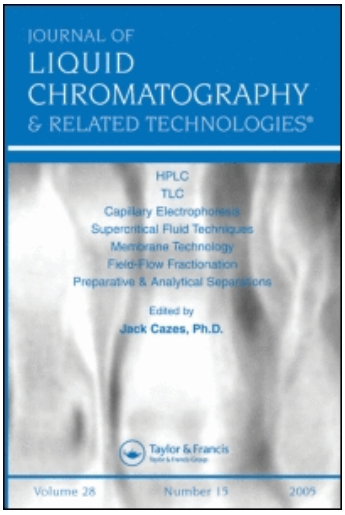

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Soontornniyomkij, B. , Chen, S. , Minnoor, E. S. and Pietrzyk, D. J.(1998) 'Application of an Electroosmotic Flow Gradient in Capillary Zone Electrophoresis Separations', Journal of Liquid Chromatography & Related Technologies, 21: 19, 2957 — 2976
To link to this Article: DOI: 10.1080/10826079808006879
URL: http://dx.doi.org/10.1080/10826079808006879

J. LIQ. CHROM. & REL. TECHNOL., 21(19), 2957–2976 (1998)

APPLICATION OF AN ELECTROOSMOTIC FLOW GRADIENT IN CAPILLARY ZONE ELECTROPHORESIS SEPARATIONS

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ABSTRACT

Resolution can be enhanced and separation time shortened in the capillary zone electrophoresis (CZE) separation of complex mixtures of acidic analytes that differ in pK_a values but have similar electrophoretic mobilities through an electroosmotic flow (EOF) gradient. The EOF gradient is created by a dynamic dilution of Mg^{2+} in the inlet reservoir, which is in the buffer as an EOF modifier, as the electrophoresis proceeds. Initially, the Mg^{2+} buffer enters the capillary by electromigration and produces a low EOF compared to the absence of Mg^{2+}. As the Mg^{2+} in the buffer in the inlet reservoir is diluted dynamically the EOF from the inlet side increases dynamically. Migration times for the fast-moving analytes are increased at a high Mg^{2+} buffer concentration in the initial stages of the separation while the migration times for the

slow-moving analytes are shortened by the EOF increase generated as Mg^{2+} is diluted during the gradient. The dynamic gradient, where EOF starts low and increases, influences analyte migration time, resolution, and separation time. The effect of initial Mg^{2+} buffer concentration, the rate of Mg^{2+} dilution, and the time delay in generating the gradient are parameters that can be altered to influence the CZE gradient separation. Several model analyte mixtures are used to illustrate the scope of the EOF gradient conditions.

INTRODUCTION

Capillary electrophoresis (CE) is an important analytical tool in many fields because of high separation efficiency, short analysis times, low reagent volume usage, and it is applicable to a broad range of analytes from small ions to biopolymers. [1] CE can be performed in several modes, the simplest and most used being capillary zone electrophoresis (CZE). A CZE separation takes place in an open tube capillary containing a homogeneous, buffer solution that serves as a background electrolyte. The separation in a constant applied field occurs because of the difference in the migration velocity of analytes, which is determined by their electrophoretic mobilities. The electrophoretic mobility (μ_{ep}) is inherently dependent on the charge to size ratio of the analytes and the viscosity and dielectric constant of the buffer solution. For a fused silica capillary migration as well as resolution of the analytes also depends on the magnitude and direction of an electroosmotic flow (EOF) or the electric-induced solvent flow, which is concurrently generated to electrophoresis. Because of an EOF flat flow profile, EOF facilitates detection as well as automation since its strong flow usually moves all species in one direction toward the cathode end of the capillary. As shown elsewhere,[1,2] EOF affects migration time, efficiency, and resolution; therefore, a well-controlled and reproducible EOF is required to obtain optimum separation results.

The effects of EOF on CZE has been studied extensively,[1] and electroosmotic velocity (ν_{eo}) can be defined as

$$\upsilon_{eo} = \frac{\varepsilon\varepsilon_o\zeta}{\eta}E \qquad (1)$$

where E is the applied electric field strength (voltage per unit length), ε_0 is the permittivity of vacuum, ε is the dielectric constant, η is the medium viscosity, and ξ is the zeta potential across the electrical double layer at the capillary wall-solution interface. Equation 1, defining ν_{eo}, is based upon two assumptions: (1) ε and η in the double layer region are the same as those in the bulk solution and

(2) the thickness of the double layer is very small compared to the radius of the capillary. Electroosmotic mobility (μ_{eo}) is defined as the electroosmotic velocity per unit field and is given by eq. 2. According to eq. 2 μ_{eo} is dependent on

$$\mu_{eo} = \frac{\varepsilon\varepsilon_o\zeta}{\eta} \qquad (2)$$

several parameters that are determined by the properties of the buffer, the capillary inner wall surface, and the temperature. Experimental variables which influence EOF include buffer components, concentration, and pH,[3-8] organic solvent for the buffer,[7,9] derivatizing silanol sites with inert polymers,[10,11] applying radial voltage to the capillary,[12-14] and using various types of buffer additives.[4,5-26] Cations as buffer additives are particularly useful to suppress EOF, since EOF affects analyte migration time and influences resolution.[15,17,18,20-26] It has been demonstrated that the cation added to the buffer undergoes cation exchange at the silanol sites in competition with the cation provided by the buffer causing a change in surface zeta potential. This causes EOF to change.[18,20,23-25] Divalent cations are more competitive in binding to the silanol sites than monovalent cations and have a greater effect on EOF as does an increase in the cation additive concentration.

In a typical CZE separation a buffer solution of known concentration and composition is placed in the inlet and outlet reservoirs, in the separation capillary, and held constant throughout the CZE run. For a given buffer condition and an applied electric field strength, a constant EOF is established. Even though CZE provides a high efficiency, small selectivity can be a serious limitation when separating a complex mixture of closely related analytes of similar electrophoretic mobilities. If a buffer that yields a low EOF is used to improve resolution of fast moving analytes, migration times for slow moving analytes may be adversely affected. Therefore, it is often impossible to find a single buffer that can provide both an optimum resolution and separation time for all analytes in a mixture containing a broad spectrum of analytes. One strategy to overcome this problem in CZE separations is to create a dynamic change in EOF through a change in buffer composition.

A pH gradient strategy has been successfully used in CZE separation of ionic species such as weak acids and bases, where buffer pH is dynamically changed in the pK_a region of the analytes. The objective was to alter buffer composition to cover a pH range that would lead to a change in the electrophoretic mobility of the analytes during electrophoresis to improve resolution while maintaining an optimum separation time. Three electrode chambers containing two different buffer systems were used to generate a pH gradient in the background buffer electrolyte by electromigration of the two solutions into the capillary at different ratios.[27,28] The same instrumental

strategy was also used to generate a pH step gradient[29] and a pulse change[30] of the buffer in CZE applications. Another approach of creating a dynamic pH gradient was to mechanically pump by syringe a second buffer solution into the inlet buffer reservoir.[31] Similarly, dynamic pH changes were obtained by directly controlling the flow of H^+ from the anode chamber[32] or by creating a step change in the electrolyte anions which determine the pH.[33] A dynamic pH change from 3.0 to 5.2, where the two modifying buffer solutions were individually pumped into a premixer at different ratios prior to introduction into the outlet reservoir, was used in the CZE separation of weak organic acids.[34] A pH gradient has been used with a chemically coated capillary[32,33] and a capillary containing a surface dynamically coated with a cationic surfactant[34] or an inert hydrophilic polymer.[31] These instrumental setups were also used to generate an ionic gradient in the buffer.[27, 29,33]

Only a few studies have been reported whereby an EOF gradient was obtained at a constant buffer pH. For example, an organic solvent step gradient was shown to be more effective than a temperature step gradient on the micellar electrokinetic capillary electrophoresis separation of a mixture of weak base organic amines.[35] As organic solvent concentration increases, the dielectric constant decreases and viscosity of the bulk solution, as well as at the interface capillary wall-solution increases, thus, the EOF will decrease. In another study analytes were injected into a buffer containing a low concentration of cationic surfactant, and as the electrophoresis proceeded a surfactant solution of higher concentration was introduced into the capillary at the anodic end.[34] As a result, the EOF decreased during the separation and the resolution of several organic acids was improved.

In this report, we demonstrate that a gradient EOF is created at a constant buffer pH by a dynamic concentration change of a metal cation additive in the buffer. As the cation additive concentration decreases, the EOF increases. Thus, the CZE separation of complex analyte mixtures is improved while maintaining an optimum separation time. Variables for the EOF gradient and their effects are identified.

EXPERIMENTAL

Instrumentation

All experiments were performed with a lab-assembled CE instrument unit. A Spellman high voltage DC power supply Model UHR (Spellman High Voltage Electronic Corp) was used in a constant voltage mode to deliver up to ±30 kV. Detection was with a Spectra Physic Spectra Focus variable wavelength detector equipped with an on-column capillary accessory Model

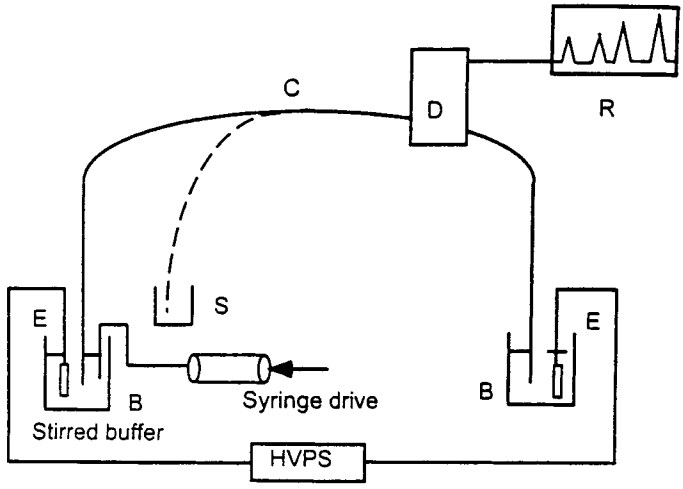

Figure 1. Instrumentation used to generate a gradient EOF in CZE separations. C = fused silica capillary, B = buffer reservoirs, E = electrodes, HVSP = high voltage power supply, D = a UV/visible detector, R = recorder/integrator, S = sample vial.

9550-01555 to accommodate a capillary. Two platinum electrodes were used to connect the power supply with two buffer reservoirs located at each end of the capillary. To prevent electrical shock, both electrode reservoirs were enclosed in a Plexiglas safety box supplied with a safety interlock system.

A syringe pump Model 341 (Sage Instruments) with a >20 mL volume disposable syringe was used to deliver a cation additive-free buffer solution to the inlet buffer reservoir. The syringe was also wrapped with a plastic sheet to prevent electrical sparking. A 15 cm long, 0.5 mm I.D. and 2.5 mm O.D. Teflon tube was used to connect the syringe with the anodic buffer reservoir, as shown in Fig. 1. All separations were carried out in a 60 cm x 50 mm I.D. (375 mm O.D.) fused silica capillary (Polymicro Technologies, Phoenix, AZ, USA.) with an effective length of 40 cm.

Chemicals

2, 4-Dinitrophenyl-amino acid (DNP-AA) and 5-(dimethylamino) naphthalene-1-sulfonyl-amino acid (DNS-AA) derivatives and L-aspartyl-L-phenylalanine methyl ester (aspartame) were purchased from Sigma Chemical Co. and used without further purification. Mesityl oxide, used to determine EOF, was purchased from Aldrich Chemical CO. Inorganic salts and organic

acids were of analytical grade and obtained from Eastman Organic Company, Mallinckrodt Chemical Works, Aldrich Co., Fisher Scientific, EM Science, and Sigma Chemical Co. Water for the preparation of solutions was freshly purified by passing in-house distilled water through a Milli-Q-Plus water treatment system and filtered through a 0.2 mm nylon filter.

An aqueous 15 mM borate buffer solution was prepared by dissolving a weighed amount of $Na_4B_4O_7$ in water and adjusting the pH of the solution to pH = 9.00 with either HCl or NaOH solution. A 12.5-20 mM NH_4OAc buffer solution was prepared with NH_4OAc and its pH was adjusted to pH = 7.0 with dilute NH_3 solution. An aqueous 0.2 M $MgCl_2$ stock solution was prepared by weight and appropriate aliquots were added to the buffer solutions. Test mixtures of carboxylic acids, phenols, and amino acid derivatives were dissolved in the buffer at a concentration of 0.1-0.2 mM in each analyte.

Procedures

The capillary was preconditioned with a 0.2 M NaOH solution for 20 min. followed by a 5 min. wash with deionized H_2O. The capillary and both buffer reservoirs were filled with the buffer solution, which contains Mg^{2+} as a buffer additive and was equilibrated for 2 hr at an applied voltage of +30kV. After equilibration the capillary and both the inlet and outlet 25 mL buffer reservoirs were filled with fresh buffer (about 2 mL in each reservoir).

The liquid level in both reservoirs was equal initially and the reservoirs were wide diameter to minimize effects of liquid height difference during the gradient run. The syringe was filled with 20 to 30 mL buffer solution which was identical to the buffer used for equilibration except that the Mg^{2+} is omitted. The syringe and the inlet buffer reservoir were connected through a 0.5 mm I.D. x 15 cm long Teflon tube (see Fig.1). Both buffer reservoirs were enclosed in the Plexiglas box and the buffer in the inlet reservoir was well mixed by magnetic stirring during the gradient step.

The sample was injected hydrostatically at the anodic end of the capillary with a height difference of 7 cm for 10 to 15 sec. The injection volume was about 10 to 15 nL. As the electric field was applied, the Mg^{2+}-free buffer was pumped into the stirred Mg^{2+} buffer solution in the inlet reservoir causing a dynamic dilution of Mg^{2+}concentration in the inlet buffer solution.

After each separation the capillary was rinsed with 0.25 M Na_2 citrate buffer for 10 to 20 min. to ensure purging Mg^{2+} from the capillary followed by rinsing with deionized water for 5 min. before switching to the new buffer.

RESULTS AND DISCUSSION

CZE Conditions

The optimum buffer and its concentration, pH, applied voltage, and the fused silica capillary dimensions were established by initial studies while the type and concentration range of metal cation additives was suggested by previous work.[23,25,26] A 15 mM borate, pH = 9.00 buffer was used for most separations because it provided adequate buffer capacity and ionic strength to maintain electrical conductance, but did not produce excess heat. Also, the borate buffer did not interfere with absorption detection at 214 nm nor did it cause precipitation when Mg^{2+} was the buffer additive. A high buffer pH was employed so that most analytes would be ionized and the EOF would be high which contributes to a fast separation. A fused silica capillary of 60 cm (40-cm effective length) by 50-μm I.D. was used throughout the studies to provide optimum migration time, efficiency, and resolution. The applied voltage of +30 kV generated a current less than 17 μA and had little adverse effect on efficiency.

Divalent cations, such as Mg^{2+}, Zn^{2+}, Cd^{2+}, and Cu^{2+}, have been used as buffer additives in a basic aqueous buffer to alter EOF.[23,25,26] As divalent cation concentration in the buffer decreases, EOF increases and the effect of the cation to reduce EOF follows the order $Cu^{2+} > Cd^{2+} > Zn^{2+}, > Mg^{2+}$. While Cu^{2+}, Cd^{2+}, and Zn^{2+} can be used at a lower concentration than Mg^{2+} to yield the same decrease in EOF as obtained with Mg^{2+}, precipitation is avoided and better resolution is obtained with Mg^{2+}as the buffer additive. Also, Mg^{2+} is less likely to form complexes or associated species with the analytes compared to the transition metal cations. For these reasons only Mg^{2+} was studied as the buffer additive to generate the EOF gradient.

As EOF decreases in the presence of the metal cation additive, the migration time and resolution of anionic analytes increases. The improved resolution can be explained by eq 3

$$R_s = \frac{1}{4}\sqrt{N}\left[\frac{\Delta\mu}{\bar{\mu} + \mu_{eo}}\right] \qquad (3)$$

where R_s is resolution, N is the column efficiency, $\Delta\mu$ is the difference in electrophoretic mobilities of the two adjacent species, $\bar{\mu}$ is the average electrophoretic mobility, and μ_{eo} is the electroosmotic flow. As EOF decreases or opposes the direction of the electrophoretic mobility, R_s as well as the migration time increases.

Table 1

Effect of Mg^{2+} as a Buffer Additive on the Migration Time of Several Analytes in the Absence and Presence of a Mg^{2+} Gradient

	Analyte Anion					
Without Gradient[a]	**Benzoate**	**2,6-Naphthalene dicarboxylate**	**Phthalate**	**DNP-L-Trp**	**DNP-L-Ala**	**DNP-Asp**
0 Mg^{2+}	3.45	5.28	6.83	---	---	---
0.20 mM Mg^{2+}	5.87	15.2	>40	3.64	4.22	10.2
0.40 mM Mg^{2+}	---	---	---	4.52	5.51	29.4
With Gradient[a]						
0.20 mM Mg^{2+} diluted at 0.65 mL/min. with buffer[b]	5.20	9.94	14.9	---	---	--
0.2mM Mg^{2+} diluted at 0.92 mL/min. with buffer[b]	4.36	7.77	11.0	---	---	---
0.40 mM Mg^{2+} diluted at 0.46 mL/min. with buffer[b]	---	---	---	3.92	4.65	11.1
0.40 mM Mg^{2+} diluted at 0.92' mL/min. with buffer[c]	---	---	---	4.09	5.00	14.5
0.40 mM Mg^{2+} diluted at 1.40 mL/min. with buffer[c]	---	---	---	4.03	4.87	13.3

[a] The buffer is 15.0 mM borate, pH = 9.00.
[b] The gradient is initiated at the start of the electrophoresis.
[c] The gradient is initiated at the 7th min. of the electrophoresis.

Table 1 shows that migration time is significantly increased for several carboxylic acid and 2,4-dinitrophenyl amino acid analytes as Mg^{2+} concentration is increased in the 15 mM borate, pH = 9.00 buffer. For divalent analyte anions in Table 1 the shift in migration times is so large that separation times for these analytes are excessive. Furthermore, selectivity is increased which leads to improved resolution.

Electropherograms for the CZE separation of complex mixtures of analyte anions of similar mobilities illustrating the enhanced resolution in the presence of Mg^{2+} is reported elsewhere.[24]

As indicated in Table 1, mixtures of analyte anions that differ widely in migration times, for example mono- and di-valent anions, would have excessively long separation times.

Gradient Conditions

EOF can be adjusted by changing the concentration of Mg^{2+} in the background buffer. If the buffer Mg^{2+} concentration is high, EOF is low, while a high EOF is favored by a low Mg^{2+} concentration. If the Mg^{2+} concentration is dynamically changed in the buffer from a high to low concentration during the CZE run, an EOF gradient of low to high EOF can be generated. Thus, the EOF systematically changes as a function of time in a controlled and reproducible way. Instrumentally, this can be accomplished by a modification in the typical CE procedure as outlined in Fig. 1. The major modification is that a syringe drive is used to deliver a Mg^{2+} free buffer to the inlet buffer reservoir at a controlled flow rate. Buffer containing Mg^{2+} is placed in both the inlet and outlet reservoirs and capillary and the sample is introduced into the capillary. When the voltage is applied, the syringe drive is started and the Mg^{2+} free buffer solution is delivered at a known, constant flow rate to the well-stirred inlet reservoir. This causes the Mg^{2+} concentration to decrease in the inlet reservoir continuously according to the rate of dilution. Thus, as the dilution occurs EOF in the capillary increases establishing an EOF gradient in the capillary.

When the gradient is started, the Mg^{2+} concentration in the inlet buffer reservoir decreases relative to the cathodic reservoir due to dilution. EOF mobility was determined[37] for a series of conditions where the outlet reservoir and the capillary contained more Mg^{2+} in the buffer than the Mg^{2+} concentration in the buffer in the inlet reservoir. As the difference in Mg^{2+} concentration (0.5 to 3.4 mM) between the two reservoirs increased (the inlet Mg^{2+} concentration in the buffer was decreased as in the gradient) EOF was found to decrease. In another set of experiments the same buffer containing Mg^{2+} was introduced into the two reservoirs and the capillary but the height difference between the two reservoirs was varied. No effect on EOF was observed for the maximum height difference studied or up to 5 cm. In the gradient experiments described here large diameter buffer reservoirs were used and the height difference that formed when the gradient was carried out was much less than the 5 cm.

Table 1 illustrates that the migration time for the analyte anions can be reduced through an EOF gradient. For example, for the carboxylate analyte anions the EOF gradient (dilution of the Mg^{2+} buffer in the inlet reservoir) is started at the same time that the voltage is applied to the capillary. The greatest effect is on the divalent carboxylate analyte anions where migration time is sharply reduced with the gradient.

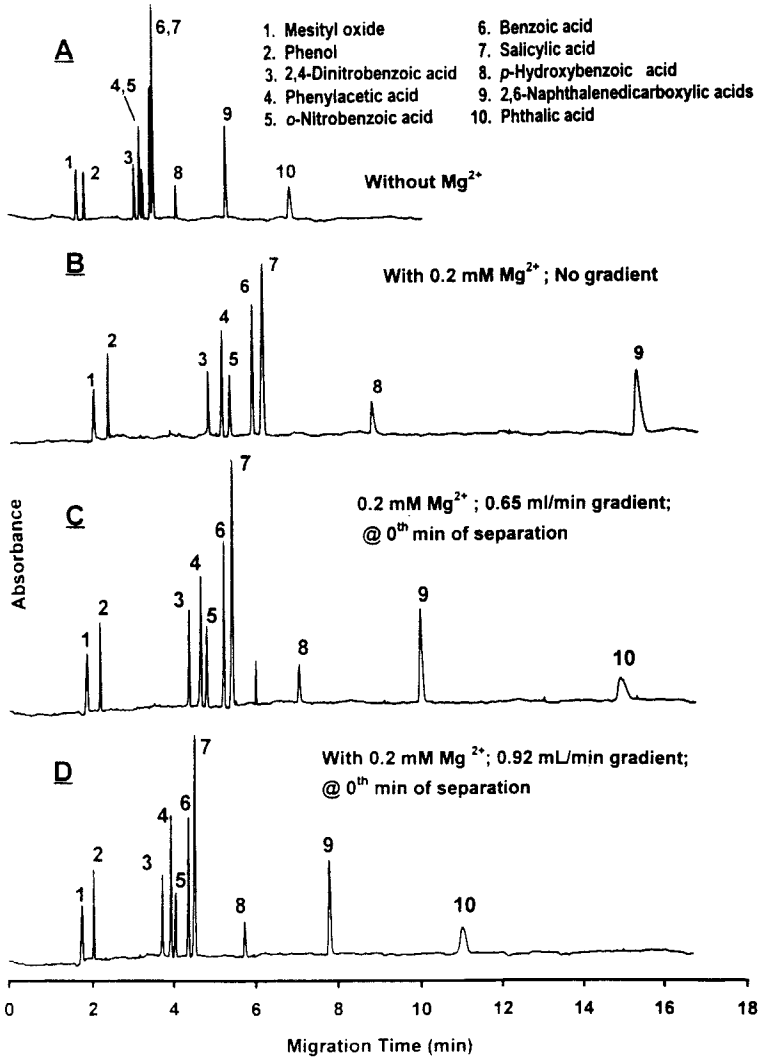

Figure 2. Effect of the EOF-Mg^{2+} concentration gradient on the CZE separation of organic acids. The conditions are a 15 mM borate, pH = 9.00 buffer, an applied voltage = + 30 kV, 15 μA current, hydrostatic injection of 10 to 15 nL, and detection at 214 nm.

Similar trends were also obtained when using DNP-amino acid analyte anions, see Table 1. Furthermore, the delivery rate of the diluent buffer at the inlet end can be varied to influence the gradient change and, therefore, the migration time for the analyte.

Effect of Mg^{2+} Concentration on EOF Gradient and Resolution

Figure 2 shows how the Mg^{2+} gradient affects the CZE separation of a mixture of 10 carboxylic acids/phenol derivatives in a 15 mM borate, pH = 9.00 buffer with and without Mg^{2+} and with an EOF gradient. These analytes differ widely in pK_a values and electrophoretic mobilities. In the absence of Mg^{2+} (see Fig. 2A) EOF is high; therefore, all the analytes have low migration times and the resolution is incomplete for several carboxylic acid derivatives particularly those that migrate the fastest. With 0.20 mM Mg^{2+} in the buffer (see Fig. 2B), EOF is decreased and resolution is significantly improved, however, migration times for 2,6-naphthalenedicarboxylate and phthalate divalent anions exceed 15 and 18 min., respectively. A successful separation of all 10 carboxylate anions including the divalent anions requires a long analysis time if an isocratic buffer condition is used. When an EOF gradient is generated (see Fig. 2C) resolution and migration time can be optimized. In this example, the separation was started by filling both buffer reservoirs, as well as the capillary, with a 15 mM borate, pH = 9.00 buffer containing 0.20 mM Mg^{2+}. The sample was then injected into the inlet. Simultaneously, the electric field was applied and the Mg^{2+}-free buffer was immediately delivered into the inlet buffer reservoir by the syringe drive to dilute the Mg^{2+} concentration. As dilution takes place in the inlet reservoir, the diluted solution continuously enters the capillary by electromigration. Thus, the EOF is dynamically reestablished as soon as the more dilute Mg^{2+}solution is in contact with the silica surface.

At the beginning of dilution, the EOF has not yet increased because the Mg^{2+}concentration is only slightly diluted. The migration time of the EOF marker (peak 1), therefore, is almost the same as found in Figure 2B. However, the rate of establishing the EOF appears to increase as the dilution takes place. This is indicated by comparing the migration times for peaks 3 to 7 in Figs. 2B and 2C. The migration times are only slightly shortened and resolution of peaks 3 to 7 is still maintained as the EOF increases.

The effect of the dynamically increased EOF (compare Fig. 2C to 2B) is more apparent when considering the migration time of the two divalent 2,6-naphthalenedicarboxylate and phthalate anions. The migration time for the former is reduced by about 5 to 6 min. while phthalate migrates even faster and it appears with a migration time of about 16 min. in Figure 2C.

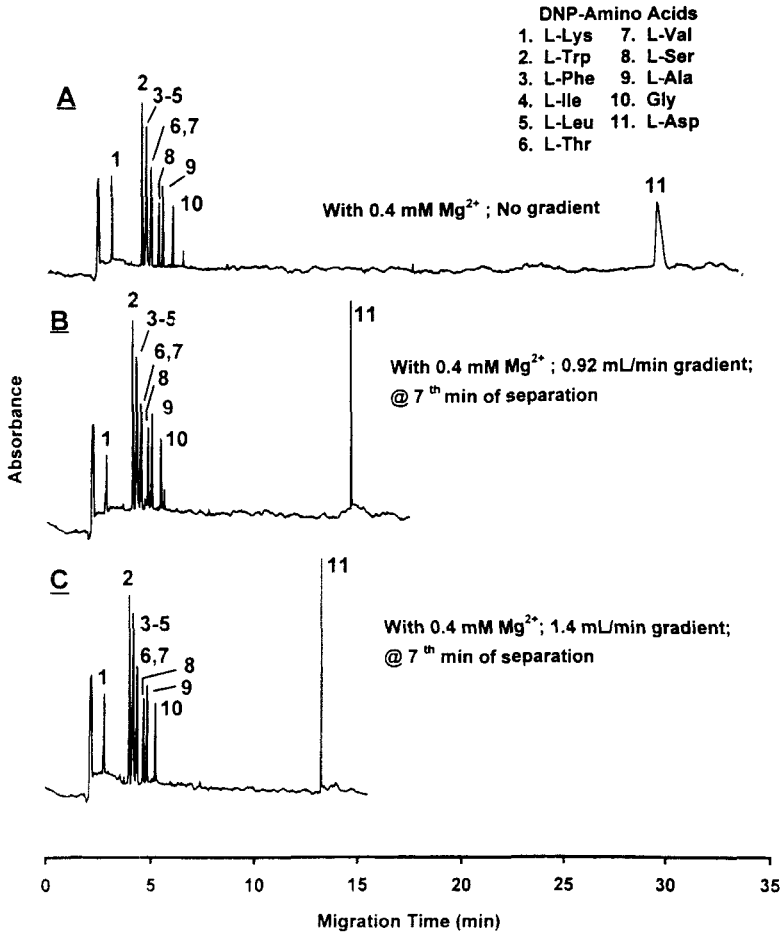

Figure 3. Effect of Mg^{2+} dilution flow rate on the EOF gradient separation of DNP-L-amino acid derivatives. Conditions are the same as Figure 2 except for the Mg^{2+} delivery.

Effect of the Dilution Flow Rate on the EOF Gradient

The analysis time can be reduced further by increasing the rate of dilution of the inlet Mg^{2+}-buffer solution. This was done by increasing the flow rate of the Mg^{2+} free buffer to the inlet reservoir. In Fig. 2D the rate was increased to 0.92 mL/min. compared to 0.65 mL/min., which was used in Fig. 2C.

The resolution of analytes 2 to 7 is still not appreciably affected although they migrate a little faster. But, the migration times for the divalent anions are reduced and the total separation time is shorter by about one third than when using the flow rate of 0.65 mL/min.

Figure 3 also illustrates the effect of the EOF gradient and dilution flow rate on the CZE separation of a mixture of eleven DNP-L-amino acid derivatives. When the 0.40 mM Mg^{2+}-buffer solution is diluted at the rate of 0.92 mL/min. (see Fig. 3B) the migration time of DNP-L-Asp acid is reduced by about 15 min. compared to the constant Mg^{2+} concentration buffer (see Fig. 3A). When the rate was increased to 1.40 mL/min. (see Fig. 3C) DNP-L-Asp appears at less than 15 min. in the electropherogram. It should be noted that in both gradients (Figs. 3B and 3C) the gradient was started at seven min. after the separation voltage was initiated. In both cases resolution of peaks 2 to 10 are not affected since these analytes have already passed the detection window before the gradient was initiated.

Effect of a Time Delay in Initiating the EOF Gradient

The starting time for the Mg^{2+} concentration gradient can be varied and therefore optimized according to the mixture being separation. In this case the gradient is initiated during the course of the electrophoresis rather than simultaneously with the start of the electrophoresis. Table 1 lists examples for DNP-L-Trp, -L-Ala, and -L-Asp analyte anions where the gradient is started during the electrophoresis. In these examples the intial Mg^{2+} concentration in the buffer was 0.40 mM and the gradient at two different delivery rates was started at 7 min. after the voltage is applied to the capillary. The L- Trp and L-Asp DNP derivatives are not appreciably affected by the gradient but the much slower moving L-Asp DNP derivative is affected. When this strategy is applied to a 11 component mixture of DNP-amino acid derivatives (see Fig. 3B and 3C) the migration times and resolution of the first 10 derivatives, several of which have migration times of 7 min. or less, are not affected by the gradient but the L-Asp derivative migration time is reduced. Thus, the total separation time is reduced by several minutes.

When the initial Mg^{2+} concentration was increased to 0.80 mM in the absence of a gradient, the higher Mg^{2+} buffer concentration (compare Fig. 4A to 3A) increases the migration time for all the DNP-amino acid derivatives because of a lower EOF. And, those that migrate with the slowest rate will be affected the most. For example, DNP-L-Asp is not detected because it migrates so slowly (or moves towards the anode) because of the low EOF in the 0.80 mM Mg^{2+}.

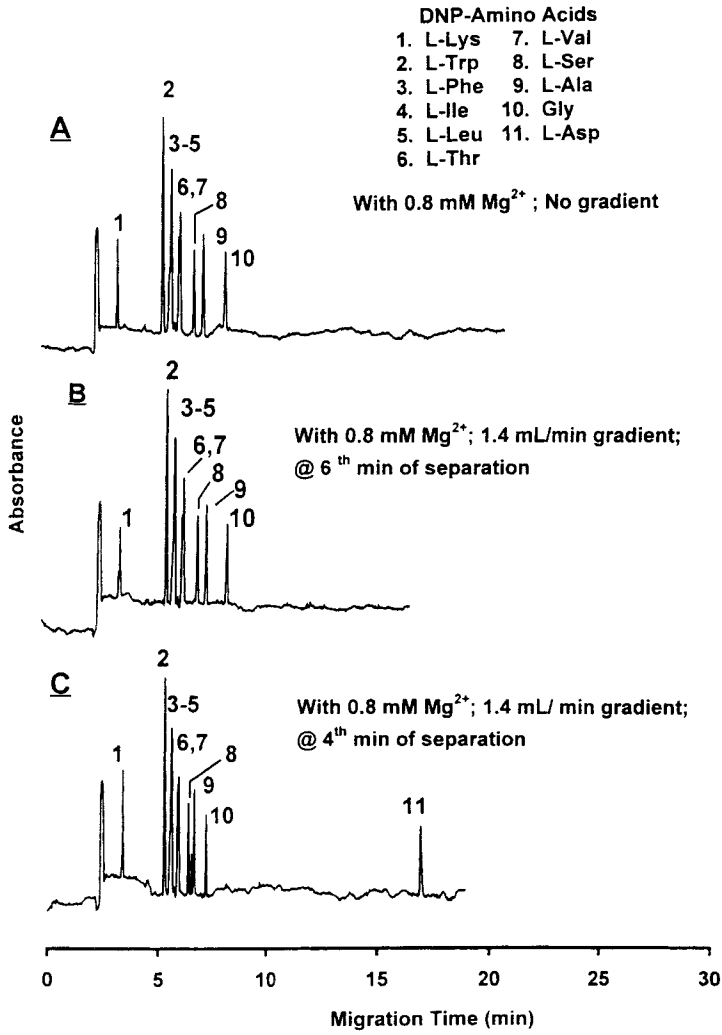

Figure 4. Effect of a time delay in initiating the EOF-Mg^{2+} concentration gradient on the CZE separation of DNP-L-amino acid derivatives. Conditions are the same as Figure 2 except for the Mg^{2+} delivery.

By using a gradient at a fast dilution rate with a delayed start only migration times for the very slow migrating analytes are appreciably affected. For example, in Fig. 3B and 3C the gradient was started at the 7 min. mark of the separation so that the gradient would only affect the migration time of the

slow moving divalent anionic DNP-L-Asp analyte. In Fig. 4B and 4C the gradient was started at 6 min. and 4 min., respectively. When compared to the absence of the gradient (see Fig. 4A), the EOF gradient is started at a time earlier than the migration times for several of the DNP-amino acid derivatives. The migration times for DNP derivatives 6 to 10 are reduced with analyte 10 being reduced the most. But, resolution for DNP derivatives 6 to 10, which migrate close together, is not appreciably affected.

When the gradient was engaged at 6 min. after the electrophoresis started (Fig.4B), the rate of reestablishing a higher EOF by dilution of the Mg^{2+} was too slow to overcome the opposing electrophoretic migration of the anionic DNP-L-Asp derivative and the DNP-L-Asp peak never appeared. Apparently, DNP-L-Asp migrated back to the anode side before the magnitude of the increasing μ_{eo} exceeded the μ_{ep} for the derivative.

When the dilution was started 2 min. earlier or at 4 min. as shown in Fig. 4C, the EOF was regenerated fast enough that it caused DNP-L-Asp to migrate past the detector within 20 min.. As the Mg^{2+} dilution was begun earlier and before the separation of peaks 2 to 10 was completed, resolution was only slightly reduced. However, what is gained is that the overall separation including the DNP-L-Asp derivative is finished within 18 min.

Effect of an EOF Gradient on the Separation DNS-Amino Acid Derivatives

Because a Mg^{2+} concentration gradient affects only the EOF, not electrophoretic mobility of analytes, this technique can be applied to other sample and buffer conditions. Figure 5 illustrates the application of the Mg^{2+}-EOF gradient to the separation of a mixture of 7 DNS-L-amino acid derivatives in a 12.5 mM NH_4OAc, pH = 7.25 buffer. In the absence of Mg^{2+} in the buffer (Fig. 5A) resolution between three acidic DNS-L-amino acid derivatives, peaks 5 to 7, was good. But, resolution among the hydrophobic side chain DNS-L-Trp, DNS-L-Val, and DNS-Gly derivatives was poor.

When 0.40 mM Mg^{2+} was included in the buffer (Fig. 5B) the resolution of the hydrophobic side chain derivatives was enhanced, but the migration times of DNS-L-Cys and DNS-L-Glu was sharply extended. Moreover, DNS-L-Asp probably traveled back to the anode or inlet end because of the low EOF and the derivative was not detected.

When the EOF gradient was engaged by dilution of the Mg^{2+} concentration at 4 min. into the electrophoretic run (Fig. 5C), resolution and migration times for all analytes were optimized and a baseline separation was obtained.

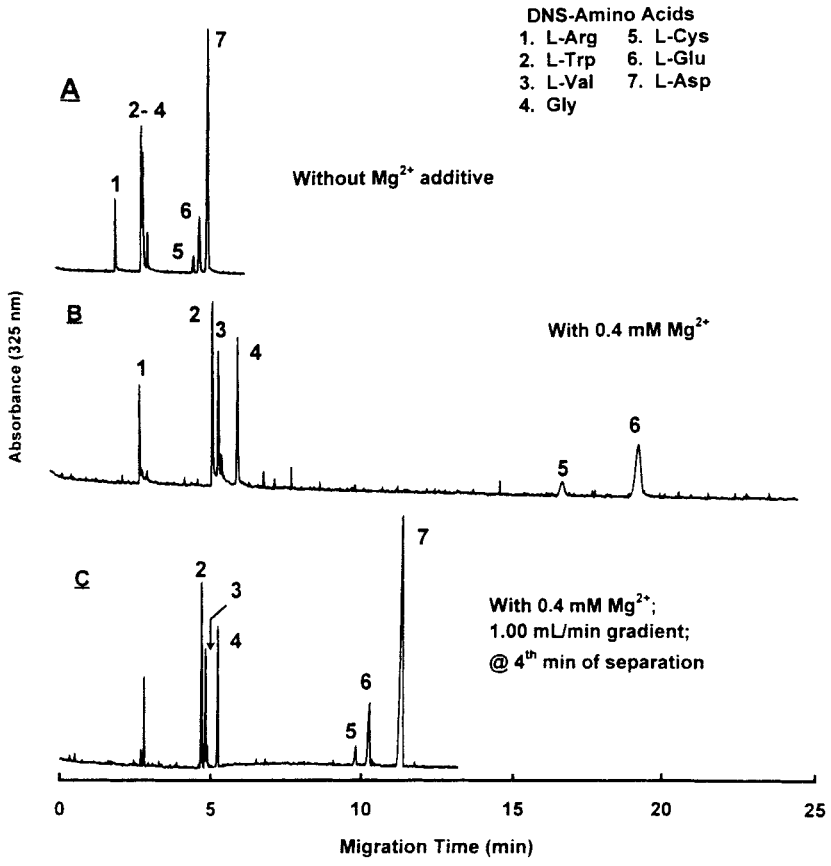

Figure 5. Effect of the EOF-Mg^{2+} concentration gradient on the CZE separation of DNS-L-amino acid derivatives. The buffer is 12.5 mM NH_4OAc, pH = 7.25 and the rest of the conditions are the same as Figure 2 except for the Mg^{2+} delivery.

The addition of Mg^{2+} to the buffer to reduce EOF was shown to be effective in improving the resolution for the chiral separation of DNS-D,L-amino acid derivatives.[26] Even through the chiral resolution was enhanced for most D,L-amino acid derivatives for a constant Mg^{2+} buffer concentration, the separation time may be long and/or incomplete depending on the DNS-D,L-amino acid derivatives in the mixture. Figure 6 compares the chiral separation of several DNS-DL-amino acid derivatives in the absence of Mg^{2+} (Fig. 6A), in the presence of 2.0 mM Mg^{2+} in the buffer (Fig.6B), and with an EOF gradient via a dilution of 2.0 mM Mg^{2+} (Fig. 6C). The buffer was composed of 10 mM

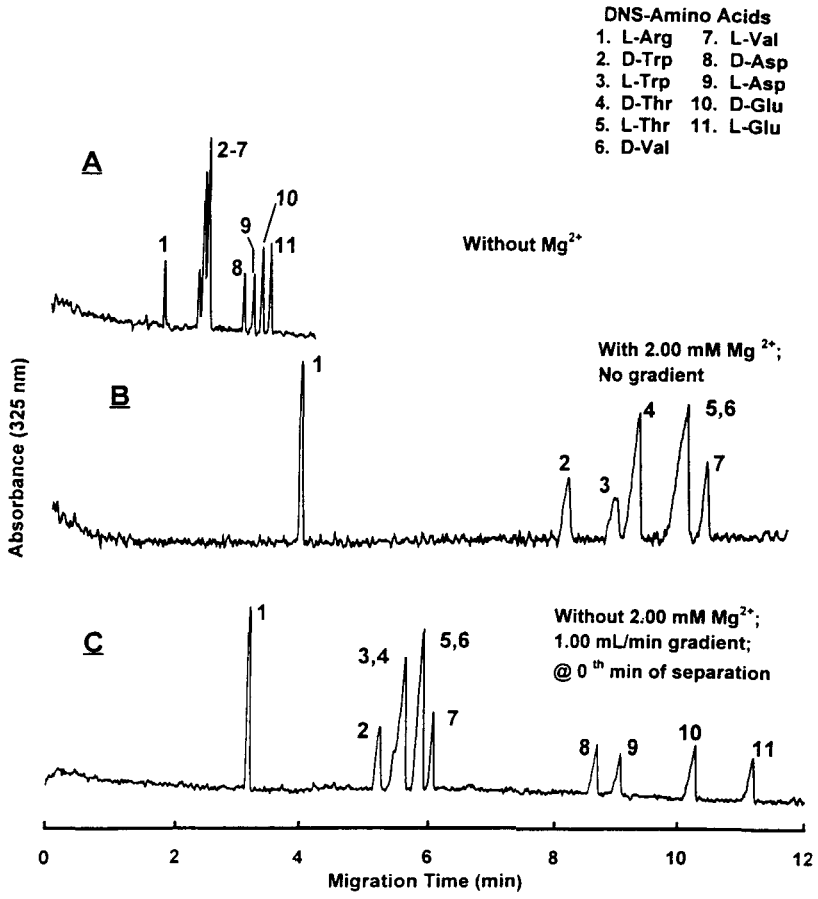

Figure 6. Effect of the EOF-Mg^{2+}concentration gradient on the CZE chiral separation of DNS-D,L-amino acid derivatives . The buffer is 10.0 mM NH_4OAc, 2.5 mM $CuSO_4$, 5.0 mM aspartame, pH = 7.25. and the rest of the conditions are the same as Figure 2 except for detection at 325 nm and Mg^{2+} delivery.

NH_4OAc, 2.5 mM $CuSO_4$, and 5.0 mM aspartame at pH = 7.40. Resolution of the neutral side chain DNS-DL-amino acid derivatives was improved in Fig. 6B when Mg^{2+} was 2.0 mM in the buffer compared to its absence (see Fig. 6A). But, the four acidic side chain DNS-DL-amino acid enantiomers migrated either too slowly or back toward the capillary inlet (the anode). As a result, their peaks (8 to 11) are absent in Figure 6B. When the Mg^{2+} concentration gradient was applied by delivering a Mg^{2+} free buffer to dilute the Mg^{2+} concentration in the inlet reservoir at the flow rate of 1.0 mL/min., migration times for all four acidic

side chain DNS-DL-AA derivatives decreased and the peaks appeared within 12 min. (see Fig. 6C). Even through the resolution of the neutral side chain DNS-D,L-AA derivatives (peaks 2 to 7) was slightly reduced due to the regenerated EOF, the resolution was still much better than the resolution obtained in the absence of the Mg^{2+} in the buffer.

SUMMARY

An EOF gradient, where EOF changes from low to high, is carried out during an electrophoresis run by a dilution of the Mg^{2+} concentration in the buffer solution in the inlet reservoir. The EOF-Mg^{2+} concentration gradient variables are the Mg^{2+} concentration in the buffer, the Mg^{2+} dilution rate in the inlet reservoir, and the time within the electrophoresis run when the gradient is initiated. These variables can be controlled individually or in combination depending on the mixture being separated as well as the difference in the electrophoretic mobility of the analytes in the mixture. Thus, analytes in a mixture that migrate quickly can be separated at low EOF for maximum resolution while migration times for analytes in the mixture that migrate slowly can be reduced by increasing the EOF. The EOF-Mg^{2+} concentration gradient allows analysis time to be reduced while still maintaining good resolution.

ACKNOWLEDGMENTS

The authors wish to thank Waters Chromatography for providing the Waters Quanta 4000 CE instrument. Part of this work was presented at the International Ion Chromatography Symposium in October 1996 at Dallas, TX as paper number 93.

REFERENCES

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1. J. P. Landers, ed., **Handbook of Capillary Electrophoresis**, 2nd Ed., CRC Press, Boca Raton, 1996.

2. J. W. Jorgenson, K. D. Lukas, Anal. Chem., **53**, 1298 (1981).

3. K. D. Lukacs, J. W. Jorgenson, J High Res. Chromatogr. Comm., **8**, 407 (1985).

4. H. H. Lauer, D. McManigill, Anal. Chem., **58**, 166 (1986).

5. K. D. Altria, C. F. Simpson, Chromatographia, **24**, 527 (1987).

6. W. J. Lambert, D. L. Middleton, Anal. Chem., **62**, 1585 (1990).

7. B. B. VanOman, G. G. Liverside, G. L. McIntire, J. Microcol. Sep., **2**, 176 (1990).

8. H. J. Issaq, I. Z. Atamna, G. M. Muschik, G. M. Janini, Chromatographia, **32**, 155 (1991).

9. C. Schwer, E. Kenndler, Anal.Chem., **63**, 1801 (1991).

10. S. Hjerten, J. Chromatogr., **347**, 191 (1985).

11. G. J. M. Bruin, R. Huisden, J. C. Kraak, H. Poppe, J. Chromatogr., **480**, 339 (1989).

12. C. S. Lee, W. C. Blanchard, C. Wu, Anal.Chem., **62**, 1550 (1990).

13. M. A. Hayes, A. G. Ewing, Anal.Chem., **64**, 512 (1992).

14. M. A. Hayes, I. Kheterpal, A. G. Ewing, Anal.Chem., **65**, 27 (1993).

15. A. S. Cohen, S. Terabe, J. A. Smith, B. L. Karger, Anal. Chem., **59**, 1021 (1987).

16. T. Tsuda, J. High Res. Chromatogr. & Chromatogr. Comm., **10**, 622 (1987).

17. K. Salomon, D. S. Burgi, J. C. Helmer, J. Chromatogr., **549**, 375 (1991).

18. S. Chen, D. J. Pietrzyk, Anal. Chem., **65**, 2770 (1993).

19. D. Corradini, A. Rhomberg, C. Corradini, J. Chromatogr. A, **661**, 305 (1994).

20. J. E. Dickens, J. Gorse, J. A. Everhart, M. Ryan, J. Chromatogr. B, **657**, 401 (1994).

21. Y.-H. Lee, T.-I. Lin, J. Chromatogr. A, **680**, 287 (1994).

22. M. F. M. Tavares, V. L. McGuffin, Anal. Chem., **67**, 3687 (1995).

23. D. J. Pietrzyk, S. Chen, B. Chanthawat, J. Chromatogr. A, **775**, 327 (1997).

24. S. Chen, Ph.D. Thesis, University of Iowa, August 1993.

25. B. Chanthawat, Ph.D. Thesis, University of Iowa, May 1997.

26. B. Soontornniyomkij, K. Scandrett, D. J. Pietrzyk, Accepted J. Liq. Chromatogr., 1998.

27. J. Pospichal, M. Deml, P. Gebauer, P. Bocek, J. Chromatogr., **470**, 43 (1989).

28. P. Bocek, M. Deml, J. Pospichal, J. Sudor, J. Chromatogr., **470**, 309 (1989).

29. J. Sudor, Z. Stransky, J. Pospichal, M. Deml, P. Bocek, Electrophoresis, **10**, 802 (1989).

30. P. Bocek, M. Deml, J. Pospichal, J. Chromatogr., **500**, 673 (1990).

31. V. Sustacek, F. Foret, P. Bocek, J. Chromatogr., **480**, 271 (1989).

32. F. Foret, S. Fanali, P. Bocek, J. Chromatogr., **516,** 219 (1990).

33. J. Sudor, J. Popichal, M. Deml, P. Bocek, J. Chromatogr., **545**, 331 (1991).

34. H.-T. Chang, E. S. Yeung, J. Chromatogr., **608**, 65 (1992).

35. A. T. Balchunas, M. J. Sepaniak, Anal. Chem., **60**, 617 (1988).

36. E. Gassmann, J. E. Kuo, R. N. Zare, Science, **230**, 813 (1985).

37. X. Huang, M. J. Gordon, R. N. Zare, Anal. Chem., **60**, 1837 (1988).

Received February 28, 1998
Accepted March 13, 1998
Manuscript 4756