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Short communication

Role of counter-ions in background electrolyte for the analysis of cationgenic weak electrolytes and amino acids in neutral aqueous solutions by capillary electrophoresis with electrokinetic injection

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ABSTRACT

We elucidated theoretically and experimentally that counter-ions in background electrolyte (BGE) play a role of booster for electrokinetic injection (EKI) for the determination of cationgenic weak electrolytes and amino acids in neutral aqueous solutions using capillary electrophoresis (CE). The pH change in the sample solution caused by the migration of counter-ions resulted in the increase of analyte mobility and hence the increase of the amount of analyte injected into the capillary. This type of EKI was named as counter-ion boosted EKI. Using the counter-ion boosted EKI-capillary zone electrophoresis (CZE), the limit of detections (LODs, $S/N = 3$) for creatinine (4.8 nM) and L-histidine (9.0 nM) were lowest ever achieved by CE with UV detection. The RSDs ($n = 3$) of the migration time for creatinine and L-histidine were obtained as 0.35% and 0.34%, for peak areas of 13% and 12%, and for peak heights of 12% and 8.5%, respectively. The concentrations of creatinine and L-histidine in a urine sample obtained by the proposed method were within those reported with a good recovery.

1. Introduction

Over the past decades of successful developments, capillary electrophoresis (CE) has become a mature separation technique and has been increasingly important for the wide range of analytical chemistry. CE has a number of advantages in terms of high separation efficiency, rapid separation, simplicity, and minor consumption of samples and reagents. However, it has disadvantages such as insufficient concentration-sensitivity and lower reproducibility compared to other separation techniques although a considerable number of studies have been reported to overcome such disadvantages.

In general, pH and compositions of background electrolyte (BGE) affect the analytical performance such as sensitivity and reproducibility in CE. The pH affects the mobilities of electroosmotic flow (EOF) and ionic analytes. The mobility of UV-absorbing probe**s** in BGE affects analyte peak shape in indirect UV detection [1,2]. In addition, co-ions in BGE can act as leading or terminating ions for transient isotachophoresis (t-ITP) depending on the analyte mobility [3,4]. Therefore, it is important to examine the effects and roles of BGE compositions to improve the analytical performance for CE analysis.

In the present study, we investigated the role of counter-ions in BGE when cationgenic weak electrolytes and amino acids in neutral aqueous solutions are analyzed by CE with electrokinetic injection (EKI). Some cationgenic weak electrolytes and amino acids in neutral aqueous solutions exist as non-ionic species and zwitterions, respectively, which depend on the pK_a and pI of the analytes. In general, it is difficult to inject above analytes into the capillary effectively by EKI without adjusting the sample pH and/or under the suppressed EOF because the analyte mobilities are extremely low in neutral aqueous solutions. It was shown theoretically using computer simulation that the counter-ions in BGE played a role of booster for EKI of the analytes (i.e. to increase the amount of the analytes injected into the capillary) by decreasing the pH of sample solution to increase the mobility. We named this type of EKI as counter-ion boosted EKI. Then, the simulation results were confirmed experimentally using a standard solution. Finally, the applicability of counter-ion boosted EKI-capillary zone electrophoresis (CZE) to real samples was demonstrated by determining creatinine and L-histidine in a diluted urine sample.

2. Materials and methods

2.1. Computer simulation

A computer simulation software, Simul 5 Complex, originally developed by Gaš [5,6] was used to simulate the concentration

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profiles for co-ion, counter-ion, and analytes as well as pH changes during EKI. The simulations were conducted on a Core i7 2.4-GHz PC. For the simulations, the capillary length, the sample-plug length, and the space step were set at 50 mm (50 μm i.d.), 1 mm, and 5 μm, respectively. A voltage (100 V) was applied for 20 s with the sample-inlet side as the anode. No EOF was assumed. The BGEs were a mixture of 10 mM Na^+ ($pK_a = 13.7$, $\mu_{ep} = 51.9 \times 10^{-5}$ $cm^2\,V^{-1}\,s^{-1}$) and 13.1 mM Cl^- ($pK_a = -2$, $\mu_{ep} = -79.1 \times 10^{-5}$ $cm^2\,V^{-1}\,s^{-1}$) or a mixture of 5.9 mM Na^+ and 13.1 mM PO_4^{3-} ($pK_a = 2.16$, 7.21, and 12.67, $\mu_{ep} = 34.6 \times 10^{-5}$, 61.4×10^{-5}, and 71.5×10^{-5} $cm^2\,V^{-1}\,s^{-1}$). The pH of these BGEs was set at 2.5. The pK_a values and absolute mobilities were quoted from the program database mainly based on the Hirokawa's table [7]. The sample was a mixture of 0.01 mM A^+ ($pK_a = 4.5$, $\mu_{ep} = 30 \times 10^{-5}$ $cm^2\,V^{-1}\,s^{-1}$) as a model analyte of cationgenic weak electrolyte and 0.01 mM $B^{\pm}$ ($pK_a = 2.0$, 5.0, and 9.0, $\mu_{ep} = 45 \times 10^{-5}$, 25×10^{-5}, and -30×10^{-5} $cm^2\,V^{-1}\,s^{-1}$) as a model analyte of amino acid.

2.2. Apparatus

All experiments were conducted using a capillary electrophoresis instrument equipped with a photodiode array detector (CAPI-3200; Otsuka Electronics, Osaka, Japan). A polyimide-coated fused-silica capillary (GL Sciences, Tokyo, Japan) with 62.4 cm total length (50 cm effective length) and 50 μm i.d. was used. The capillary was thermostated at 25 °C. The detection wavelength was set at 200 nm. The pH measurements were conducted using a pH meter (F-22; Horiba, Kyoto, Japan).

2.3. Chemicals and reagents

All reagents were of analytical-reagent grade. Sodium chloride and creatinine were purchased from Nacalai Tesque (Kyoto, Japan). Hydrochloric acid and L-histidine was the product of Wako Pure Chemical Industries (Osaka, Japan). Hydroxypropyl methylcellulose (HPMC) was obtained from Sigma-Aldrich (St. Louis, MO, USA). The BGE was 10 mM NaCl solution containing 0.03% (w/v) HPMC to suppress EOF, adjusted to pH 2.5 with 1 M HCl. The individual stock solutions (5 mM) of creatinine and L-histidine were prepared in water and serially diluted as required. A urine sample was collected from a healthy male volunteer. All solutions were filtered through a 0.45 μm membrane filter (Advantec Toyo Kaisha, Tokyo, Japan) before use. Distilled, demineralized water, obtained from an automatic still (WG220; Yamato Kagaku, Tokyo, Japan) and a Simpli Lab-UV high-purity water apparatus (Merck Millipore, Tokyo, Japan) was used throughout.

2.4. Experimental procedure

A new capillary was flushed with water for 5 min, followed by 1 M NaOH for 40 min, water for 10 min, and BGE for 10 min. Before the first analysis of each day, the capillary was flushed with water for 5 min and BGE for 10 min. Between runs, the capillary was flushed with BGE for 3 min. The sample solution was injected by EKI (10 kV) with the sample-inlet side as the anode for a designated time. A positive voltage of 20 kV was applied for separation.

3. Results and discussion

3.1. Computer simulation

To explore the role of counter-ions in BGE when cationgenic weak electrolytes and amino acids in neutral aqueous solutions are injected by EKI, computer simulations were conducted using two kinds of counter-ions with different effective mobility (-79.1×10^{-5} (Cl^-) or -23.8×10^{-5} $\left(PO_4^{3-}\right)$ $cm^2\,V^{-1}\,s^{-1}$) in BGE. Fig. 1 depicts the simulation results of the concentration profiles for co-ion (Na^+), counter-ion (Cl^- or PO_4^{3-}), and analytes (A^+ and $B^{\pm}$) and the pH profiles in the sample (between anode and capillary inlet in a sample vial) and BGE (in capillary) zones. Fig. 1(A) and (B) are the results when the counter-ion was Cl^-. Fig. 1(C) and (D) are the results when the counter-ion was PO_4^{3-}. In the initial states (Fig. 1(A) and (C)) before the injection voltage was applied, the concentrations of the analytes A^+ and $B^{\pm}$ were 0.01 mM. Most of the analytes A^+ ($pK_a = 4.5$) and $B^{\pm}$ ($pI = 7.0$) existed as non-ionic species and zwitterions, respectively, because the sample zone pH was 7.0. Therefore, the analytes A^+ and $B^{\pm}$ had almost no mobilities (respectively, 0.09×10^{-5} and -0.09×10^{-5} $cm^2\,V^{-1}\,s^{-1}$). When the counter-ion was Cl^-, the maximum concentrations of the analytes A^+ and $B^{\pm}$ were, respectively, 0.38 and 0.83 mM after the voltage was applied for 20 s (Fig. 1(B)). The concentration of Cl^- in the sample zone increased because of the electrophoresis of Cl^- from the BGE zone. With the increase of Cl^- concentration, the pH in the sample zone decreased because H^+ was generated from H_2O to meet the electroneutrality law. The pH at 0.5 mm point from the left side (the middle point in the sample zone) was 3.6. As a result, the cationic species of the analytes increased, and hence the analyte mobilities increased. At the point, the mobilities of the analytes A^+ and $B^{\pm}$ were, 26.6×10^{-5} and 24.5×10^{-5} $cm^2\,V^{-1}\,S^{-1}$, respectively. Therefore, the analytes could be injected into the capillary by EKI.

When the counter-ion was PO_4^{3-}, the maximum concentrations of the analytes A^+ and $B^{\pm}$ were, respectively, 0.26 and 0.58 mM after the voltage was applied for 20 s (Fig. 1(D)). These values were lower than those when the counter-ion was Cl^-. This was because the effective mobility of PO_4^{3-} was smaller than that of Cl^-. In the sample zone, the amount of counter-ion $\left(PO_4^{3-}\right)$ migrating from the BGE zone was less than that for Cl^-, and hence H^+ was less generated than the case of Cl^-. At 0.5 mm point from the left side, the pH was 4.2 and the mobilities of the analytes A^+ and $B^{\pm}$ were, 19.7×10^{-5} and 21.6×10^{-5} $cm^2\,V^{-1}\,S^{-1}$, respectively. Therefore, the smaller amount of analytes was injected into the capillary by EKI. In addition, the computer simulation using the model counter-ion X^- with the negligible effective mobility (-0.1×10^{-5} $cm^2\,V^{-1}\,s^{-1}$) was conducted (data not shown). The maximum concentrations of the analytes A^+ and $B^{\pm}$ were, respectively, 0.02 and 0.01 mM after the voltage was applied for 20 s. The analytes could be little injected into the capillary by EKI.

From these simulation results, it was revealed that the counter-ions in BGE boosted EKI of cationgenic weak electrolytes and amino acids in neutral aqueous solutions. We named this type of EKI as counter-ion boosted EKI.

3.2. Experiment using standard solutions

To confirm the simulation results experimentally, a standard solution containing 0.1 μM creatinine and L-histidine was analyzed with negligibly weak EOF. The pH of the standard solution was 8.2 in which most of creatinine ($pK_a = 4.5$) and L-histidine ($pI = 7.6$) existed as non-ionic species and zwitterions, respectively. The mobilities of creatinine and L-histidine in pH 8.2 were, respectively, 0.0158×10^{-5} and -1.768×10^{-5} $cm^2\,V^{-1}\,S^{-1}$, obtained using simulation software, Peakmaster 5.3 Complex [8,9]. As the counter-ion in BGE, Cl^- was used because it was revealed that Cl^- was more effective than PO_4^{3-} for counter-ion boosted EKI by the computer simulations. Fig. 2 depicts an electropherogram of the standard solution. In general, the analytes with low mobility or opposite directional (toward the anode) mobility cannot be injected into the capillary by EKI if EOF is negligibly weak. However, the peaks of creatinine and L-histidine were clearly observed in this case. This is