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Influence of sample injection time of ions on migration time in capillary zone electrophoresis

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Abstract

It was found that the sample injection time of ions during hydrodynamic injection affects the migration time in capillary zone electrophoresis (CZE). The relationship between sample injection time and migration time is different depending on the conditions, *i.e.*, in stacking and non-stacking runs, because the dominant factors that determine the relationship are different. In non-stacking runs, theoretically, a simple relationship is obtained, *i.e.*, the migration time decreases linearly with increasing injection time, which is identical with experimental results for different ions. For stacking runs, a new mathematical model is developed to account for the increase in migration time with increasing sample injection time. The predictions of the model agree well with experimentally determined profiles for different ions.

1. Introduction

The use of capillary electrophoresis (CE), because of its high efficiency and separation power, is growing dramatically. It was introduced by Mikkers *et al.* [1,2] and developed by Jorgenson and Lukacs [3,4]. In particular, capillary electrophoresis holds the promise of becoming the separation method of choice for bio-related compounds such as amino acids, peptides, nucleic acid and proteins.

Much work has been done to determine the influence of various factors on migration times and apparent mobilities. Issaq and co-workers [5,6] investigated the effect of the buffer type and concentration on analyte mobility and directly related the separation factor to analysis time. Jones and Jandik [7] established an empirical correlation between ionic equivalent conductance and analyte migration time in CE. Atamna *et al.* [8] investigated the influence of the buffer cation on mobility. Mizukami *et al.* [9] investigated the relationship between migration time and pK_a in CE. Huang and Ohms [10] discussed the effect of a non-uniform electrical field on the migration behaviour of different species. Beckers and Ackermans [11] developed a model for the calculation of migration behaviour and discussed the effect of sample stacking on resolution, calibration graphs and pH shifts in capillary zone electrophoresis (CZE). Gebauer *et al.* [12] described sample self-stacking in zone electrophoresis and discussed the effect of the concentration of the major component in the sample on the elution times of the individual minor compounds. The experimental phenomenon de-

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scribed by Mikkers *et al.* [2] (Fig. 1 in ref. 2) indicated that the amount of sample injected influenced the migration behaviour. It is the aim of this paper to present a theoretical description to show how and why the sample injection length influences the migration time under stacking and non-stacking conditions.

We investigated the relationship between sample injection time of ions during hydrodynamic injection and migration time under two different conditions: stacking and non-stacking runs. Sample stacking (concentration of the analyte zone) is the process that occurs when a voltage is applied along a capillary tube containing a sample plug with a specific conductivity lower than that of the surrounding running buffer. In other words, the resistivity (ρ_1) of the sample zone is higher than that (ρ_2) of the surrounding running buffer. Moreover, because electric field strength is inversely proportional to the specific conductivity of the liquid, the field strength is higher along the sample plug compared with the running buffer. Consequently, the analyte ions in the sample plug will migrate rapidly towards the steady-state boundary between the low-concentration plug and the surrounding running buffer. Once the ions have passed the concentration boundary between the sample plug and the rest of the capillary, they immediately experience a lower electric field and slow down. As a result, the ionic analyte zone becomes narrow. This phenomenon is termed sample stacking. When the resistivity (ρ_1) of sample zone is equal to that (ρ_2) of surrounding running buffer, the field strength along the sample zone is equal to that of the running buffer. To distinguish it from the sample stacking process, we call this process non-stacking. The stacking mechanism occurs for both positive and negative ions.

The aims of this study were twofold. First, in non-stacking runs, a linear relationship between sample injection time and migration time was found. This theoretical relationship is identical with the experimental results. Second, we developed a mathematical model accounting for the changes in analyte zone length in a stacking run. From this model, the experimental relationship between sample injection length and migration time can be explained reasonably well.

2. Experimental

2.1. Instrumentation

The CE system employed was the Quanta 4000 (Waters Chromatography Division of Millipore, Milford, MA, USA) with a negative power supply. Indirect UV detection was achieved with the use of a mercury lamp and a 254-nm optical filter. Waters AccuSep fused-silica capillaries are used throughout. The capillary dimensions were 60 cm total length with a 52-cm distance from point of injection to the centre of detector cell. Both 75 and 100 μm I.D. capillaries were used. Data acquisition was carried out with a Maxima 820 Chromatography Workstation (Waters) with a System Interface module (SIM) connecting the CE system to the station. The detector time constant was set at 0.1 s and the data acquisition rate was 20 points s^{-1}. Collection of electrophoretic data was initiated by a signal cable connection between the Quanta 4000 and the SIM.

2.2. Preparation of support electrolytes

The chromate electrolytes used for CE were prepared from a concentrate containing 100 m*M* Na_2CrO_4 (analytical-reagent grade) and 0.68 m*M* H_2SO_4. When preparing both a 5 m*M* chromate and a 10 m*M* chromate supporting electrolyte, the concentration of electroosmotic flow modifier (OFM Anion-BT; Waters) was 0.5 m*M*. In both instances, the dilute sulphuric acid was added to the chromate concentrate to pre-adjust the electrolyte pH to 8.0.

2.3. Standard solutions

Experiments were divided into two series, with stacking and non-stacking runs.

Stacking

The concentration of the supporting electrolyte was 5 m*M* (pH 8.0). The samples of $NaNO_3$, Na_2SO_4 and KBr were dissolved in and diluted with distilled, deionized water to $5.0 \cdot 10^{-5}$ *M*. The conductivity of the supporting electrolyte was $10.5 \cdot 10^2$ μS cm^{-1} and the con-

ductivities of the $NaNO_3$, Na_2SO_4 and KBr sample solutions were 11.0, 12.1 and 9.7 μS cm^{-1}, respectively.

Non-stacking

Sample solutions of $NaNO_3$, Na_2SO_4 and KBr were prepared in a buffer identical with that used as the supporting electrolyte. The concentration of the supporting buffer was increased to 10 m*M* and the concentration of sample ions was chosen to be sufficiently low ($2.5 \cdot 10^{-5}$ *M*) that the relative difference in conductivity between the buffer and the sample ions was small enough to be negligible. The conductivity of the supporting electrolyte was $18.9 \cdot 10^2$ μS cm^{-1} and the conductivities of the sample solutions of $NaNO_3$, Na_2SO_4 and KBr were 1950, 1930 and 1910 μS cm^{-1}, respectively.

2.4. System operation

Gravity injection was used in the experiments because of its simplicity and because it does not have the bias involved in electromigration injection. A 3-min capillary purge was performed prior to all injections. The purge was accomplished with a 12–15 p.s.i. vacuum (1 p.s.i. = 6894.76 Pa) applied to the receiving electrolyte vial.

3. Results and discussion

3.1. Non-stacking

Under non-stacking conditions, with a short injection plug, the electropherogram displays a sharp peak, and with a long injection plug, the electropherogram displays a peak with characteristic flat-topped shape, which is shown in Fig. 1. In the latter instance, it is assumed that on the electropherogram the migration time is the time to which the centre of the flat top of the "trapezoid" corresponds (see Fig. 1). For non-stacking runs, there is no concentration stacking and the diffusion is towards both sides of the sample zone; this means the diffusion does not influence migration time. Hence, with the above

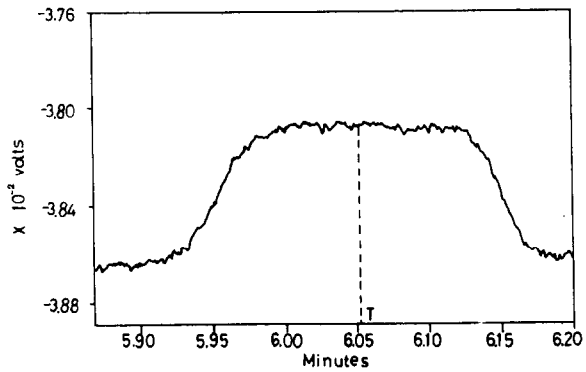

Fig. 1. Electrophoretogram of SO_4^{2-} in a non-stacking run with a 30-s injection length. The time to which the centre of the flat top of the "trapezoid" corresponds is the migration time of SO_4^{2-}. Experimental conditions are given in Fig. 3.

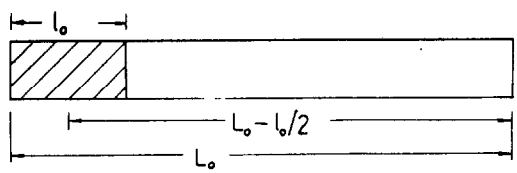

Fig. 2. Effect of injection length on the distance from the centre of the initial sample injection plug to the centre of the detector.

assumption, only one factor needs to be considered when investigating the influence of sample injection length on migration time, namely that different initial sample injection lengths cause different effective distances from the centre of the initial sample injection length to the detector. This effect is shown schematically in Fig. 2, where L_0 is the length of the capillary to the optical centre of detector, l_0 is the initial sample length and $L_0 - l_0/2$ is the distance from the centre of the initial sample injection to detector. The initial sample length l_0 is related to the migration time T by the equation

$$T = \frac{L_0 - l_0/2}{(\mu_e + \mu_{eo})E} = \frac{(L_0 - l_0/2)V}{(\mu_e + \mu_{eo})L} \quad (1)$$

where V = applied voltage, L = total length of capillary, μ_e = electrophoretic mobility and μ_{eo} = coefficient of electroendosmotic flow. Moreover, l_0 is expressed by the equation

$$l_0 = t_i V_i \quad (2)$$

where t_i is the sample injection time and V_i is the average injection velocity, which is assumed to be a constant for different injection times. Eq. 2 can be substituted into Eq. 1 to yield

$$T = \frac{(L_0 - V_i t_i/2)V}{(\mu_e + \mu_{eo})L} \quad (3)$$

For a given system, L_0, V, μ_e, μ_{eo}, L and V_i are constants, so from Eqs. 1 and 3, it can be concluded that the migration time T decreases linearly with increasing sample injection length l_0 or sample injection time t_i.

To confirm this analysis, we carried out the following experiment. Migration times T were measured with different injection times t_i for three different anions in non-stacking runs. Fig. 3 shows T as a function of t_i for three different anions, Br^-, SO_4^{2-} and NO_3^-. At first glance there may be a false impression that the migration velocity of analyte ions in a capillary increases with increasing sample injection length. Actually, the essence of the plots is that the sample injection length does not affect the migration velocity of analyte ions or, in other words, the migration velocity of an ion is the same at different injection times. The plots are not horizontal lines, but sloping, for the same reason as above, *i.e.*, that different initial sample injection lengths lead to different distances from the centre of the initial sample injection length to the detector. In our experiments, obviously larger injection times (60 s or 4.2 cm or 330 nl) were included, because our interest was in the relationship between t_i and T rather than peak spreading. The same applies to the experiments with stacking runs. We observed that the migration time decreases approximately linearly with increasing injection time t_i in all instances. These experimental results are in agreement with theoretical predictions. Regression analysis was performed for experimental data on t_i and T, and the correlation coefficients were 0.996 for Br^-, 0.995 for NO_3^- and 0.98 for SO_4^{2-}. These correlation coefficients provide a good confirmation of Eq. 3. It also holds for different electrolytes and different experimental conditions such as capillary dimensions, applied voltages and electric field strength.

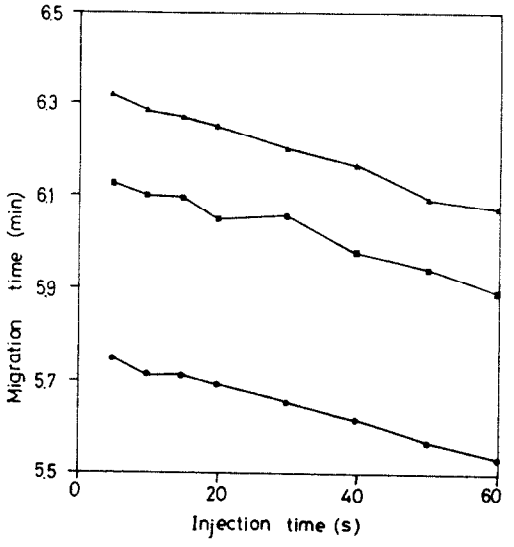

Fig. 3. Measured relationship between migration time and injection time for (▲) nitrate, (■) sulphate and (●) bromide in non-stacking runs. The samples ($2.5 \cdot 10^{-5}$ *M*) were prepared in the supporting electrolyte, which is 10 m*M* chromate–0.5 m*M* OFM Anion-BT (pH 8.0). Capillary: I.D. 100 μm, $L = 60$ cm and $L_0 = 52.5$ cm. Applied negative voltage, 10 kV. Detection at 254 nm.

3.2. Stacking

In stacking runs, how does the sample injection time influence the migration time? First, we carried out the following experiments. We measured the migration times T with different injection times t_i for different ions in stacking runs. The migration time T was plotted as a function of the sample injection time t_i for three anions, Br^-, SO_4^{2-} and NO_3^-, as shown in Figs. 4, 5 and 6, respectively. T increases non-linearly with increasing t_i. Evidently, the relationship is completely different and the trend is opposite to those for non-stacking runs. In an attempt to describe and explain these experimental results, a theoretical approach to simulate the stacking process is presented below.

Description of migration in stacking runs

In the whole process of a sample zone running from the inlet to the outlet of the capillary, the

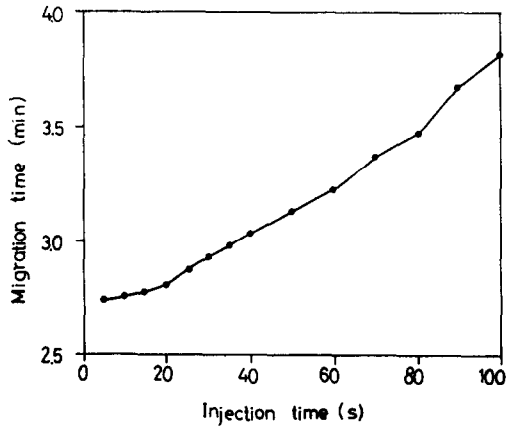

Fig. 4. Measured relationship between migration time and injection time for bromide ($5.0 \cdot 10^{-5}$ M) in stacking runs. Supporting electrolyte, 5 mM chromate–0.5 mM OFM Anion-BT (pH 8.0). Capillary: I.D. 75 μm, $L = 60$ cm and $L_0 = 52.5$ cm. Applied negative voltage, 20 kV. Detection at 254 nm.

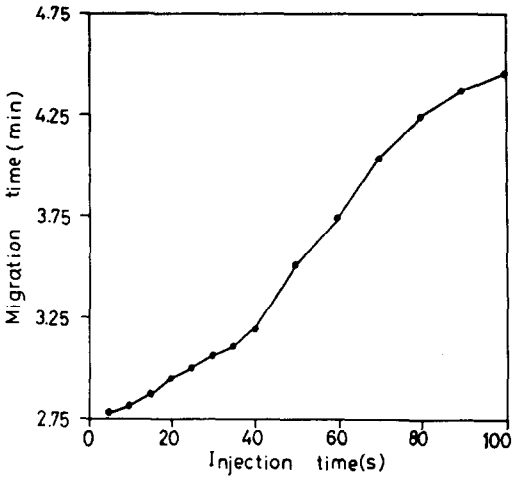

Fig. 6. Measured relationship between migration time and injection time for nitrate ($5.0 \cdot 10^{-5}$ M) in stacking runs. Conditions as in Fig. 5.

sample zone length changes all the way; in other words, the sample zone length is a function of time. The concentration of the sample in the capillary also changes with time owing to the changes in the sample zone length, so the electric resistivity of the sample zone is also a function of time. In the same way, the changes in the sample zone length lead to corresponding changes in the electric resistance, the voltage and the electric field strength of the sample zone, so these parameters are also a function of time. For the running buffer, it is assumed that its resistivity is approximately constant, *i.e.*, not a function of time. This assumption holds because the properties and concentration of the buffer hardly change with time, whereas the electric resistance, the voltage and the electric field strength of the buffer zone are all functions of time, owing to the changes in the sample zone length.

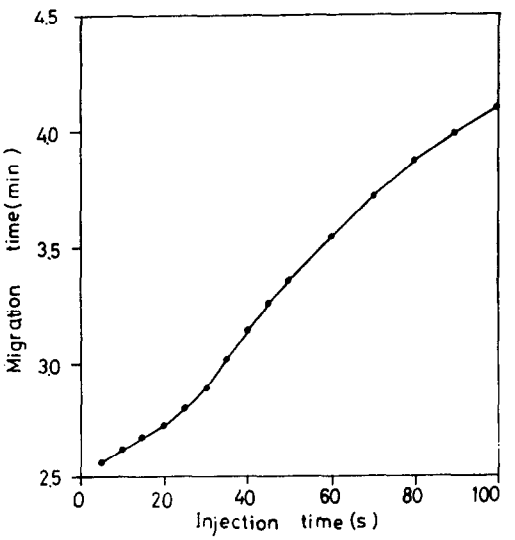

Fig. 5. Measured relationship between migration time and injection time for sulphate ($5.0 \cdot 10^{-5}$ M) in stacking runs. Conditions as in Fig. 4, except for the capillary I.D. (100 μm).

The following symbols corresponding to the above physical parameters are used in the subsequent mathematical treatment: $l(t)$ = length of sample zone at the moment t; $\rho_1(t)$ = electric resistivity of sample zone at moment t; $R_1(t)$ = electric resistance of sample zone at moment t; $V_1(t)$ = voltage of sample zone at moment t; $E_1(t)$ = electric field strength of sample zone at moment t; $R_2(t)$ = electric resistance of buffer zone at moment t; $V_2(t)$ = voltage of buffer zone at moment t; $E_2(t)$ = electric field strength of buffer zone at moment t; $L - l(t)$ = length of buffer zone at moment t; ρ_0 = resistivity of sample zone when $t = 0$ or $l(t) = l_0$; and ρ_2 = resistivity of buffer.

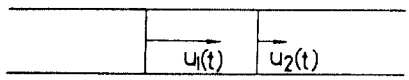

Fig. 7. Schematic representation of sample stacking in a stacking run. In the mathematical model it is assumed that the terminating edge of the analyte zone migrates faster than the leading edge, thus reducing the zone length.

In our model, it is assumed that the total amount of analyte is conserved in the sample zone during the stacking period [13]. Moreover, it is assumed that the coefficient of electroendosmotic flow (μ_{eo}) in the sample is equal to that in buffer zone and both of them are constants. The electrophoretic mobility (μ_e) of the sample is also constant. Sample stacking is shown schematically in Fig. 7 [13]. The leading edge of the analyte zone migrates with a velocity $u_2(t)$ in an electric field of strength $E_2(t)$ at moment t. The terminating edge of the analyte zone migrates with a velocity $u_1(t)$ in an electric field of strength $E_1(t)$ at moment t. The velocity of the analyte between the two edges is also $u_1(t)$. These two velocity expressions are, respectively,

$$u_2(t) = (\mu_{eo} + \mu_e)E_2(t) \tag{4}$$

$$u_1(t) = (\mu_{eo} + \mu_e)E_1(t) \tag{5}$$

The resistances of the analyte and buffer zones are given by

$$R_1(t) = \rho_1(t) \cdot \frac{l(t)}{s} \tag{6}$$

$$R_2(t) = \rho_2 \cdot \frac{L - l(t)}{s} \tag{7}$$

respectively, where s is the cross-sectional area of the capillary. From Eqs. 6 and 7, the voltages of the analyte and buffer zones can be expressed by, respectively,

$$V_1 = \frac{\rho_1(t) \cdot \dfrac{l(t)}{s}}{\rho_1(t) \cdot \dfrac{l(t)}{s} + \rho_2 \cdot \dfrac{L - l(t)}{s}} \cdot V \tag{8}$$

$$V_2 = \frac{\rho_2 \cdot \dfrac{L - l(t)}{s}}{\rho_1(t) \cdot \dfrac{l(t)}{s} + \rho_2 \cdot \dfrac{L - l(t)}{s}} \cdot V \tag{9}$$

Then the expressions of the electric field strengths of analyte and buffer zones are, respectively [11],

$$E_1(t) = \frac{\rho_1(t)}{\rho_1(t)l(t) + \rho_2[L - l(t)]} \cdot V \tag{10}$$

$$E_2(t) = \frac{\rho_2}{\rho_1(t)l(t) + \rho_2[L - l(t)]} \cdot V \tag{11}$$

Obviously, before sample stacking finishes, $\rho_1(t) > \rho_2$, so $E_1(t) > E_2(t)$ and, as a result, $u_1(t) > u_2(t)$. The terminating edge migrates faster than the leading edge, so the sample zone becomes narrow.

In a stacking run, because of sample stacking, the peak shape, unlike the flat-topped shape in a non-stacking run, is sharp. The measured migration time T to which the maximum response of the detector system corresponds is the time required for the leading edge of the analyte zone to migrate in the capillary (see Fig. 7). Based on this approximation, the migration time T of analyte zone is determined by the following integral equation:

$$\int_0^T (\mu_{eo} + \mu_e)E_2(t)\,dt = L_0 \tag{12}$$

Eq. 11 can be substituted into Eq. 12 to yield

$$\rho_2 V(\mu_{eo} + \mu_e) \int_0^T \{[\rho_1(t) - \rho_2]l(t) + \rho_2 L\}^{-1}\,dt = L_0 \tag{13}$$

Further, because the resistivity of strong electrolyte solution is inversely proportional to the solution concentration, $\rho_1(t)$ can be expressed as

$$\rho_1(t) = \frac{A}{\dfrac{n}{l(t)s}} = \frac{Asl(t)}{n} \tag{14}$$

where A is a constant and n is total number of moles of electrolyte in the analyte zone. For a given system, n and s are also constants, so Eq. 14 can be simplified to

$$\rho_1(t) = kl(t) \tag{15}$$

where $k = As/n$. The constant k can be determined by the following equation:

$$k = \rho_0/l_0 \quad (16)$$

The combination of Eqs. 13, 15 and 16 leads to

$$\rho_2 V(\mu_{eo} + \mu_e) \int_0^T \{[\rho_0 l(t)/l_0 - \rho_2]l(t) + \rho_2 L\}^{-1} dt = L_0 \quad (17)$$

It is difficult to determine how the sample injection length l_0 influences the migration time T from Eq. 17, because $l(t)$ is also related to l_0, so it is necessary to establish the mathematical model for $l(t)$.

Mathematical model for $l(t)$

In investigating the influence of the sample injection length l_0 on the migration time T in stacking runs, the dominant factor that needs to be considered is that different injection times lead to different electric field strengths in the buffer zone, which affects the migration velocity of the analyte zone, whereas the diffusional effects on the migration velocity of the analyte zone are negligible. Therefore, in the following mathematical model, we consider only the effect of sample stacking on $l(t)$ in order to simplify the process. We define $\Delta u(t)$ as

$$\Delta u(t) = u_1(t) - u_2(t) \quad (18)$$

The combination of Eqs. 4, 5, 10 and 11 now provides an expression for $\Delta u(t)$:

$$\Delta u(t) = \frac{(\mu_{eo} + \mu_e)V}{\rho_1(t)l(t) + \rho_2[L - l(t)]}[\rho_1(t) - \rho_2] \quad (19)$$

Eq. 19 can be arranged and rewritten as

$$\Delta u(t) = \frac{(\mu_{eo} + \mu_e)V}{l(t) + \rho_2 L[\rho_1(t) - \rho_2]^{-1}} \quad (20)$$

where $(\mu_{eo} + \mu_e)V$ is a constant for a given system. Combining Eqs. 15 and 20 gives

$$\Delta u(t) = \frac{(\mu_{eo} + \mu_e)V}{\frac{1}{k}\cdot\rho_1(t) + \rho_2 L[\rho_1(t) - \rho_2]^{-1}} \quad (21)$$

In Eq. 21, as $\rho_1(t)$ decreases and approaches ρ_2, the term $\rho_1(t) - \rho_2$ decreases and tends to zero, which leads to the term $\rho_2 L[\rho_1(t) - \rho_2]^{-1}$ increasing and approaching infinity as a limit, and the term $(1/k)\rho_1(t)$ in the denominator decreases and tends to $(1/k)\rho_2$, which is a constant. Therefore, the conclusion can be drawn that $\Delta u(t)$ decreases and approaches zero as a limit with decrease in $\rho_1(t)$. Moreover, because of the relationship in Eq. 15, the same conclusion for $l(t)$ can be drawn: $\Delta u(t)$ decreases and tends to zero with shortening of $l(t)$.

Now we analyse the change in $l(t)$ in a stacking run to establish its mathematical model. At the beginning of analyte zone stacking, the analyte zone l_0 is longest, so the initial rate $\Delta u(t)$ of sample stacking is highest, which leads to the analyte zone $l(t)$ becoming shorter extremely rapidly. In turn, the shorter $l(t)$ causes a decrease in $\Delta u(t)$, which in turn generates a slower shortening of $l(t)$. Consequently, the overall effect is that $l(t)$ becomes shorter extremely rapidly at the beginning, but considerably more slowly after a few seconds. With the shortening of $l(t)$, $\Delta u(t)$ becomes smaller and smaller, and finally tends to zero, which in turn makes $l(t)$ becoming shorter more and more slowly, finally approaching a limit. The limit is represented by a horizontal asymptote: $l(t) = l_{min}$. When $t = \infty$, then $l(t) = l_{min}$. The sample stacking model is simulated as shown in Fig. 8. The profile tends towards the exponential decay form as l_{min}:

$$l(t) = (l_0 - l_{min})\, e^{-at} + l_{min} \quad (22)$$

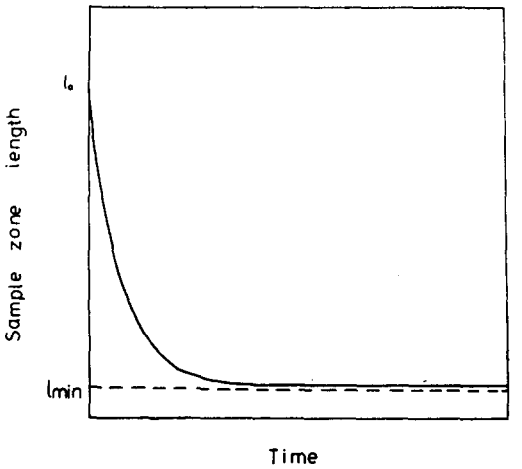

Fig. 8. Mathematical model, $l(t)$ *vs.* t, in a stacking run. The units are arbitrary.

where a is a positive constant. It can be noted from Eq. (22) that

when $t = 0$, $l(t) = l_0$

when $t = \infty$, $l(t) = l_{min}$

We observe that $l(t)$ becomes short initially very rapidly, but soon slows considerably and finally approaches a limit. The simulated equation is identical with the above analysis.

Relationship between l_0 and T

From Eq. 15, the following relationship can be obtained

$$\rho_2 = kl_{min} \tag{23}$$

The combination of Eqs. 15, 23 and 22 leads to

$$l(t) = [(1 - \rho_2/\rho_0)\, e^{-at} + \rho_2/\rho_0]l_0 \tag{24}$$

Now we have the relationship between $l(t)$ and l_0. On substitution of Eq. 24 into Eq. 17, the following relationship can be obtained:

$$\rho_2 V(\mu_{eo} + \mu_e) \int_0^T \{(\rho_0 - \rho_2 e^{-at}) [(1 - \rho_2/\rho_0)\, e^{-at} + \rho_2/\rho_0]l_0 + \rho_2 L\}^{-1} dt = L_0 \tag{25}$$

Eq. 25 is the final equation describing the relationship between the length of sample injection l_0 and migration time T. The parameters ρ_2, V, μ_{eo}, μ_e, ρ_0, L and a are constants. The term $\rho_0 - \rho_2 e^{-at}$ is positive at any moment t of a stacking run because $\rho_0 > \rho_2$ and $\rho_2 e^{-at}$ decreases with time t; the term $(1 - \rho_2/\rho_0) e^{-at}$ is also positive at any moment t of a stacking run because $\rho_0 > \rho_2$, and the term $\rho_2 L$ is a constant, so the overall term in the denominator $(\rho_0 - \rho_2 e^{-at})[(1 - \rho_2/\rho_0) e^{-at} + \rho_2/\rho_0]l_0 + \rho_2 L$ increases with increasing l_0 at any moment t. That is, the integrand in Eq. 25 decreases with increasing l_0. Therefore, it can be concluded that with increase in l_0, the migration time T increases, which is in agreement with the experimental results. Our model is also confirmed by the experimental results of Mikkers *et al.* [2] (Fig. 1 in ref. 2) which indicated that with increasing injection amount, the migration time also increases. It should be emphasized that our theoretical model is developed for strong electrolytes. Eq. 25 is complicated and its further detailed mathematical solution and confirmation are currently being investigated.

Under non-stacking conditions, from Fig. 3, if the difference in two injection times is 40 s, the migration time changes by 9 s for Br^-, 10 s for SO_4^{2-} and 11 s for NO_3^- (about 3% of the migration time). Under stacking conditions, from Figs. 4, 5 and 6, if the injection time changes from 0 to 40 s, the migration time changes by about 15 s for Br^- (about 9% of the migration time), 33 s for SO_4^{2-} (about 20% of the migration time) and 27 s for NO_3^- (about 15% of the migration time). Obviously, the influence of injection time on migration time for a stacking run is greater than that for a non-stacking run. In the present experiments, in a non-stacking run, if the injection time exceeds 40 s it significantly influences the migration time; in a stacking run, if the injection time exceeds 20 s it significantly influences the migration time.

Although large injection volumes lower the separation performance, in practical analyses by CZE for the determination of compounds present in samples at very low concentrations (*e.g.*, in the EC drinking-water directive, the concentration of any pesticide should be lower than 0.1 ppb) [14], large injection volumes have to be applied in order to introduce a detectable amount of the analytes. Moreover, in CZE, special injection methods have been developed and allow the introduction of samples as large as the entire volume of the separation capillary [14–16]. Therefore, a large injection length is sometimes necessary. From our work, however, it should be noted that a large injection length leads to changes in migration time compared with a small injection length, so qualitative evaluations of electropherograms based on migration time must be performed with great care when the injection lengths vary.

4. Conclusions

In non-stacking runs, because a different initial sample injection length causes different distance from the centre of the initial sample

injection length to the centre of the detector cell, consequently the migration time decreases with increasing sample injection time, which exactly reflects that the sample injection time does not affect the migration velocity of analyte ions. In stacking runs, the opposite experimental phenomenon was observed compared with non-stacking runs, because an increase in sample injection time decreases the migration velocity of analyte ions. In both instances, the experimental results and theoretical predictions are in agreement.

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