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Numerical simulation for capillary electrophoresis II. Relaxation effect of potential gradient in capillary zone electrophoresis

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Abstract

Using our simulation program for CE based on partial differential equations of unsteady state, we found the phenomena at the initial stage of the capillary zone electrophoretic process that a transient zone with very high potential gradient was produced at the front-end of the sample plug and consequently the potential gradient of the rest part without samples decreased. The potential gradient of the zone and the rest part gradually reached the averaged potential gradient (relaxation of potential gradient). This phenomena causes delay of migration time to some degree affecting its reproducibility. This effect is due to discontinuities of the CE system and low mobility of a counter ion in a background electrolyte (BGE). Therefore, in order to improve the delay, discontinuities of solution should be minimized or a counter ion with high mobility in BGE should be selected.

Keywords: Computer simulation; Potential gradients

1. Introduction

Capillary zone electrophoresis (CZE) is a significant analysis method because of its high separability and its high absolute sensitivity. However, CZE is still less popular than liquid chromatography. One of the reasons might be low reproducibility of migration times which are frequently used as qualitative indices. Obviously reproducibility of migration times also influence peak areas as quantitative indices. From the above view-point, high reproducibility of migration times in CZE is crucial to obtain accurate analytical results.

The low analytical reproducibility of CZE is partly originated from experimental factors such as change of electroosmotic flow (EOF) caused by hysteresis of a capillary inner wall, change of a sample amount introduced to a separation capillary, etc. These experimental problems have been solved by using automated equipment with an appropriately pretreated capillary. However, even under ideal experimental conditions, migration time is often not reproducible enough for identification.

Although the CZE system seems quite simple from its principle, Mikkers et al. [1] had showed theoretically that CZE system could be very complex in the sense that the potential gradient in the capillary might be easily perturbed by introduced samples and/or sample zones themselves. For example, it was reported that electrophoretic dispersion was caused by local changes in potential gradient

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[1]. In fact, if the potential gradient was exactly constant through an separation experiment, no problems occurred including reproducibility of migration time.

Thus, time and/or location dependence of potential gradient is very important in CZE, but detailed studies on the behavior of potential gradient in the separation capillary are not so many. Using a model based on Kohlrausch's regulating function [2], Mikkers et al. [1] discussed concentration distribution and electric field distribution in relation to the mobility ratio of the sample to the carrier constituent. Using a simpler model for the case that samples could be stacked, Beckers and Ackermans [3] succeeded to explain quantitatively that migration times were influenced by relative length of injection plug to the whole capillary. In spite of simplicity of their model, the calculated migration times agreed surprisingly well with experimental ones when the sample concentration was low and the plug was long. However, when the relative plug length was smaller than 1% as in usual experiments, the experimental migration times were larger than the predicted ones. This disagreement was explained as the result that the effect of the low concentration sample is partly canceled by diffusion.

Obviously, migration time is closely related with potential gradient. Although they assume constant potential gradient for the sampling zone and background electrolyte during migration process, the former may change at the initial stage of migration and accordingly the latter should change.

In this paper, from the above viewpoint, the CZE process was simulated using our computer program [4] to discuss quantitatively how the migration times of test mixture depends on the concentration. We also discussed how we can improve the reproducibility of migration time among the samples with different concentrations.

2. Theoretical

As described by Mikkers et al. [1], the sample peak may have a Gaussian shape when the sample mobility is equal to that of the carrier constituent. However except for such a special case, the sample peaks show asymmetric profiles with tailing or fronting. Potential gradient at the edge of these peaks can be approximated to that of background electrolyte without sample ions, which affects directly migration times of the sample peaks.

The potential gradient of background electrolyte without sample ions in CZE is expressed as the first approximation, by the averaged potential gradient E_{av}, i.e., the applied voltage divided by whole length of the capillary. This is valid when no samples are contained in the system. However, when the samples are introduced and the composition in the separation system is not homogeneous, the potential gradient of a background electrolyte (BGE) without sample ions is different from the averaged one.

In order to study potential gradient in detail using computer simulation, we used the following mathematical model: supposing that diffusion current is negligibly small, conductivity at a given place x and time t, $\Lambda(x,t)$, is expressed as follows:

$$\Lambda(x,t) = F\left(\sum_i |\bar{m}_i| C_i + |m_{H^+}|[H^+] + |m_{OH^-}|[OH^-]\right) \tag{1}$$

where F, m_H and m_{OH} are Faraday constant and mobility of H^+ and OH^-, respectively.

Then, specific resistance at the place, $\rho(x,t)$, can be obtained as follows:

$$\rho(x,t) = 1/\Lambda(x,t) = \frac{1}{F\left(\sum_i |\bar{m}_i| C_i + |m_{H^+}|[H^+] + |m_{OH^-}|[OH^-]\right)} \tag{2}$$

The resistance of whole capillary system at a given time, $R(t)$, can be expressed in a following integral form:

$$R(t) = \int_L \frac{\rho(x,t)}{\pi r^2} dx = \int_L \frac{dx}{\pi r^2 F\left(\sum_i |\bar{m}_i| C_i + |m_{H^+}|[H^+] + |m_{OH^-}|[OH^-]\right)} \tag{3}$$

where r is a radius of a separation capillary. When

voltage V is applied to the system using a constant voltage supplier, current through the system, I, is expressed as:

$$I(t) = \frac{V}{R(t)} = \frac{V}{\int_L \frac{dx}{\pi r^2 F\left(\sum_i |\bar{m}_i| C_i + |m_{H^+}|[H^+] + |m_{OH^-}|[OH^-]\right)}} \quad (4)$$

Potential gradient at a given position x is expressed as follows:

$$E(x,t) = I\rho(x,t)/\pi r^2 = \frac{V}{\int_L \frac{dx}{\left(\sum_i |\bar{m}_i| C_i + |m_{H^+}|[H^+] + |m_{OH^-}|[OH^-]\right)}} \times \frac{1}{\left(\sum_i |\bar{m}_i| C_i + |m_{H^+}|[H^+] + |m_{OH^-}|[OH^-]\right)} \quad (5)$$

Therefore, if potential gradient were extremely high in a certain zone, potential gradient at the other position is lower than the averaged potential gradient.

3. Experimental

All simulations were performed using our program on a 433au (DEC, Nashua, NH, USA). Details of the developed program using ''shifted up-winding method'' were already reported [4].

The analyzed samples were equimolar mixtures of KCl, LiCl and ϵ-aminocaproic acid (0.3 m*M*, 3 m*M*, 30 m*M*). The used BGE was 30 m*M* creatinine and pH of the solution was adjusted by adding capric acid to 4.8. Besides capric acid, some model anions of strong electrolyte and weak electrolyte with various mobilities were also used in the simulation. Table 1 shows physico-chemical constants of the used electrolytes. Mobility of EOF was assumed to be $30 \cdot 10^{-5}$ cm^2 V^{-1} cm^{-1}.

The other simulation condition was that capillary length $L = 60$ cm, injection plug length is 0.2 cm, applied voltage was 30 kV (averaged potential gradient = 500 V/cm), and the space step and the time step were 0.02 cm and 0.001 s, respectively. It took 1 to 4 h for the simulation of one run.

In order to confirm the simulation results, migration times were evaluated experimentally. The samples were equimolar mixtures of KCl, LiCl and ϵ-aminocaproic acid (0.3–30 m*M*). Both of the samples dissolved in water and BGE were used. The used BGE was 30 m*M* creatinine and 30 m*M* isobutylic acid, and resultant pH was 4.8. A used apparatus was CAPI-3100 (Otsuka Electronics, Osaka, Japan). The fused silica capillary (Otsuka Electronics) of 60 cm (effective length, $l = 47.7$ cm) × 75 μm I.D. was used. The capillary chamber was thermostatted at 25°C using a cooling fan. The applied voltage was 20 kV. The sample solutions were injected hydrodynamically for 60 s at 1 cm.

3.1. Results and discussion

Fig. 1 shows simulated concentration profiles of the samples in the capillary (50s after starting migration). Obviously from Figs. 1a–c, it was pre-

Table 1
Physico-chemical constants for the simulation (25°C)

Material	Mobility ($\cdot 10^{-5}$ cm^2/V s)	pK_a
KOH	40.1	13.8
LiOH	76.2	13.0
ϵ-Aminocaproic acid	28.8	4.4
HCl	−79.1	−2.0
Creatinine	37.2	4.8
Capric acid	−22.1	5.0
Strong electrolytes	−5 to −80	<0
Weak electrolytes	−10 to −50	4.8

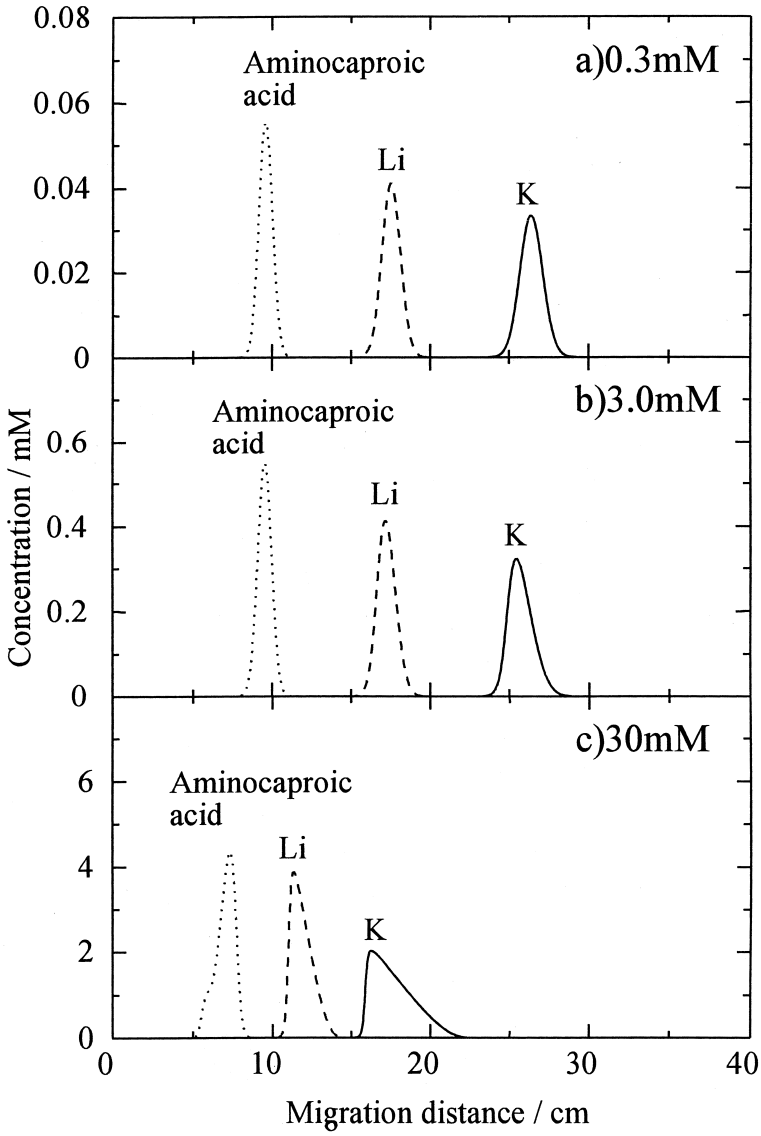

Fig. 1. Change in electrophoretic behaviors of K, Li and ϵ-aminocaproic acid according to their concentration. The values in the figures are the sample concentration. The BGE was 30 m*M* of creatinine–capric acid (pH 4.8). The other conditions are described in the text.

dicted that migration times may change according to the concentration of the sample. It had been reported by Mikkers et al. that the front peak edge of ions with higher mobility than that of BGE, such as K and Li, might be reproducible when the injection plug lengths were constant. However, their prediction did not hold in these simulations. Consequently, we should consider the other reason for the change of migration time than electrophoretic dispersion. So, potential gradient in the system should be discussed in more detail.

Fig. 2 shows time dependence of potential gradient profile of a transient zone formed at the leading edge of the injection plug. In the case of the low

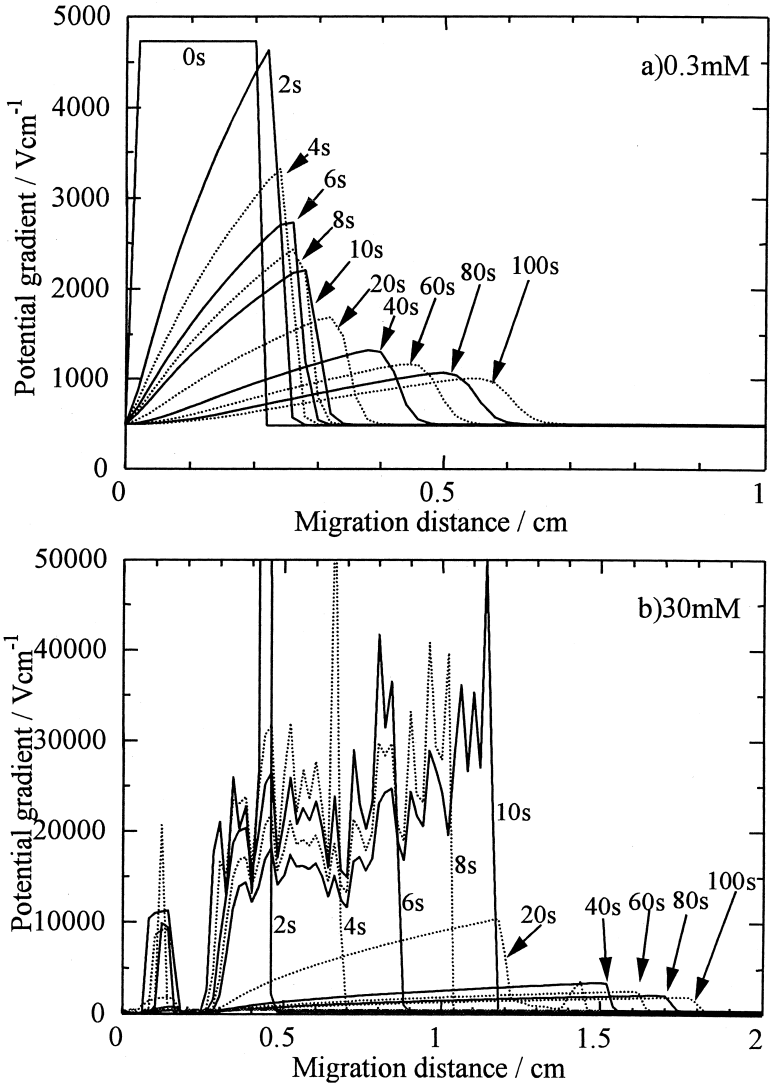

Fig. 2. Time dependence of potential gradient profile near the injection plug. Sample concentration was (a) 0.3 m*M* and (b) 30 m*M*. The numbers are time after start of migration.

concentration sample (Fig. 2a), initial potential gradient at the transient zone was higher than in the other zone, potential gradient at the zone decreased by diffusion and approached to the averaged value (in this case 500 V/cm) in the course of time. On the other hand, in the case of the high concentration sample(Fig. 2b), a transient zone with much higher potential gradient than that of the low concentration case was formed at the leading edge of the injection plug and it was retained for a while. Then the potential gradient at the zone gradually decreased by diffusion again.

As the result, potential gradient of whole capillary depended on time as shown in Fig. 3. Obviously from comparison of Figs. 3a and b, the change of potential gradient was serious in the case of samples with high concentration. Such a process that the generation of the transient zone with high potential

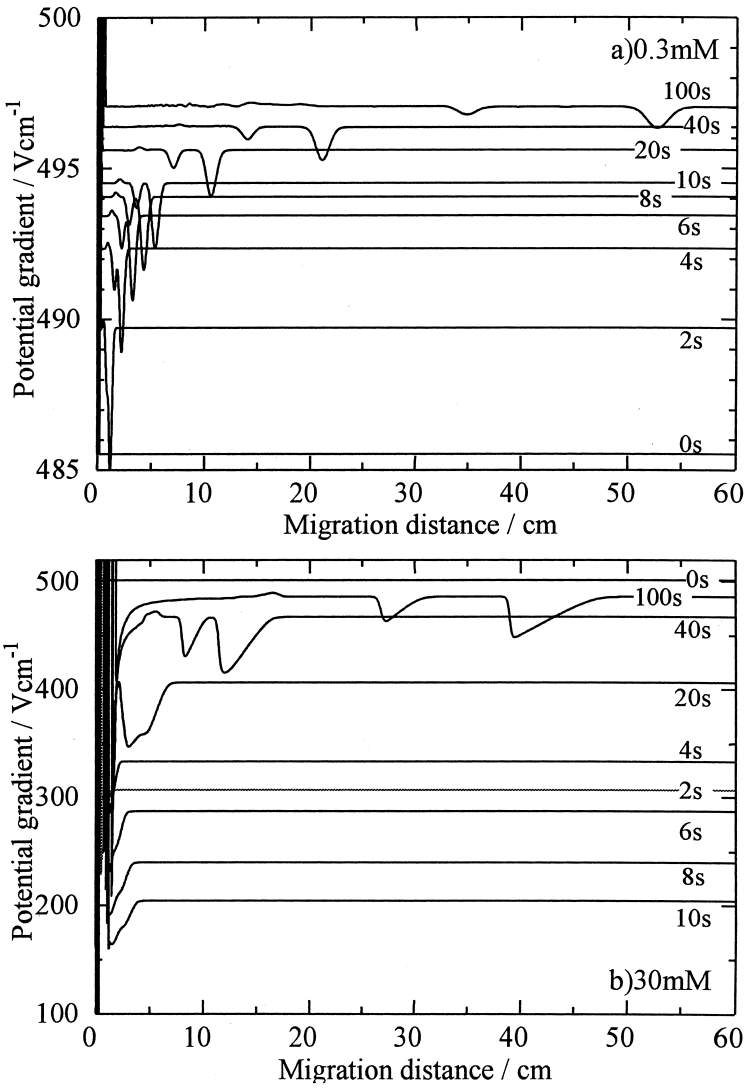

Fig. 3. Time dependence of potential gradient profile of whole capillary. Sample concentration was (a) 0.3 m*M* and (b) 30 m*M*. The numbers in the figure are time after start of migration.

gradient and the gradual decrease of the potential gradient approaching to the average one is named ''relaxation of potential gradient'' (RPG).

In a constant voltage mode, migration time is influenced by RPG because electromigration velocity depends on potential gradient. Especially, the potential gradient of BGE without sample ion is significant to reproducibility of migration time, because the potential gradient at the leading edge of a fronting zone with highest reproducibility can be approximated to the potential gradient. Consequently, the migration time of high concentration sample is behind that of low concentration sample due to RPG effect. The decrease of the potential gradient of BGE without sample is caused by the decrease of the current passing through the capillary, because the resistance of the whole capillary system increases due to the increase of the specific resistance at the transient zone. In constant current mode, therefore, migration time may not be affected by RPG effect in

principle. However, in the actual run, the use of constant current mode does not always ensure no RPG effect, because the used current source has the upper limit of the suppliable voltage. Furthermore, even if the constant current was kept, the bubbles may be formed due to Joule heating resulting discontinuation of migration.

Fig. 4 shows time dependence of the potential gradient of BGE without samples. The potential gradient was kept constant approximately when the low concentration sample was injected. On the other hand, the potential gradient decreased once at the initial stage and then gradually approached the average potential gradient E_{av} asymptotically when the high concentration sample was injected. The migration time of the high concentration sample was delayed by this effect in comparison with the low concentration one. Delay time τ of any ions detected after the potential gradient reaching E_{av} may be estimated to be constant. τ can be obtained as follows (see Fig. 2):

$$\tau = \frac{A}{E_{av}} \tag{6}$$

where E_{av} is average potential gradient (V/cm) and A is the area surrounded by E_{av} and potential gradient at the zone without sample ion (V s/cm).

In order to clarify the reason for this phenomenon, the relation between the potential gradient and the ion concentration near the sample plug was studied in detail. As shown in Fig. 5 a zone with extremely high potential gradient was formed at the boundary between the injection plug and BGE. It is clear from Fig. 5 that the potential gradients at BGE without sample ions decreases with spreading of the zone. This zone with high potential gradient was caused by concentration decrease of BGE and counter ion: concentration of chloride ion decreased at the boundary between the plug and BGE. The region with low total ionic concentration was formed because the effective mobility of capric acid was lower than that of chloride ion. For this reason, potential gradient of the zone began to increase just after applying migration voltage as shown in Fig. 5 and the resultant high potential gradient swept out the ions from the zone. Consequently, the zone with low concentration of total ions with high potential gradient expanded as shown in Fig. 5 for 0.1–0.9 s. The zone persisted for a while and then disappeared because of the diffusion.

Thus, the decrease of total ion concentration at the

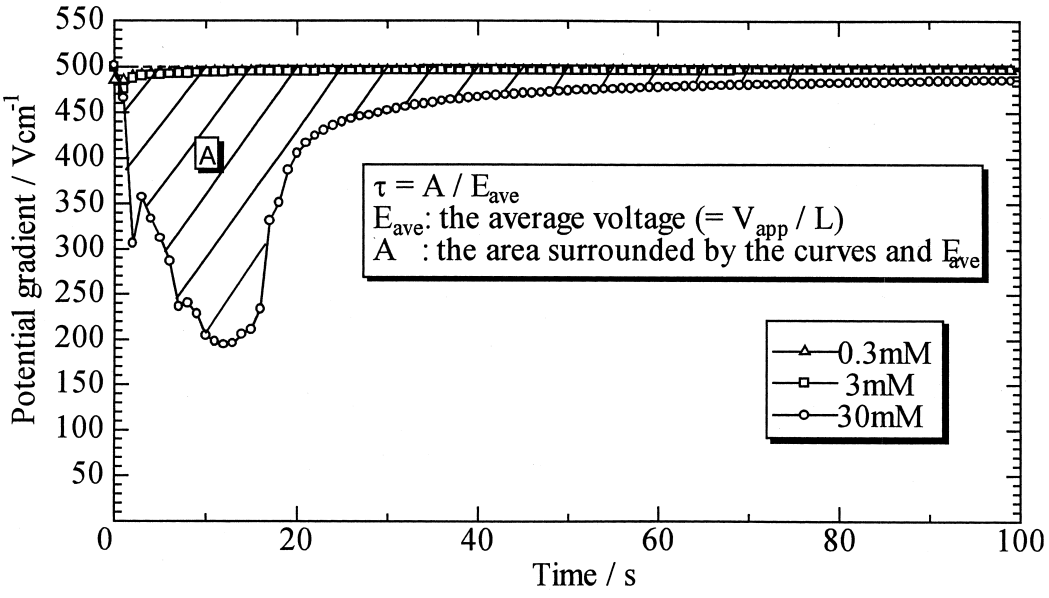

Fig. 4. Time dependence of the potential gradient of BGE. The experimental conditions are the same as in Fig. 1. The delay time τ can be obtained by dividing the area, surrounded by the average potential gradient 500 V and these curves, by the average potential gradient.

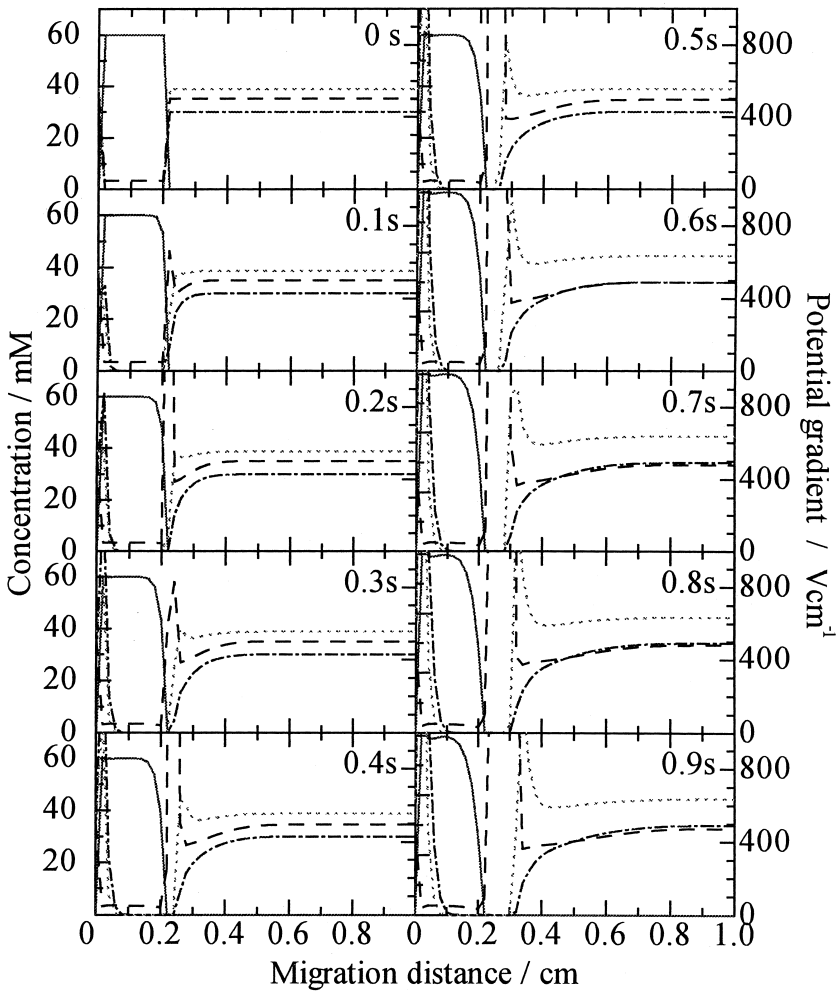

Fig. 5. Concentration profiles of chloride ion (solid line), creatinine (dashed line), capric acid (dotted line), and potential gradient (broken line) near the injection plug. The sample was the 30 m*M* equimolar mixture of KCl, LiCl and ϵ-aminocaproic acid.

sample plug causes RPG. The fundamental causes of occurrence of the transient zone are electrolyte discontinuity in the separation system and/or low mobility of the counter ion in BGE. Therefore, if the discontinuity and the counter ion with low mobility will not be provided, RPG will be significantly suppressed.

Fig. 6 shows simulated time dependence of the potential gradient of BGE when the samples (30 m*M* KCl, LiCl, ϵ-aminocapronic acid) were dissolved in BGE. This sample minimizes the discontinuity of the separation system. Obviously, the influence of RPG decreased in comparison with the sample dissolved in water in accordance with our prediction. The delay time was improved 34.0% from 13.0 to 8.6 s, which were evaluated by using Eq. 6. Migration time necessary for the detection of all sample components was 320 s according to the present simulation. RPG remained still because discontinuities of potential gradient and ion components were not completely removed.

Then, the effect of the effective mobility of the counter ion on the delay time τ was simulated and the result was shown in Fig. 7. The delay time

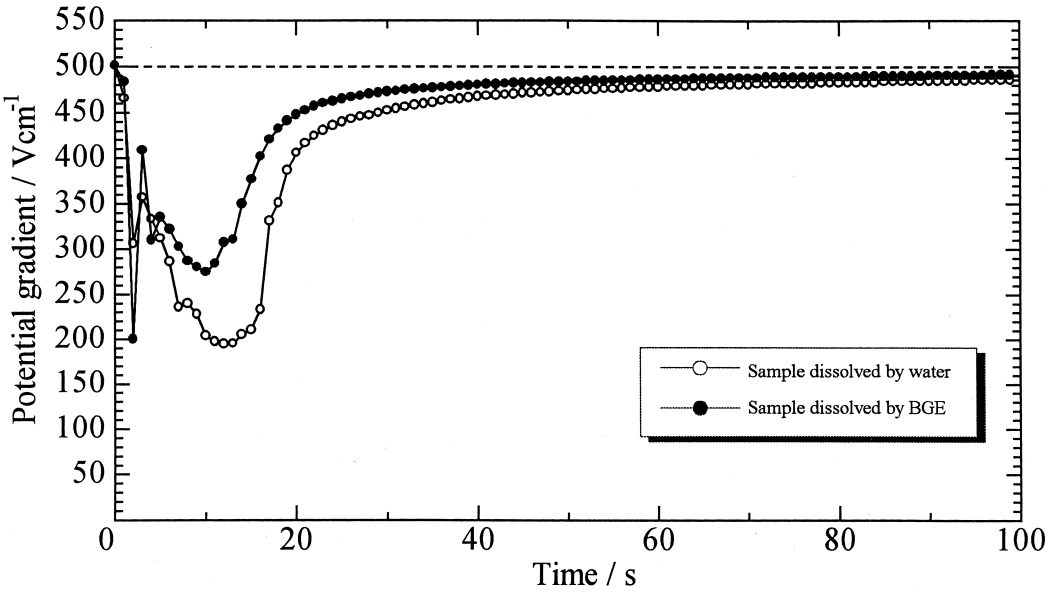

Fig. 6. Potential gradient of BGE. The sample was dissolved in water (○) and in BGE (●). The sample was the 30 m*M* equimolar mixture of KCl, LiCl and ε-aminocaproic acid.

decreased with the increase of the mobility of the counter ion in accordance with our prediction, and the relaxation effect was suppressed more efficiently than using the sample dissolved in BGE. It is interesting to note that the counter ions of strong electrolytes suppressed RPG more effectively than

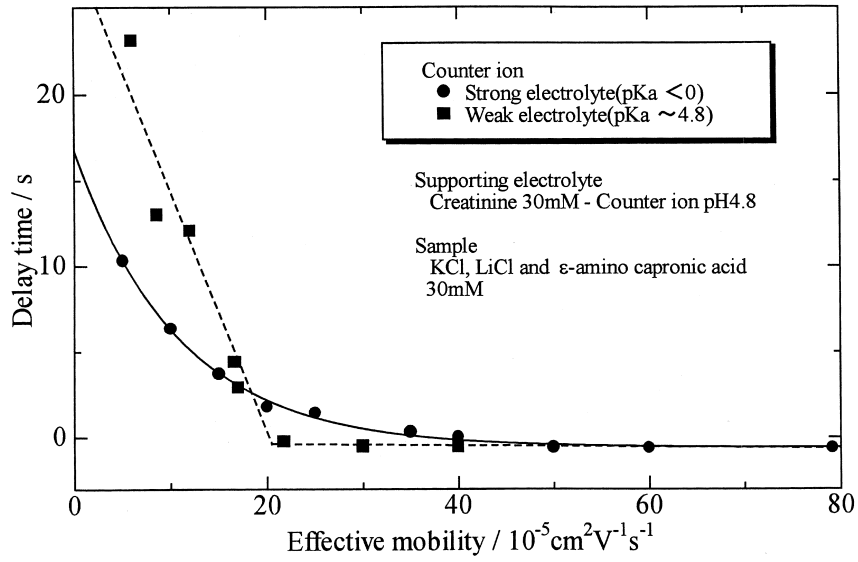

Fig. 7. Dependence of the delay time τ on the effective mobility of the counter ions (●, strong electrolytes; ■, weak electrolytes).

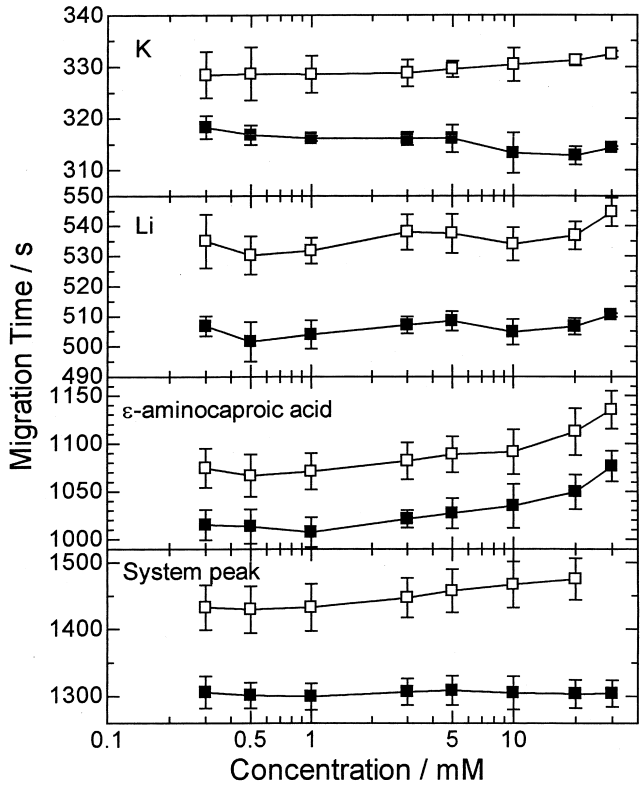

Fig. 8. Dependence of experimentally determined migration time on the sample concentration. The migration times of the K, Li and system peak were taken from the leading edges, while the rear edge was used for ε-aminocaproic acid.

those of the weak electrolytes. This was because the relaxation effect was enhanced due to the small effective mobility of the counter ions at the leading edge of the transient zone, where the pH is lower than that of BGE.

Fig. 8 shows the experimental migration times of the sample ions at the various concentrations. Obviously, the migration times delayed slightly but significantly when the sample concentration was high. However, the delay of the migration time was smaller than estimated suggesting that RPG was overestimated by the present simulation because no temperature change in the separation capillary was considered. In the actual experiment, the temperature of the transient zone will increase considerably due to Joule heating. This overestimation does not deny the concept of RPG, since the migration times of the samples dissolved in BGE were shortened as predicted.

4. Conclusions

When high concentration samples were injected in CZE, migration times delay even if the length of the sample plug was practical and appropriate. This delay was caused by the relaxation of potential gradient of the transient zone formed at the leading edge of the sample plug. The transient zone is formed by discontinuities of the electrolyte system in the separation system and low mobility of counter ion in BGE. It is possible to eliminate this effect by using the counter ion with high effective mobility or operating in a constant current mode if the voltage of

the current source can follow without forming bubbles.

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