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Large-Volume Sample Sweeping with a High Theoretical Plate Number Using a Coupled-Capillary in Capillary Electrophoresis

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A large-volume sample injection (> 5 μL) with an extremely high theoretical plate number ($N > 10^7$) was achieved when the sweeping-MEKC mode and a coupled-capillary (100 – 50 μm i.d.) were simultaneously used in a capillary electrophoresis (CE) separation. A low-cost and compact violet-LED (~2 mW) was used as the fluorescence excitation source. As a result, the theoretical plate numbers of the detected peaks (two model compounds: naphthalene-2,3-dicarboxaldehyde derivatized-dopamine and -norepinephrine) were 1.0×10^7 and 7.4×10^6, respectively. The limits of detection (at $S/N = 3$) of these were determined to be 2.8×10^{-10} M (92 ppt) and 2.3×10^{-10} M (83 ppt), respectively.

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Introduction

On-line sample concentration techniques are widely employed in capillary electrophoresis (CE) to improve the limit of detection (LOD). Most of these techniques were developed to accommodate a larger volume of sample injection, since the LOD is proportional to the amount of sample injected. Indeed, an improvement in the detection limit could theoretically be achieved by simply increasing the volume of the sample solution, if individual electrophoretic parameters, such as the solvents, buffer conductivities, pH values, the magnitude and direction of electroosmotic flow (EOF), the concentration of surfactants (if needed) used, and even the polarity of the electrode, could all be optimized. The so-called large-volume sample stacking (LVSS) method[1–4] is a generally accepted and well known technique that can be used for a larger sample injection in CE separations. However, in the "stacking" process, a proportionally greater electric field develops across the sample zone, causing the cationic and anionic analytes to migrate faster in different directions. Once these ions reach the boundaries between the sample zone and the background solutions, the electric field strength suddenly decreases and migration becomes slower, causing the ions to be concentrated near the boundaries. Due to the effect of diffusion and the distribution of the electric field strength along the capillary axial, it is, in principle, difficult to focus the individual analyte-ion into a very narrow zone. This is one of the major reasons for the poorer (10^4 – 10^5) theoretical plate numbers that are typical, when the "stacking" mode is used. In contrast, when the "sweeping" mode is applied, the sample is concentrated by means of the moving micelles.

Meanwhile, the analytes are almost under a static state before being swept by the micelles. Hence, diffusion does not have a dramatic effect and, as a result, the peak broadening can be suppressed. Thus, a higher (10^5 – 10^6) theoretical plate number can be obtained. A number of efforts have been made to permit the use of a larger sample volume in CE. Table 1 summarizes some of these methods and some of the compounds examined.[1-12] Each method has unique advantages and disadvantages with respect to the sensitivity, precision and simplicity of use. In fact, the influence of the analyte plug width on the plate number in CE has been discussed previously.[13] The injection of a larger volume always results in a decrease in the theoretical plate number. For this reason, it is difficult to improve the theoretical plate number from 10^6 to 10^7, if experimental factors, such as the physical (capillary style) or chemical (solvent system) conditions, are not also changed. We previously reported on a very high value ($9.4 \pm 0.9 \times 10^6$) for the theoretical plate number, when a 75 – 25 μm coupled-capillary (sample injection, 0.9 μL; pathway of detection window, 25 μm) was used.[10] In this study, in an attempt to acquire a larger volume of sample injection with a high theoretical plate number, we report on the optimized conditions for achieving this. Two NDA (naphthalene-2,3-dicarboxaldehyde) derivatized compounds, (NDA-dopamine and NDA-norepinephrine) were selected as model compounds. Several electrophoretic parameters, such as the organic solvent used, the resolution (R_s) of the two compounds under different separation conditions, and the injection length required for sample concentration were optimized. These data are also reported herein.

Experimental

Apparatus

The violet-LED (light-emitting diode) light source (~2 mW), CE set-up and data-acquisition system used were similar to that described previously,[10] and are abbreviated herein.

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Abbreviations: BGS, background solution; LED, light emitting diode; NDA, naphthalene-2,3-dicarboxaldehyde; N, the number of theoretical plate; SDS, sodium dodecyl sulfate; Sweeping-MEKC, sweeping-micellar electrokinetic chromatography.

Table 1 Comparisons of large-volume sample injection methods, analytes, injected volume of sample solution, limit of detection (LOD), and theoretical plate number (*N*)

Method	Analyte	Injected volume/μL	LOD	*N*	Ref.
LVSS	PTH-Asp	2.0	—	~3×10^{4}[a]	1
	PTH-Glu			~2×10^{4}[a]	
LVSS	Carbovir triphosphate	1.4	20 nM	~3.2×10^{3}[a]	2
LVSS	Nitrotyrosine	1.8	2.5 – 10 nM	~2.3×10^{4}[a]	3
	Chlorotyrosine			~2.1×10^{4}[a]	
	ortho-Tyrosine			~3.4×10^{4}[a]	
	meta-Tyrosine			~3.7×10^{4}[a]	
LVSEP	Weak acids	0.5	10 – 80 nM	2.7×10^{5}	4
	Chlorinated phenols				
	Aromatic amino acids				
Stacking	Methylmercury (Me-Hg)	3.6	12 ng/g	~1.2×10^{5}[a]	5
Counterflow-ITP-CZE	Neostigmine bromide	21	2.5 nM	—	6
	Propantheline bromide				
Counterflow-ITP-CZE	Neostigmine bromide	8.3	2.5 nM	3.1×10^{5}	7
	Propantheline bromide			1.9×10^{5}	
ITP-CZE	Succinate	1.2	8 – 13 nM	—	8
	Acetate				
Sweeping-MEKC	Progesterone	0.8	8.3 nM	1.3×10^{6}	9
	Testosterone		5.9 nM	8.1×10^{5}	
	Fluocinolone		3.6 nM	4.9×10^{5}	
	Dexamethasone	1.8	24.5 nM	9.4×10^{4}	
Sweeping-MEKC	Dopamine	0.9	1.0 nM	9.4×10^{5}	10
	Dopamine	2.0	0.6 nM	9.4×10^{6}	
LTB/sweeping-MEKC	Dopamine	2.8	0.7 nM	2.5×10^{6}	11
FCSS/sweeping-MEKC	Tryptophan		1.4 nM	1.1×10^{5}	12
	Isoleucine		1.1 nM	1.2×10^{5}	
Sweeping-MEKC	Dopamine	5.2	0.28 nM	1.0×10^{7}	In this work
	Norepinephrine		0.23 nM	7.4×10^{6}	

Abbreviations: LVSS, large volume sample stacking; LVSEP, large volume stacking using the electroosmotic flow (EOF) pump; ITP-CZE, isotachophoresis-capillary zone electrophoresis; LTB/sweeping-MEKC, low temperature bath assisted sweeping-MEKC; FCSS/sweeping-MEKC, full capillary sample stacking/sweeping-MEKC; PTH-Asp, phenylthiohydantoin-aspartic acid; PTH-Glu, phenylthiohydantoin-glutamic acid. a. Theoretical plate number (*N*) calculated directly from the published data using the definition of $N{:}N = 5.545(t_R/w_{1/2})^2$, where *t* is the time axis, t_R is the migration time (time of the peak maximum), and $w_{1/2}$ is the peak width (in time) at half-height.

Reagents

All chemicals used were of analytical grade. Dopamine, norepinephrine and NDA (naphthalene-2,3-dicarboxaldehyde) were purchased from Sigma (St. Louis, MO, USA). SDS (sodium dodecyl sulfate), sodium tetraborate, methanol and phosphoric acid were purchased from Acros (Geel, Belgium).

Derivatization procedure of NDA derivatized dopamine and norepinephrine

The derivatization procedure was modified from the original literature description,[14] and is abbreviated herein.

Results and Discussion

In general, in the sweeping-MEKC mode the sweeping step and the MEKC separation step must be considered separately, that is, in the sweeping step, the interaction between the analyte and the micelle must be maximized, so as to increase the concentration efficiency, whereas in the MEKC step the interaction between the two must be optimized to give good resolution or suitable retention factors. Therefore, the addition of an organic solvent to the sample matrix, which reduces the interaction between the analyte and the micelle, and hence deteriorates the sweeping efficiency, is not recommended. In Fig. 1A (MEKC mode), the CE buffer consisted of 100 mM SDS and 30 mM H_3PO_4 in a mixed acetonitrile–water solution (electropherograms a – e, ACN%: 0, 5, 10, 15 and 20%, respectively (v/v)); the percentages (%) at the top of each electropherogram indicate the ratio of the organic solvent used. The concentrations of NDA derivatized-dopamine (NDA-Dop) and -norepinephrine (NDA-Nor) were 5.0×10^{-5} M each. The diameter and the length of the capillary used were 50 μm i.d. and 100 cm (effective length: 93 cm), respectively; the applied voltage was –20 kV (current, –30 – –50 μA; depending on the various solutions used). The two analytes could be separated more completely when the percentages of acetonitrile were increased. However, this was not true when the sweeping-MEKC was used. In the case of sweeping-MEKC (Fig. 1B), the background solution (BGS) consisted of 100 mM SDS and 30 mM H_3PO_4 in a mixed acetonitrile–water solution (electropherograms f – j, ACN%: 0, 5, 10, 12.5 and 15%, respectively (v/v)). The analytes (5.0×10^{-7} M each) were dissolved in the same solution (without SDS), resulting in a non-micelle buffer. After completion of the injection, a negative charge (high voltage, –20 kV; current, –21 – –30 μA) power supply was used for the CE separation. In this case, the length of the sample matrix injected was 25 cm. It could be seen that the two analytes were not separated when only an aqueous solution was used (0% organic solvents). However, when the amount of acetonitrile was increased, the separations appeared to be good, since the distribution of analytes between the micelles and the aqueous phase were changed. In the case of acetonitrile, the optimal ratio was determined to be 12.5% (R_s = 1.5). On the other hand, the optimal ratios for acetone, methanol and ethanol were 15 (R_s = 2.4), 22.5 (R_s = 2.5) and

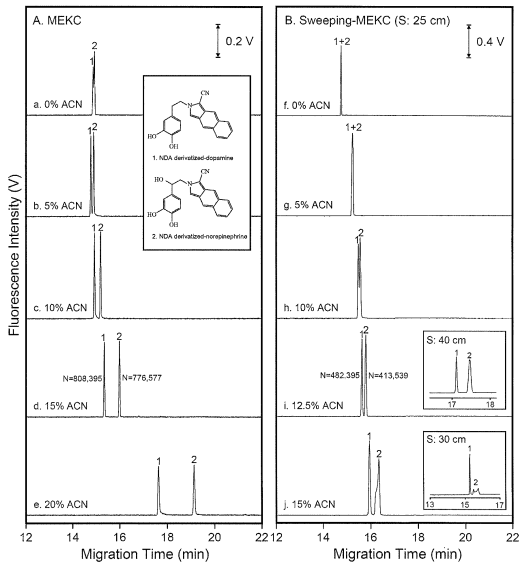

Fig. 1 CE electropherograms of NDA derivatized-dopamine (peak 1), norepinephrine (peak 2) standards obtained by the MEKC mode (frame A) and the sweeping-MEKC mode (frame B) using different percentages of acetonitrile. In frame A, electropherograms a – e, acetonitrile %: 0, 5, 10, 15 and 20%, respectively. CE conditions: 100 mM SDS and 30 mM H_3PO_4; applied voltage, –20 kV; current, –30 – –50 μA. Sample concentration, both 5.0×10^{-5} M. In frame B, background solution (BGS): 100 mM SDS and 30 mM H_3PO_4 in a mixed acetonitrile-water solution (electropherograms f – j, acetonitrile %: 0, 5, 10, 12.5 and 15%, respectively, v/v); applied voltage, –20 kV; current, –21 – –30 μA; sample injected length, 25 cm. Sample concentration, both 5.0×10^{-7} M. Insets above the electropherograms i and j, results obtained when the length of the injected sample zone were increased to 30 and 40 cm, respectively.

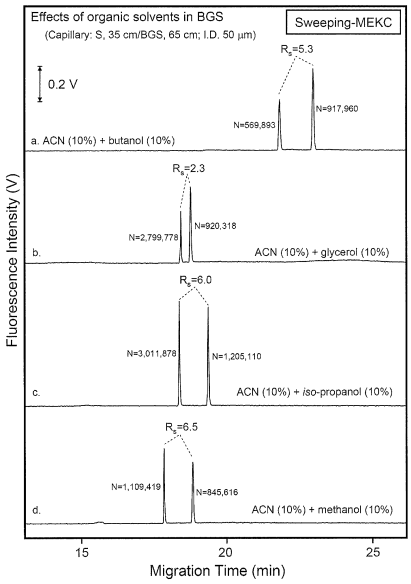

Fig. 2 CE electropherograms show the effects of the solvent when two types of solvents were used (A-solvent, acetonitrile/B-solvent, alcohol). The ratios of acetonitrile, alcohol and water were 10/10/80, v/v. The types of alcohols in electropherograms a – d: butanol, glycerol, *iso*-propanol and methanol, respectively.

17.5 (R_s = 1.9) %, respectively (data not shown). However, such optimized solvent ratios do not guarantee that they will continue to be functional when more sample solution is injected. It can be seen from the insets above electropherograms i and j (Fig. 1B) that when the sample zone was shorter, the separation appeared to be acceptable. However, when the length of the injected sample zone was increased, the shape of the NDA-Nor peak was distorted. The focus of this study was to investigate the effects of the solvent on achieving a high separation efficiency (greater number of theoretical plates). Of course, the choice of an appropriate solvent continues to be difficult, because each analyte has its own unique physical and chemical characteristics. For separating analytes with different properties (in this case, the hydrophilicity of NDA-Nor is greater than NDA-Dop), suitable separation conditions still need to be determined by trial-and-error. We selected acetonitrile as the model solvent (as the A-solvent, conforming to the usage in HPLC), because of its shorter migration time and higher detection sensitivity. Furthermore, in order to examine the separation efficiency, four types of alcohols (butanol, glycerol, *iso*-propanol and methanol) were selected as the B-solvent, each of which was added to the BGS to form a mixed ACN-alcohol solvent (A/B/water, 10/10/80, v/v). In Fig. 2, electropherograms a – d show the various solvent effects when 10% butanol, glycerol, *iso*-propanol and methanol were added to the BGS. As excepted, the results show that a mixed organic solvent provides more latitude for improving the separation efficiency. Since the use of *iso*-propanol provides better efficiency for both the detection sensitivity and resolution (Fig. 2, electropherogram c), we selected this as the buffer of choice in subsequent experiments. The relative standard deviations (RSD %) of the peak area for NDA-Dop/NDA-Nor of this buffer system were 1.3/1.0% (intra-day) and 22.7/12.2% (inter-day), respectively. In order to investigate the optimal sample injection length, various injection lengths were tested, as shown in Fig. 3 (electropherograms a – c; sample zone, 70, 65 and 55 cm, respectively; total length, 100 cm). The same experimental conditions, as described in Fig. 2c, were used in these experiments. As the sweeping-process proceeds, the sample-zone containing NDA-Dop and NDA-Nor can be focused into a narrow zone irrespective of the amount of sample solution injected into the capillary. The inset-plots show the results including the plate number, peak area and resolution when various sample zones (in length, cm) were injected. The number of theoretical plates can all be maintained at over ~10^6. It is clear that when the injection length is longer, the peak area is increased, but the separation resolution deteriorates. This is because, when a larger volume of the sample solution is injected, the remaining portion of the capillary becomes shorter, leading to an incomplete separation. The inset above the electropherogram b (Fig. 3) shows the result obtained for a lower sample concentration (5.0×10^{-9} M). In this case, the LODs for NDA-Dop and NDA-Nor were calculated to be 1.6×10^{-9} M and 9×10^{-10} M, respectively. In an attempt to further increase the sample volume, we applied these conditions to a coupled-capillary. The test length was 65 cm (volume, 5.2 μL) in the wider portion. Herein, the length of each section of the capillary used was 70 (100 μm i.d.) and 30 cm (50 μm i.d.), respectively; total length/effective length = 100/93 cm. Figure 4 shows a typical CE electropherogram of a mixture of NDA-Dop and NDA-Nor, using a coupled-capillary based on the sweeping-MEKC using the optimized solvent system, as described in Fig. 2c. It can be seen that the theoretical plate numbers (N) for the detected peaks are 1.0×10^7 and 7.4×10^6, respectively. The limits of detection (LOD at S/N = 3) of these analytes were determined to be 2.8×10^{-10} M (92 ppt) and 2.3 ×

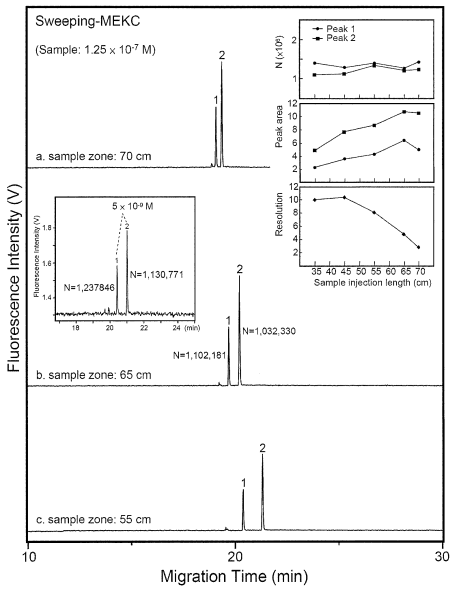

Fig. 3 CE electropherograms obtained using different sample injection lengths when a mixed acetonitrile-*iso*-propanol (10/10%, v/v) solution was used based on the sweeping-MEKC mode. Electropherograms a – c; sample zone, 70, 65 and 55 cm, respectively. The separations were performed under the same experimental conditions, as described in Fig. 2c. The plot-inset shows the relationships between various sample zones (in length, cm) and the plate number/peak area/resolution. Inset above the electropherogram b, results obtained using a lower concentration (5.0×10^{-9} M).

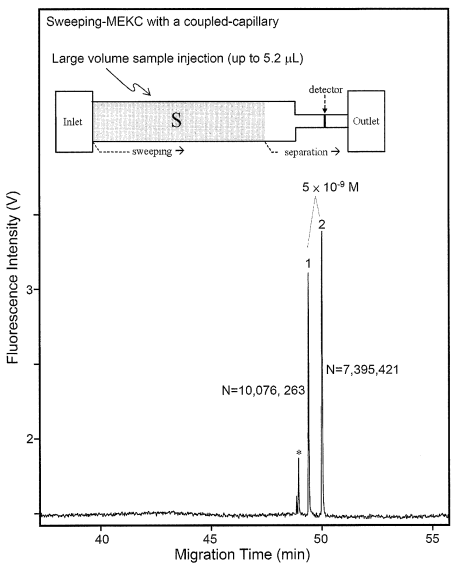

Fig. 4 CE electropherogram obtained under the optimal conditions described in Fig. 2 (electropherogram c) when a coupled-capillary was used based on the sweeping-MEKC mode. The asterisk indicates the system peak.

10^{-10} M (83 ppt), respectively; 1600 and 2560-folds improvements can be achieved. In fact, in a previous study, based on the MEKC mode we reported that the limit of detection for NDA-Dop was 2×10^{-7} M when a regular capillary (50 μm i.d.) and a normal sample injection method (~3 nL) were applied, respectively.[10] Compared to the data obtained in the previous study, the use of the coupled-capillary/sweeping-MEKC mode not only resulted in an enhanced sensitivity, but

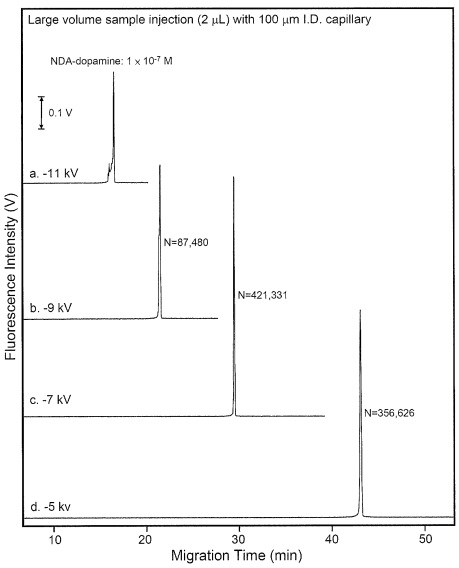

Fig. 5 CE electropherograms obtained under different applied voltages (electropherogram c) when a wider capillary (100 μm i.d.; total/effective length, 80/74 cm) was used based on the sweeping-MEKC mode. Electropherograms a – d: –11, –9, –7 and –5 kV, respectively; currents: –70 – –110, –50 – –72, –40 – –50, and –30 – –33 μA, respectively). CE conditions: as described in Fig. 1, frame B (electropherogram i).

the theoretical plate number was also dramatically improved from ~10^5 to ~10^7. Figure 5 shows a negative example, which demonstrates that a single wider capillary (100 μm i.d.) cannot provide a high theoretical plate number. In this case, although more sample solution can be injected (in this case, sample zone: 25 cm in length/2 μL in volume), Joule-heating would cause the sweeping step to be incomplete (as shown in electropherogram a; applied voltage, –11 kV). This could be improved when the applied voltage (electropherograms b – d: –9, –7 and –5 kV, respectively) was decreased, leading to lower Joule-heating. However, the theoretical plate number was found to be only in the range of ~10^4 – 10^5. Thus, when a coupled-capillary was used, the sweeping-MEKC mode under optimized buffer conditions, both the limit of detection and the theoretical plate number could be dramatically improved. This is because, when a capillary consisting of two portions (wide portion, lower field strength; narrow portion, higher field strength) having different inside diameters is used, the field strength inside the capillary must be different. Hence, the electrophoretic migration velocities of the analytes and electroosmotic flow (EOF) must also be different (wide portion, analytes moving slower; narrow portion, analytes moving faster). Since strong acidic conditions were employed, the EOF should be extremely small; the SDS micelles would migrate very slowly (toward the outlet) inside the capillary, since the field strength (in the wide portion) was very low. As a result, the analytes were gradually and slowly swept without peak broadening. Furthermore, since a comparatively large amount of accumulated SDS-analytes suddenly flowed into the narrow capillary (from 100 to 50 μm i.d.), this process permits the SDS-analytes to further collect around the boundary. As a result, an extremely sharp peak was obtained.

Conclusions

This work describes the successful application of a coupled-

capillary (100 – 50 μm i.d.) for use in large volume sample injections in CE separations, providing an extremely high theoretical plate number. Although the utility of the coupled-capillary was investigated using the sweeping-MEKC method in this study, it would be possible to extend the performance to real samples, since the detected peak is much more sharp. The method is a sensitive, rapid, simple, reproducible and economic technique, and, also suggests that the use of a wide-to-narrow channel configuration in a microchip would have great potential for use. Further applications of this technique are currently under investigation.

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