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Unlimited-volume Electrokinetic Stacking Injection in Sweeping Capillary Electrophoresis Using a Cationic Surfactant

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Abstract

Sweeping is an effective and convenient way for online sample preconcentration in micellar electrokinetic chromatography (MEKC). The usual procedure includes a hydrodynamic injection step carried out by applying pressure to the sample vial followed by the subsequent sweeping and separation processes. The injected sample volume is limited by the dimensions of the capillary because a part of the capillary has to be left free of sample solution for the subsequent sweeping and separation steps. In addition, when a short capillary, such as 4–10 cm, is used for sweeping, the injected sample volume is small even if the entire capillary is filled with sample solution. In order to solve this problem, an electrokinetic stacking injection (EKSI) scheme was developed by using a cationic surfactant, dodecyltrimethylammonium bromide, for sweeping in capillary electrophoresis. An experimental model was proposed, and the entire process was theoretically analyzed. According to the theoretical discussion, the optimal conditions for two model analytes, 5-carboxyfluorescein (5-FAM) and sodium fluorescein (FL), were experimentally determined. The injected sample plug lengths for 5-FAM and FL under 20.1 kV for 60 min were experimentally estimated as 836 and 729 cm, corresponding to 28- and 24-fold the effective capillary length, respectively. The EKSI scheme resulted in increased detection factors for 5-FAM and FL of 4.5×10^3 and 4.0×10^3 using 60-minute injection relative to a traditional pressure injection.

As one of the most powerful separation techniques, capillary electrophoresis (CE) has been rapidly developed and extensively utilized in several major fields, such as pharmaceuticals^{1, 2} and environmental,³ as well as in forensic, clinical and food analysis.^{4–6} However, the detection sensitivity by optical methods is a drawback for the use of CE in trace analysis due to the small loaded sample volume and the short light path defined by the capillary diameter.⁷ The usual way to solve this problem is to do online sample preconcentration sample stacking or sweeping to introduce a large volume of sample solution into the capillary.^{8, 9}

Sweeping is an effective and convenient way for doing online sample preconcentration in micellar electrokinetic chromatography (MEKC).^{9, 10} The concept is based on the accumulation and isolation of analytes, injected in a large sample volume, by micelles so as to concentrate the analytes into a narrow zone and enhance the detection sensitivity.¹¹ The basic condition for sweeping is that the separation buffer contains a surfactant at a concentration above its critical micelle concentration (CMC), while the sample solution is free of the surfactant.⁹ The micelles in the separation buffer move through the large injected volume and essentially “sweep” the analyte distributed in the injected volume into a narrow zone. The final magnitude of concentration enhancement is determined by the analyte’s retention factor, which is the ratio of the number of the analyte molecules distributed in the micellar phase to those in

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the aqueous phase.¹² The most commonly used sweeping carriers are micelles of sodium dodecyl sulfate (SDS)⁹ which is a good choice for sweeping neutral and cationic analytes. Other kinds of surfactants used for sweeping include cationic,¹³ nonionic¹⁴ and polymeric,¹⁵ as well as mixed micelles.¹⁶ In general, ionic micelles promote sweeping of analytes with opposite charge. For instance, cationic surfactants, such as dodecyltrimethylammonium bromide (DTAB) and tetradecyltrimethylammonium bromide, may be selected for sweeping anionic analytes.¹³

The usual procedure for sweeping includes a hydrodynamic injection step carried out by applying pressure to the sample vial followed by the subsequent sweeping and separation processes. The injected sample volume is limited by the dimensions of the capillary. Moreover, a part of the capillary prior to the detector must be left free of sample solution for the subsequent sweeping and separation processes. Longer capillaries allow larger volumes of sample solution to be injected so that detection enhancement is improved. However, with short capillaries, e.g. 4-10 cm, the injected sample volume would be small even if the entire capillary were filled with sample solution. To overcome this sweeping barrier, cation- and anion-selective exhaustive injection and sweeping (C/ASEI-sweeping) has been developed, which can produce a million-fold detection enhancement for some analytes.^{17, 18} However, the C/ASEI-sweeping scheme requires several initial liquid plugs in the capillary before sample injection and needs polarity changes during stacking and sweeping, which makes the process complicated. The exhaustive nature of C/ASEI-sweeping introduces significant sample bias such that a given sample can only be injected once or twice from the same sample solution. Palmer et al.¹⁹ developed a relatively simple large-volume injection process for neutral analytes by electrokinetic stacking injection (EKSI) for sweeping with sodium dodecylsulfate (SDS), which has the capability of injecting 7-fold the effective capillary length of sample solution. However, their scheme was based on decreasing the velocity of the analyte/micelle complex without substantially reducing the EOF, which essentially causes longer migration times for the analytes.

Electrokinetic injection in CE is based on electroosmotic pumping, referred to as electroosmotic flow (EOF), as well as the electrophoretic velocities of analytes. Under normal polarity conditions, the injected analytes are biased, i.e., the cationic analytes are favorably introduced into the capillary because their electrophoretic velocities are in the same direction as the EOF, while anionic analytes are rejected completely or partially, depending on the relative magnitude of the EOF and their individual electrophoretic velocities. Neutral analytes are carried into the capillary by the EOF and are distributed throughout the volume of the injected sample solution. In sweeping CE using SDS under normal polarity conditions, the micelles migrating against the EOF sweep the analytes into a narrow zone which forms the basis of EKSI for neutral analytes.¹⁹ When cationic surfactants are used for sweeping, the EOF is reversed relative to the bare silica due to the dynamic coating of the capillary walls by the positively charged surfactants. However, the cationic micelles also migrate against EOF and so EKSI also can be conducted by using cationic surfactants as sweeping carriers.

Here we developed a new EKSI scheme to preconcentrate anionic analytes by sweeping with cationic surfactants in a dynamically-coated capillary. The bulk flow is controlled by the dynamic coating of cationic surfactants on the capillary walls, which can keep the surfactant penetrating front at a steady state with the EOF for essentially an unlimited time. Consequently, the injection flow is still continuing while the length of the sample plug in the capillary is relatively constant, and the remaining part of the capillary is left for the subsequent sweeping and separation.

EXPERIMENTAL SECTION

Apparatus

All electrophoresis experiments were performed with a P/ACE MDQ instrument from Beckman Coulter, Inc. (Fullerton, CA, USA). Fused silica capillaries with 50 μm i.d. and 362 μm o.d. were obtained from Polymicro Technologies (Phoenix, AZ, USA). The total length of the capillary was 40.2 cm and the effective length was 30.0 cm from the inlet to the detection point. Laser-induced fluorescence detection (LIF) was used with an excitation wavelength of 488 nm and an emission wavelength of 520 nm. The capillary was thermostated at 25 °C. Buffer conductivities were measured with an ExStik EC400 Conductivity/TDS/Salinity meter manufactured by Extech Instruments (Waltham, MA, USA).

Chemicals and Reagents

DTAB, 5-carboxyfluorescein (5-FAM), 0.1 M NaOH and 1.0 M NaOH were purchased from Sigma-Aldrich, Inc. (St. Louis, MO, USA). Fluorescein disodium salt (FL) was from ICN Biomedicals, Inc. (Aurora, OH, USA). BODIPY 505/515 (4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3 α ,4 α -diazas-indacene) was obtained from Molecular Probes (Eugene, OR, USA). Monobasic and dibasic sodium phosphate was obtained from Fisher Scientific (Fair Lawn, NJ, USA). Distilled-deionized water (dd-water) was prepared with a Milli-Q system (Millipore, Bedford, MA, USA). All chemicals and reagents were used without further purification. Stock solutions of DTAB (200 mM) and mono and dibasic phosphate solutions (200 mM) were prepared in the dd-water. A pH 8.0 phosphate stock buffer solution (200 mM) was prepared by mixing the 200 mM monobasic and 200 mM dibasic phosphate solutions in a volume ratio of 1/9 (v/v). A stock solution of BODIPY 505/515 (2.5 mM) was prepared in methanol, while stock solutions of FL (1.0 mM) and 5-FAM (0.44 mM) were prepared in methanol/water (30:70; v/v). The final sample solutions of FL and 5-FAM were prepared by a series of dilutions with dd-water and phosphate buffer (pH 8.0) with the conductivity adjusted to be similar to the selected separation buffer. Separation buffers usually consisted of 20.0 mM DTAB, 15% (v/v) acetonitrile and 40.0 mM phosphate (pH 8.0); however, for certain experiments, the molarity of the phosphate buffer and the percentage of acetonitrile in the separation buffer were varied as indicated in the appropriate portions of the text. The separation buffer was degassed and filtered through 0.45 μm syringe filters purchased from Gelman Laboratory, Pall Corp. (Ann Arbor, MI, USA).

Procedure

New capillaries were conditioned sequentially with 1.0 M sodium hydroxide, dd-water and separation buffer for 10 min each at 40 psi. At the beginning of each analysis day, the capillary was washed sequentially with 1.0 M NaOH, water and separation buffer for 5 min each at 40 psi. Before a run, the capillary was flushed at 40 psi sequentially with 1.0 M sodium hydroxide (2 min), water (2 min) and separation buffer (3 min).

Sample injection was performed by applying 20.1 kV voltage at the reversed polarity across the capillary through the sample vial and the separation buffer vial for a period of time. The separation voltage was set at 20.1 kV at the reversed polarity giving an electric field strength of 500 V/cm in the continuous buffer system. When the separation buffer involved DTAB, EOF mobilities were determined with the current monitoring method²⁰ by using a lower concentration of phosphate in the replacing buffer. Otherwise, BODIPY 505/515 was used as a neutral marker for the determination of EOF mobilities. The injection length under a pressure was determined by a triple-injection method, in which a separation buffer plug was introduced into the capillary between two short dye plugs.²¹

RESULTS AND DISCUSSION

Preconcentration Model with the Unlimited-volume EKSI

EKSI is carried out by inserting one end of the capillary, which is already precoated with separation buffer containing cationic surfactants, into a sample solution free of surfactant. A reversed-polarity voltage is then applied across the capillary through the sample and the separation buffer vials (Figure 1a). Due to the dynamic coating process by the cationic surfactant, the capillary walls have positive charges that cause a reversal of the EOF, relative to bare glass, resulting in a bulk flow toward the anode. Analytes along with the sample buffer are electrokinetically pumped into the capillary while the positively charged micelles migrate toward the cathode. However, when the sample buffer flows into the capillary, the capillary wall contacts buffer free of surfactant. Consequently, the sorbed surfactants desorb from the capillary wall and diffuse into the bulk sample solution along the sample front that is moving into the capillary, leading to a decrease in the local EOF. Thus, the bulk flow in the entire capillary column slows down and the bulk fluid velocity eventually reaches the same magnitude as that of the oppositely migrating cationic micelles. Consequently, the incoming micelle front zone stays in the steady state with an apparent velocity of zero (Figure 1b). This steady state can be maintained for an extended period of time so that an essentially unlimited volume of sample solution can be injected into and flow through the capillary. After an appropriate time duration, the sample vial is exchanged with the separation buffer vial (Figure 1c) and the same voltage is applied between the two separation vials leading to introduction of the separation buffer into the capillary from the cathode side. The cationic surfactants in the injected separation buffer recoat the capillary previously stripped of surfactants, resulting in an increase of the local EOF and accordingly an increase of the bulk fluid flow in the capillary. The bulk fluid flow destroys the steady state of the micelle front and pumps the micelle boundary toward the detector (Figure 1d). For the negatively charged analytes, the completed concentration zone is in the middle of the sample buffer plug due to their electrophoretic velocity towards the detector (Figure 1d) while for neutral analytes, sweeping is completed at the end of the sample buffer plug as shown in Figure 1e. Finally, the analyte zone enters the separation buffer containing organic additives, and the analytes are separated and detected (Figure 1f).

Theoretical consideration

After a sample buffer plug is introduced into the capillary, the bulk velocity (V_b) is the sum of the local EOF in the sample buffer (V_{eo2}) and the local EOF in the separation buffer (V_{eo1}) zones and these are weighted by the ratio of the sample plug to the total capillary length as in Equation 1.²²

$$V_b = xV_{eo2} + (1 - x)V_{eo1} \quad (1)$$

where $x = \frac{l_{SB}}{L}$ is the ratio of the sample buffer plug length (l_{SB}) relative to the total capillary length (L), as shown in Figure 1a. The micelles are positively charged, so they have cathodic electrophoretic velocities ($V_{ep,m}$) under the applied voltage. The effective velocity ($V_{eff,m}$) of micelles can be determined by Equation 2.

$$V_{eff,m} = V_b - V_{ep,m} \quad (2)$$

For convenience, we suppose that migration in the direction to the detector is positive. If $V_{eff,m} > 0$, the sample buffer plug length (l_{SB}) increases with time, i.e. the boundary plane of micelles is moving to the detector. When $V_{eff,m} = 0$, the boundary plane of micelles is at a steady state although the bulk flow is still going on, and the sample buffer plug length reaches its maximum length ($l_{SB,max}$). By substituting V_b from Equation 1 into Equation 2, the maximum ratio (x_{max}) for the sample buffer front divided by surfactant front is determined by Equation 3.

$$x_{\max} = \frac{V_{eo1} - V_{ep,m}}{V_{eo1} - V_{eo2}} \quad (3)$$

The magnitude of V_{eo1} depends on the electroosmotic mobility (μ_{eo1}) and the local electric field strength (E_1). Similarly, the magnitude of $V_{ep,m}$ is determined by $\mu_{ep,m}$ and E_1 . However, the magnitude and direction of V_{eo2} depends on the surface conditions of the capillary wall and the composition of the buffer in contact with the capillary wall. The desorption process of surfactants is time-dependent. With the sample injection going on, V_{eo2} would be decreasing and possibly, even reversed to be negative, resulting in a decrease of the bulk velocity V_b . This situation could make $V_{eff,m}$ less than zero, resulting in a decrease in $l_{SB,max}$ and correspondingly an increase of the capillary length occupied by the separation buffer ($L - l_{SB}$). As the separation buffer occupies portions of the capillary previously stripped of surfactants by the sample buffer, the surfactants in the separation buffer will then dynamically repair the capillary walls coated with less surfactants. This situation results in a decrease in x and correspondingly an increase in the bulk velocity V_b , so as to keep $V_{eff,m} = 0$ again. This process becomes a self-adjusting mechanism, which theoretically can permit unlimited injection volume of sample solution. As can be seen in Figure 2a, analyte was not detected during the 60-min ESKI period and only was detected after the 60-min ESKI period once the sweeping and separation voltages were applied.

However, there are two potentially limiting factors for the large-volume sweeping. First, there is the potential for a pressure-driven effect due to the velocity mismatch of the two buffer plugs involved. The pressure-driven effect could produce a parabolic flow profile causing peak broadening. However, this effect was apparently negligible for the model analytes with DTAB sweeping since as shown in Figure 2b the FL and 5-FAM peaks were still very sharp even after a 60-min ESKI period. As pointed out by Ramsey et al.,²³ ionic transport number mismatch during sweeping may play a role for the final stacked peaks. The other potential issue is the limited retention factor (k) of analytes in the micellar phase since the swept zone is controlled by Equation 4,²⁴

$$l_{sweep} = \left(\frac{1}{1+k} \right) l_{inj} \quad (4)$$

where l_{sweep} and l_{inj} are the lengths of the swept zone and the injected sample solution, respectively. When the interaction between the analyte and micelles is too weak, the analyte may 'leak' from the concentrated zone and flow through the detection window during the ESKI process. In practical applications, the ESKI duration needs to be optimized through experiments to determine the optimal values according to the analyte retention factor.

The goal of sample preconcentration is to obtain higher detection sensitivity while maintaining resolution during the separation. If the swept zone in the capillary during the injection passes as far as the detection window, then there would be no ability to separate the sample components. Moreover, to separate the concentrated analytes from one another after sweeping, a sufficient length of the capillary from the detection point to the sample front needs to be left free of analytes for subsequent sweeping and separation in the separation buffer. Generally, the resolution of the analyte peaks is poor if there is no organic additive added in the separation buffer for MEKC,^{21, 25, 26} while the sample buffer is usually free of the organic additive for the sweeping step. Therefore, the analyte zone concentrated by sweeping needs to enter the separation buffer zone containing the organic additive to improve the resolution. After the injection process is completed, the time (t_s) required for the micelles to sweep across the sample plug length l_{SB} is determined by Equation 5.

$$t_s = \frac{l_{SB}}{V_{ep,m}} \quad (5)$$

On the other hand, the micelles are moving at an apparent velocity ($V'_{eff,m}$) determined by Equation 6.

$$V'_{eff,m} = V'_b - V_{ep,m} \quad (6)$$

where V'_b is the bulk velocity during the final sweeping process after the injection. Then, the analyte migration time is determined by Equation 7.

$$t_m = \frac{l - l_{SB}}{V'_{eff,m}} \quad (7)$$

Before the sample zone reaches the detection point, the concentrated analyte zone needs to arrive at the rear boundary of the sample plug or already enter the separation buffer zone containing the organic additive. Therefore, the sweeping time t_s and the migration time t_m need to satisfy the following condition,

$$t_m \geq t_s \quad (8)$$

By substituting Equations 5-7 into Equation 8, l_{SB} needs to satisfy the following condition,

$$l_{SB} \leq \frac{lV_{ep,m}}{V'_b} \quad (9)$$

For a long injection time, the maximum of sample solution length may have been reached, and then l_{SB} in Equation 9 is replaced by $l_{SB,max}$, which is equal to Lx_{max} .

$$x_{max} \leq \beta = \frac{lV_{ep,m}}{LV'_b} \quad (10)$$

where x_{max} is determined by Equation 3, and β is the critical ratio of the sample plug length to the entire capillary length. That is to say, the sample plug length cannot exceed βL , otherwise the concentrated sample zone has not come out of the sample buffer zone when it passes through the detection point and hence analyte resolution may not be obtained. Because V'_b must be larger than $V_{ep,m}$ so as to make sure the sample zone finally passes through the detection point, the magnitude of β must be less than l/L .

To obtain the best sweeping result and good resolution, our goal is to make x_{max} (above zero) as small as possible and/or β as large as possible. There are several ways to realize this objective. First, the ratio l/L could be increased. The P/ACE MDQ instrument uses standard capillary cartridges with a constant length of 10.0 cm from the detection point to the outlet. Therefore, by using a longer capillary, the ratio l/L could be increased to some extent. If the detection point can be freely moved, such as in microchip CE, the l/L ratio can reach the maximum value '1'. Second, one could decrease the bulk flow velocity. The bulk flow velocity is determined (Equation 11) by the individual local EOFs weighted by the plug length ratio in a fashion similar to Equation 1.

$$V'_b = x_{max} V'_{eo2} + (1 - x_{max}) V'_{eo1} \quad (11)$$

This relationship is maintained until part or the entire buffer plug devoid of surfactants has come out of the capillary along with the bulk flow. For simplicity, we suppose the adsorption process is very fast, so that with the separation buffer replacing the sample buffer from the inlet side, the part of the capillary containing separation buffer has constant average electroosmotic mobility and the corresponding velocity as V'_{eo1} , whose magnitude is close to that of V_{eo1} . On the other hand, when the buffer devoid of surfactants replaces the separation buffer containing surfactants, the desorption process is generally much slower than the adsorption process,²⁷ so that V'_{eo2} is larger than V_{eo2} , which has experienced a possibly long

injection time. The total result is that V'_b can reach a much larger magnitude when the analyte zone reaches the detection window than that of V_b at the steady state of the sample injection process where $V_{eff,m} = 0$, i.e. $V_b = V_{ep,m}$. The most effective way to decrease V'_b is to decrease V'_{eo1} , or V_{eo1} . This could be done by decreasing the electric field strength, but this would cause a similar decrease in $V_{ep,m}$. An alternative way of decreasing V'_b is to use a low pH buffer which decreases the number of ionized silanols on the capillary wall and accordingly decreases the adsorption of the cationic surfactants leading to a decrease in the EOF. Lucy et al.²⁸ have investigated the characteristics of cationic surfactant induced reversal of EOF and found that EOF velocity is independent of pH above a pH of 4 when the cationic surfactant concentration is above the CMC.

A third approach is by increasing $V_{ep,m}$. This method seems promising, since it can increase β and simultaneously decrease x_{max} , while keeping EOF relatively stable. Increasing the ionic strength of the buffer usually produces a complicated effect in regard to the relative velocities and the separation, which was experimentally studied below.

Effect of Ionic Strength

An increase of ionic strength in capillary zone electrophoresis (CZE) usually causes a decrease in EOF and a variable decrease in the electrophoretic mobility of analytes,²⁹ which accordingly leads to the potential for modulating the separation selectivity and resolution by varying the buffer concentration. On the other hand, the increase of ionic strength also produces higher current and joule heating, which is deleterious to CE separation and therefore ionic strength increases must be balanced against joule heating.

The electrophoretic mobilities in several buffers of differing ionic strength were determined by using the current monitoring method and are summarized in Table 1. As can be seen, the magnitude of the EOF mobility is essentially constant between the buffer concentrations from 5 to 60 mM. The electrophoretic mobilities of FL in the different buffer systems also remained relatively constant, while those for 5-FAM decreased from $1.17 \times 10^{-4} \text{ cm}^2/\text{Vs}$ in 5.0 mM phosphate buffer to $4.38 \times 10^{-5} \text{ cm}^2/\text{Vs}$ in 60.0 mM phosphate. It has been previously reported²⁹ that ions of higher charge experience greater ionic strength effects and consequently the difference of the electrophoretic mobilities between 5-FAM (-3)³⁰ and FL (-2)³¹ increased with the increase of ionic strength leading to improved resolution as shown in Figure 3a in a non-sweeping mode. These separation buffers also were tested in sweeping experiments under the EKSI condition of 20.1 kV for 20 min, and the electropherograms obtained demonstrated improved resolution at higher buffer concentration except for 20.0 mM phosphate buffer where no resolution between the 5-FAM and FL peaks was obtained (Figure 3b). Considering this higher current effect at higher buffer concentration, 40.0 mM phosphate (pH 8.0) was selected to do the remaining sweeping experiments.

Conductivity Ratio Effect

The ratio of conductivities between sample buffer and separation buffer affects the distribution of electric field strength across the capillary and correspondingly influences the sweeping results.^{32, 33} We used a fixed separation buffer (40.0 mM phosphate + 20.0 mM DTAB + 15% ACN) and various concentrations of sample buffer from 35-55 mM phosphate (pH 8.0) to study the sweeping results. As shown in Figure 4, the sample buffers with lower ionic strength produced higher swept peak height. The low-conductivity sample buffer may have triple effects: more injected analyte, stacking after injections, and faster migration due to the increased apparent velocity of analyte. Therefore, peak broadening was reduced and peak height increased. Thus, the large-volume injection scheme was valid at different ionic strengths

of the sample buffer, but for the convenience of discussion, the sample (in 45 mM phosphate, pH 8.0) and separation buffers with similar conductivities were used for the other experiments.

Effect of Acetonitrile

Organic additives in separation buffers can increase buffer viscosity, accordingly causing a decrease in the electrophoretic mobilities of analytes and improving selectivity and resolution.²⁶ In addition, the existence of organic additives also can modify k values by shifting the ratio of analyte in the micelles versus the separation buffer.³⁴ By using current monitoring, the EOF mobilities for buffers with various volumes of acetonitrile were determined and are summarized in Table 2. As can be seen, EOF mobilities changed little with increases in acetonitrile volume in the separation buffers. The electrophoretic mobilities of both 5-FAM and FL decreased but the electrophoretic mobility difference of 5-FAM and FL increased with the increases in acetonitrile volume up to 15% (v/v) in the separation buffer. A dramatic decrease in both the electrophoretic mobilities of the two analytes and the mobility difference was observed with 20% acetonitrile in the separation buffer. The electropherograms obtained under non-sweeping CE conditions shown in Figure 5a confirmed the mobility changes. The mobility changes are attributed to the presence of acetonitrile which might weaken the interaction between FL/5-FAM and DTAB micelles. These buffers were also used for the sweeping experiments with electropherograms shown in Figure 5b. For the sweeping experiments, 5% acetonitrile in the separation buffer only produced one sharp peak without separations, while 10% acetonitrile produced a separation but the 5-FAM peak was broadened and 20% produced poor resolution of the analytes. Only 15% acetonitrile produced completely resolved peaks with excellent resolution. Therefore, 15% acetonitrile was chosen as the organic additive for the separation buffer.

Effect of High Voltage on Sample Solution

Hydrodynamic injection by pressure or vacuum produces an injection without analyte bias, while electrokinetic injection has bias for charged analytes. It favors analytes with high electrophoretic mobilities in the same direction of EOF. Only neutral analytes are introduced into the capillary without discrimination. In the previously reported cation- or anion- selective exhaustive stacking injection and sweeping approaches,^{17, 18} analyte depletion occurred with successive injections from the sample solution, so that fresh samples are required for each injection. For the scheme discussed here, sample consumption is not a serious issue since the continuous buffer system is used although injection bias still exists. However, the high voltage and the long voltage application time could lead to depletion of the sample solution. Successive injections from the same sample (0.4 mL) vials were conducted to study the change in the sample solutions. When 20.1 kV for 20 min was applied for the EKSI, the peaks were distorted after the fourth injection as shown in Figure 6. Similarly, peak distortion after the third injection was noted when 20.1 kV for 30 min was used, and the second peak was distorted when 20 kV for 60 min was tried. To further study this effect, various sample volumes were used for the successive injections. When the sample volumes of 0.6 and 1.2 mL were used to conduct successive injections under 20.1 kV for 30 min each, the 5th and the 7th injections, respectively, led to distorted peaks. When 1.6 mL sample volume was used, the peak shape still looked good after the 8th injection. This peak distortion is attributed to sample buffer depletion due to the electrolysis of water which produced OH^- and altered the pH of the sample solutions. The original pH of the sample buffer was 8.0. For a sample solution with a volume of 0.4 mL, the pH was measured as 8.9, 10.0 and 10.7 after 20-, 60- and 100-min cumulative injections by EKSI, respectively. Therefore, a large volume of sample needs to be prepared when the high voltage is applied on the sample solution for a relatively long period of time.

Estimation of Injected Sample Volume

The effective sample volume injected into the capillary by the Eksi for sweeping is directly related to the apparent velocity (V_a) of the analyte and the time duration (t). Since the diameter of the capillary is fixed, the injected amount of an analyte can be quantified by the comparatively occupied capillary length (l_{inj}). The electrophoretic mobility of anions is in the same direction as the bulk flow, therefore it can be determined by Equation 12,

$$V_a = V_{ep} + V_b \quad (12)$$

where V_{ep} is the electrophoretic velocity of the analyte. At the very beginning of the injection process, the bulk flow is at the maximum V_{eo1} , which is produced when the entire capillary is filled with the separation buffer. With the sample solution entering the capillary, the bulk flow is decreasing as expressed in Equation 1. Finally, the micelle boundary reaches the steady state where the effective velocity of micelles is zero, i.e. the bulk flow is equal to the electrophoretic velocity of micelles $V_{ep,m}$, and the time duration required is t_0 . The average analyte velocity (V_a^{ave}) during a time duration of t_0 can be estimated as shown in Equation 13a and the apparent velocity of an analyte can be expressed in Equation 13b.

$$V_a^{ave} = \frac{1}{2} \left[(V_{eo1} + V_{ep}) + (V_{ep,m} + V_{ep}) \right] \quad (13a)$$

$$V_a^{steady} = V_{ep,m} + V_{ep} \quad (13b)$$

The injected length is determined by the following equation,

$$l_{inj} = V_a^{ave} t_0 + V_a^{steady} (t - t_0) \quad (14)$$

By substituting Equations 13a and 13b into Equation 14, the injected sample solution length can be determined by Equation 15.

$$l_{inj} = \frac{1}{2} (V_{eo1} - V_{ep,m}) t_0 + (V_{ep,m} + V_{ep}) t \quad (15)$$

For a long injection time, the first part of the right side of Equation 15 is relatively small and negligible. Therefore the effective length of the injected sample plug length can be estimated by the following equation,

$$l_{inj} = (V_{ep,m} + V_{ep}) t \quad (16)$$

Under the experimental conditions, $V_{ep,m}$ ($\mu_{ep,m} = 2.0 \times 10^{-4} \text{ cm}^2/\text{Vs}$) was estimated by the velocity of FL and 5-FAM determined in the separation buffer (45.0 mM phosphate + 20.0 mM DTAB) free of acetonitrile. V_{ep} ($\mu_{ep}^{FL} = 2.74 \times 10^{-4} \text{ cm}^2/\text{Vs}$, $\mu_{ep}^{FAM} = 3.66 \times 10^{-4} \text{ cm}^2/\text{Vs}$) was determined in the sample buffer (45.0 mM phosphate) with BODIPY 505/515 as the neutral marker at the normal voltage polarity. The estimated sample solution lengths for neutral analytes, FL and 5-FAM can be determined with Equations 17a-c, respectively.

$$l_{inj}^{neutral} = 6.0t \quad (17a)$$

$$l_{inj}^{FL} = 14.2t \quad (17b)$$

$$l_{inj}^{FAM} = 17.0t \quad (17c)$$

where the unit of time t is minute. For 60 min Eksi, the sample buffer entering the capillary is approximately 360 cm, equivalent to 12-fold the effective capillary length (30.0 cm), and the lengths of the comparative 5-FAM and FL solution plugs are approximately 1020 cm and

852 cm, corresponding to 34- and 28-fold the effective length of the capillary, respectively. These injection lengths can also be estimated by their peak area enhancements. The normal injection of 0.2 psi for 50.0 s in CE produced 1.12 cm length of sample solution determined by the multiple injection method. The peak area enhancements of FL and 5-FAM under 20.1 kV for 60 min of the EKSI relative to those obtained under the pressure injection of 0.2 psi for 50.0 s are 746- and 651-fold, respectively. Therefore, the injected lengths of 5-FAM and FL are calculated as 836 and 729 cm, respectively, corresponding to 28- and 24- fold the effective capillary length. There is a difference between the theoretical and the experimental estimated results for either 5-FAM or FL. On one hand, the values of EOF and the electrophoretic mobilities used for the theoretical estimation are approximated from the comparative conditions to the real ones under which the EKSI is performed, and the electric field strength is considered as uniform across the entire capillary; however, the true conditions cannot be directly determined. On the other hand, this EKSI process has a recovery ratio of less than '1'. Thus, the actual injection length is above the experimental result.

DETECTION ENHANCEMENT

The detection enhancement was tested by using a 60-min EKSI for 250 pM 5-FAM and 250 pM FL relative to the normal pressure injection for 250 nM 5-FAM and 250 nM FL at 0.2 psi for 5 s. The enhancement factor was calculated by simply using the peak height ratio multiplied by the dilution factor. Signal enhancements of 4.5×10^3 and 4.0×10^3 -fold were achieved for 5-FAM and FL, respectively. The limit of detection (LOD) for FL under EKSI of 20.1 kV for 60-min was found to be 75 fM, which experimentally produced a signal-to-noise ratio of 3. The LOD for FL under the standard injection protocol (0.2 psi for 5 s) was found to be 300 pM.

CONCLUSIONS

Sweeping using cationic surfactants is promising for the on-line preconcentration of negatively charged analytes CE. The dynamic coating process of cationic surfactants on the negatively charged capillary wall can be employed to control the bulk flow velocity. The desorption of dynamic cationic coating of the capillary decreases the bulk flow velocity to the magnitude of the electrophoretic velocity of micelles under the applied voltage, leading to a large-volume injection by EKSI; on the other hand, the timely recoating of the bare capillary walls when contacted with the surfactant containing separation buffer speeds up the bulk flow so that the concentrated analyte zones are separated and detected rapidly. The ionic strength of the separation buffer and the acetonitrile concentration can be adjusted to improve selectivity and resolution. Care must be taken to adjust sample volumes to avoid depletion of the sample by the extended application of the high voltage.

The EKSI process with DTAB micelles is straightforward and easy to perform relative to the C/ASEI-sweeping scheme. The injection time can be easily adjusted depending on the practical demand of sensitivity enhancement. The running conditions, such as ionic strength and organic additives in the buffer, are easy to optimize. The EKSI scheme described here may provide prospects for sample preconcentration in miniaturized CE systems.

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Figure 1.

Evolution of the EKSI and sweeping. The dense-dot part represents sample solution in phosphate buffer. The blank part refers to the separation buffer containing DTAB, phosphate and acetonitrile. The sparse-dot part denotes the phosphate sample buffer. l and L are the effective and total capillary lengths, respectively. l_{SB} and $l_{SB, max}$ are the sample buffer plug length and its maximum, respectively. V_{eo1} (V'_{eo1}) and V_{eo2} (V'_{eo2}) are the local EOF velocities, respectively.

Figure 2.

Electropherogram monitoring the EKSI, sweeping and separation. Injection was performed under 20.1 kV for 60 min followed by the subsequent sweeping and separation. The time for vial exchange from the sample vial to the separation buffer is about 8.0 s. The separation buffer consisted of 40.0 mM phosphate, 20.0 mM DTAB and 15% acetonitrile; the sample buffer contained 45.0 mM phosphate with the similar conductivity to that of the separation buffer. Peaks 1 and 2 are 5-FAM (0.25 nM) and FL (0.25 nM), respectively. b) is the scale-up of a).

Figure 3.

Ionic strength effect on the resolution and migration of 5-FAM and FL. The separation buffers consisted of 20.0 mM DTAB, 15% acetonitrile and phosphate buffers of various concentrations. Peaks 1 and 2 are 5-FAM and FL, respectively. a) with the normal injection under a pressure of 0.4 psi for 5 s; the sample solution contained 100 nM 5-FAM and 100 nM FL dissolved in the separation buffer. b) with the EESI under 20.1 kV for 20 min; sample solution contained 1.0 nM 5-FAM and 1.0 nM FL dissolved in phosphate buffer with the same conductivity as that of the separation buffer.

Figure 4.

Conductivity ratio effect on swept peaks. The separation buffer consisted of 20.0 mM DTAB, 40.0 mM phosphate and 15% acetonitrile. Sample buffers were as shown in the figure. Peaks 1 and 2 are 5-FAM (1.0 nM) and FL (1.0 nM), respectively. Injection was performed at 20.1 kV for 20 min.

Figure 5.

Acetonitrile effect on the resolution and migration of 5-FAM and FL. The separation buffers consisted of 40.0 mM phosphate, 20.0 DTAB and various volumes of acetonitrile. Peaks 1 and 2 are 5-FAM and FL, respectively. a) with the normal injection under a pressure of 0.4 psi for 5 s; the sample solution contained 100 nM 5-FAM and 100 nM FL dissolved in the separation buffer. b) with the EESI under 20.1 kV for 20 min; the sample solution contained 1.0 nM 5-FAM and 1.0 nM FL dissolved in phosphate buffer with the same conductivity as that of the separation buffer.

Figure 6.

Voltage effect on the sample solution. Conditions were as in Figure 2a. Injection was performed under 20.1 kV for 20 min for several successive runs. Peaks a and b are 1.0 nM 5-FAM and 1.0 nM FL respectively.

Table 1

Mobility changes with buffer ionic strength

Phosphate (mM), ^a	Mobilities, 10 ⁻⁴ , cm ² /Vs			
	EOF ^b	5-FAM	FL	Difference ^c
5.0	4.01	-1.17	-1.33	0.16
20.0	3.86	-1.12	-1.48	0.36
30.0	3.84	-0.93	-1.43	0.51
40.0	3.91	-0.82	-1.41	0.59
60.0	3.96	-0.44	-1.26	0.82

^a Buffer consisted of phosphate, 20.0 mM DTAB and 15% acetonitrile, pH 8.0.^b Determined by using current monitoring method.^c Electrophoretic mobility difference of 5-FAM and FL.

Table 2

Mobility changes with acetonitrile volume

Acetonitrile (v/v), ^a	Mobilities, 10 ⁻⁴ , cm ² /Vs			
	EOF ^b	5-FAM	FL	Difference ^c
0	3.90	-1.95	-2.02	0.07
5%	4.01	-1.89	-2.09	0.11
10%	4.02	-1.76	-2.00	0.24
15%	3.93	-0.80	-1.41	0.61
20%	3.89	-0.07	-0.02	0.05

^a Buffer consisted of 40.0 mM phosphate, 20.0 mM DTAB and acetonitrile, pH 8.0.

^b Determined by using current monitoring method.

^c Electrophoretic mobility difference between 5-FAM and FL.