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Methanol plug assisted sweeping-micellar electrokinetic chromatography for the determination of dopamine in urine by violet light emitting diode-induced fluorescence detection

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Abstract

The use and limitations of a methanol plug assisted sweeping-micellar electrokinetic chromatography (sweeping-MEKC) method is described. Using naphthalene-2,3-dicarboxaldehyde (NDA)-labeled dopamine as a model compound, this new method was also used in the determination of dopamine in actual urine samples. An inexpensive violet light emitting diode (LED) was used for the light source, because this is suitable for fluorescence excitation. The number of theoretical plates of the analyte was determined to be $\sim 1 \times 10^5$ and $\sim 2 \times 10^5$ by means of MEKC and sweeping-MEKC and this was improved to $\sim 1 \times 10^6$ when the methanol plug assisted mode was applied. In addition, the detection limit of NDA-labeled dopamine was determined to be 9.1×10^{-7} and 1.2×10^{-8} M by means of MEKC and sweeping-MEKC and this was improved to 4.7×10^{-9} M when the methanol plug assisted sweeping-MEKC mode was applied.

Keywords: Methanol plug; Violet light emitting diode; Capillary electrophoresis; Sweeping-MEKC; Dopamine

1. Introduction

Capillary electrophoresis (CE) has developed into a versatile and powerful tool in the area of separation science. A high detection sensitivity and high separation efficiency are goals in chromatographic separations. On-line sample concentration techniques, such as the so-called "stacking", "pH junction" and "sweeping" techniques [1–25], have rapidly grown in popularity over the past few years because they achieve this goal. Dramatic increases in sensitivity can be obtained when these techniques are used. Except for some on-line sample concentration techniques that involve different mechanisms such as methods based on a Hadamard transform [26] or liquid-phase micro-extraction [27], most on-line sample concentration techniques were developed to accommodate a large volume injection, since the limit of detection is proportional to the injected sample zone. Unfortunately, an increase in detection limit cannot be achieved by simply increasing the injection time (for electrokinetic injection) or the length of the sample plug, because individual electrophoretic parameters such as the injection length required for the separation, the concentration of surfactant used, buffer conductivity and even the pH value must be optimized. Furthermore, in a real sample analysis, the application of such techniques sometimes continues to be a challenge since numerous unknown matrix effects could lead to the appearance of many unidentified peaks when the on-line sample concentration techniques are applied. Thus, a high separation efficiency is also important. In this study, we report on the feasibility of using a methanol plug, in an attempt to improve separation efficiency. Based on the sodium dodecyl sulfate (SDS) sweeping-MEKC mode, a portion of the methanol (near the junction between the sample solution and the background solution) is injected into the capillary. This methanol plug serves as a micelle destruction zone. When the sweeping-MEKC mode is applied, the analytes become concentrated in the SDS-micelles from the inlet reservoir along the capillary

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axis. Once these SDS-micelle-analytes enter the methanol plug, most of the SDS-micelle-analytes are freed since SDS does not form complete micelles in methanol. Thus, the methanol plug becomes a "barrier" that interrupts the micelle-analytes when they pass through the methanol plug. As a result, the injected analytes are further concentrated, leading to a higher separation efficiency. We selected naphthalene-2,3-dicarboxaldehyde (NDA)-labeled dopamine as a model compound since it is not only suitable for detection by fluorescence excitation using an inexpensive violet light emitting diode (LED) as the light source (instead of a laser) but also it represents a continuation of our previous research [28]. The results obtained by normal MEKC, sweeping-MEKC and the methanol plug assisted sweeping-MEKC mode are reported and compared. Several electrophoretic parameters such as SDS concentration, the length of the methanol plug, the injection length required for sample concentration and separation were optimized and these data are also reported herein. In addition, the concentrations of dopamine in actual urine samples from a volunteer were investigated and these data are also reported.

2. Experimental

2.1. Apparatus

The CE set-up was fabricated in-house and is identical to that described previously [28]. Briefly, a high-voltage power supply (model RR30-2R, 0–30 kV, 0–2 mA, Gamma, FL, USA) was used to drive the electrophoresis and a 50 μm i.d. fused silica capillary (J&W Scientific, CA, USA) was used for the separation. The sample was injected by raising the reservoir 40 cm relative to the exit reservoir (at this height, the flow speed for the sample injection was 0.344 mm/s) to provide the injection length (depending on the specific situations). A violet LED (InGaN; Type No. M053UVC, Monarchal Electronics Co. Ltd.) with a luminous intensity of 300 mcd (peak emission wavelength: 410 nm) was purchased on the Taipei electronic market. A microscope objective (40×) was used for focusing on the capillary. Fluorescence emission was collected by means of a microscope eyepiece (10×), passed through a green-yellow cut filter (cut-off wavelength: 475 nm) and a slit (0.3 mm), focused by a second microscope eyepiece (10×), and then detected by a photomultiplier tube. The analog signal was converted to a digital signal by an A/D converter (9724-1 module, Scientific Information Service Co. Ltd., Taiwan). Electropherograms were collected at a speed of 5 points/s with a data acquisition system connected to a personal computer.

2.2. Reagents

All chemicals used were of analytical grade. Dopamine ($C_8H_{11}NO_2$), naphthalene-2,3-dicarboxaldehyde, sodium dodecyl sulfate, sodium tetraborate, methanol and phosphoric acid were purchased from Sigma (St. Louis, MO, USA). Diphenyl boric acid ethanolamine complex and ammonium hydroxide were purchased from Acros (New Jersey, USA). Acetonitrile, ammonium acetate, ammonium chloride and EDTA were obtained from Alps Chem Co. Ltd. (Taiwan) and RdH Laborchemikalien GmbH & Co. KG, respectively.

2.3. Derivatization procedure of NDA-labeled dopamine

The derivatization procedure was modified from the original literature description [29]. To 1.0 mL of a solution containing 0.7 mL of an aqueous sodium tetraborate buffer (0.1 M, pH 9) was added 0.1 mL of dopamine (10^{-3} M in MeOH) and the same volume of KCN (10^{-3} M in tetraborate aqueous buffer). The reaction was initiated by the addition of 0.1 mL of NDA (10^{-3} M in MeOH) to give concentrations of [dopamine] = 10^{-4} M, [CN] = 10^{-4} M, and [NDA] = 10^{-4} M. After mixing, the reaction solution was allowed to stand at room temperature in the dark for 20 min. The derivative was directly used for mass spectrometric analysis and in subsequent CE separations.

2.4. Solid-phase extraction for dopamine form urine samples

Urine samples I and II were obtained from a volunteer at 3 p.m. within different 2 days. The subject was allowed to function in a normal manner during the day with free access to food and water. Before urine extraction, the cartridges (Bakerbond spe Octadecyl (C_{18}), 1 mL/100 mg, J.T. Baker 7020-01) were conditioned with 2 mL of methanol and 2 mL of 0.2 M ammonium chloride (pH 8.5). A 1 mL aliquot of urine was pretreated by hydrolysis with acid (50 μL of 6 M HCl, 100 °C, 30 min) and then 2 mL of 2 M ammonium chloride (containing 0.2% diphenyl boric acid ethanolamine complex and 0.5% EDTA), the pH was adjusted to 8.5 by the addition of 30% ammonium hydroxide. Following this, a 1 mL mixed solution was poured into the cartridge, rinsed with 2 mL of 0.2 M ammonium chloride (pH 8.5), followed by 2 mL of 0.2 M ammonium chloride–methanol (8:2, v/v; pH 8.5), followed by drying under vacuum for 2 min. Finally, the dopamine was eluted with 1 mL of 1 M acetic acid solution (freshly prepared). This organic phase was then evaporated to dryness for subsequent derivatization and CE separation.

3. Results and discussion

Fig. 1 shows schematic diagrams of a normal MEKC (A), sweeping-MEKC (B) and the methanol plug assisted sweeping-MEKC (C) used in the CE separations, respectively. Based on the normal MEKC mode, only a short plug of sample is injected. However, a longer sample injection can be achieved when the sweeping-MEKC mode is applied because the process of "sweeping" involves the collection and accumulation of analyte molecules by the pseudostationary

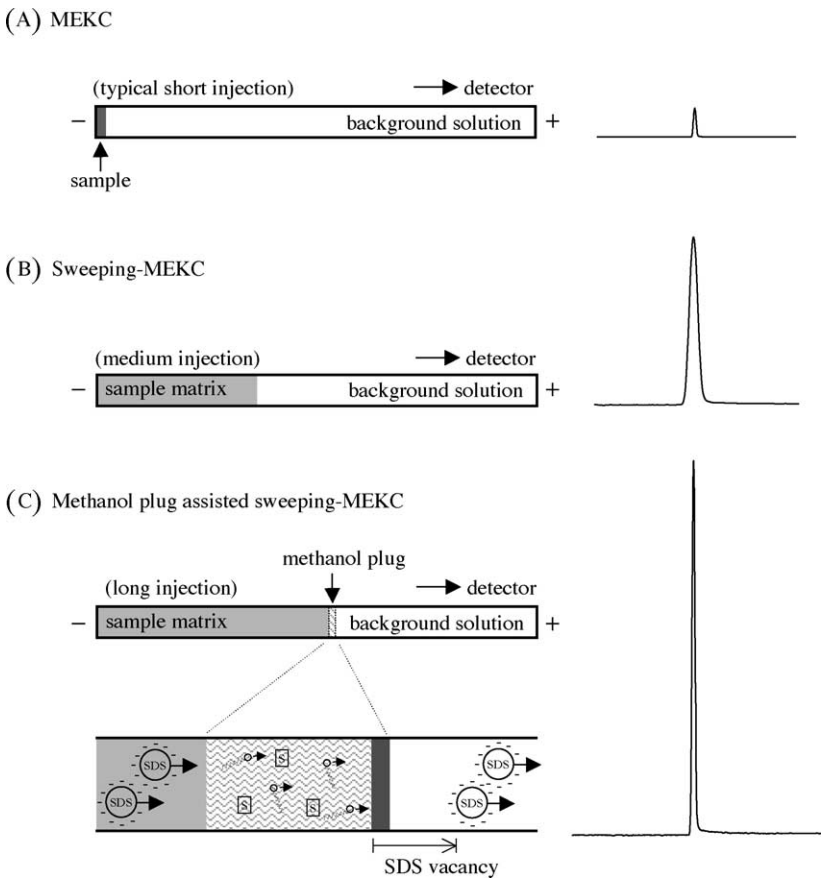

Fig. 1. Schematic diagrams of a normal MEKC (A), sweeping-MEKC (B) and methanol plug assisted sweeping-MEKC (C) used in the CE separation as well as their expected peak shapes, respectively.

(PS) phase that penetrates the sample zone. The length of the injected neutral analyte zone was found to be theoretically narrowed by a factor equal to $1/(1+k)$ (k, retention factor) and the concentration can be increased approximately by a factor, $1+k$ [12,13]. In contrast to this, a methanol plug assisted sweeping-MEKC (C) permits the length of the sample injection to be extended, thus improving the separation efficiency by injecting a plug of methanol between the sample matrix and background solution (Fig. 1C). The impurities in methanol serve as a source of electrolytes to maintain the current when a high voltage is applied. In the starting situation when a negative charge is applied to the cathode at the inlet end, the negatively charged SDS-micelles (both in the inlet reservoir and in the background solution zone) migrate toward the anode at the outlet and, up to this point, follows the same behavior as the normal sweeping-MEKC mode. However, when the SDS-micelle/SDS-analytes enter the methanol plug, they are destroyed, since SDS does not form complete micelles in methanol. In addition, the conductivity of the methanol plug is lower than that of the sample matrix and SDS would move faster. As a result, the analytes can be focused into a narrow zone. Of course, the other possibility is that the methanol plug may form a mixed methanol–water zone since, the diffusion phenomenon may be present, which permit several SDS-micelles to be formed. Nevertheless, the methanol plug acts as a "barrier" to slow down the speed of SDS-micelles as well as the SDS-analytes. Compared to the normal sweeping-MEKC, this makes the length of sample injection longer and is useful for sample focusing.

The inset in Fig. 2 shows the excitation and fluorescence spectra of NDA-labeled dopamine; the dashed line shows the wavelength range of the violet LED used. This light source is particularly well matched to excite NDA-labeled dopamine. In Fig. 2, electropherograms a–c show typical CE electropherograms for the NDA-labeled dopamine standard obtained by the MEKC (electropherogram a), sweeping-MEKC (electropherogram b) and methanol plug assisted sweeping-MEKC (electropherogram c) modes, respectively. The total length and effective length of the capillary were 100 and 93 cm. Herein, the CE conditions for MEKC consisted of 50 mM SDS and 30 mM H_3PO_4 in water. The test concentration and sample injection length were 5×10^{-5} M and ~1.1 mm, respectively. For the sweeping-MEKC method, the background solution consisted of 100 mM SDS and 30 mM H_3PO_4 in a mixed acetonitrile–water solution (15:85, v/v), the pH of which was 1.5. NDA-labeled dopamine was dissolved in the same solution (without SDS) resulting in a non-micelle buffer. Hydrodynamic injection was achieved by raising the sample reservoir to a height of 40 cm rela-

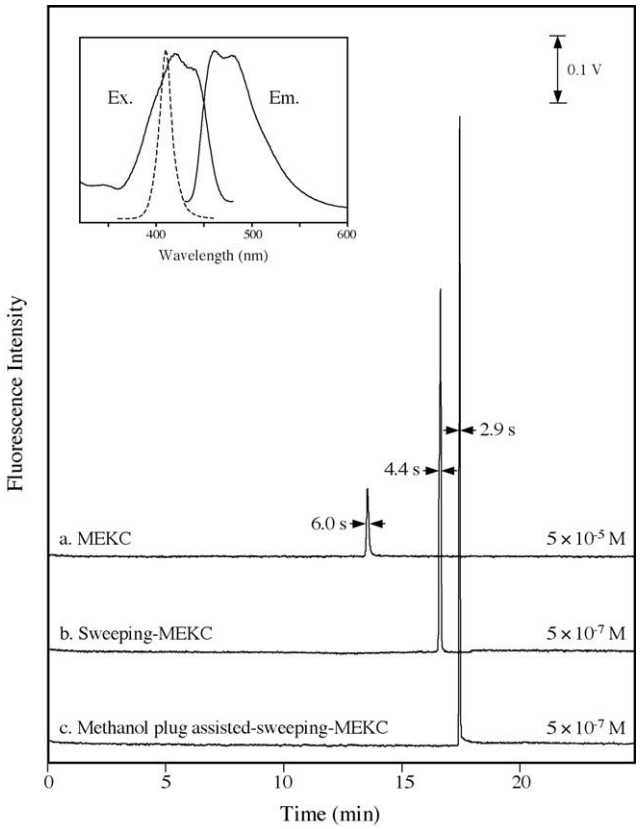

Fig. 2. CE electropherograms obtained using different separation modes (electropherograms a–c; MEKC, sweeping-MEKC and methanol plug assisted sweeping-MEKC; test concentration, 5×10^{-5}, 5×10^{-7} and 5×10^{-5} M, respectively). CE conditions: MEKC, 50 mM SDS and 30 mM H_3PO_4 in a mixed acetonitrile–water solution (15:85, v/v); the sweeping-MEKC, background solution consisted of 100 mM SDS and 30 mM H_3PO_4 in a mixed acetonitrile–water solution (15:85, v/v), sample matrix, same solution (without SDS) resulting in a non-micelle buffer; methanol plug assisted sweeping-MEKC, same as the sweeping-MEKC mode, but a 1 mm in length of methanol plug was injected between the sample matrix and the background solution. The injected length for each was 1.1, 326 and 490 mm, respectively. The inset shows the excitation and fluorescence spectra of NDA-labeled dopamine; the dashed line shows the wavelength range of the violet LED used.

tive to the exit reservoir, thus generating an injection length of 0.344 mm/s (for a normal 50 μm i.d. fused silica capillary), where the effects of temperature, atmospheric pressure and solution viscosity were neglected. The test concentration and injected length were 5×10^{-7} M and 326.6 mm, respectively. The experimental conditions for the methanol plug assisted sweeping-MEKC mode were the same as the sweeping-MEKC mode, in addition to the injection to a 1 mm in length of methanol plug (hydrodynamic injection was achieved by raising the methanol reservoir to a height of 40 cm for 3 s) between the sample matrix and the background solution. The test concentration and injection length were 5×10^{-7} M and 489 mm, respectively. As a result, the signal intensity (*V*)/theoretical plate number (*N*)/time of the peak pass through the detector for these detected peaks (electropherograms a–c) correspond to 0.11, 0.56 and 0.98 V (background signal, ∼5 mV)/100,708, 274,950 and 836,225/6.0, 4.4 and 2.9 s, respectively. This suggests that, with the assistance of a methanol plug, this method provided an improved sensitivity and was a benefit for high efficiency separation.

In order to investigate the results obtained from different methanol plug lengths corresponding signal intensity and separation efficiency, various lengths (1.1, 2.4 and 4.0 mm) of methanol plug, under exactly the same experimental conditions, were examined and these results are shown in Fig. 3 (electropherograms a–c). The findings show that a longer methanol plug did not lead to a better result; the intensity of the detected peaks also was decreased. This suggests that a long methanol plug would destroy the micelles completely and that separation efficiency would not be improved. Thus, we selected a 1 mm methanol plug in subsequent experiments. Table 1 summarizes these results as well as the calibration curve, coefficient of correlation, detection range, limit of detection (LOD) values (at a 92.1% confidence level) and the number of theoretical plates for NDA-labeled dopamine by the normal MEKC, the sweeping-MEKC and the methanol plug assisted sweeping-MEKC modes, respectively, for the above experiments.

In order to compare the two separation modes (sweeping-MEKC and the methanol plug assisted sweeping-MEKC)

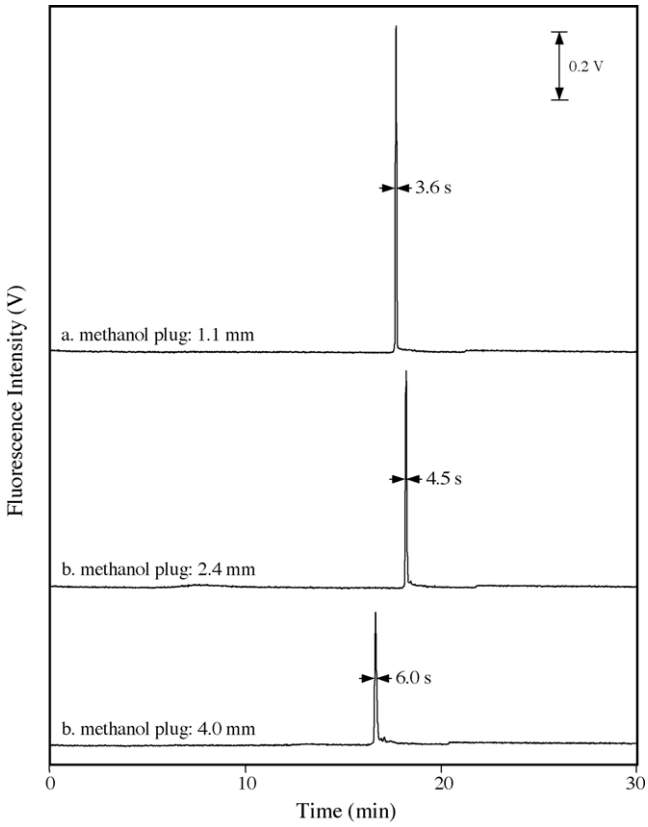

Fig. 3. CE electropherograms obtained using different methanol injection lengths (electropherograms, a–c; methanol injected lengths, 1.1, 2.4 and 4.0 mm.

Table 1
Sample injected length, calibration curve, coefficient of correlation, detection range, limit of detection (LOD) values (at a 92.1% confidence level) and the number of theoretical plates for NDA-labeled dopamine by normal MEKC, sweeping-MEKC and methanol plug assisted sweeping-MEKC modes

(A) Normal MEKC	
Sample injected length	1.1 mm
Equation of the line	$y = 48380x - 14814$
Coefficient of correlation	$r^2 = 0.9989$
Detection range	5.0×10^{-5} to 0.0 M
LOD	9.1×10^{-7} M
Plate number (N)	100,708–188,974
(B) Sweeping-MEKC	
Sample injected length	326.6 mm
Equation of the line	$y = 15746x + 2901$
Coefficient of correlation	$r^2 = 0.9984$
Detection range	5.0×10^{-7} to 0.0 M
LOD	1.2×10^{-8} M
Plate number (N)	123,919–275,995
(C) Methanol plug assisted sweeping-MEKC	
Sample injected length	489.0 mm
Methanol plug length	1 mm
Equation of the line	$y = 24551x + 20970$
Coefficient of correlation	$r^2 = 0.9997$
Detection range	5.0×10^{-7} to 0.0 M
LOD	4.7×10^{-9} M
Plate number (N)	833,119–1,100,503

Capillary: total length/effective length = 100/95 cm; i.d.: 50 μm. Exciting source: violet LED (peak emission wavelength, 410 ± 7 nm; power, ~2 mW). Applied voltage: −20 kV.

the corresponding the peak height (signal intensity, in mV), peak area (arbitrary unit) and theoretical plate number (N), respectively, various sample injected lengths (163.3, 245.0, 326.6 and 408.3 mm for sweeping-MEKC; 326.6, 408.3, 490.0 and 571.6 mm for the methanol plug assisted sweeping-MEKC) were examined and these results are summarized in Fig. 4 (frames A–C, respectively). In these experiments, the test concentration was 5×10^{-7} M. For the sweeping-MEKC mode, the signal intensity (frame A, solid line) and peak area (frame B, solid line) were improved when a longer sample matrix was injected, whereas the theoretical plate number was decreased (frame C, solid line). In contrast to this, when a methanol plug was used, a further longer sample injection became possible. As a result, the signal intensity was further improved although it still has limitations (frame A, dashed line). It should be noted that the peak area did not increase when the sample injection was longer (frame B, dashed line). It is possible that the analytes (dopamine–NDA) were tightly focused in a narrow zone where the molecular density was too high to support the phenomenon of fluorescence self-quenching. The theoretical plate number was clearly increased (frame C, dashed line) and this is useful for the analysis of a complicated actual sample.

For the determination of dopamine in urine, we selected dopamine and norepinephrine for initial comparison because they would need to be separated in an actual analysis. In

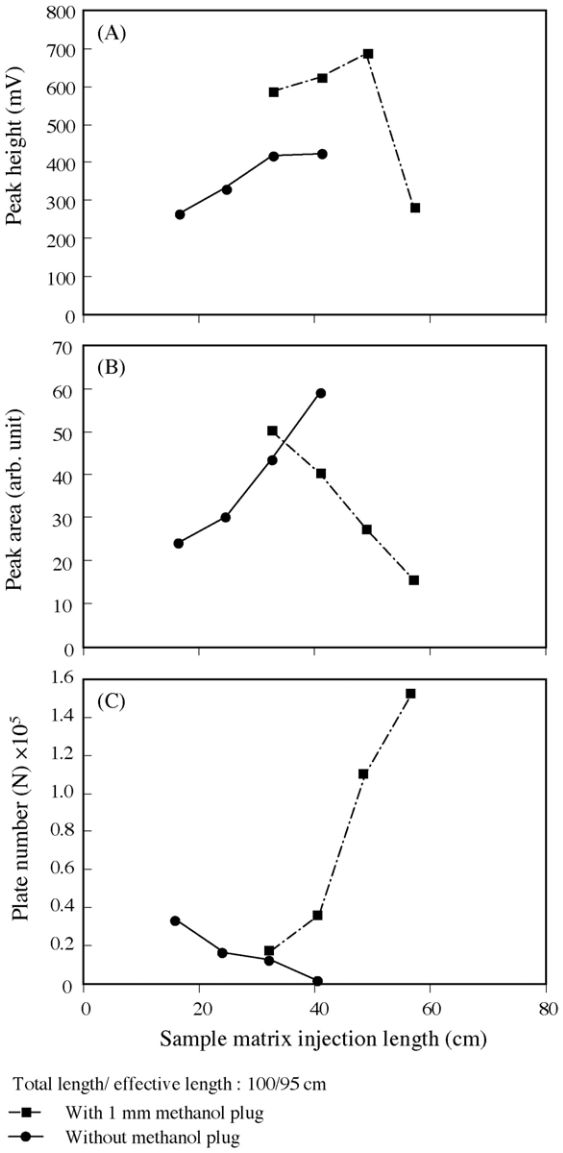

Fig. 4. The sweeping-MEKC and the methanol plug assisted sweeping-MEKC modes corresponding the peak height (signal intensity, in mV), peak area (arbitrary unit) and/theoretical plate number (N), respectively, various sample injected lengths (163.3, 245.0, 326.6 and 408.3 mm for sweeping-MEKC; 326.6, 408.3, 490.0 and 571.6 mm for methanol plug assisted sweeping-MEKC).

Fig. 5, electropherograms a and b show typical CE separation results of dopamine–NDA and norepinephrine–NDA standards (peaks 1 and 2) obtained by the sweeping-MEKC (electropherogram a) and the methanol plug assisted sweeping-MEKC (electropherogram b) modes, respectively. These findings show that the methanol plug assisted method provides a superior separation efficiency.

Fig. 6 shows the results obtained from the actual urine samples (frame A, by means of sweeping-MEKC; frame B, methanol plug assisted sweeping-MEKC). In frame A, electropherogram a shows a typical CE electropherogram of urine extract I after NDA labeling by the normal sweeping-MEKC

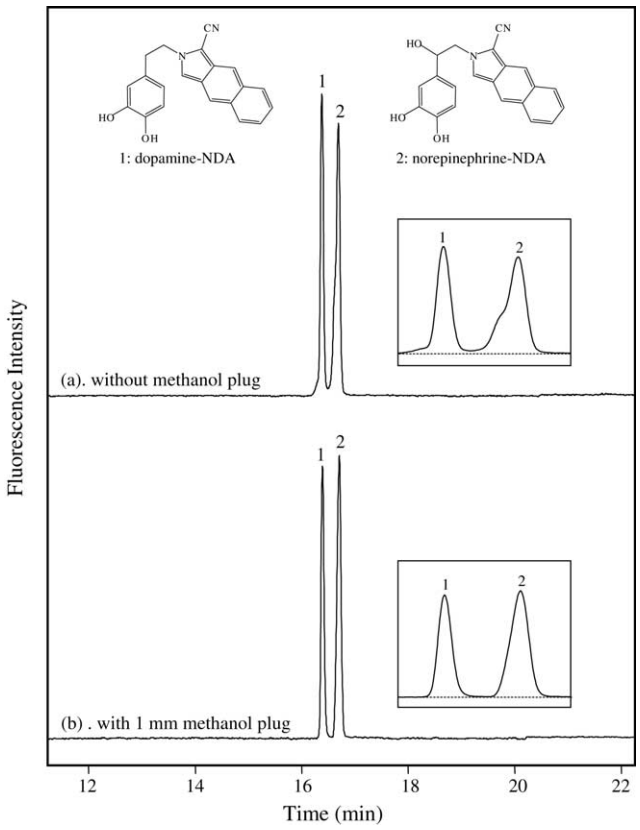

Fig. 5. Electropherograms a and b, typical CE separation results for the dopamine–NDA and norepinephrine–NDA standards (peaks 1 and 2) obtained by the sweeping-MEKC (electropherogram a) and methanol plug assisted sweeping-MEKC (electropherogram b) modes, respectively. The inset shows the chemical structures of dopamine–NDA and norepinephrine–NDA standards (peaks, 1 and 2), respectively.

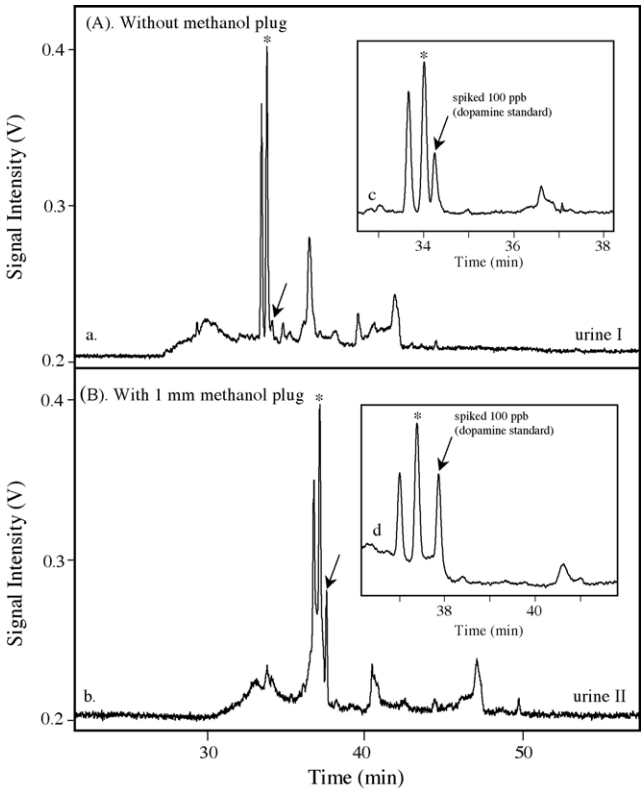

Fig. 6. CE electropherograms of urine I and II extracts obtained using the sweeping-MEKC (frame A) and methanol plug assisted sweeping-MEKC (frame B), respectively. The CE conditions were similar to those described earlier, but the solvent was adjusted to a mixed acetonitrile–water–methanol solution (15:65:25, v/v/v). Insets in frames A and B, comparison by standard addition method by spiking 100 ppb dopamine standard before solid-phase extraction, respectively.

mode. The qualitative and quantitative analysis of dopamine in urine could be achieved by a comparison of the migration time and the peak area. The CE conditions were similar to those described earlier, but the solvent was adjusted to a mixed acetonitrile–water–methanol solution (15:60:25, v/v/v). The peak with a 34.13 min migration time (arrow) is assigned to dopamine. With a standard addition method (100 ppb dopamine was spiked before the extraction and the NDA labeling), the results were compared and the findings show that the peak (arrow in electropherogram a in the inset, in frame A) has clearly increased when compared with the peak marked by an arrow in electropherogram c. The concentration was determined to be 2.3×10^{-7} M (35 ppb). Using the same experimental procedures, the electropherogram b (in frame B) shows the results obtained for the urine extract II. We assigned this peak (arrow in electropherogram d) to dopamine and its concentration was determined to be 9.0×10^{-7} M (137 ppb). For a comparison of the separation efficiency, an indicate peak (marked with an asterisk in frames A and B) was selected for evaluation. As shown in electropherograms c and d, the methanol assisted method provided better separation efficiency. The methanol plug assisted method as well as sweeping-MEKC provides sufficient sensitivity and separation efficiency for the detection of low concentration level of dopamine in urine.

4. Conclusions

This work represents the first use of a methanol plug in a sweeping-MEKC separation. With the assistance of a methanol plug, the injection length can be longer and the separation efficiency can also be improved because it easily tolerates a large volume sample injection, compared to a normal sweeping-MEKC mode and it could also be useful for other types of on-line sample concentration techniques, such as the pH junction-sweeping mode or cation-selective exhaustive injection-sweep-micellar electrokinetic chromatography (CSEI-sweep-MEKC), where a large volume sample injection may be needed.

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