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Approaching a Million-Fold Sensitivity Increase in Capillary Electrophoresis with Direct Ultraviolet Detection: Cation-Selective Exhaustive Injection and Sweeping

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A novel method that combines two on-line concentration techniques in capillary electrophoresis (CE), namely, sample stacking with electrokinetic injection (field-enhanced sample injection, FESI) and sweeping, afforded the detection of positively chargeable analytes in parts per trillion (ppt) levels. The main idea is to selectively introduce by FESI as many molecules of cationic analytes as possible from a very dilute sample solution and focus the resulting zone by sweeping. Limit of detection values (signal-to-noise ratio 3) of 4.1 and 8.0 ppt—the lowest concentration reported by direct UV detection in CE—with average plate numbers of 3.6×10^5 and 4.4×10^5 are obtained for laudanosine and naphthylamine (standard solutions), respectively. This translates to improvements in peak heights compared with usual injection approaching a million-fold. Optimization schemes and application to quantitative and qualitative analyses are also investigated.

Diverse organic cations are vital in industry and medicine, for example, aromatic amines, peptides, and alkaloids. For the analysis of such samples, high-performance liquid chromatography (HPLC) is the most popular method. Capillary electrophoresis (CE), a family of high-resolution separation techniques, can be advantageous over HPLC in terms of simplicity, resolution, and economy. However, CE suffers from low concentration sensitivity as a consequence of the limited sample volume and short path length for absorbance-related detection. Electrochemical, fluorometric, and conductometric detectors can provide better sensitivity but are not universal nor easily affordable compared to the more popular UV detector.[1,2] Mass spectrometry (MS), although universal and mass sensitive, is expensive, and more studies are needed in coupling MS to CE.[3] In addition, off-line preconcentration methods that include liquid−liquid extraction and on-line solid-phase preconcentration have provided significant improvement in concentration detection limits.[4,5]

Among the various modes of CE, capillary zone electrophoresis (CZE)[6] and micellar electrokinetic chromatography (MEKC)[7] are the more prevalent ones. Charged analytes are easily separated by CZE on the basis of differences in mobility or velocity under an applied electric field. Neutral analytes, which cannot be separated by CZE, are easily separated by MEKC on the basis of differences in analyte affinities between the micellar and aqueous phases. Separation selectivity of charged analytes is also usually enhanced by MEKC.[8,9]

Considerable interest to improve concentration sensitivity in CE is the development of on-line concentration techniques. These include isotachophoresis (ITP), sample stacking, and more recently sweeping. From around 10- to 5000-fold increases in detection sensitivity have been documented.[10−15] ITP preconcentration is performed by the proper choice of leading and terminating background electrolytes. This is usually followed by separation using the CZE mode. Specific discussions regarding ITP preconcentration are given in the works of some research groups.[10,11] Sample stacking is performed by preparing the sample in a matrix having a resistance considerably higher than that of the background solution.[12] Sample stacking can be performed in both the CZE and electrokinetic chromatography (EKC) modes of CE.[12,13] Also, hydrodynamic or electrokinetic injection modes can be utilized. The concentrating effect basically relies on the change in electrophoretic velocities when the analyte molecules reach the interface between the high-resistance sample solution or water zone and low-resistance background solution zone. The higher the difference in resistance or conductance, the greater the concentrating effect. The concentrating effect is directly propor-

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tional to the enhancement factor (γ) or ratio between the conductivity of the background and sample or water zones.

Sweeping in EKC was initially described by preparing the sample in a matrix having a resistance similar to that of the background solution. Also, the matrix is void of the pseudostationary phase used. The concentration effect relies on the pseudostationary phases that enter the sample solution zone. The pseudostationary phases entrap and accumulate the analytes as they pass the sample solution zone. The higher the affinity of the analyte toward the pseudostationary phase, the greater the concentrating effect.[14,15] The cumulative effect of sweeping and sample stacking was also shown to narrow the lengths of analyte zones prepared in a low-conductivity aqueous matrix void of the pseudostationary phase.[16] Furthermore, the effect of a high-conductivity sample matrix on sweeping was also investigated.[17,18]

In this paper, we show more than 5 orders of magnitude increase in concentration detection sensitivity or 10^2- to almost 10^6-fold increases in UV detector response for positively chargeable analytes in CE. This was accomplished by the proper combination of sample stacking with electrokinetic injection (field-enhanced sample injection, FESI)[12] and sweeping,[14] which we name as cation-selective exhaustive injection and sweeping (CSEI-sweep). MEKC was utilized for the separation; thus, the total procedure is termed as CSEI-sweep-MEKC. FESI and sweeping had been shown to provide 1000-fold and 5000-fold increases in UV detector response, respectively. To avoid confusion, it is emphasized that sample stacking and sweeping are two different on-line concentration, enrichment, or focusing techniques. Briefly, samples prepared in a low-conductivity matrix or water are injected using voltage at positive polarity for a very long time (e.g., 10 min) into a capillary previously conditioned with a low-pH buffer. This is performed after injection of a high-conductivity zone and a short water plug. Immediately after FESI, vials containing low-pH micellar solutions were connected at both ends of the capillary, and voltage was applied at negative polarity to effect sweeping and MEKC separation. Discussions regarding the principle of concentration, strategies for improvement of concentration, and analytical performance are also conferred.

EXPERIMENTAL SECTION

Apparatus. All capillary electropherograms were recorded with a Hewlett-Packard 3D capillary electrophoresis system (Waldbronn, Germany). Electrophoresis was performed in fused silica capillaries of 50 μm i.d. and 365 μm o.d. obtained from Polymicro Technologies (Phoenix, AZ). All capillaries were 64.5 cm long having an effective length of 56 cm and were thermostated at 20 °C unless otherwise stated. An optimum detection wavelength was selected for each analyte on the basis of the spectra recorded by a diode array detector. Conductance was measured using a Horiba ES-12 conductivity meter (Kyoto, Japan). Water was purified with a Milli-Q system (Millipore, Bedford, MA). Absorbance measurements were done with a Hitachi U-2000 spectrophotometer (Japan) for the determination of molar absorptivity (ϵ).

Reagents and Solutions. All reagents were purchased in the highest grade possible from Nacalai Tesque (Kyoto, Japan). Stock solutions of 0.5 M sodium dodecyl sulfate (SDS) and 0.2 M triethanolamine were prepared every two weeks in purified water. Nonmicellar background solutions (nonmicellar BGSs) were prepared by dilution of 0.5 M phosphoric acid stock solution, triethanolamine stock solution, and additives in water. Micellar background solutions (micellar BGSs) were prepared by dilution of the SDS stock solution, 0.5 M phosphoric acid stock solution, and additives in water. The nonmicellar and micellar BGSs were prepared every day to prevent reproducibility problems.

Stock solutions of 1-naphthylamine, metoprolol, acebutolol, and laudanosine were prepared in methanol. Proper care should be taken when the above compounds are handled, as they can be toxic. The concentrations ranged from 1000 to 3000 ppm. Portions of selected stock solutions were generally combined and diluted with 50 mM phosphoric acid and 30% methanol solution, water, nonmicellar BGS, or the micellar BGS. In some cases, only one stock solution was used (see the figures). Resulting concentrations ranged from 100 to 300 ppm. Other dilutions were done in water. All solutions were filtered through 0.45 μm filters (Toyo Roshi, Japan) prior to capillary electrophoresis experiments.

General Concentration and Electrophoresis Procedure. The capillary was conditioned prior to use with 1 M NaOH (30 min), followed by methanol (30 min), purified water (30 min), and finally the nonmicellar BGS (10 min). A high-conductivity buffer devoid of organic solvent (HCB), for example, 100 mM phosphoric acid, was injected using pressure followed by a short water plug. To approximate the lengths of the injected zones, a UV-absorbing sample (e.g., 1-naphthylamine) was injected into the capillary, and then the desired pressure was applied until a response was obtained. From the velocity that was obtained, the lengths of the zones were computed given the injection time. The velocities of a liquid at ~1 bar and 50 mbar of pressure were 1.1 cm/s (% RSD = 2.3 with $n = 20$) and 0.06 cm/s (% RSD = 1.8 with $n = 20$), respectively. The RSD of the liquid velocities computed is quite good, implying that injection lengths are reproducible. Thereafter, electrokinetic injection of the sample was performed at positive polarity with the nonmicellar BGS at the outlet end of the capillary. Voltages were then applied at negative polarity with the micellar BGS at both ends of the capillary for sweeping and separation. The capillary was flushed at ~1 bar, between consecutive analyses to ensure repeatability, with methanol (1 min), purified water (1 min), and finally the nonmicellar BGS (3 min). Other conditions are specified in the text or figures.

RESULTS AND DISCUSSION

CSEI-sweep−MEKC Model. The main idea of this work is illustrated in Figure 1. The column is a bare fused silica capillary that is initially filled or conditioned with a low-pH buffer or nonmicellar BGS. A zone of a high-conductivity buffer devoid of organic solvent (HCB) followed by a short zone of water is injected hydrodynamically (Figure 1A). The cationic sample prepared in a low-conductivity solution (or simply water) is injected using voltage at positive polarity (Figure 1B) for a period much longer than usual (e.g., 10 min). This procedure creates long zones of cationic analytes (Figure 1C), which have concentrations greater than that in the original. The direction of the electroosmotic flow (considered small due to suppressed dissociation of the silanol groups) and the cationic analytes is toward the cathode. The next step is to focus the injected zones by sweeping. This is ac-

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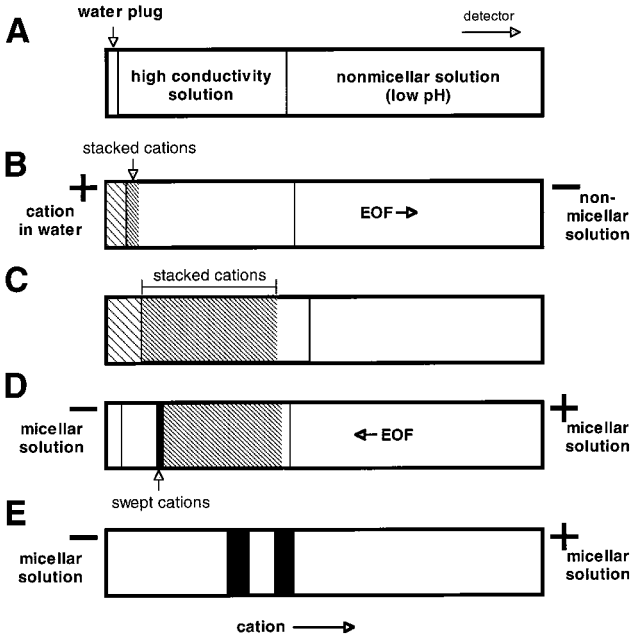

Figure 1. Evolution of analyte zones in CSEI-sweep-MEKC: (A) starting situation, conditioning of the capillary with a nonmicellar background buffer, injection of a high-conductivity buffer void of organic solvent, and injection of a short water plug; (B) electrokinetic injection at positive polarity (FESI) of cationic analytes prepared in a low-conductivity matrix or water, nonmicellar background buffer found in the outlet end, cationic analytes focus or stack at the interface between the water zone and high-conductivity buffer void of organic solvent zone; (C) injection is stopped and the micellar background solutions are placed at both ends of the capillary, shows the profile of the analytes after FESI; (D) application of voltage at negative polarity that will permit entry of micelles from the cathodic vial into the capillary and sweep the stacked and introduced analytes to narrower bands; (E) separation of zones based on MEKC. Other explanations are given in the text.

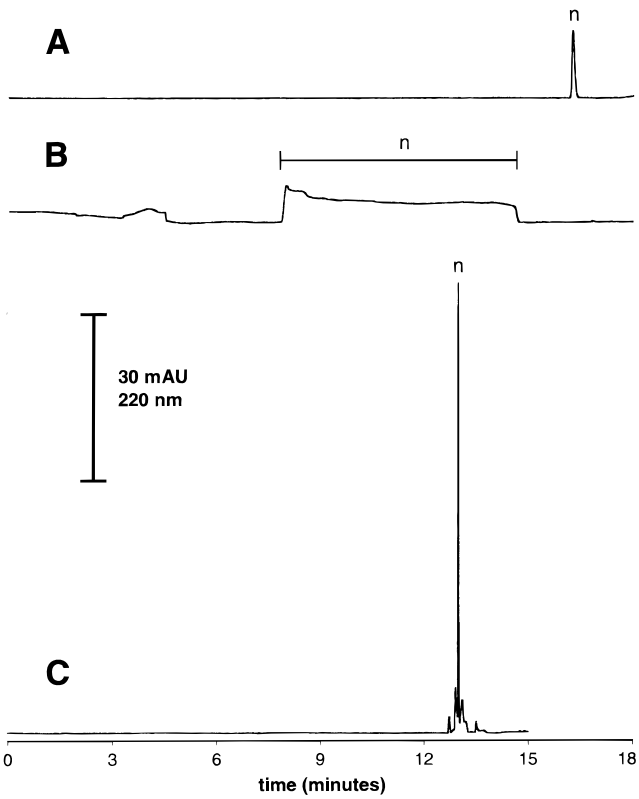

Figure 2. Peak shapes with different types of injections. Conditions: (A) CZE mode; nonmicellar BGS, 50 mM phosphoric acid/10% MeOH; sample solution, 29.1 ppm 1-naphthylamine in nonmicellar BGS/water (1:1); injection scheme, 0.6 mm of the sample solution (50 mbar); CZE separation voltage, 18 kV with the nonmicellar BGS at both ends of the capillary; (B) CZE mode; nonmicellar BGS, same as in (A); sample solution, 582 ppb 1-naphthylamine in water; injection scheme, 3 mm of water followed by 18 kV electrokinetic injection of the sample solution for 600 s; CZE separation voltage, 18 kV with the nonmicellar BGS at both ends of the capillary; (C) CSEI-sweep-MEKC mode; nonmicellar BGS, 50 mM phosphoric acid/10% MeOH; micellar BGS, 50 mM SDS/25 mM phosphoric acid/10% MeOH; sample solution, 2.91 ppb 1-naphthylamine in water; injection scheme, 3 mm of water followed by 18 kV electrokinetic injection of the sample solution for 600 s; sweeping and MEKC voltage, −18 kV with the micellar BGS at the inlet end and the nonmicellar BGS at the outlet end of the capillary. Other conditions: identity of peak, 1-naphthylamine (n); capillary temperature, 16 °C.

complished by placing a low-pH buffer solution containing anionic micelles or micellar BGS in the inlet vial followed by application of voltage at negative polarity (Figure 1D). Separation is then achieved via MEKC (Figure 1E).

In Figure 1B, the molecules enter the capillary through the water plug with high velocities. Once the molecules cross the stacking boundary or interface between the water and HCB zones, they will slow and focus at this interface. In Figure 1C, part of the sample matrix enters the capillary since the electroosmotic flow is directed toward the cathode. The area with more diagonal lines depicts the injected and stacked cations (contained in the HCB area), while the area with less diagonal lines depicts the injected but not stacked cations (contained in the water plug and injected sample matrix).

In Figure 1D, once voltage is applied at negative polarity with the micellar BGS in the inlet vial, anionic micelles will enter the capillary and sweep the analytes that were injected whether they are stacked or not. The micelles enter the low-conductivity zone consisting of the sample matrix introduced during FESI and the water plug, and then stack at the interface between the water plug and HCB. The stacked micelles then sweep the stacked cations. In Figure 1E, once the stacked cations are completely swept, they separate by MEKC in the reverse migration mode. The mechanism of separation is due to partitioning of the focused analytes between the fast moving micellar phase and very slow moving aqueous phase. The micellar phase carries the analytes toward the detector. The water zone and the sample matrix that was introduced are consequently removed from the column by the slow bulk electroosmotic flow, which is now directed toward the inlet vial (from part D to part E of Figure 1).

Injected Length of the Cation and Comparison of CSEI-sweep with FESI or Sweeping Alone. The long length of the stacked zone shown in Figure 1C is depicted in Figure 2B. FESI of the test analyte (1-naphthylamine) is performed for a much longer time in Figure 2B. For example, FESI injection is usually done for less than 60 s, whereas in this experiment it was done for 600 s. If a micellar BGS is placed in the inlet vial after the long FESI, sweeping will occur as described above. The micelles by virtue of sweeping will then narrow the long length of the stacked zone (including the injected but not stacked molecules). This is seen in Figure 2C, showing a sensitivity enhancement factor [SEF, peak heights obtained with CSEI-sweep-MEKC

divided by peak heights obtained with 1 s (50 mbar) of usual MEKC or CZE (e.g., Figure 2A), and the quotient multiplied by the dilution factor] of 60 000. Note that the concentration of 1-naphthylamine in parts B and C of Figure 2 is a 50-fold and a 10000-fold dilution of that used in Figure 2A, respectively. For presentation, the concentration used in Figure 2B is higher than that used in Figure 2C such that the long zone of stacked analyte could be detected by the UV detector. In this experiment, no HCB was used, showing that this procedure also works without a HCB. Furthermore, SEF obtained by CSEI-sweep is greater than that obtained by FESI or sweeping alone; optimized FESI and sweeping methods for 1-naphthylamine yielded SEFs of 138 and 854 (data not shown), respectively.

In Figure 2C, the sweeping step was conducted with a micellar BGS in the inlet vial and a nonmicellar BGS in the outlet vial. To obtain a very stable current, it is suggested that the micellar BGS should be found in both ends of the capillary. Additionally, it is suggested that the concentration of the buffer component (e.g., phosphate) in the micellar BGS should be lower compared to that in the nonmicellar BGS to lessen the difference in conductivity between these two solutions.

Effect of a Water Plug on the FESI Step. Several groups studied the effect of a water plug in sample stacking by electrokinetic injection.[12,19−21] Chien noted that the water plug provides a higher electric field at the tip of the capillary, which will eventually improve the sample stacking procedure.[19] The sample ions will stack at the injection point if there is no water plug and cause degradation in the field enhancement. Here, the water plug may not be extremely necessary since the electroosmotic flow although small during FESI is directed toward the detector. In effect a small volume of the low-conductivity sample matrix will enter the capillary and provide the field enhancement.

We briefly studied the amount of sample that can be injected into the capillary under CZE conditions in the presence and absence of a water plug. The nonmicellar BGS was 50 mM phosphoric acid/20% acetonitrile. The sample was 1-naphthylamine in water (291 ppb). The FESI time was 500 s, the water plug length was 3 mm, and the voltage applied was 25 kV. The peak shapes and corrected peak areas obtained with or without the water plug were comparable, implying that the water plug is not very necessary in this focusing procedure. Similar results occurred with the use of a HCB zone. However, when some salts are present in the sample matrix, a water plug may be helpful in maintaining the field enhancement. Moreover, Wey and Thormann noted that a water plug is necessary for reproducibility purposes.[21] With this in mind, a water plug was still used in this study and was kept at 3 mm. Although not so relevant, a 3 mm plug of water showed the best peak shape in the usual FESI injection of 1-naphthylamine. A more systematic study will be performed in the future.

Effect of a High-Conductivity Buffer Devoid of Organic Solvent. Figure 3 shows some electropherograms illustrating the effect of a HCB that was injected before the water plug. Two β-receptor adrenergic blockers (metoprolol and acebutolol) were used as test analytes. Figure 3A is for a 0.6 mm injection as a standard to show the sensitivity enhancements obtained. The

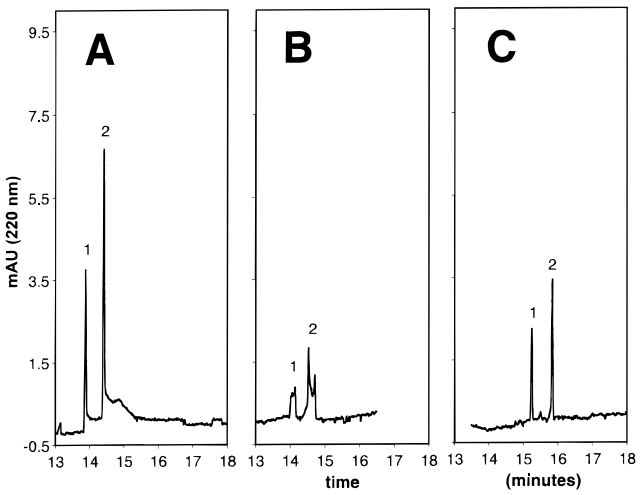

Figure 3. Effect of injecting a high-conductivity buffer devoid of organic solvent (HCB) zone before the water plug on CSEI-sweep-MEKC. Conditions: nonmicellar BGS, 1 mM triethanolamine/15% acetonitrile/100 mM phosphoric acid; micellar BGS, 100 mM SDS/1 mM triethanolamine/15% acetonitrile/50 mM phosphoric acid; HCB, 100 mM phosphoric acid; samples, metoprolol (1), acebutolol (2); sample solution, ~240 ppm of samples in 50 mM phosphoric acid and 30% methanol (A), ~24 ppb in water (B, C); injection schemes, 0.6 mm of the sample solution (A), 3 mm of water followed by 20 kV electrokinetic injection of the sample solution for 220 s (B), 54 mm of HCB and then 3 mm of water followed by 20 kV electrokinetic injection of the sample solution for 220 s (C); sweeping and MEKC voltage, −20 kV with the micellar BGS at both ends of the capillary.

sample solutions in Figure 3B,C were a 10000-fold dilution of the sample solution in Figure 3A. Peak shapes were improved when a HCB was injected before the water plug (Figure 3C). Peak heights can be improved further by increasing the FESI time and the length of the HCB zone (data not shown). The effect of the HCB will be discussed below.

After FESI, the analyte molecules are found in the HCB that is devoid of the micelle. These analyte molecules will be focused for the second time by sweeping. Figure 4 shows the effect of different sample matrixes having different conductivity values on peak shapes in sweeping-MEKC. The sample matrixes were different concentrations of phosphoric acid having conductivity values 1× (A), 2.1× (B), and 5.7× (C) compared to that of the micellar BGS. The injection length was kept at 14.4 cm, and the test sample was 1-naphthylamine. As seen in the electropherograms, preparing the sample in a high-conductivity matrix does not have a profound effect on peak shapes. It is assumed here that the sample prepared in matrixes with a high concentration of phosphoric acid depicts the HCB zone containing the analytes after FESI. Therefore, a HCB zone should not affect the sweeping step in this procedure. On the contrary, another group reported that sample matrix conductivity 2−3× greater than the separation solution conductivity and devoid of the micelle improves the focusing effect in MEKC.[17] A more detailed study on the effects of a high-conductivity sample matrix on sweeping will be reported in a separate paper.[18]

Now we discuss the effect of a HCB on the FESI step. Actually, Zhang and Thormann have shown that sample focusing by FESI is further increased about 2-fold by introduction of a short solution plug of high conductivity, high pH, and high viscosity at the

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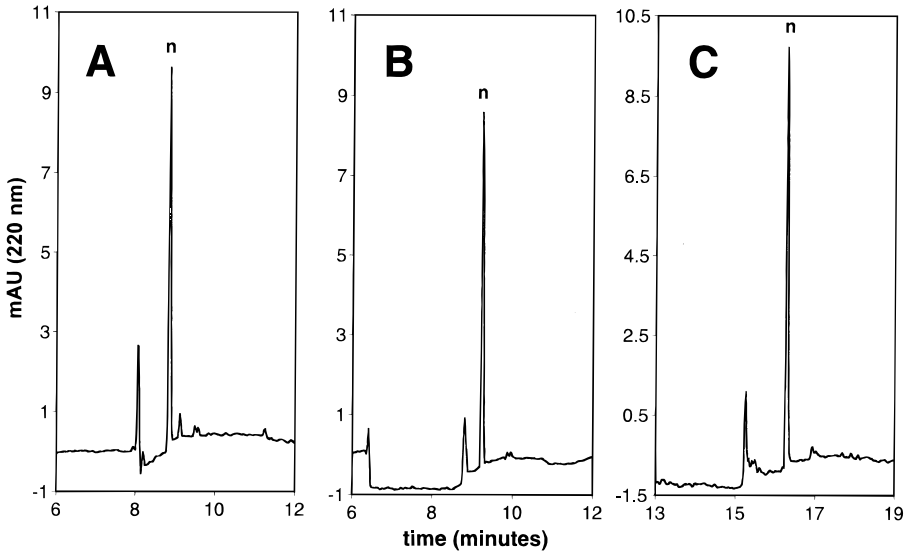

Figure 4. Sweeping effects using high-conductivity sample matrixes. Conditions: micellar BGS, 50 mM SDS/25 mM phosphoric acid/10% acetonitrile (5.4 mS/cm); sample, 146 ppb 1-naphthylamine; sample matrixes, phosphoric acid solutions having conductivity values of 5.4 mS/cm (A), 11.6 mS/cm (B), and 31.0 mS/cm (C); injected length, 14.4 cm; applied voltage, −20 kV.

capillary tip before injection.[22] In the presence of a HCB, the resulting concentration of analyte zones will be higher after FESI. This is compared to when no HCB is introduced. The resulting concentration of analyte zones will be higher because the Kohlrausch value at the HCB is higher than that at the nonmicellar BGS. The higher concentration can also be explained by the higher γ. The above discussion is illustrated in Figure 5. Parts A and B of Figure 5 are 500 s FESI experiments of 1-naphthylamine without and with a HCB, respectively. Pressure was applied to mobilize the 1-naphthylamine zone to the detector. As seen in Figure 5A,B, the peak height in the presence of a HCB is almost 3 times higher compared to that in the absence of a HCB. Furthermore, the peak width is 2 times narrower in the presence of HCB. The narrower peak in Figure 5B is due to the slower migration of the analyte molecules in the HCB due to low field strength. Note that the field in the HCB is lower compared to that in the nonmicellar BGS.

The stacked molecules inside the HCB will move after some time to the nonmicellar BGS and adjust to the lower Kohlrausch value of the nonmicellar BGS. This results in dilution of the stacked zone or destacking at the interface between the HCB and nonmicellar BGS zones. This is illustrated in Figure 6. Figure 6A is the same electropherogram as that in Figure 5B. Figure 6B is an experiment similar to Figure 6A but performed without the electrokinetic injection step to observe the HCB zone (shallow negative peak). From the concentration profiles, the stacked cation zone length in Figure 6A corresponds to the shallow negative peak obtained in Figure 6B. Note that the concentration profiles were drawn on different scales to emphasize the results. The difference in migration time between the two concentration profiles is due to the migration of the high-conductivity zone during the electrokinetic injection step in Figure 6A. This result suggests that the stacked cations have higher concentration when inside the high-conductivity zone and strongly implies the destacking of the stacked analyte molecules. Furthermore, the stacked cations in Figure 6A can be completely destacked by applying voltage to detect peaks instead of pressure (Figure 6C). Note that the concentration profile of the destacked cations in Figure 6A is similar to that found in Figure 6C.

Given a fixed FESI time, increasing the HCB zone length could hinder destacking. This is shown in Figure 5C where the HCB zone length was increased twice compared to that in Figure 5B, the destacked zone in Figure 5B disappeared, and the total stacked analyte zone became wider. The FESI time did not allow any stacked molecules to leave the longer HCB zone. An increase in corrected peak area was also observed, which suggests that the number of injected molecules increased; this will improve the focusing effect. In conclusion, the lengths of the stacked zones should be shorter than the length of the higher conductivity zone. This is to provide zones that are narrower for the sweeping step. Therefore, the FESI time and length of the higher conductivity zone should be adjusted accordingly to improve the total focusing procedure.

Effect of FESI Time on CSEI-Sweep-MEKC. Using 1-naphthylamine as the test analyte and without a HCB zone, SEFs increased with the increase in the FESI time. This is because the amount injected naturally increases with the increase in injection time. However, as the injection time was increased further, the SEF ceased to increase and peak widths became broad. This is explained by the fact that sweeping is limited by the retention factor (k), wherein the length of the zone injected can only be narrowed by a factor equal to $1/(1 + k)$.[14,15] Remember that a longer FESI time results in longer analyte zone lengths. An example of a long injected and focused zone from a diluted sample solution was shown earlier in Figure 2B. On another hand, peak areas still increased because peak area is directly related to the amount of sample molecules that were injected. Therefore and as stated earlier, to maximize the effect of sweeping, the FESI time should only be performed to an optimum.

Effect of the Concentration of the Sample on CSEI-Sweep-MEKC. Figure 7 is a stepwise illustration of the concentration

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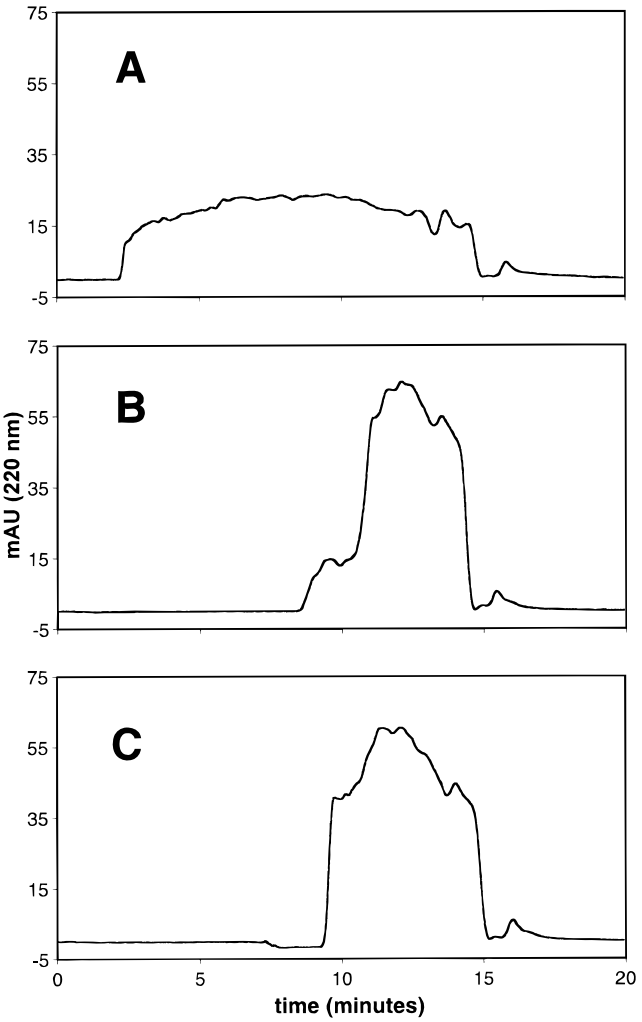

Figure 5. Effect of a high-conductivity zone on the FESI step observed through pressure-driven migration. Conditions: nonmicellar BGS, 50 mM phosphoric acid/20% acetonitrile; sample solution, 291 ppb 1-naphthylamine in water; HCB, 100 mM phosphoric acid; injection schemes, 3 mm of water followed by electrokinetic injection of the sample solution (A), 13.2 cm of HCB and then 3 mm of water followed by electrokinetic injection of the sample solution (B), 26.4 cm of HCB and then 3 mm of water followed by electrokinetic injection of the sample solution (C); electrokinetic injection, 25 kV for 500 s; mobilization of zones, pressure was applied at 50 mbar after the injection scheme with the nonmicellar BGS at both ends.

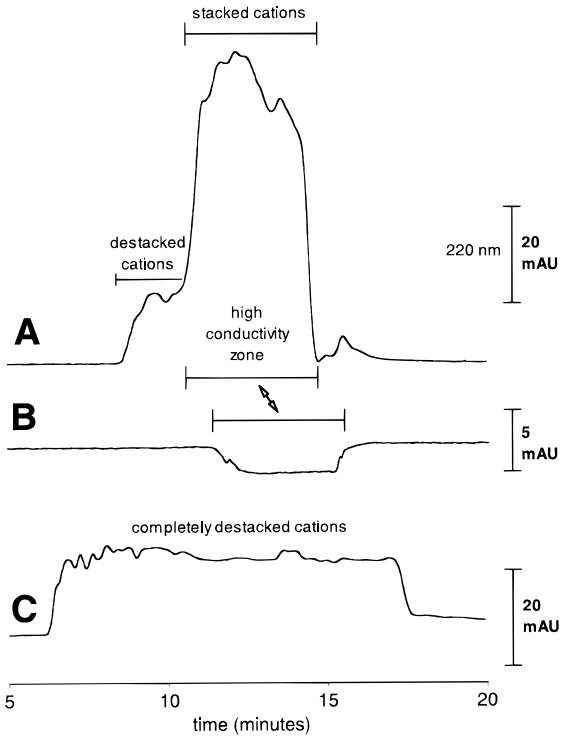

Figure 6. Stacked and destacked cationic zones after the FESI step in the presence of a high-conductivity zone observed through pressure-driven migration. Conditions: injection schemes, 13.2 cm of HCB and then 3 mm of water followed by electrokinetic injection of the sample solution (A, C), 13.2 cm of HCB and then 3 mm of water (B); electrokinetic injection, 25 kV for 500 s; mobilization of zones, pressure at 50 mbar (A, B) or voltage at 25 kV (C) was applied after the injection scheme with the nonmicellar BGS at both ends. Other conditions are similar to those of Figure 5.

effect of CSEI-sweep at different orders of concentration magnitude. Figure 7A is for a usual injection of a 291 ppm sample solution of 1-naphthylamine. The FESI step was performed for 1000 s in Figure 7B−F. Note that the concentration of the sample used in Figure 7B is 1000 times less compared to the concentration of the sample used in Figure 7A. The sample solution used in Figure 7C was a 10-fold dilution of the sample solution used in Figure 7B. A similar analogy (Figure 7C) was done in the preparation of sample solutions used in Figure 7D−F. On the basis of the results shown in Figure 7, the proposed technique could not provide a linear response in terms of peak height over 4 orders of concentration magnitude. However, the FESI time can be reduced for the more concentrated samples to get acceptable peak shapes. It is evident from part B or C of Figure 7 that overloading is present. With the proper FESI time, calibration curves may then be constructed on the basis of the concentration range. The molar absorptivity of 1-naphthylamine in the micellar BGS used in Figure 7 was found to be high ($\epsilon = 57\,300$ at 220 nm), which could partly explain the detection of this sample in the parts per trillion level. However, the improvement in detection is clearly seen by comparing the electropherograms obtained with CSEI-sweep and the electropherogram obtained with the usual injection procedure.

Detecting Parts per Trillion Levels of Analytes, and Qualitative and Quantitative Aspects. Figure 8 shows the ~240 ppt level detection of two cationic analytes using the present method. Although signal-to-noise (S/N) ratios were a bit low, the identities of peaks were confirmed using an on-line-recorded UV spectrum. SEFs were around 900 000 and 550 000 for laudanosine and 1-naphthylamine, respectively. Note that, under the conditions in Figure 8, the sensitivity enhancement for 1-naphthylamine was improved compared to that in Figure 2 or 7. The highest ever reported improvement in detector response was 5000.[14] However, enhancements would be less if longer injection times were possible in conventional CE. The system peaks that appeared before the peaks of interest result from the change in composition of the liquid that passed the detector window. Note that the capillary was initially filled with a solution void of the pseudostationary phase, which is also the case with the usual injection procedure.

An important precaution in performing CSEI-sweep-MEKC is that fresh samples should always be used for each injection. This is because of the decrease in the concentration of the sample after

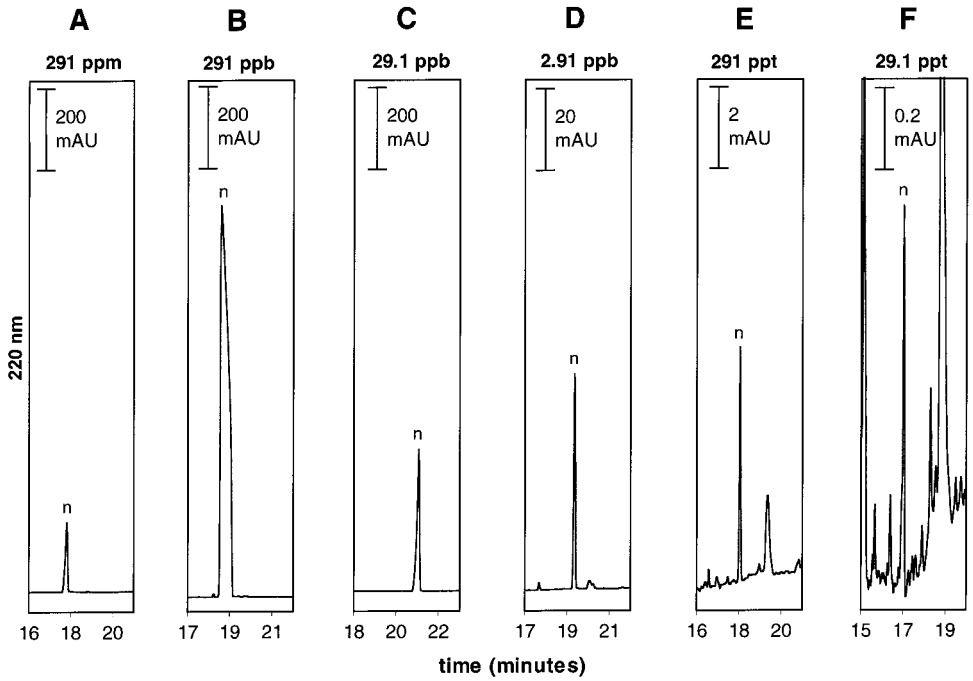

Figure 7. Effect of sample concentration on CSEI-sweep-MEKC. Conditions: nonmicellar BGS, 50 mM phosphoric acid/20% acetonitrile; micellar BGS, 50 mM SDS/25 mM phosphoric acid/20% acetonitrile; HCB, 100 mM phosphoric acid; sample solution, 1-naphthylamine in water (concentration in the figures); injection scheme, 0.7 mm of the sample solution (A), 13.2 cm of HCB and then 3 mm of water followed by 25 kV electrokinetic injection of the sample solution for 1000 s (B−F); sweeping and MEKC voltage, −25 kV.

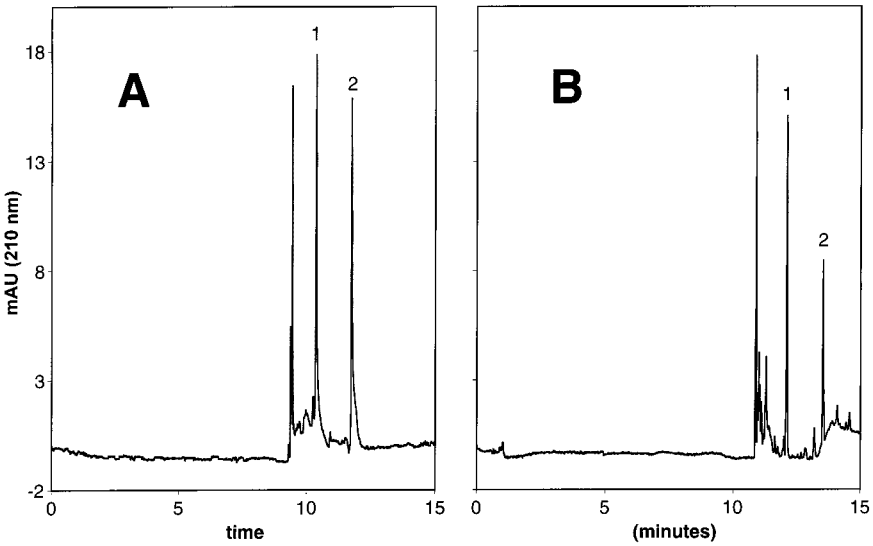

Figure 8. Almost a million-fold concentration of dilute cations by CSEI-sweep. Conditions: nonmicellar BGS, 1 mM triethanolamine/15% acetonitrile/100 mM phosphoric acid; micellar BGS, 100 mM SDS/1 mM triethanolamine/15% acetonitrile/50 mM phosphoric acid; HCB, 100 mM phosphoric acid; sample solution, laudanosine (1) and 1-naphthylamine (2) in water; sample concentration, ∼240 ppm (A), ∼240 ppt (B); injection scheme, 0.6 mm of the sample solution (A), 30 cm of HCB and then 3 mm of water followed by 23 kV electrokinetic injection of the sample solution for 1000 s (B); sweeping and MEKC voltage, −23 kV with the micellar BGS at both ends of the capillary.

injection from a single sample vial (Figure 9). In the experiments performed in Figure 9, the sample vial contents were mixed after each injection. It can also be inferred from Figure 9 that FESI can deplete the sample solution of the analyte molecules after several injections (for example, after more than 10 injections), which lead us to using the exhaustive term in CSEI. In addition, the name includes electrokinetic injection selectivity toward positive species, which is considered a benefit when anionic and neutral compounds are a major interference or when only cationic analytes are targeted. Although neutral and anionic (with low electrophoretic mobilities) analytes can be injected by virtue of the electroosmotic flow, only a small amount of sample can be injected since the electroosmotic flow is very small and thus lessens possible interference.

With the conditions stated in Figure 8, limit of detection values, linearity, and percentage relative standard deviations (%RSD) of peak heights and migration times were calculated and are summarized in Table 1. Compared to the usual injection (0.6 mm), limit of detection values were improved from around 5 orders of magnitude with CSEI-sweep. Limit of detection values ($S/N = 3$)

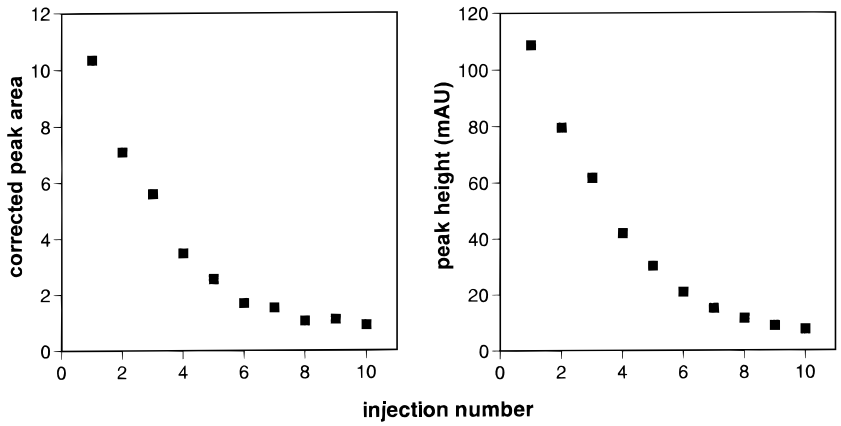

Figure 9. Depletion of a cationic analyte (1-naphthylamine) in the sample solution after several CSEI-sweep-MEKC analyses. Conditions: concentration of 1-naphthylamine, 2.91 ppb; sample solution volume in the vial, 0.3 mL. Other conditions are similar to those in Figure 2C. Corrected peak area = peak area/migration time.

Table 1. Limit of Detection Values, RSD Values, and Plate Numbers of Laudanosine and 1-Naphthylamine with CSEI-sweep-MEKC[a]

	laudanosine	1-naphthylamine
equation of the line	$y = 54.9x + 1.5$	$y = 28.1x - 0.9$
coefficient of variation	$r^2 = 0.992$	$r^2 = 0.996$
limit of detection (S/N = 3)		
(ppt)	4.1	8.0
(M)	1.2×10^{-11}	5.6×10^{-11}
% RSD		
migration time ($n = 14$)	2.7	4.2
peak height ($n = 5$)	13.8	21.6
corrected peak area ($n = 5$)	14.6	29.6
plate number	3.6×10^5	4.4×10^5

[a] Equation of the line: concentration (ppb) = slope peak height (mAU) + *y*-intercept. Conditions: same as those in Figure 8B, concentration range, 21.0 ppt to 2.91 ppb.

for a 0.6 mm injection were 2.0 ppm (5.6×10^{-6} M) and 0.7 ppm (4.9×10^{-6} M) for laudanosine and 1-naphthylamine, respectively. The RSD values are quite acceptable showing the applicability of the method to qualitative and quantitative analyses. Linearity of response spans notable 2 orders of magnitude. The poor reproducibility in peak heights and corrected peak areas is probably caused by the long FESI time. Mixing of the sample solution during FESI is a possible way of improving reproducibility. Use of the internal standard method might be very useful to improve %RSD of migration times and especially peak heights and corrected peak areas. When the FESI time and the HCB zone length were reduced (for example, 50% of the time and length given in Table 1), better RSD values were obtained. However, SEFs were less (~50% also), and thus a compromise between sensitivity and reproducibility should also be considered.

CONCLUSION

In the present paper we describe a new, simple, selective, sensitive, and high-resolution procedure to analyze trace amounts of positively chargeable analytes. Modification of present commercial CE instrumentation is unnecessary. A few compounds have been concentrated from a thousand- to almost a million-fold compared to a less than 1 mm injection in the usual CE. The method would be useful for most positively chargeable analytes by simply reducing the FESI time to obtain an acceptable length of the stacked zone for sweeping. The presence of a water plug during the FESI step will help maintain the field enhancement at the tip of the capillary especially when the sample matrix contains some salt. The water plug may also improve reproducibility.[21] The presence of a high-conductivity buffer void of organic solvent, which improves the total focusing protocol, affects the FESI step and not the sweeping step.

One difficulty will be preparing the sample in a low-conductivity matrix. This is especially true in real world analysis. Sample preparation schemes compatible with the proposed method will be investigated in the future. Another concern is total analysis time especially when greater enhancements in detector response are desired. More than 15 min is needed for the FESI step alone, on top of the 20−30 min sweeping and separation time and 5 min for conditioning (total CE analysis ~50 min). However, tedious off-line sample concentration schemes require much longer times compared to the proposed on-line sample concentration method. The transfer of this technology to the chip format would be very interesting, especially with fabrication of specific channels for injection, sweeping, and separation. On top of that, this is very timely because sensitive high-resolution methods are becoming very essential to solving many problems in various scientific fields.

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