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**Highly sensitive tITP-CZE determination of L-histidine and creatinine in human blood plasma using field-amplified sample injection with mobility-boost effect**

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**Abbreviations:**  $D_{ec}$ , distance between the end of capillary inlet and electrode; **EKI**, electrokinetic injection; **EKS**, electrokinetic supercharging; **FASI**, field-amplified sample injection; **HPMC**, hydroxypropyl methylcellulose; **MB effect**, mobility-boost effect; **SysT**, system-induced terminator; **tITP**, transient ITP

**Keywords:** Amino acid / Capillary electrophoresis / Counter-ion / Mobility-boost effect / transient ITP /

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## **Abstract**

Two-dimensional (2-D) computer simulation revealed that amino acids and weak electrolytes were cationized because of the migration of counter-ion from a BGE zone to a sample zone, which encouraged electrokinetic injection (EKI) of these analytes (by the mobility-boost (MB) effect). To investigate the effects of kinds and concentrations of counter-ions on the MB effect and the analyte amount injected into the capillary, experiments and one-dimensional (1-D) computer simulations were performed. When acetate was used as the counter-ion, the LODs ( $S/N = 3$ ) of L-histidine and creatinine respectively reached 0.10 and 0.25 nM because of the concentration effect by transient ITP (tITP). The concentrations of L-histidine and creatinine in human blood plasma obtained using the proposed method were agreed with those obtained using the conventional methods. The proposed method can be applied to the analysis of amino acids and weak bases which have similar  $pI$  and  $pK_a$  to L-histidine and creatinine.

## **1 Introduction**

By virtue of the past several decades of continuous improvement, CE has become a mature separation technique that is increasingly important for analytical chemistry. CE presents numerous benefits in terms of high separation efficiency, rapid separation, simplicity, and minimum consumption of samples and reagents. However, CE with conventional UV detection has the important shortcoming of relatively low concentration sensitivity because of its small sample-injection

volume into the capillary and its short light pathway. Various on-line concentration procedures have been developed to overcome this shortcoming: field-amplified sample injection (FASI) [1, 2], transient ITP (tITP) [3, 4], large volume sample stacking [5, 6], dynamic pH junction [7, 8], and sweeping [9, 10]. In CE, samples are usually injected into the capillary either by hydrodynamic injection or by electrokinetic injection (EKI) [11]. EKI is used in CGE [12] and for on-line concentration, e.g. FASI, selective exhaustive injection-sweeping [13, 14] and electrokinetic supercharging (EKS) [15–17]. A report of our previous study described that counter-ion in BGE plays a role as a booster for EKI of cationogenic weak electrolytes and amino acids in neutral aqueous solutions [18].

For this study, 2-D computer simulations were conducted to elucidate the process of the mobility-boost (MB) effect. Then, the effects of counter-ion (weak or strong electrolyte) and concentration in BGE on the MB effect and the analyte amount injected were investigated using experiments and 1-D computer simulations. The BGE conductivity, sample-injection time, and sample pH were optimized to improve the proposed method. The concentrations of L-histidine and creatinine in a diluted human blood plasma sample were ascertained using the proposed method. Histidine and creatinine in plasma or serum are a biomarker or an indicator for some diseases [19, 20]. These analytes are usually determined using LC-MS and enzymatic method, respectively. Recently, CE methods have been developed for the determination of these analytes [21, 22].

The proposed tITP-CZE method, based on the FASI with mobility-boost effect, is an ultrahigh-sensitive analytical method for amino acids and cationogenic weak electrolytes in neutral aqueous solutions which contain low concentrations of

co-existing substances. It is not necessary to adjust the sample pH to ionize analytes because the sample pH decreases spontaneously. Also the sensitivity is higher than that for adjusting the sample pH. Because of its high sensitivity, low concentrations of analytes in samples with high concentrations of co-existing substances can be determined without interferences by diluting the samples. In addition, ultralow volume of samples is required because the samples must be diluted. Therefore, the proposed method is suitable for the determination of L-histidine and creatinine in blood samples as shown in this study. Generally, deproteinization process is essential for blood analysis. It is not necessary because the samples must be highly diluted and the BGE contains hydroxypropyl methylcellulose (HPMC) [23].

## **2 Materials and methods**

### **2.1 MB effect in EKI**

Figure 1 presents the MB effect to increase the effective mobilities ( $\mu_{\text{eff}}$ ) of amino acids and cationogenic weak electrolytes. For simplification, EOF is not considered. The capillary is filled initially with the BGE ( $\text{Ci}^-$ : counter-ion, Fig. 1A). One capillary end is dipped into the sample vial, which contains amino acid ( $\text{A}_1^\pm$ ) and cationogenic weak electrolyte ( $\text{A}_2$ ) as analytes. These amino acid and cationogenic weak electrolyte exist respectively as a zwitterion and a non-ionic species in aqueous sample solutions, depending on the  $pI$  and  $pK_a$  of the analytes. In general, it is difficult to inject these analytes into the capillary using EKI because of the extremely low  $\mu_{\text{eff}}$  of these analytes. When the voltage is applied with the

sample-inlet side as the anode,  $\text{Cl}^-$  in the BGE migrates to the sample vial by electrophoresis (Fig. 1B). Accompanying the migration of  $\text{Cl}^-$ , the pH in the sample zone decreases because  $\text{H}^+$  is generated from  $\text{H}_2\text{O}$  to meet the electroneutrality law. Thereby, the cationic species of these analytes increase so that the  $\mu_{\text{eff}}$  of these analytes increases (MB effect, Fig. 1C). Consequently, these analytes are injected into the capillary by electrophoresis (Fig. 1D).

## 2.2 Computer simulation

Confirming the MB effect experimentally is difficult because the phenomenon would happen in a local and small area around the inlet capillary end. To elucidate the MB effect two-dimensionally, 2-D computer simulation (a finite element method) was conducted using CFD-ACE+software (version 2006, CFDRC, Huntsville, AL, USA) [15–17]. Although the software enabled three-dimensional simulation, a 2-D model was used in this study. Figure 2A shows the basic model of a capillary and a cylindrical sample reservoir used in the present simulation. In this model, the cylindrical electrode is on the reservoir wall. The assumed  $od$  and  $id$  of capillary were 500 and 100  $\mu\text{m}$ , respectively. The whole length of the capillary was 10 mm and half of the capillary was set in the sample reservoir. The  $id$  and length of the sample reservoir were 4 and 7.5 mm, respectively. The size of meshes in the reservoir was  $50 \times 50 \mu\text{m}$  and that in the capillary was  $10 \times 10 \mu\text{m}$ . A BGE was 10 mM acetic acid ( $\text{pH} = 3.4$ ,  $\text{p}K_{\text{a}} = 4.756$ , limiting ionic mobility ( $\mu_{\text{lim}}) = -42.4 \times 10^{-9} \text{ m}^2\text{V}^{-1}\text{s}^{-1}$ ). A sample was a mixture of 1  $\mu\text{M}$  of histidine ( $\text{p}K_{\text{a}} = 1.82, 6.04, \text{ and } 9.33$ ,  $\mu_{\text{lim}} = 59.2 \times 10^{-9}, 29.6 \times 10^{-9}, \text{ and } -28.3 \times 10^{-9} \text{ m}^2\text{V}^{-1}\text{s}^{-1}$ ), glycine ( $\text{p}K_{\text{a}} = 2.35$  and

9.78,  $\mu_{\text{lim}} = 37.4 \times 10^{-9}$  and  $-37.4 \times 10^{-9} \text{ m}^2\text{V}^{-1}\text{s}^{-1}$ ), and creatinine ( $\text{p}K_{\text{a}} = 4.828$ ,  $\mu_{\text{lim}} = 37.2 \times 10^{-9} \text{ m}^2\text{V}^{-1}\text{s}^{-1}$ ). The respective  $\mu_{\text{eff}}$  of histidine, glycine, and creatinine were  $2.0 \times 10^{-9}$ ,  $-0.1 \times 10^{-9}$ , and  $0.2 \times 10^{-9} \text{ m}^2\text{V}^{-1}\text{s}^{-1}$  at the initial pH (7.1) of the sample solution. The applied voltage for injection was set at 0 V at the electrode and 100 V at the end of the capillary. No EOF was assumed. This simulation was performed for 1.775 s. The software was executed on a PC (Dual Pentium Xeon 3 GHz processor; Intel, CA, USA).

To investigate the effects of kinds and concentrations of counter-ion in BGE on the MB effect and the analyte amount injected, 1-D computer simulations were performed using Simul 5 Complex, originally developed by the Gaš group [24]. For the simulations, the total capillary length, id of the capillary, the sample-plug length, and the space step were set, respectively, as 50 mm, 50  $\mu\text{m}$ , 1 mm, and 5  $\mu\text{m}$ . The simulations were conducted on a PC (Core i7 2.4 GHz processor; Intel, CA, USA). Two kinds of BGE (A and B) with equal conductivity (0.57 S/m) were used. This equalizes the initial potential gradient in the sample zone. The BGE (A) was a mixture (pH = 4.8) of 100 mM acetic acid ( $\text{p}K_{\text{a}} = 4.756$ ,  $\mu_{\text{lim}} = -42.4 \times 10^{-9} \text{ m}^2\text{V}^{-1}\text{s}^{-1}$ ) and 50 mM aqueous ammonia ( $\text{p}K_{\text{a}} = 9.25$ ,  $\mu_{\text{lim}} = 76.2 \times 10^{-9} \text{ m}^2\text{V}^{-1}\text{s}^{-1}$ ). The BGE (B) was a mixture (pH = 2.5) of 30 mM HCl ( $\mu_{\text{lim}}(\text{Cl}^-) = -79.1 \times 10^{-9} \text{ m}^2\text{V}^{-1}\text{s}^{-1}$ ) and 25.53 mM aqueous ammonia. A sample was a mixture of 0.1  $\mu\text{M}$  histidine and creatinine. Voltage (100 V) was applied for 100 s with the sample-inlet side as the anode. No EOF was assumed.

### 2.3 Instrumentation

The capillary-electrophoresis instrument (CAPI-3200; Otsuka Electronics, Osaka, Japan) was equipped with a photodiode array detector. A polyimide-coated fused-silica capillary (GL Sciences, Tokyo, Japan) with 62.4 cm total length (50 cm effective length) and 50  $\mu\text{m}$  id (375  $\mu\text{m}$  od) was used. The capillary was thermostated at 25  $^{\circ}\text{C}$ . The detection wavelength was set at 210 nm. The distance between the end of the capillary inlet and electrode ( $D_{\text{ec}}$ ) in a sample vial was set to 15 mm (Reportedly, the sensitivity and repeatability were improved using the longer  $D_{\text{ec}}$  [16, 17, 25]). This experiment used a pH meter (F-22; Horiba, Kyoto, Japan) and conductivity meter (DS-71; Horiba, Kyoto, Japan).

### 2.4 Chemicals and reagents

All reagents used were of analytical-reagent grade. Acetic acid and HCl were obtained from Wako Pure Chemical Industries (Osaka, Japan). Aqueous ammonia, L-histidine, and creatinine were obtained from Nacalai Tesque (Kyoto, Japan). Hydroxypropyl methylcellulose (HPMC) was obtained from Sigma-Aldrich (St. Louis, MO, USA). BGEs were a mixture of acetic acid and aqueous ammonia containing 0.03% (m/v) HPMC or a mixture of HCl and aqueous ammonia containing 0.03% (m/v) HPMC. The stock solution of L-histidine and creatinine was prepared in water at a concentration of 5 mM and was serially diluted to prepare standard solutions. All solutions were filtered through a 0.45  $\mu\text{m}$  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. To assess the MB effect,  $\mu_{\text{eff}}$  of analytes must be low in standard solutions with suppressed EOF. The pH of the standard solutions used in the experiments was about 6.5. The  $\mu_{\text{eff}}$  of L-histidine and creatinine in the standard solutions were found to be sufficiently low:  $6.9 \times 10^{-9}$  and  $0.8 \times 10^{-9} \text{ m}^2\text{V}^{-1}\text{s}^{-1}$ , respectively, as obtained using simulation software (Peakmaster 5.3 Complex) [26]. The pH of BGEs used was 4.6 or below, and HPMC was added to the BGEs. Therefore, EOF was fully suppressed in these BGEs. A blood plasma sample was given by a healthy male volunteer.

## 2.5 Experimental procedure

A new capillary was flushed with water for 5 min, then with 1 M NaOH for 20 min, water for 10 min, and BGE for 15 min (50 kPa vacuum pressure). When the counter-ion in BGE was changed, a new capillary was used. When the counter-ion concentration in BGE or the conductivity of BGE was changed, the capillary was flushed with water for 5 min, and then with BGE for 15 min. Before the first analysis of each day, the capillary was flushed with water for 5 min and BGE for 15 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. Voltage (20 kV) was applied for separation with the sample-inlet side as the anode. When the effects of kinds and concentrations of counter-ion were examined, the BGE conductivity was adjusted to the same value to equalize the

potential gradient between the end of the capillary inlet and the electrode.

### **3 Results and discussion**

#### **3.1 2-D computer simulation of the MB effect**

Figures 2B–2D depicts 2-D computer simulation results of EKI for histidine, glycine, and creatinine. Around the capillary inlet end in the sample vial, the pH became lower (Fig. 2B) because of the migration of acetate from the BGE. Because of the pH decrease, analytes around the capillary inlet end were cationized, and the  $\mu_{\text{eff}}$  of analytes increased (MB effect). Consequently, the cationized histidine and creatinine migrated into the capillary toward the cathode (Fig. 3C). Figure 3D presents the 2-D concentration profile of histidine. These results elucidated that the MB effect occurs around the capillary inlet end in a sample vial. As observed in Fig. 3C, because of the lower  $pK_a$  (2.35) compared to those of histidine (6.04) and creatinine (4.828), glycine did not migrate into the capillary. Therefore, glycine was excluded from subsequent experiments and simulations.

#### **3.2 Kind of counter-ions**

The effects of kind of counter-ion (acetate as anion of a weak acid and chloride as anion of a strong acid) in BGE on the MB effect and the analyte amount injected into the capillary were investigated. The BGE (A) was a mixture (pH = 4.6) of 100

mM acetic acid, 50 mM aqueous ammonia, and 0.03% (m/v) HPMC. The BGE (B) was a mixture (pH = 2.5) of 30 mM HCl and 0.03% (m/v) HPMC. The conductivity of the BGE (B) was adjusted to 0.42 S/m to be equal to the conductivity for the BGE (A) with 1 M aqueous ammonia to equalize the initial potential gradient in the sample. A mixture of 0.1  $\mu$ M L-histidine and creatinine was injected by EKI (+10 kV for 100 s). Figures 3A and 3B respectively depict electropherograms obtained using the BGEs (A) and (B). The peaks of L-histidine and creatinine obtained using the BGE (A) were higher and sharper than those for the BGE (B). The following equation was used for rough calculations of the injection amount ( $I_{\text{EKI}}$ ) of analytes into the capillary by EKI.

$$I_{\text{EKI}} = A_{\text{EKI}}I_{\text{vac}}/A_{\text{vac}} \quad (1)$$

Therein,  $A_{\text{EKI}}$  is the peak area of analytes when the sample (a mixture of 0.1  $\mu$ M L-histidine and creatinine) is injected by EKI.  $I_{\text{vac}}$  and  $A_{\text{vac}}$  respectively denote the injection amount (645 fmol) and the peak area of analytes when 50  $\mu$ M L-histidine and creatinine is vacuum injected (50 kPa) for 1.0 s (12.9 nL). In the case of the BGE (A), the respective injection amounts of L-histidine and creatinine into the capillary were 5.3 and 3.6 amol. In the case of the BGE (B), the respective injection amounts of L-histidine and creatinine were 2.5 and 1.0 amol.

The experimentally obtained results were confirmed using 1-D computer simulations. Figures S1 and S2 (Supporting Information) present simulation results of concentration profiles for the counter-ions (acetate and chloride), co-ion ( $\text{NH}_4^+$ ), and analytes (histidine and creatinine), potential gradient profile, and pH profile in

the sample (between anode and capillary inlet in the sample vial) and the BGE (in capillary) zones. In the figures, (A) and (C) depict the distributions at 0 s and (B) and (D) the distributions at 100 s. When the counter-ion was acetate, the sharp peaks for histidine and creatinine were observed (Fig. S1B). The respective injection amounts of histidine and creatinine were 98 and 69 fmol. The pH in the sample zone decreased from 7.0 to 3.0–3.6 because of the MB effect (Fig. S1D). The potential gradient in the sample zone decreased from 101 kV/m to 11.8–44.4 kV/m by the migration of acetate from the BGE zone (Fig. S1D). When the counter-ion was chloride, broad peaks were observed (Fig. S2B). The respective injection amounts of histidine and creatinine were 41 and 28 fmol. The pH in the sample zone decreased from 7.0 to 1.6–2.0 (Fig. S2D). The potential gradient in the sample zone decreased from 101 kV/m to 1.8–4.3 kV/m (Fig. S2D): the MB effect of chloride counter-ion was stronger than that of acetate counter-ion. However, the potential gradient in the sample zone for chloride counter-ion decreased more rapidly than that for acetate counter-ion. The rapid decrease of the potential gradient resulted from the rapid increase of the conductivity in the sample zone because of the migration of chloride from the BGE zone and the generation of  $H^+$  from  $H_2O$  to meet the electroneutrality law. As a result, the injection amount of analytes for acetate counter-ion was larger than that for chloride counter-ion. In both BGEs, FASI was realized because of the differences in conductivity between the sample zone and the BGE zone. Furthermore, tITP was realized in the ammonium acetate BGE. In this case,  $NH_4^+$  in the BGE acted as the leading ion.  $H^+$  generated by the dissociation of acetic acid acted as the terminating ion (system-induced terminator, SysT) [17, 27]. Therefore, the analytes were more

concentrated and provided the sharper peaks. Results show that the MB effect played an important role in enhancing the  $\mu_{\text{eff}}$ , but other factors affected the amount of analyte injected (e.g. potential gradient). Its enrichment (tITP) is a concern. Therefore, acetate was adopted as the counter-ion in BGE in subsequent experiments.

### **3.3 Concentration of counter-ions**

The effects of counter-ion concentration in BGE on the MB effect and the amount of analytes injected into the capillary were investigated. The BGEs were mixtures of 100, 200, or 500 mM acetic acid, 50 mM aqueous ammonia, and 0.03% (m/v) HPMC. The conductivity of these BGEs was 0.47 S/m. The mixture of 0.1  $\mu\text{M}$  L-histidine and creatinine was injected (+10 kV for 100 s). The injection amounts of L-histidine and creatinine into the capillary were calculated using equation (1). When the concentrations of acetic acid were 100, 200, and 500 mM, the respective injection amounts of L-histidine and creatinine were 6.8, 5.7, and 4.7 amol and 3.6, 3.2, and 3.0 amol. Results of 1-D computer simulations revealed that the potential gradient in the sample zone decreased concomitantly with increasing concentration of acetic acid in the BGE; no significant difference was found in the tendency of pH decrease in a sample zone (data not shown). The higher the acetic acid concentration, the more the acetate amount migrated to the sample zone, causing less potential gradient in the sample zone. Therefore, the injection amounts of analyte decreased with an increase in the concentration of acetic acid. The LODs ( $S/N = 3$ ) of L-histidine and creatinine were, respectively, 0.8, 0.9, and 1.0 nM and

1.5, 1.4, and 1.6 nM. No difference was found between the LODs because the conductivities of the BGEs were equal. Therefore, the FASI stacking effect was the same. When the lower concentration of counter-ion was used, slightly broader peaks of the analytes were observed. It was elucidated that the counter-ion concentration in BGE did not affect the MB effect but affected the injection amount of analyte because of the potential gradient in the sample zone.

### **3.4 Approaches to improve the performance of the proposed method**

In FASI, BGE conductivity affects the injection amount of analyte into the capillary and the stacking effect. The BGE conductivity was optimized in these respects. The BGEs consist of acetic acid (25, 50, 100, or 200 mM), aqueous ammonia (12.5, 25, 50, or 100 mM), and 0.03% (m/v) HPMC. The BGE conductivities were, respectively 0.11, 0.23, 0.46, and 0.87 S/m. The mixture of 0.1  $\mu$ M L-histidine and creatinine was injected (+10 kV for 100 s). The injection amount of L-histidine and creatinine was calculated using equation (1). When the conductivities were 0.11, 0.23, 0.46, and 0.87 S/m, the respective injection amounts of L-histidine and creatinine into the capillary were 2.0, 4.7, 4.9, and 4.7 amol and 0.71, 2.0, 3.3, and 3.3 amol (Fig. S3). The respective LODs ( $S/N = 3$ ) of L-histidine and creatinine were 2.0, 1.4, 1.0, and 1.0 nM and 5.5, 2.5, 1.6, and 1.5 nM. The injection amounts of analyte increased and the LODs improved with increasing conductivity of BGE. The LODs for 0.87 S/m were not significantly different from the LODs for 0.46 S/m. Therefore, the BGE containing 100 mM acetic acid, 50 mM aqueous ammonia, and 0.03% (m/v) HPMC was adopted in subsequent experiments.

The sample-injection time was optimized between 100 and 1000 s to improve the sensitivity of the proposed method. A mixture of 10 nM L-histidine and creatinine was injected (10 kV with the sample inlet side as the anode). The peak area and height of L-histidine and creatinine increased linearly with an increase in the injection time (Fig. S4). For injection times longer than 800 s, both peaks were insufficiently separated. Therefore, 500 s was adopted as the optimum sample-injection time in subsequent experiments. Under the optimal conditions, the LODs ( $S/N = 3$ ) for L-histidine and creatinine were, respectively, 0.10 and 0.25 nM. The LODs were respectively improved 23,000 (2.3  $\mu\text{M}$ ) and 13,000 times (3.3  $\mu\text{M}$ ) compared to those obtained using the vacuum injection method (50 kPa for 1.0 s, 12.9 nL). The LODs were the lowest ever achieved by CE with UV detection. The RSDs ( $n = 4$ ) of migration times for L-histidine (10 nM) and creatinine (10 nM) were obtained respectively as 0.42 and 0.24%, for peak areas of 2.5 and 1.7%, and for peak heights of 5.2 and 1.4%.

Another method to increase the  $\mu_{\text{eff}}$  of analytes for EKI is to lower the sample pH directly before analysis. The pH of the mixture of 0.1  $\mu\text{M}$  L-histidine and creatinine (pH = 6.5) was adjusted to 4.0 by adding acetic acid to the sample. According to the sample pH decrease, the  $\mu_{\text{eff}}$  of L-histidine and creatinine resulted in  $26.8 \times 10^{-9} \text{ m}^2\text{V}^{-1}\text{s}^{-1}$  ( $6.9 \times 10^{-9} \text{ m}^2\text{V}^{-1}\text{s}^{-1}$  at pH 6.5) and  $32.4 \times 10^{-9} \text{ m}^2\text{V}^{-1}\text{s}^{-1}$  ( $0.8 \times 10^{-9} \text{ m}^2\text{V}^{-1}\text{s}^{-1}$  at pH 6.5), respectively (calculated using Peakmaster 5.3 Complex). The sample was injected (+10 kV for 100 s). Figures 4A and 4B respectively depict the electropherograms for the sample of pH 6.5 and the sample of pH 4.0. The injection amount of L-histidine and creatinine calculated using equation (1) decreased respectively from 4.9 to 0.84 amol and from 3.3 to 1.4 amol. Two reasons

explain the decrease. (1) The sample conductivity increased from 0.166 to 3.32 S/m by the addition of acetic acid. As a result, the potential gradient in the sample zone decreased and the injection amounts of analyte decreased. (2) The transference number of  $H^+$  increased by adding acetic acid, causing the decrease of the transference number of analytes. Thereby, the injection amounts of analyte decreased [16]. The sample zone pH was lowered spontaneously because of the MB effect without adjusting the pH before analysis. A lower probability of contamination and labor are the benefits of using the MB effect.

### 3.5 Application to real samples

Using the proposed method, L-histidine and creatinine in a human blood plasma sample from a healthy male volunteer were determined. Figure 5 depicts an electropherogram of a 10,000-fold diluted plasma sample. The RSDs ( $n = 4$ ) of the migration times for L-histidine and creatinine were obtained respectively as 0.67 and 0.68%, for peak areas of 1.7 and 5.7%, and for peak heights of 3.0 and 3.8%. Calibration graphs were established by spiking L-histidine (5–20 nM) and creatinine (5–20 nM) in 10,000-fold diluted plasma samples. Regression equations relating the area response to concentration for L-histidine and creatinine were  $y = 2.62x + 20.9$  ( $R^2 = 0.9869$ ) and  $y = 1.36x + 8.60$  ( $R^2 = 0.9997$ ). The concentrations of L-histidine and creatinine in the 10,000-fold diluted plasma sample were, respectively, 8.0 and 6.3 nM. The respective recoveries of L-histidine and creatinine spiked into the plasma sample (5 nM L-histidine and creatinine) were 109 and 102%. Therefore, the concentrations of L-histidine and creatinine in the sample



were, respectively, 80 and 63  $\mu\text{M}$ . The results agreed with those obtained using the conventional methods (LC-MS for L-histidine and enzymatic method for creatinine), 79 and 61  $\mu\text{M}$ , respectively. Other amino acids except for arginine could not be detected because they are not cationized in the BGE pH for the proposed method. Arginine could be migrated between L-histidine and creatinine, but its absorbance at 210 nm is much lower than those for L-histidine and creatinine.

#### **4 Concluding remarks**

This study demonstrated that the MB effect occurred around the inlet end of the capillary in a sample zone using 2-D computer simulation. Results of experimentation and 1-D computer simulations revealed that the MB effect for acetate (weak electrolyte counter-ion) was weaker than that for chloride (strong electrolyte counter-ion). However, acetate should be adopted because of the realized tITP and the higher potential gradient in the sample zone. The concentration of acetic acid in the BGE did not affect the MB effect, but the lower concentration was preferred because the higher potential gradient in the sample zone caused more analyte amount injected into the capillary. As a result of optimization of other factors such as BGE conductivity and sample-injection time, the LOD of L-histidine and creatinine reached the sub-nanomolar level. The proposed tITP-CZE method using FASI with MB effect is useful for evaluating low concentrations of analytes with similar  $pI$  and  $pK_a$  to L-histidine and creatinine.

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The authors have declared no conflict of interest.

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**Figure 1.** Mobility-boost effect in EKI. (A) initial state (the capillary is filled with BGE; a sample vial is set), (B) migration of the counter-ion ( $\text{Cl}^-$ ) in the BGE to the anode by electrophoresis, (C) dissociation of  $\text{H}^+$  from  $\text{H}_2\text{O}$  and cationization of analytes (from  $\text{A}_1^\pm$  to  $\text{A}_1\text{H}^+$  and  $\text{A}_2$  to  $\text{A}_2\text{H}^+$ ), and (D) injection of the cationized analytes into the capillary by electrophoresis. Other explanations are given in the text.

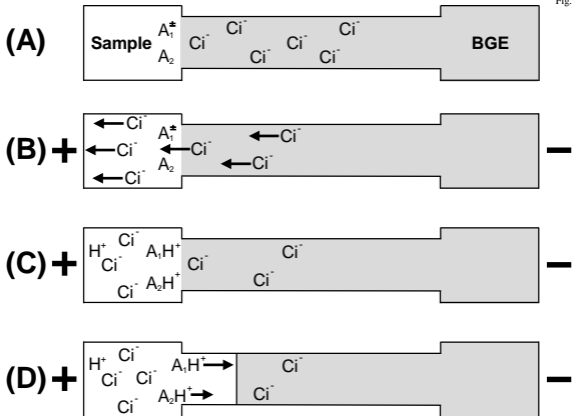
**Figure 2.** Schematic of a 2-D simulated model and simulation results at 1.775 s after the injection voltage was applied: (A) 2-D simulated model, (B) 2-D profile of pH, (C) concentration profile of analytes (histidine, glycine, and creatinine), and (D) 2-D profile of histidine concentration. The color corresponds to the values as scaled in (B) and (D). Other conditions are described in the text.

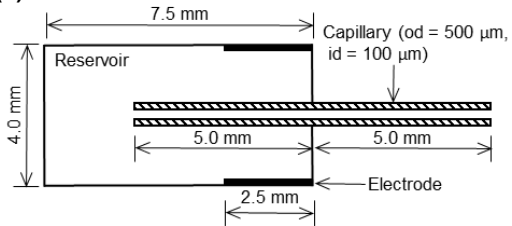
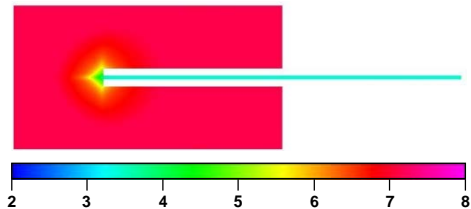
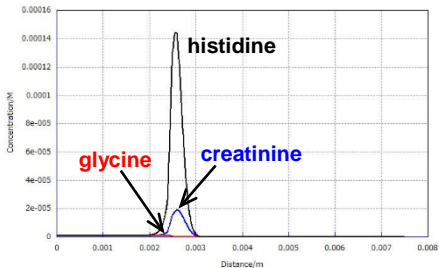
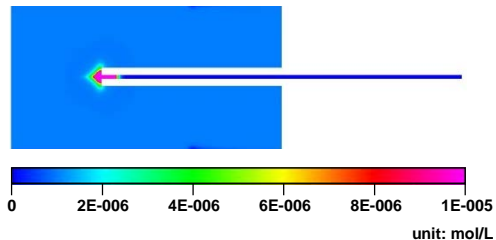
**Figure 3.** Electropherograms obtained using different counter-ions: (A) BGE, a mixture of 100 mM acetic acid, 50 mM aqueous ammonia, and 0.03% (m/v) HPMC (conductivity = 0.42 S/m, pH = 4.6); (B) a mixture of 30 mM HCl and 0.03% (m/v) HPMC (conductivity = 0.42 S/m adjusted by aqueous ammonia, pH = 2.5). Electrophoretic conditions: capillary, 62.4 cm total length (50 cm effective length) and 50  $\mu\text{m}$  id (375  $\mu\text{m}$  od); sample, a mixture of 0.1  $\mu\text{M}$  L-histidine and creatinine; sample injection, 10 kV with the sample inlet side as the anode for 100 s; separation voltage, 20 kV; wavelength for detection, 210 nm.

**Figure 4.** Effect of the sample pH on the injection amounts for L-histidine and creatinine: (A) sample pH, 6.5; (B) sample pH, 4.0 (adjusted by adding acetic acid

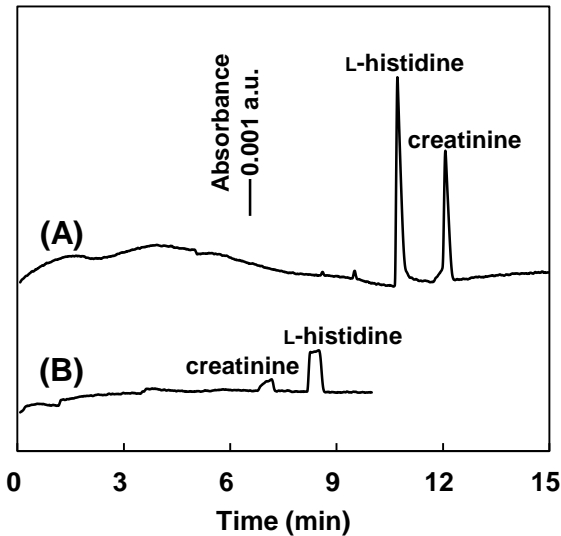
to the sample). Electrophoretic conditions: BGE, a mixture of 100 mM acetic acid, 50 mM aqueous ammonia, and 0.03% (m/v) HPMC (conductivity = 0.42 S/m, pH = 4.6); sample, a mixture of 0.1  $\mu$ M L-histidine and creatinine; Other electrophoretic conditions are identical to those in Fig. 3.

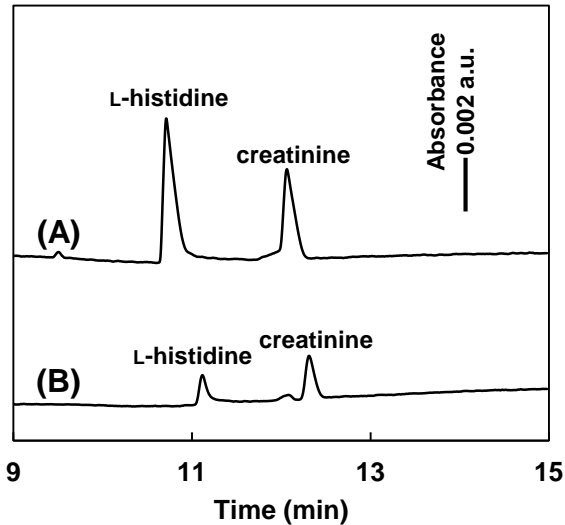
**Figure 5.** Electropherogram of a 10,000-fold diluted plasma sample using the proposed method. Electrophoretic conditions: sample injection, 10 kV with the sample inlet side as the anode for 500 s. Other electrophoretic conditions are identical to those in Fig. 4.

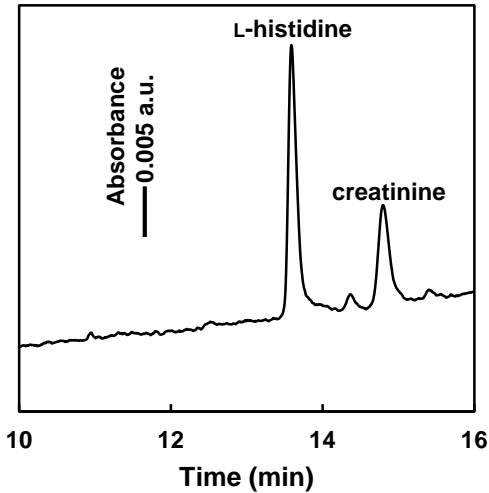


**(A) 2-D simulated model****(B) pH****(C) analytes conc.****(D) histidine conc.**







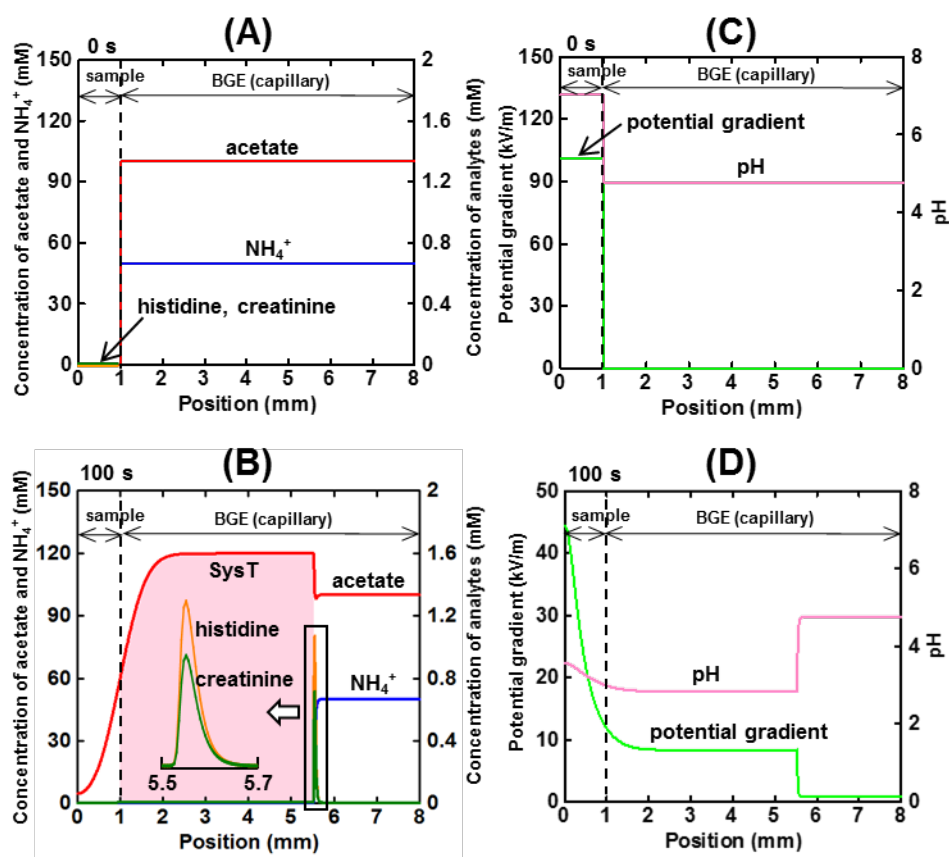


# Highly sensitive tITP-CZE determination of L-histidine and creatinine in human blood plasma using field-amplified sample injection with mobility-boost effect

Takanari Hattori and Keiichi Fukushi\*

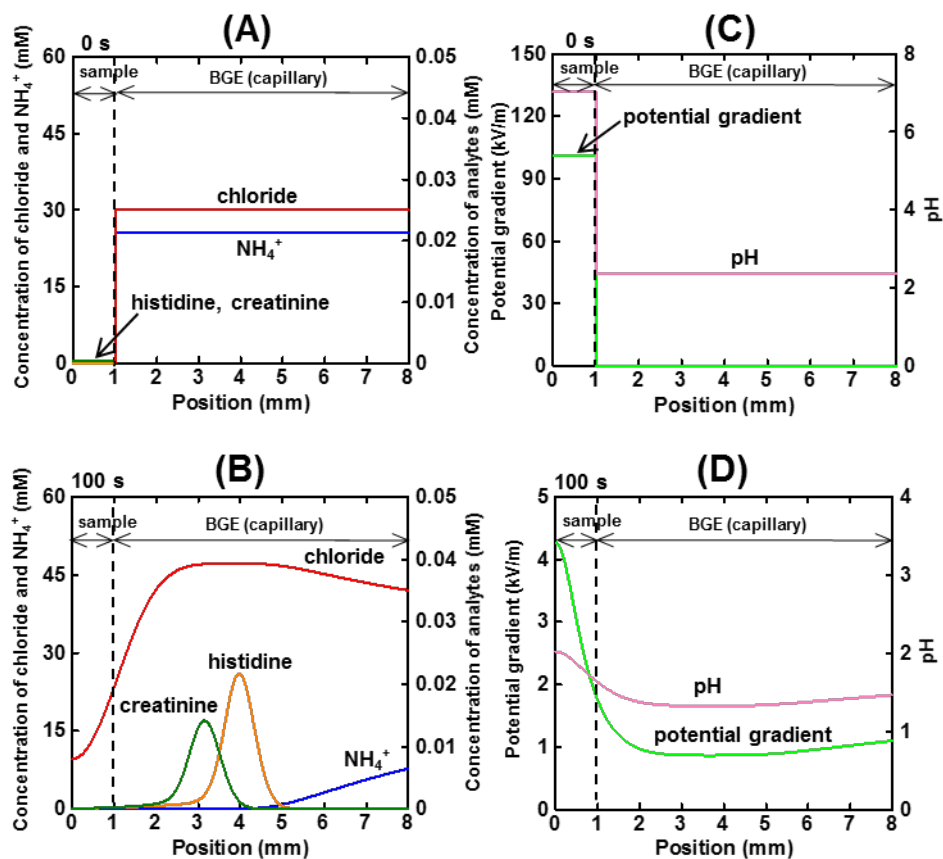
Graduate School of Maritime Sciences, Kobe University, Kobe, Japan

## Supporting information

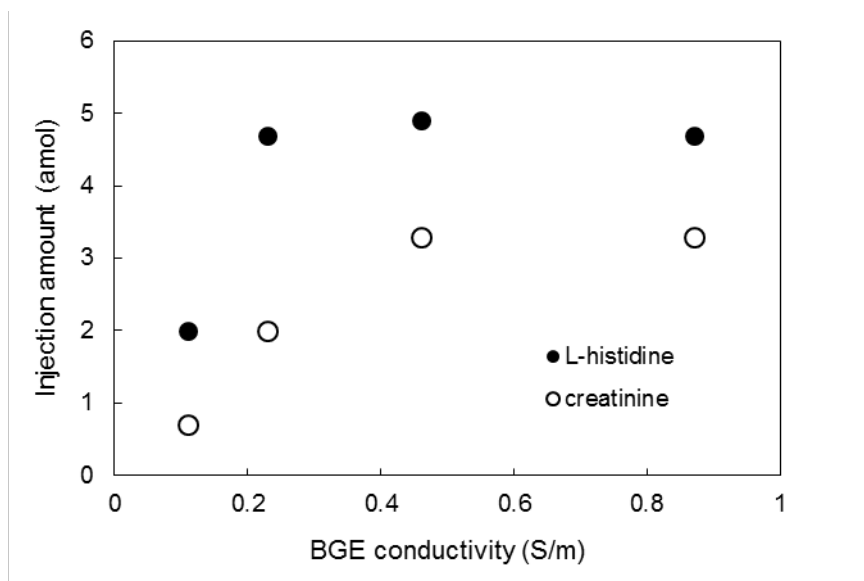


**Figure S1.** 1-D simulation results obtained using the BGE of acetate counter-ion at 0 s (A) and (C) and 100 s (B) and (D) after the injection voltage was applied: (A) and (B) concentration profiles for counter-ions (acetate), co-ion ( $\text{NH}_4^+$ ), and analytes (histidine and creatinine) in the sample and the BGE zones; (C) and (D) potential gradient and pH profiles in the sample and the BGE zones. SysT:

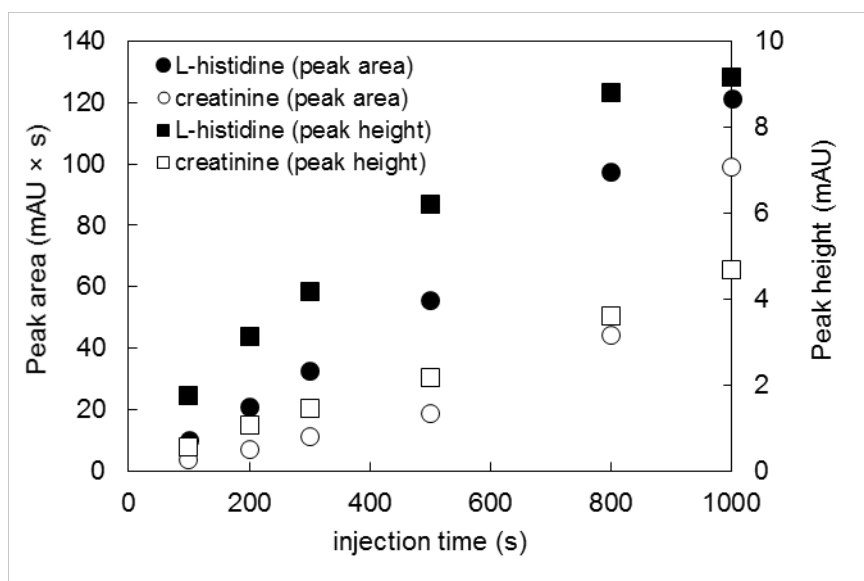
system-induced terminator. Other conditions are described in the text.



**Figure S2.** 1-D simulation results obtained using the BGE of chloride counter-ion at 0 s (A) and (C) and 100 s (B) and (D) after the injection voltage was applied: (A) and (B) concentration profiles for counter-ions (chloride), co-ion (NH<sub>4</sub><sup>+</sup>), and analytes (histidine and creatinine) in the sample and the BGE zones; (C) and (D) potential gradient and pH profiles in the sample and the BGE zones. Other conditions are described in the text.



**Figure S3.** Effect of BGE conductivity on the injection amount of L-histidine and creatinine into the capillary. (●) L-histidine and (○) creatinine. Electrophoretic conditions: BGE, a mixture of acetic acid (25, 50, 100, or 200 mM), aqueous ammonia (12.5, 25, 50, or 100 mM), and 0.03% (m/v) HPMC; capillary, 62.4 cm total length (50 cm effective length) and 50  $\mu\text{m}$  id (375  $\mu\text{m}$  od); sample, a mixture of 0.1  $\mu\text{M}$  L-histidine and creatinine; sample injection, 10 kV with the sample inlet side as the anode for 100 s; separation voltage, 20 kV; wavelength for detection, 210 nm.



**Figure S4.** Effect of sample-injection time on the peak area and peak height of L-histidine and creatinine. (●) peak area of L-histidine, (○) peak area of creatinine, (■) peak height of L-histidine, (□) peak height of creatinine. BGE, a mixture of 100 mM acetic acid, 50 mM aqueous ammonia, and 0.03% (m/v) HPMC; sample, a mixture of 10 nM L-histidine and creatinine; sample injection, 10 kV with the sample inlet side as the anode for 100–1000 s. Other electrophoretic conditions are identical to those in Fig. S3.