 mM in terms of separation and sensitivity enhancement.

4.4. Method validation under optimum conditions

From the viewpoint of separation and focusing, the optimum conditions were 250 mM acetate (pH 4.5) containing 2 mM CTAC for the BGE and a 100 mM phosphate (pH 2.0) for the sample matrix.

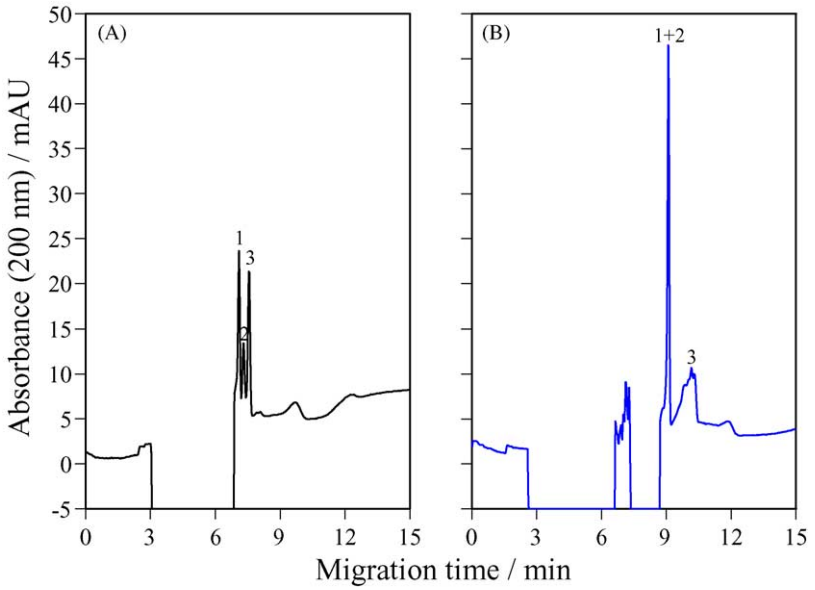

Fig. 3. Effect of sample matrix concentration on separation and focusing. Sample matrix (phosphate, pH 2.0): (A) 50 mM; (B) 150 mM. Other conditions are as in Fig. 2.

The results of the linearity of calibration lines, limits of detection (LOD), relative standard deviations (R.S.D.) and sensitivity enhancement factor in terms of peak heights (SEF_{height}) obtained for *m*-anisidine, *p*-bromoaniline, and aniline are summarized in Table 1. Calibration lines of peak height against concentration showed good linearity. The LODs of the test analytes calculated at S/N = 3 were in the range of 1.9–3.7 ppb or 1.5×10^{-8} to 3.3×10^{-8} M with UV detector. Good reproducibility was achieved, as R.S.D. obtained with three consecutive runs in migration times, corrected peak areas (peak area divided by the migration time), and peak heights were less than 2.3% for all analytes. Detection sensitivity of

Table 1
LOD, R.S.D., and SEF_{height} for anilines in dynamic pH junction[a]

	m-Anisidine	*p*-Bromoaniline	Aniline
Calibration line[b]	$y = 29.07x + 0.482$	$y = 15.02x + 0.201$	$y = 18.12x + 0.223$
Correlation coefficient (r^2)	0.9954	0.9974	0.9978
LOD (S/N = 3)			
(a) ppb	1.9	3.7	3.1
(b) $\times 10^{-8}$ M	1.5	2.1	3.3
R.S.D. (%, $n = 3$)			
(a) Migration time	1.0	1.1	1.0
(b) Corrected peak area[c]	1.0	0.67	1.1
(c) Peak height	0.87	2.3	1.3
SEF_{height}[d]	160	140	100

[a] Conditions: Fig. 2B.
[b] Calibration line: peak height (mAU) = slope × concentration (ppm) + *y*-intercept. Concentration range: 0.1–1.0 μg/ml.
[c] Corrected peak area = peak area/migration time.
[d] SEF_{height} = (peak height obtained with pH junction (350 s)/peak height obtained with normal junction (1 s)) × dilution factor.

m-anisidine, *p*-bromoaniline, aniline were improved about 160-, 140-, and 100-fold, respectively. Sensitivity enhancement factors were calculated by simply calculating the ratio of the peak heights obtained from dynamic pH junction and normal injection and correction by the dilution ratio.

In conclusion, we have shown that the dynamic pH junction can be used as an on-line sample preconcentration technique to enhance detection sensitivity of cationic analytes. Dynamic pH junction of some cationic analytes gave high concentration factors up to 160-fold without any pretreatment step.

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