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Standardization of capillary zone electropherograms obtained by using field enhanced sample stacking

Takeshi Hirokawa*, Natsuki Ikuta, Tatsuya Yoshiyama

Applied Physics and Chemistry, Faculty of Engineering, Hiroshima University, 1-4-1 Kagamiyama, Higashi-Hiroshima 739-8527, Japan

Abstract

Migration times in a capillary zone electropherogram obtained by using the field enhanced sample stacking technique are strongly affected by the injected sample volume. That is, the migration times significantly decrease with the increase of the sample volume. To avoid inaccurate qualitative analysis due to the above phenomena, the time axis of the electropherograms was converted into an effective mobility axis using our conversion method taking account of the temperature increase in the separation tube and relaxation of the potential gradient of the separation field. After the conversion, accurate qualitative analysis was possible in spite of drastic change of the migration time, suggesting our conversion method could be successfully used for the standardization of electropherograms obtained even by using the stacking effect. The cause of the decrease of the migration time in the stacking process was briefly discussed.

Keywords: Capillary electrophoresis; Sample stacking; Standardization; Field enhanced sample injection; Metal cations; Aminocaproic acid; Tris

1. Introduction

Although chromatography is widely utilized, it is behind the other analytical methods from the viewpoint of the standardization of the data. This is because usual chromatograms are strongly dependent on the used hardware. Capillary electrophoresis (CE) methods are the exceptions either: the obtained electropherograms are also dependent on hardware such as capillary length, capillary inner surface, applied voltage, and thermostatting capacity, even if the same supporting electrolyte (SE) and the same sample is used. However, the separation principle of capillary zone electrophoresis (CZE) is the difference of effective mobilities among the sample components, which should be constant as long as the same SE at the same temperature was used. Therefore, such hardware dependence may be removed, since in the case of CE methods the separation field is the free solution system and therefore complex liquid–solid interaction should not be considered.

However, it should be noted that the temperature rise in a separation capillary could not be avoided due to Joule heating, and the potential gradient of the separation field varies depending on the sample constituent, which sometimes causes bad reproducibility of migration time. The latter is due to the fact that a CZE system may be easily perturbed by the sample constituent, because the electrolyte system is simple and it has no concentrating effect.

*Corresponding author. Tel.: +81-824-227-111; fax: +81-824-227-192.

E-mail address: hiro77@hiroshima-u.ac.jp (T. Hirokawa).

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We have developed a conversion method of the time-based electropherograms of CZE into mobility-based ones by removing the contribution of electroosmotic flow (EOF) considering temperature rise caused by Joule heating such as ''hypothetical electroosmotic flow method'' [1,2]. The developed conversion method also contained a correction for delay of migration time caused by relaxation of the potential gradient at the sample plug which was found by our numerical simulations of the CE process: a zone with high potential gradient was formed in the initial stage of migration and the gradient gradually decreased to the mean value (the applied voltage/capillary length). This phenomenon is remarkable, especially when the low-concentration sample is analyzed by using the stacking effect. The electropherograms with effective mobility axis can considerably eliminate hardware dependence and even sample dependence, and they will be useful for standardization of the CZE data.

In this paper, adaptability of our method was tested for the CZE analysis of a dilute sample, where on-line sample concentration by a field enhanced stacking effect [3] was utilized. In this case migration time may vary seriously and therefore a standardization procedure for the observed electropherograms is necessary for accurate analysis.

2. Theoretical

We have proposed some conversion methods to obtain electropherograms with a mobility axis from the usual ones with a time axis. The comparison among the proposed methods was detailed in Ref. [2]. In this section, the used conversion method ''temperature coefficient method'' is briefly described.

In the conventional conversion method, effective mobility (m) is described as follows:

$$\overline{m} = v_{\text{ion}}/\overline{E} = \frac{l/t - v_{\text{eof}}}{V/L} \quad (1)$$

where v_{ion} is the electrophoretic velocity of an ion, $\overline{E}$ the average potential gradient ($\overline{E} = V/L$), l the effective capillary length, t the migration time, v_{eof} the electroosmotic flow velocity from nonion or a system peak ($v_{\text{eof}} = l/t_{\text{eof}}$, t_{eof} the migration time), V the applied migration voltage and L the whole capillary length.

The effective mobility obtained by Eq. (1) is that at the temperature of the SE in the capillary, which is always higher than the thermostatted temperature. Since it is influenced by the migration current and the cooling system, such a conversion method is not useful for standardization of the electropherograms. As an option of the conductivity measurement system to convert the observed conductivity to that of the standard temperature e.g. 25°C, it is very convenient and useful if the effective mobility at the observed temperature can be converted to that at the standard temperature.

The other defect of Eq. (1) is that the potential gradient of the separation system is assumed to be constant as $\overline{E} = V/L$ during migration. However, as we have pointed out [4], the potential gradient of the SE controlling electrophoretic velocity of the sample components is frequently smaller than the average value at the initial stage of migration. The value gradually approaches the average value in the time course of migration (relaxation of potential gradient). Consequently, migration time delays in comparison with the ideal state (the potential gradient of the SE is always equal to the average value). For protection of the electric circuit, sometimes the migration voltage was programmed to increase slowly to the set value. This also causes a delay of migration time.

Our conversion method took account of the above and it is expressed as follows:

$$\overline{m} = l/[t - \tau)E] - m_{\text{eof}} \quad (2)$$

where τ is the delay time to correct the relaxation of the potential gradient and m_{eof} is the electroosmotic flow mobility.

On the other hand, the following equation is valid since the mobility is an approximately linear function of temperature:

$$\overline{m} = m_0(1 + \alpha\Delta T) \quad (3)$$

where m_0 is the effective mobility at reference temperature, α the temperature coefficient (ca. 0.02). The value can be assumed to be constant, although it is slightly different among the ions. Then, combining Eqs. (2) and (3) gives the following equation:

$$m_0 = \{l/[t-\tau)E] - m_{eof}\}/(1+\alpha\Delta T) \quad (4)$$

where τ, $1+\alpha\Delta T$ and m_{eof} are unknown constants and should be obtained from the added internal standard ions with known effective mobility and system peak (or the nonion's peak). The details are discussed in Ref. [2].

3. Experimental

The used sample was an equimolar mixture of KCl, NaCl, LiCl, Tris and ϵ-aminocaproic acid dissolved in purified water (3 m*M* and 0.06 m*M*). The used SE was a solution of 30 m*M* creatinine and 30 m*M* isobutylic acid (pH 4.8). The used apparatus was a CAPI-3200 system (Otsuka Electronics, Japan) and indirect UV absorption at 220 nm was utilized to obtain the electropherograms. A fused-silica capillary (Otsuka Electronics) of 40 cm (effective length 27.7 cm)×75 μm I.D. was used and the applied voltage was 10 kV. The capillary chamber was thermostatted at 25°C using a cooling fun. The sample solutions were injected hydrodynamically for 20, 100, 180, 260 s at 25 mm. The injected volumes of the sample solution were estimated from the Hargen–Poiseuille equation as 11, 53.5, 96.2 and 139 nl, the plug lengths were 0.24, 1.21, 2.18 and 3.15 cm, and the amounts of each sample component were 0.64, 3.21, 5.77 and 8.34 pmol, respectively.

All simulations were carried using our software on some personal computers (Pentium III, 450 MHz clock). The analyzed samples were the mixtures of K^+, Li^+, $m15S^+$, ϵ-aminocaproic acid (AMC^+) and a nonion, where $m15S^+$ is a model cation with effective mobility of $15\cdot10^{-5}$ cm^2 V^{-1} cm^{-1} (strong electrolyte). The concentration of the sample components was varied from 0.3 to 30 m*M*.

The used SE for simulation was the same with that used in the experiment. Physicochemical constants of the used samples and electrolyte were taken from [5]. Mobility of electroosmotic flow was assumed to be $30\cdot10^{-5}$ cm^2 V^{-1} cm^{-1}.

The other simulation conditions were: capillary length 40 cm, injection plug length was varied from 0.2 to 3 cm, applied voltage was 30 kV (averaged potential gradient 750 V/cm), and the space step and the time step were 0.025 cm and 0.002 s, respectively. It took 8 h for the simulation of one run (200 s).

4. Results and discussion

4.1. Electropherogram conversion for the 3 mM sample

Fig. 1 shows electropherograms for the 3 m*M* test mixture (applied voltage 10 kV over 40 cm capillary). Since the observed conductivity of the test mixture was 1.021 mS cm^{-1} and that of the SE was 0.861 mS cm^{-1}, a stacking effect was not expected. Fig. 1b shows the observed electropherograms obtained after hydrostatic injection for 10–100 s.

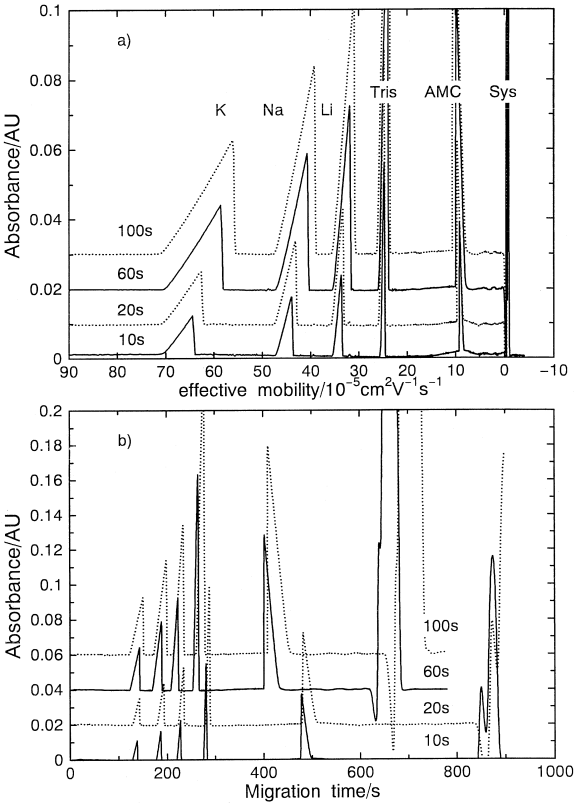

Fig. 1. Converted (a) and observed electropherograms (b) for the 3 m*M* test mixture. Time of hydrostatic injection was varied from 10 to 100 s. The capillary length was 40 cm and the applied voltage was 10 kV. The capillary was not pretreated with alkaline solution before each run.

Obviously the migration time of K^+ agreed well among the observed electropherograms, that of Tris and AMC changed significantly. As a matter of course, qualitative analysis according to migration time becomes a subject of discussion when the number of samples increases.

Fig. 1a was obtained from Fig. 1b by using the temperature-coefficient method (Eq. (4)), where the used effective mobility of the front end of K^+, Li^+ and the system peak for the conversion was $70.9 \cdot 10^{-5}$, $35.7 \cdot 10^{-5}$, $0 \cdot 10^{-5}$ $cm^2\ V^{-1}\ s^{-1}$. The former two values were obtained from the absolute mobility at 25°C [5] using Onsager's equation [6]. Obviously from Fig. 1a, the front ends of the peaks for the same kind of sample component in each electropherogram agreed very well. In the present experiment (Fig. 1b), the capillary was not pretreated. When the capillary was pretreated with alkaline solution (10 m*M* NaOH, 120 s), the migration time was shortened but a similar change of migration time was observed.

4.2. Electropherogram conversion for the 0.06 mM sample (with stacking)

Fig. 2 shows electropherograms obtained for the 0.06 m*M* test mixture (applied voltage 10 kV over 40 cm capillary). Since the observed conductivity of the test mixture was 25.6 μS cm^{-1}, a significant stacking effect was expected. Fig. 2b shows the observed electropherograms obtained after hydrostatic injection for 20–260 s. Obviously the migration time of K^+ agreed well among the observed electropherograms, that of the system peak and slower ions such as Tris and AMC was shortened dramatically with increase of the injected sample volume. In spite of the use of the capillary without alkaline pretreatment, when the time for hydrostatic injection was 260 s, baseline separation of Li and Tris was not obtained because the migration time was not sufficient for effective separation. We will try to interpret such shortening of the migration time in the later section.

Conversion of the observed electropherograms shown in Fig. 2b gave electropherograms with an effective mobility axis as shown in Fig. 2a. Although the agreement among the peaks decreased with the increase of the sample volume, it could not disturb qualitative analysis.

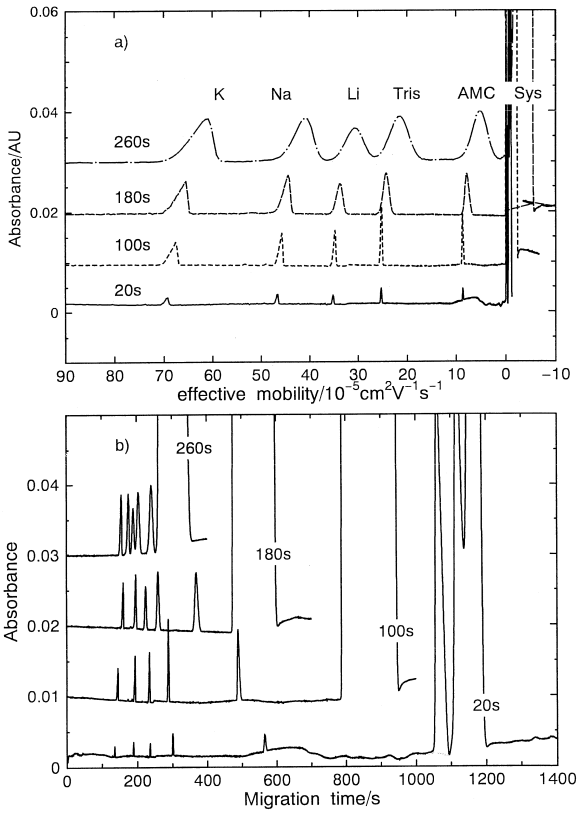

Fig. 2. Converted (a) and observed electropherograms (b) for the 0.06 m*M* test mixture. Time of hydrostatic injection was varied from 20 to 260 s. Other conditions as in Fig. 1.

Since the observed phenomena (the migration time of system peak and slow cations became short) seemed to be enhanced when the EOF of the separation capillary was large, similar capillary zone electropherograms were obtained by using another capillary pretreated by alkaline solution. To keep the activated surface, the capillary was pretreated with 10 m*M* NaOH solution for 120 s and rinsed with water and the SE before each run. Consequently, electropherograms were obtained as shown in Fig. 3. Because of increased EOF, the migration time in Fig. 3b decreased to 1/3 of that in Fig. 2b. However similar phenomena were observed as in Fig. 2b. In this case, the converted electropherograms in Fig. 3a agreed well until 200 s injection, confirming utility of our conversion method.

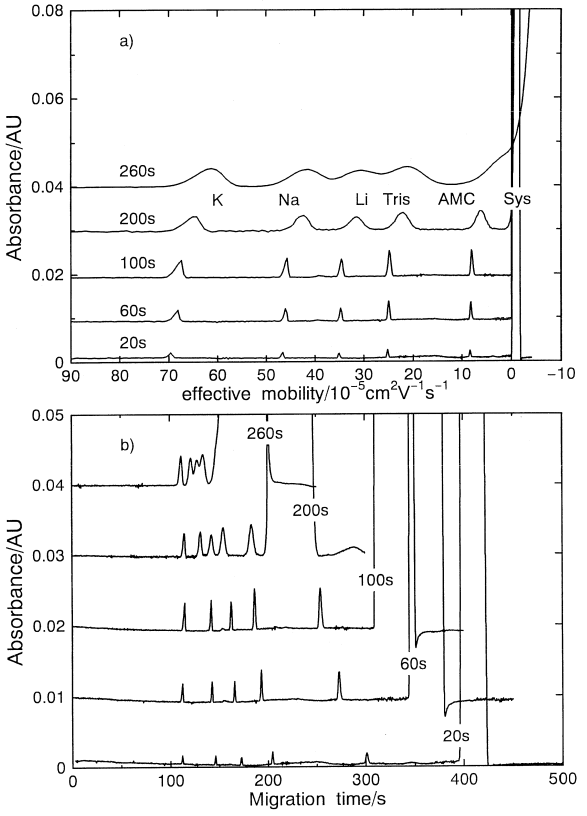

Fig. 3. Converted (a) and observed electropherograms (b) for the 0.06 m*M* test mixture. Time of hydrostatic injection was varied from 20 to 260 s. The capillary was not pretreated with alkaline solution before each run. Other conditions as in Fig. 1.

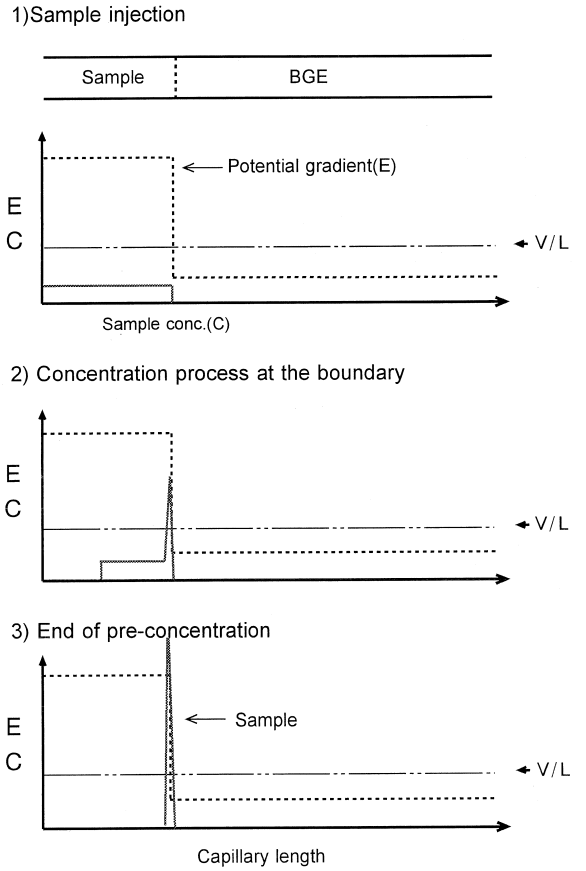

Fig. 4. Schematic diagram for concentration process in field enhanced sample stacking.

4.3. Shortening of migration time by introducing large sample volume and the cause

Fig. 4 shows the scheme for the concentration process in field enhanced sample stacking. Obviously from Fig. 4 the conductivity of the sample solution is lower than that of the SE, therefore the potential gradient at the sample plug is higher than that of the SE, and the potential gradient at the SE is much smaller than the average potential gradient. Consequently, sample components are concentrated at the boundary between the sample plug and the SE. As far as the potential gradient of the sample plug is high after sample concentration, electrophoretic velocity is low and this may cause delay of migration time. However, the shortening of the migration time for the system peak and slow cations with increase of the sample volume in Figs. 2b and 3b could not occur except for the increase of migration velocity, which might be caused by, for example, increase of EOF velocity.

For the detailed discussion, a computer simulation of CZE was carried out at first for three cases: the sample of the first case was the 3 m*M* test mixture and plug length was 0.3 cm. The sample of the second and the third cases was the 0.3 m*M* test mixture and plug lengths were 1 cm and 3 cm, respectively. In the latter two cases, a stacking process was expected.

Fig. 5 shows simulated potential gradient change of the SE and concentration profile at the end of capillary. The capillary length and applied voltage

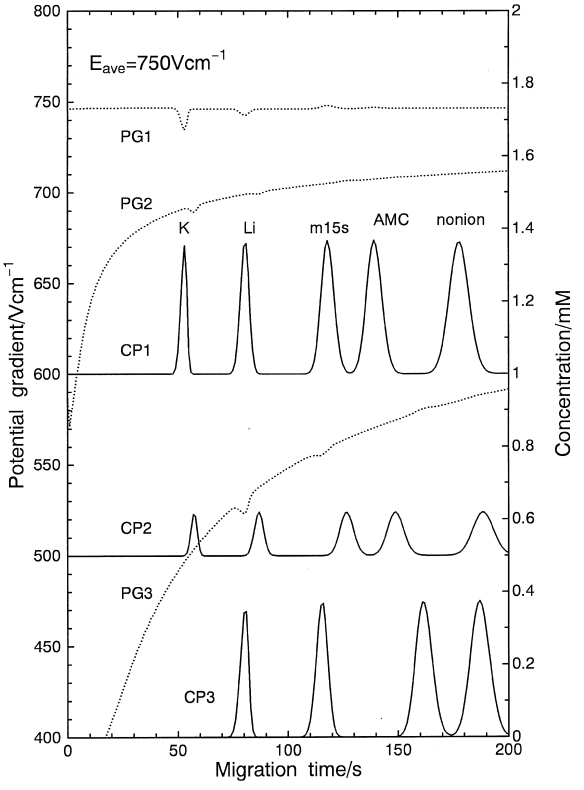

Fig. 5. Simulated potential gradient change of the SE (dotted lines) and concentration profile (solid lines) at the end of capillary. PG1, PG2 and PG3 show the potential gradient change when the plug lengths were 0.3 cm, 1 cm and 3 cm, and the sample concentrations were 3 m*M*, 0.3 m*M* and 0.3 m*M*, respectively. CP1, CP2 and CP3 show the concentration profiles corresponding to PG1, PG2 and PG3, respectively. The capillary length and the applied voltage was 40 cm and 30 kV, respectively. $m_{eof} = 30 \cdot 10^{-5}$ $cm^2\ V^{-1}\ s^{-1}$.

was 40 cm and 30 kV, respectively (average potential gradient 750 V/cm) and m_{eof} was $30 \cdot 10^{-5}$ $cm^2\ V^{-1}\ s^{-1}$. Obviously from Fig. 5, when stacking phenomena occurred (Fig. 5b and c), the potential gradient of the SE is significantly smaller than the average value, causing delay of migration time. The migration time of Fig. 5b and c was larger than that of Fig. 5a (3 m*M* sample, plug length 0.3 cm) by ca. 7% and ca. 40%, respectively. Thus, computer simulation predicted migration time would delay according to the plug length.

Becker and Ackermans discussed the shortening of migration time in stacking for the first time [7] and concluded that this was due to the fact that EOF velocity in the sample plug is larger than that in the other part of the capillary. Although their explanation is very probable, according to our simulation it was concluded that the increase in EOF does not explain the shortening of the migration time as detailed below.

In our simulation program, electroosmotic mobility at the sample plug can be set to a different value from that at the other part of the capillary. So, computer simulation was done on the basis of the following two models: the first model assumed EOF mobility was constant through the whole capillary and the second model assumed EOF mobility in the sample plug was twice as large as that in the rest of the capillary. In the first model such an increase in EOF mobility is very plausible because of the low ionic strength of the sample solution and temperature increase at the plug due to Joule heating. However the first model caused more delay of migration time in comparison to the second model.

Consequently, we could not explain the shortening of migration time observed in the stacking process by the increase of the mobility of electroosmotic flow in the sample plug. Our physical model used in simulation including EOF might not be valid, or we have to conclude that an electrokinetically-driven hydrodynamic flow swept away the sample components and sample plug. In the latter case, migration velocity of sample components (v_{mig}) may be expressed as follows:

$$v_{mig} = v_{ep} + v_{eof} + v_{ehf} \quad (5)$$

where v_{ep} is electrophoretic velocity, v_{eof} the velocity of electroosmotic flow, v_{ehf} the velocity of electrokinetic hydrodynamic flow (EHF). The cause of EHF can be the difference of the transportation number between the sample plug and the SE as reported by Stedry et al. [8] (v_{conc} in the paper). Details of the cause of the shortening of migration time are still under investigation.

Acknowledgements

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