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New method for standardization of electropherograms obtained in capillary zone electrophoresis

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Abstract

A new method for standardization of electropherograms obtained by capillary zone electrophoresis was proposed, where the migration time axis was replaced by the effective mobility axis. The mobility increase due to temperature increase by Joule heating and the relaxation effect of the potential gradient were eliminated successfully by introducing a temperature coefficient for mobility expression and a delay time, respectively. The precision of the mobility evaluated by the proposed conversion methods was evaluated for a model sample. By using the conversion method, almost the same electropherograms could be obtained even from the electropherograms originally obtained by using different hardware conditions. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Migration time; Electrophoretic mobility; Capillary electrophoresis

1. Introduction

The reproducibility of migration time in capillary electrophoresis (CE) is very important for accurate analysis. However as mentioned in the previous papers [1,2], even if the same sample and supporting electrolyte are used, migration time in capillary zone electrophoresis (CZE) is affected by three major factors: change of electroosmotic flow (EOF) of the separation system [3], relaxation of the potential gradient (RPG, potential gradient of supporting electrolyte was not constant during migrational [2]) and temperature increase due to Joule heating caused by applying high voltage to the system [3]. To put it concretely, the change of EOF depends on the history of the used capillary, RPG depends on the

constituent of the sample solution, and the temperature increase depends on both the thermal property of the separation system and the applied voltage and driving current. Since these phenomena are caused by complex reasons and are difficult to control, the same migration time often cannot be obtained depending on the hardware used even if the same sample and supporting electrolyte were used. Thus, standardization of electropherograms is necessary so that the data obtained by using different hardware may be meaningfully compared with each other.

Then, we proposed methods to obtain electropherograms with mobility axis from the usual ones with time axis, because the effective mobility depends on only the sample ion and the supporting electrolyte. For this purpose, however, the change of the mobility caused by Joule heating and RPG should be considered as already reported in our previous paper [1], where hypothetical time-dependent EOF (HEOF) was introduced to correct tem-

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perature increase and a delay time was introduced to correct RPG. Although the proposed method was very effective, the physical meaning was not clear and the adaptable window of an electropherogram was limited to the range between the internal standards. In this paper, we propose a new conversion method to improve such defects.

2. Theoretical

The effective mobility, \bar{m} , can be calculated by the conventional method as follows:

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

where v_{ion} , E , l , t , v_{eof} , V and L are the migration velocity, the potential gradient, the effective capillary length, the migration time, the velocity of EOF, the voltage applied to the system and the whole capillary length, respectively. The velocity of EOF, v_{eof} , can be obtained by using the migration time of a non-ion peak or a system peak (t_{eof}) as follows:

$$v_{\text{eof}} = l/t_{\text{eof}} \quad (2)$$

Temperature dependence of effective mobility should be divided into the influence of sample ion, $f(T)$, and that of the supporting electrolyte $g(T)$ in order to discuss the change of the mobility exactly.

$$\bar{m} = f(T) \cdot g(T) \quad (3)$$

The Taylor expansion of this equation around T_0 , which is usually the temperature of the thermostatted oven for the separation chamber, can be described as follows:

$$\begin{aligned} \bar{m} &= (f_0 + f_1 \Delta T + f_2 \Delta T^2 + \dots)(g_0 + g_1 \Delta T \\ &\quad + g_2 \Delta T^2 + \dots) \\ \Delta T &= T - T_0, f_0 = f(T_0), g_0 = g(T_0) \end{aligned} \quad (4)$$

where T is the temperature of the supporting electrolyte in the capillary and ΔT means the temperature increase. Since the temperature dependence of ion size is negligible but that of viscosity and dielectric constant of the supporting electrolyte is significant, effective mobility can be described as follows:

$$\begin{aligned} \bar{m} &= f_0(g_0 + g_1 \Delta T + g_2 \Delta T^2 + \dots) \\ &= f_0 g_0 \left(1 + \frac{g_1}{g_0} \Delta T + \frac{g_2}{g_0} \Delta T^2 + \dots \right) \end{aligned} \quad (5)$$

When ΔT is sufficiently small, the terms of secondary and higher order in Eq. (5) can be neglected. Substituting $\bar{m}_0 = f_0 g_0$ and $\alpha = g_1/g_0$ in Eq. (5), the mobility of the target ion at $T_0 + \Delta T$, \bar{m} , can be described as follows:

$$\bar{m} = (1 + \alpha \Delta T) \bar{m}_0 \quad (6)$$

\bar{m}_0 and α are the effective mobility of the target ion at T_0 and the temperature coefficient independent of ion species, respectively. Generally, the value of α is approximately 0.02 at 25°C.

3. Conversion method using temperature coefficient (TC)

The value of $(1 + \alpha \Delta T)$ can be obtained as follows:

$$1 + \alpha \Delta T = \bar{m}_s / \bar{m}_{0,s} \quad (7)$$

where \bar{m}_s is the effective mobility of an internal standard at elevated temperature (T) and $\bar{m}_{0,s}$ is that at the reference temperature T_0 . The $\bar{m}_{0,s}$ can be evaluated theoretically on the basis of reported absolute mobility and Onsager's equation.

From Eqs. (6) and (1), the effective mobility of a certain sample ion at T_0 , \bar{m}_0 , can be obtained by using the following equation:

$$\begin{aligned} \bar{m}_0 &= \frac{\bar{m}}{1 + \alpha \Delta T} = \frac{l/t - v_{\text{eof}}}{(1 + \alpha \Delta T)E} \\ &= \frac{t_{\text{eof}}/t - 1}{t_{\text{eof}}/t_s - 1} \cdot \bar{m}_{0,s} \end{aligned} \quad (8)$$

where t_s is the migration time of the standard. Obviously from Eq. (8), the hardware depending parameters such as E , V , l , L and v_{eof} could be eliminated.

4. Conversion method using temperature coefficient and delay time (TCDT)

The migration times of all ions delay due to RPG

by a certain time τ . Supposing that the velocity of EOF is constant during one run, the effective mobility of the sample ion can be described by subtracting τ from the migration time as follows:

$$\bar{m} = \frac{l}{(t - \tau)E} - m_{\text{eof}} \quad (9)$$

where $m_{\text{eof}} = v_{\text{eof}}/E$ is the mobility of EOF. The value obtained from Eq. (9) is the mobility at the inner temperature (T). Then, substituting \bar{m} in Eq. (8) by Eq. (9), the mobility at the reference temperature T_0 can be described as follows:

$$\frac{\bar{m}}{m_0} = \frac{\frac{l}{(t - \tau)E} - m_{\text{eof}}}{1 + \alpha \Delta T} \quad (10)$$

where the parameters τ , m_{eof} and $1 + \alpha \Delta T$ are unknown. These parameters are calculated from the migration time of the internal standards A and B with the known mobility and the system peak in an indirect UV system or a peak of a non-ion. The mobility of the standards A and B and EOF can be described as follows:

$$\frac{\bar{m}}{m_{0,A}} = \frac{\frac{l}{(t_A - \tau)E} - m_{\text{eof}}}{1 + \alpha \Delta T} \quad (11)$$

$$\frac{\bar{m}}{m_{0,B}} = \frac{\frac{l}{(t_B - \tau)E} - m_{\text{eof}}}{1 + \alpha \Delta T} \quad (12)$$

$$m_{\text{eof}} = \frac{l}{(t_{\text{eof}} - \tau)E} \quad (13)$$

From Eqs. (11)–(13), the following equations can be obtained:

$$\tau = \frac{\bar{m}_{0,A} t_A (t_{\text{eof}} - t_B) - \bar{m}_{0,B} t_B (t_{\text{eof}} - t_A)}{\bar{m}_{0,A} (t_{\text{eof}} - t_B) - \bar{m}_{0,B} (t_{\text{eof}} - t_A)} \quad (14)$$

$$1 + \alpha \Delta T = \frac{\frac{l}{(t_A - \tau)E} - m_{\text{eof}}}{\bar{m}_{0,A}} \quad (15)$$

$$\frac{\bar{m}}{m_0} = \frac{t_A - \tau}{t - \tau} \cdot \frac{t_{\text{eof}} - t}{t_{\text{eof}} - t_A} \cdot \bar{m}_{0,A} \quad (16)$$

Since the parameters such as E , V , l , L and v_{eof} could be eliminated, the electropherogram obtained from Eq. (16) is independent of the hardware conditions.

That is, the obtained electropherograms are standardized and can be compared with each other, even if the hardware is different.

5. Experimental

All experiments were carried out with capillary electrophoretic analyzer CAPI-3100S (Otsuka Electronics, Osaka, Japan). The separation capillary used for measurements was a fused-silica capillary with 75- or 50- μm I.D. The whole length and effective length were 40 cm and 27.7 cm, respectively. The applied voltage was 15 or 30 kV. The preset of the

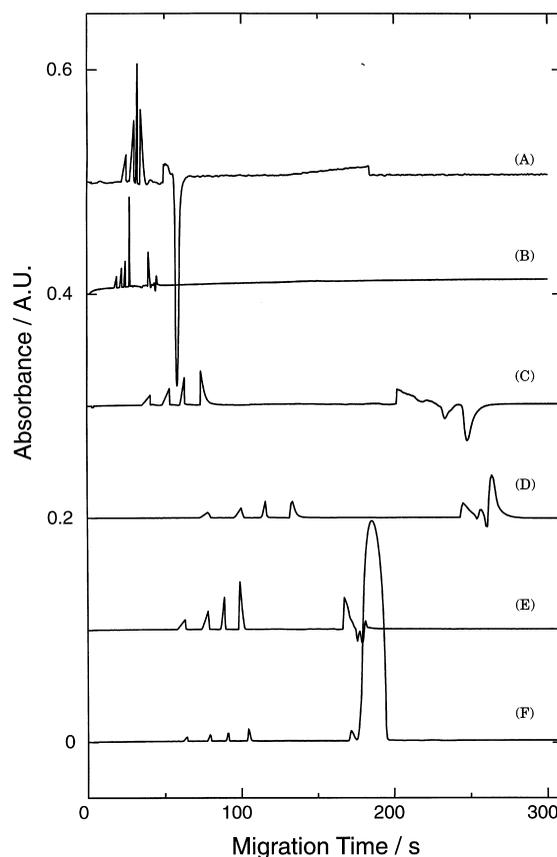


Fig. 1. Observed electropherograms with migration time axis: (A) 30 mM sample, SE 20, capillary 75- μm I.D., applied voltage=30 kV, (B) 30 mM sample, SE 40, 75- μm I.D., 30 kV, (C) 30 mM sample, SE 40, 50- μm I.D., 30 kV (D) 30 mM sample, SE 40, 50- μm I.D., 15 kV, (E) 30 mM, sample, SE 40, 75- μm I.D., 15 kV, (F) sample 0.3 mM, SE 40, 75- μm I.D., 15 kV.

separation chamber and reference temperature was 25°C. The wavelength used for indirect detection was 265 nm. The analyzed samples were 30 mM and 0.3 mM equimolar mixtures of KCl, LiCl, Tris, ϵ -aminocaproic acid and sodium octylsulfonate. The conditions of hydrodynamic injections for the 30 mM and 0.3 mM sample solutions were (2 cm, 60 s) and (2 cm, 75 s), respectively. The supporting electrolyte (SE) was a 40 mM lutidine solution buffered by adding acetic acid (pH 4.8, abbreviated as SE 40) or a 20 mM lutidine solution whose pH was adjusted to 4.8 by adding HCl (SE 20).

6. Result and discussions

Fig. 1 shows the electropherograms measured in the following conditions: (A) 30 mM sample, SE 20, capillary 75 μm , I.D. applied voltage 30 kV (B) 30 mM sample, SE 40, 75 μm I.D., 30 kV (C) 30 mM sample, SE 40, 50 μm I.D., 30 kV, (D) 30 mM sample, SE 40, 50 μm I.D., 15 kV, (E) 30 M sample, SE 40, 75 μm I.D., 15 kV, (F) 0.3 mM sample, SE 40, 75 μm I.D., 15 kV. A new capillary without pretreatment of the inner wall was used in condition (C), while an aged capillary was used to obtain the other electropherograms. Obviously from Fig. 1, the various electropherograms were obtained in the different instrumental conditions even if the same sample and the same electrolyte were used. Correspondingly, different electropherograms will be obtained by using different apparatuses. Thus, a useful standardization method is expected to obtain compatibility among the data.

Fig. 2 shows the electropherograms transformed by using the adjusted migration index (AMI) proposed by Lee et al. [4]. The electropherograms were similar to one another in the conditions using the same electrolyte (B)–(F). The broken lines in the figure show the mean AMI values of the peaks in the electropherograms (B)–(F). The relative standard deviations (RSD) of peak positions of the K^+ , Na^+ , Li^+ and Tris^+ evaluated for AMI were 4.8%, 3.8%, 4.7% and 7.1%, respectively. The electropherogram (A) in Fig. 2 was different from the others in spite of the same mobilities of sample ions as those in the other electropherograms (B)–(F). The difference was caused by the difference of the conductivity of the

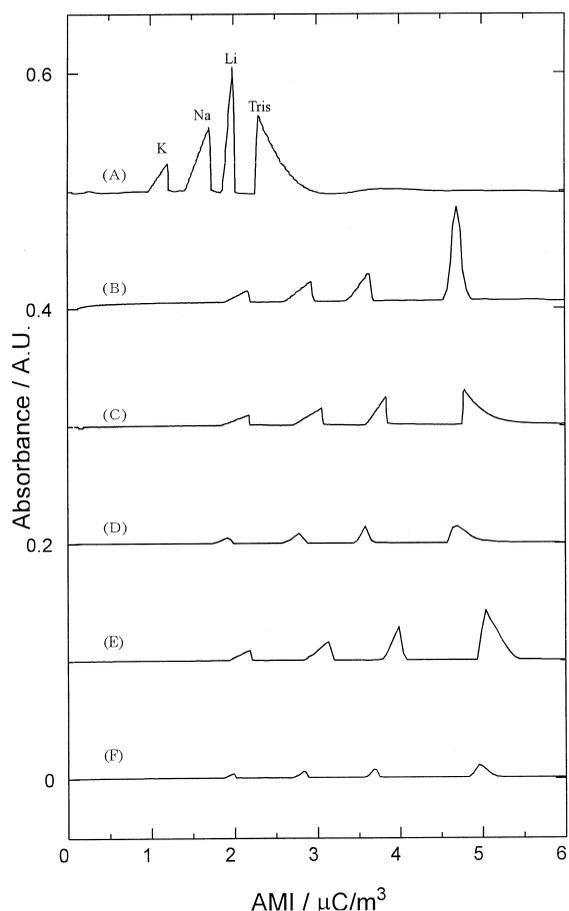


Fig. 2. Electropherograms with the adjusted migration index (AMI) axis [4]. The broken lines indicate the mean AMI values of K^+ , Na^+ , Li^+ and Tris^+ in the conditions (B)–(F).

supporting electrolyte. Fig. 3 shows the electropherograms with mobility axis obtained by using the conventional method [Eq. (1)]. The broken lines in Fig. 3 at 69.9 , 46.3 , 34.9 and $24.4 \cdot 10^{-5} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ show the theoretical effective mobilities at 25°C for K^+ , Na^+ , Li^+ and Tris^+ , respectively [5]. Obviously the converted mobilities were overestimated and RSD values were in the range of 17.5% and 26.0%. Additionally, the effective mobilities in (A)–(C) with 30 kV operation were estimated larger than those in (D)–(F) with 15 kV operation, suggesting the temperatures of the supporting electrolyte in the capillary for (A)–(C) were higher than those for (D)–(F) because of larger electric powers. The tendency was especially re-

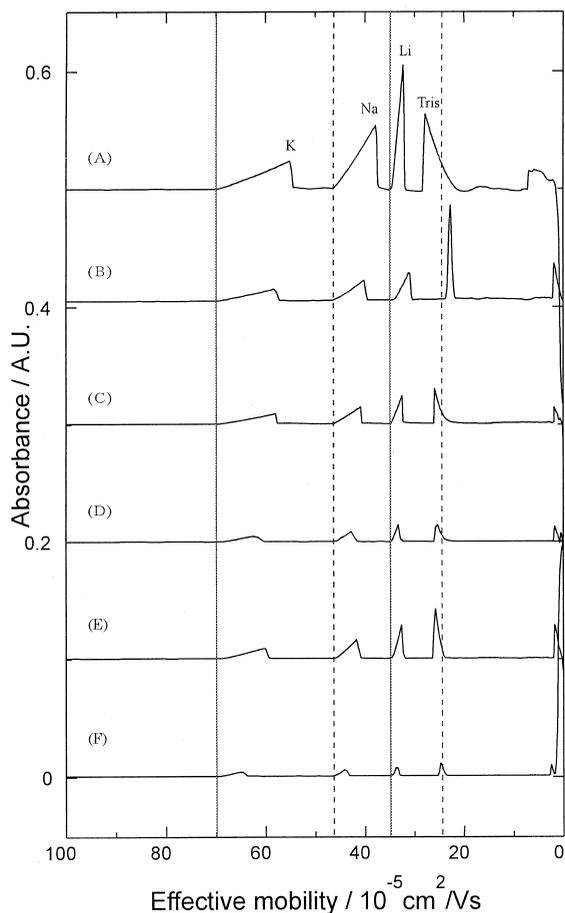


Fig. 3. Electropherograms with effective mobility axis obtained by the conventional method. The broken lines indicate the theoretical effective mobilities of K^+ , Na^+ , Li^+ and $Tris^+$ at the reference temperature.

markable in (B) obtained by using the wide-bore capillary and the concentrated supporting electrolyte. Anyway all estimated mobility was larger than the reported value.

Fig. 4 shows the electropherograms with mobility axis converted by using the HEOF method [1] to remove hardware dependence. The solid lines in Fig. 4 show the value of K^+ and Li^+ which were used as the internal standard at 25°C. The broken lines at 46.3 and $24.4 \cdot 10^{-5} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ indicate the theoretical value of Na^+ and $Tris^+$ at 25°C, respectively. The mean mobilities (RSD) of Na^+ and $Tris^+$ were 46.9 (1.1%) and $21.8 \cdot 10^{-5} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ (8.1%), respectively, and the dispersion of the mobility

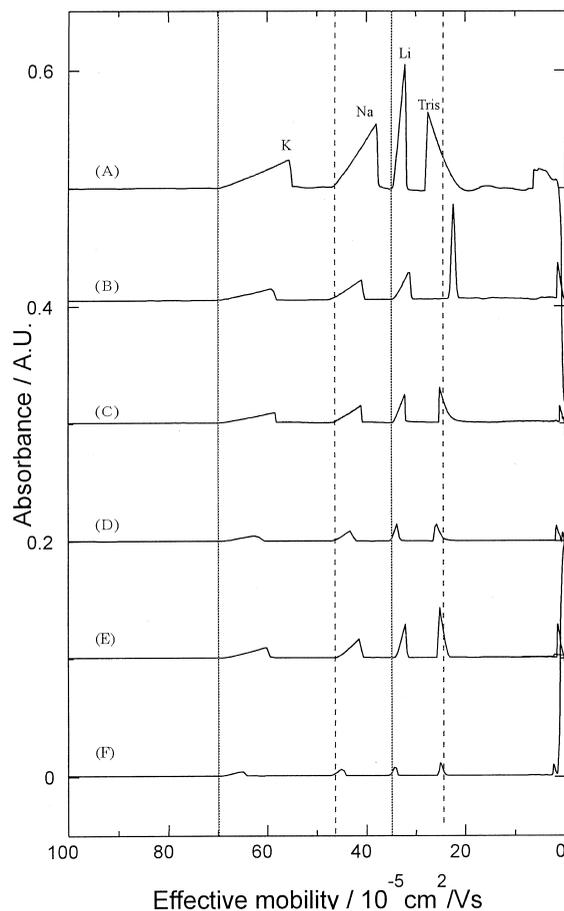


Fig. 4. Electropherograms with effective mobility axis obtained by the HEOF method [1]. The solid lines indicate the theoretical mobilities of K^+ and Li^+ , which were used as the internal standard. The broken lines indicate the theoretical mobilities of Na^+ and $Tris^+$.

decreased. The differences of these values from the theoretical ones were 1.4% and 10.7%. The mobility of $Tris^+$ was significantly different from the theoretical one because the peaks were tailing. This method is useful since the mean mobility of Na^+ , whose peaks were fronting, was almost the same as the theoretical value. However, the problem is that the delay times, τ , were negative. The delay τ should be positive in principle.

Fig. 5 shows the electropherograms with mobility axis which were converted by the TCDT method. The solid lines show the theoretical values of K^+ and Li^+ at 25°C which were used as $m_{0,A}$ and $m_{0,B}$ in

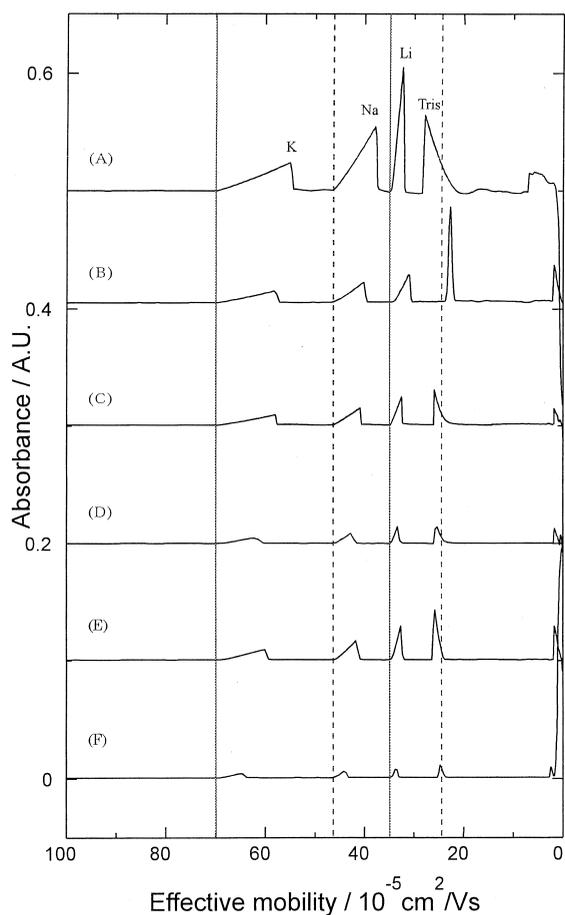


Fig. 5. Electropherograms with effective mobility axis obtained by the TCDT method. The solid lines indicate the theoretical mobilities of K^+ and Li^+ which were used as the internal standards. The broken lines indicate the theoretical mobilities of Na^+ and $Tris^+$.

Eqs. (14) and (16), respectively. The broken lines at 46.3 and $24.4 \cdot 10^{-5} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ figure are the value of Na^+ and $Tris^+$, respectively. Table 1 shows the evaluated mobilities, RSD and the parameters ($1 + \alpha \Delta T$ and z). The dispersion of data was best improved by this method. The mean mobility (RSD) of Na^+ and $Tris^+$ were 46.4 (0.3%) and $22.2 \cdot 10^{-5} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ (7.7%), respectively. The differences of the values from the theoretical values were 0.3% and 9.2%. The estimated values of Tris were different from the theoretical ones, since the peaks of $Tris^+$ were tailing. On the other hand, the mobility of Na^+ could be determined within the RSD of 0.3% even if the condition were varied. Thus, the effective mobility of ionic components in a sample mixture at the reference temperature could be determined from the migration time obtained from the CZE electropherogram rapidly and accurately by the TCDT method. When the delay time was not considered (TC method), the mean values of mobility (RSD) of Na^+ , Li^+ and $Tris^+$ were 66.6 (3.6%), 45.7 (1.2%) and 22.6 (8.1%) and the differences between them from the theoretical values were -4.7% , -1.4% and -7.5% , respectively.

Although migration time varied depending on the hardware used, TCDT conversion enables standardization of electropherograms. If a database of absolute mobility and pK_a evaluated by using the CZE method can be created in future, unknown samples may be automatically determined. A quantitative feature of the present conversion is under investigation. It will be reported in due course.

Table 1
Effective mobility obtained by the TCDT method and the parameters

Experiment	Mobility/ $10^{-5} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$					$1 + \alpha \Delta T$	τ/s
	K^a	Na	Li^a	Tris	EOF		
(A)	69.89	46.37	34.86	19.95	67.24	1.87	3.87
(B)	69.89	46.64	34.86	23.82	102.19	2.81	5.56
(C)	69.89	46.54	34.86	21.91	15.90	1.38	2.75
(D)	69.89	46.49	34.86	20.38	28.32	1.08	1.84
(E)	69.89	46.26	34.86	23.79	42.40	1.32	3.14
(F)	69.89	46.34	34.86	23.13	41.95	1.47	10.88
Average	69.89	46.44	34.86	22.16			
RSD (%)	0.00	0.30	0.00	7.68			
Error (%)	0.00	0.28	0.00	-9.17			

^a Internal standards.

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