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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.

Keywords: Amino acid; Capillary electrophoresis; Counter-ion; Electrokinetic injection; Cationgenic weak electrolyte

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 probes 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 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, μ_{ep} = 51.9×10^{-5} $cm^2V^{-1}s^{-1}$) and 13.1 mM Cl^- (pK_a = -2, μ_{ep} = -79.1×10^{-5} $cm^2V^{-1}s^{-1}$) or a mixture of 5.9 mM Na^+ and 13.1 mM PO_4^{3-} (pK_a = 2.16, 7.21, and

12.67, μ_{ep} = 34.6×10^{-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, μ_{ep} = 30×10^{-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, μ_{ep} = 45×10^{-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 25ºC. 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,

139 computer simulations were conducted using two kinds of counter-ions with
140 different effective mobility (-79.1×10^{-5} (Cl^-) or -23.8×10^{-5} (PO_4^{3-}) $cm^2V^{-1}s^{-1}$) in
141 BGE. Fig. 1 depicts the simulation results of the concentration profiles for co-ion
142 (Na^+), counter-ion (Cl^- or PO_4^{3-}), and analytes (A^+ and $B^{\pm}$) and the pH profiles in
143 the sample (between anode and capillary inlet in a sample vial) and BGE (in
144 capillary) zones. Fig. 1(A) and 1(B) are the results when the counter-ion was Cl^-.
145 Fig. 1(C) and 1(D) are the results when the counter-ion was PO_4^{3-}. In the initial
146 states (Fig. 1(A) and 1(C)) before the injection voltage was applied, the
147 concentrations of the analytes A^+ and $B^{\pm}$ were 0.01 mM. Most of the analytes A^+
148 (pK_a = 4.5) and $B^{\pm}$ (pI = 7.0) existed as non-ionic species and zwitterions,
149 respectively, because the sample zone pH was 7.0. Therefore, the analytes A^+ and
150 $B^{\pm}$ had almost no mobilities (respectively, 0.09×10^{-5} and -0.09×10^{-5} $cm^2V^{-1}s^{-1}$).
151 When the counter-ion was Cl^-, the maximum concentrations of the analytes A^+
152 and $B^{\pm}$ were, respectively, 0.38 and 0.83 mM after the voltage was applied for 20 s
153 (Fig. 1(B)). The concentration of Cl^- in the sample zone increased because of the
154 electrophoresis of Cl^- from the BGE zone. With the increase of Cl^- concentration,
155 the pH in the sample zone decreased because H^+ was generated from H_2O to meet
156 the electroneutrality law. The pH at 0.5 mm point from the left side (the middle
157 point in the sample zone) was 3.6. As a result, the cationic species of the analytes
158 increased, and hence the analyte mobilities increased. At the point, the mobilities
159 of the analytes A^+ and $B^{\pm}$ were, 26.6×10^{-5} and 24.5×10^{-5} $cm^2V^{-1}S^{-1}$, respectively.
160 Therefore, the analytes could be injected into the capillary by EKI.

161 When the counter-ion was PO_4^{3-}, the maximum concentrations of the analytes
162 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 (PO_4^{3-}) 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} $cm^2V^{-1}S^{-1}$, 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} $cm^2V^{-1}s^{-1}$) 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} $cm^2V^{-1}S^{-1}$, obtained using simulation

187 software, Peakmaster 5.3 Complex [8,9]. As the counter-ion in BGE, Cl^- was used
188 because it was revealed that Cl^- was more effective than PO_4^{3-} for counter-ion
189 boosted EKI by the computer simulations. Fig. 2 depicts an electropherogram of
190 the standard solution. In general, the analytes with low mobility or opposite
191 directional (toward the anode) mobility cannot be injected into the capillary by
192 EKI if EOF is negligibly weak. However, the peaks of creatinine and L-histidine
193 were clearly observed in this case. This is because these analytes were injected
194 into the capillary by counter-ion boosted EKI as shown in the simulation results.
195
196 *3.3. Optimization of sample-injection time*
197
198 The sample-injection time was varied between 50 and 200 s with the injection
199 voltage set at 10 kV to examine its effect on the analyte peak-height. The standard
200 solution of 0.1 μM creatinine and L-histidine was used as the sample. The both
201 peak heights increased with the injection time up to 100 s and almost leveled off
202 when going to 150 s. When the injection time was 200 s, the peaks of creatinine
203 and L-histidine were split into two peaks, although the reason was not clear now.
204 Therefore, the optimum sample-injection time adopted in the subsequent
205 experiments was 100 s. Under the condition of the optimum injection time (100 s),
206 the limit of detections (LODs, S/N = 3) for creatinine and L-histidine were,
207 respectively, 4.8 and 9.0 nM. The LODs were improved 230 and 180 times for
208 creatinine and L-histidine compared to those obtained using the conventional
209 vacuum injection method (50 kPa for 1.0 s). In addition, the LOD for creatinine
210 was improved 92 times compared to the lowest LOD (0.44 μM) ever reported in

CE [10]. The LOD for L-histidine was comparable to the lowest LOD (3.8 nM) obtained in CE with lamp-induced fluorescence detection [11]. 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.

3.4. Application to real samples

To demonstrate the applicability of counter-ion boosted EKI-CZE to real samples, creatinine and L-histidine in a urine sample were determined. Fig. 3 depicts an electropherogram of 20000 fold diluted urine sample. The RSDs (n = 4) of the migration time for creatinine and L-histidine were obtained as 0.62 and 0.63%, for peak areas of 5.7 and 4.6%, and for peak heights of 2.8 and 4.5%, respectively. Calibration curves were established by spiking creatinine (0.2–0.6 μM) and L-histidine (0.2–0.6 μM) in 20000 fold diluted urine sample. Regression equations relating the area response to concentration for creatinine and L-histidine were $y = 41.3x + 36.1$ (correlation coefficient, 0.9993) and $y = 36.3x + 3.81$ (0.9993). The concentrations of urinary creatinine and L-histidine were 17.5 and 2.1 mM, respectively. The recoveries of creatinine and L-histidine spiked into the urine sample (0.2 μM creatinine and L-histidine) were 88 and 105%, respectively. Also, these concentration values were within the values reported by Liotta et al. [12] and Langley [13], 2.5–23 mM for creatinine and 0.26–6.6 mM for L-histidine, respectively. It was demonstrated that counter-ion boosted EKI-CZE has the applicability to real samples.

4. Conclusions

In the present work, we elucidated theoretically and experimentally that the counter-ions in BGE boost EKI of cationgenic weak electrolytes and amino acids in neutral aqueous solutions. The counter-ion boosted EKI could be combined with various on-line pre-concentration procedures and separation modes. Therefore, the proposed EKI has the potential to revolutionize the CE analysis for low concentrations of cationgenic weak electrolytes and amino acids in a neutral aqueous solution. Only counter-ion boosted EKI for cationgenic weak analytes and amino acids were demonstrated here. However, it is also possible to conduct counter-ion boosted EKI for aniongenic weak analytes with larger pKa (>> 7). In this case, the counter-ions (e.g. Na^+) in BGE zone migrate to the sample zone after the injection voltage is applied with the sample-inlet side as the cathode. Then, the pH in the sample zone increases because OH^- is generated from H_2O to meet the electroneutrality law. The resultant high pH increases the anionic species of analytes, and hence the analyte mobilities increase. We are currently investigating the effects of mobility and concentration of counter-ion in BGE on the efficiency of counter-ion boosted EKI by computer simulations and experiments in detail. In addition, we intend to apply counter-ion boosted EKI to other analytes and/or samples.

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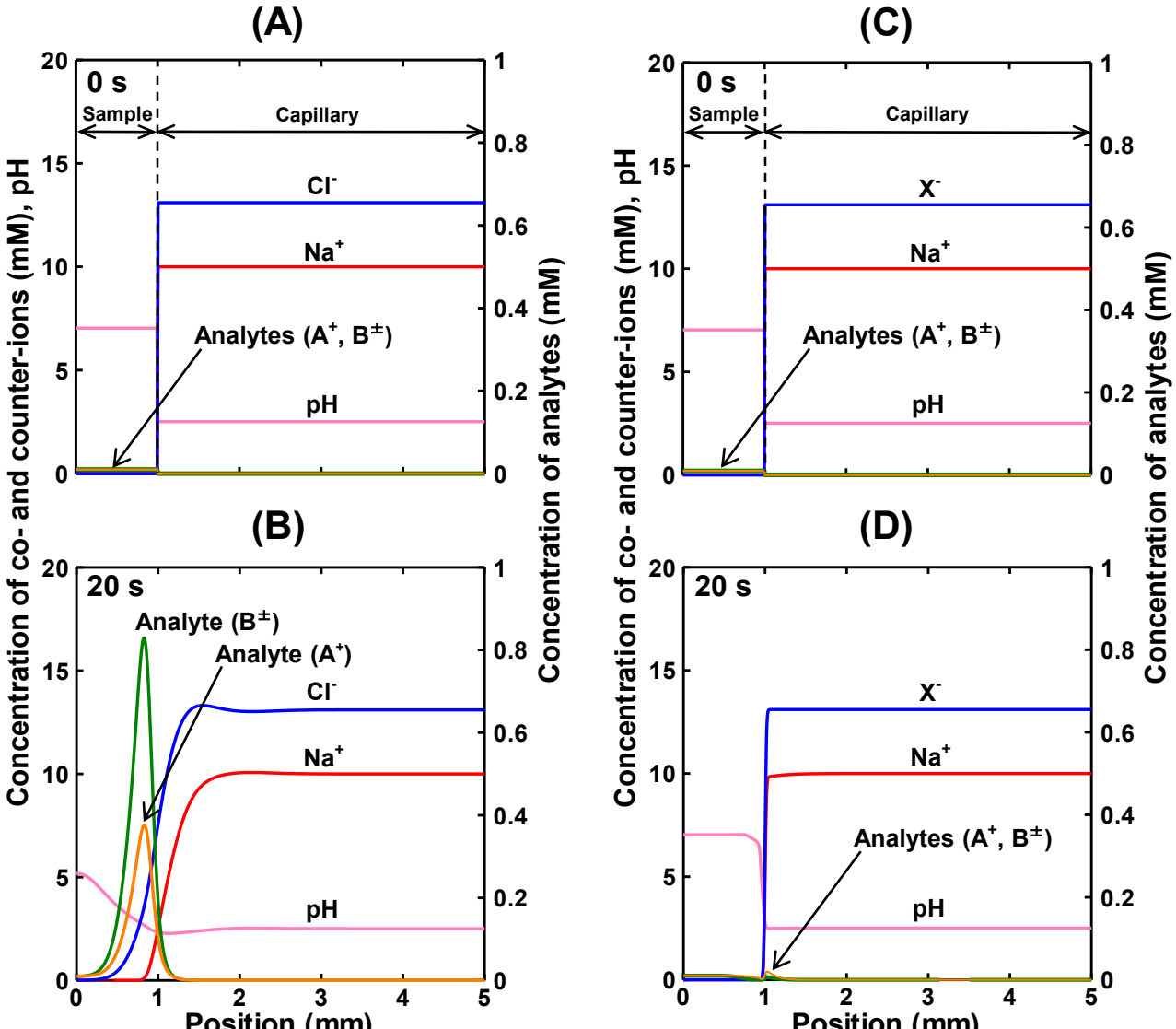
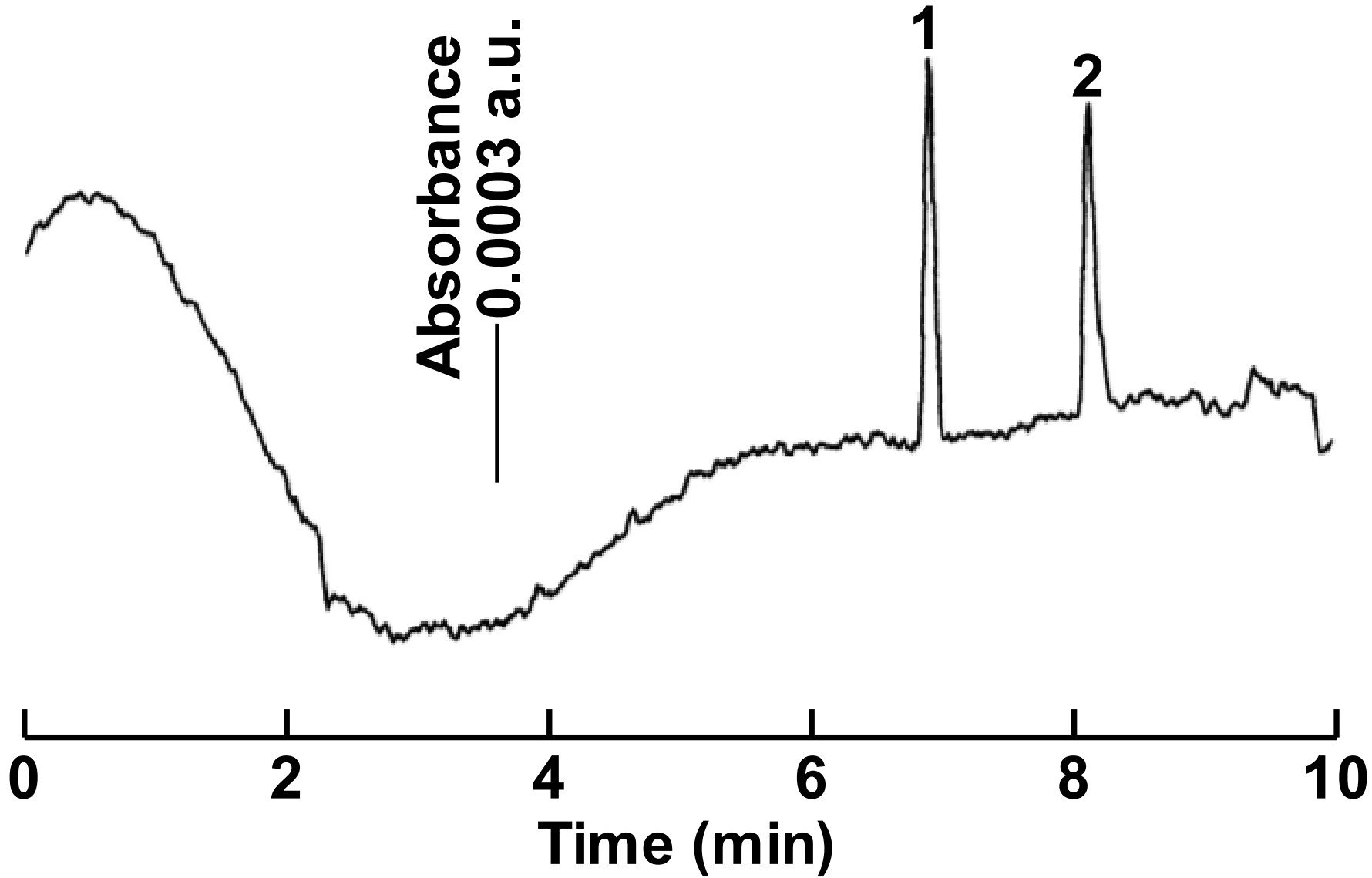
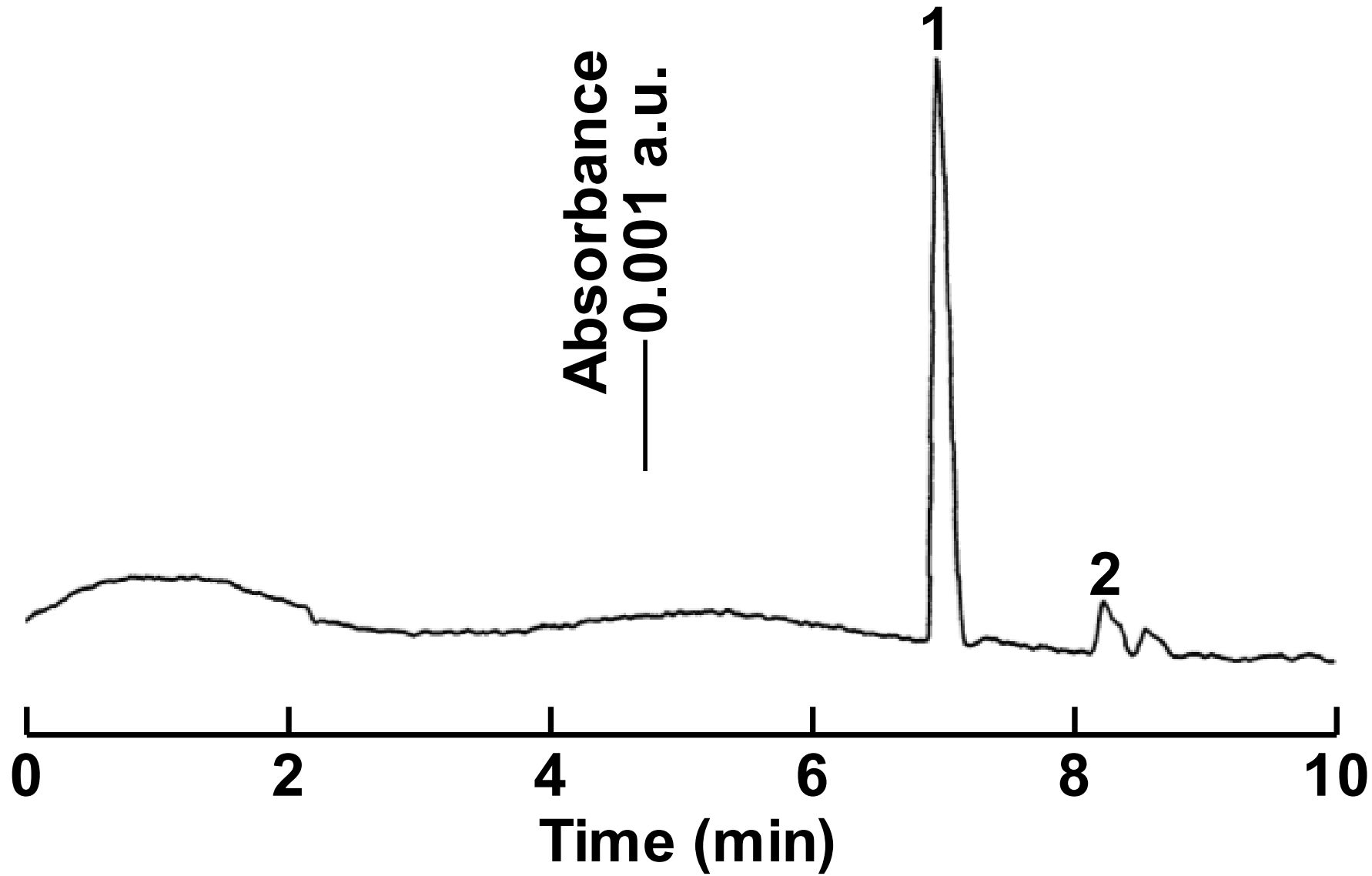
Fig. 1. Simulation results of the concentration profiles for co-ion (Na^+), counter-ion (Cl^- or PO_4^{3-}), and analytes (A^+ and $B^{\pm}$) and the pH profiles in the sample and the BGE zones at 0 s (A, C) and 20 s (B, D) after the injection voltage was applied. (A) and (B) counter-ion, Cl^-, (C) and (D) counter-ion, PO_4^{3-}. Other conditions are described in the text.

Fig. 2. Electropherogram of a standard solution of creatinine and L-histidine by counter-ion boosted EKI-CZE. Analytical conditions: capillary, 62.4 cm total length (50 cm effective length) and 50 μm i.d.; BGE, 10 mM NaCl solution containing 0.03% (w/v) HPMC adjusted to pH 2.5 with 1 M HCl; sample solution, 0.1 μM creatinine and L-histidine; injection, 10 kV for 100 s; separation voltage, 20 kV; wavelength for detection, 200 nm. Analyte peak numbering: 1, creatinine; 2, L-histidine.

Fig. 3. Electropherogram of 20000 fold diluted urine sample by counter-ion boosted EKI-CZE. Analytical conditions and analyte peak numbering as in Fig. 2.


(A)
0 s
Sample
Capillary
Cl-
Na+
Analytes (A+, B±)
pH
(B)
20 s
Analyte (B±)
Analyte (A+)
Cl-
Na+
pH
(C)
0 s
Sample
Capillary
X-
Na+
Analytes (A+, B±)
pH
(D)
20 s
X-
Na+
Analytes (A+, B±)
pH
Concentration of co- and counter-ions (mM), pH
Concentration of analytes (mM)
Position (mm)


Absorbance
0.0003 a.u.
1
2
0
2
4
6
8
10
Time (min)


1
2
Absorbance
0.001 a.u.
0
2
4
6
8
10
Time (min)

- Counter-ions boosted electrokinetic injection of weak electrolytes and amino acids.
- The counter-ions changed the sample pH to fulfill the electroneutrality requirement.
- The pH change in the sample vial increased the analyte mobility.
- The effectiveness of the method was confirmed theoretically and experimentally.
- The proposed method determined creatinine and L-histidine in a urine sensitively.