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Comparison of the use of single capillaries and coupled capillaries based on micellar electrokinetic chromatography (MEKC) and sweeping-MEKC modes

The use of single capillaries (25 and 50 μm inner diameter (ID)) and coupled capillaries of different diameters (100–50 and 75–25 μm ID) based on micellar electrokinetic chromatography (MEKC) and sweeping-MEKC modes is compared and reported. Naphthalene-2,3-dicarboxaldehyde (NDA)-derivatized dopamine was selected as the model compound by examining the fluorescence intensity when a violet (410 ± 7 nm, ~2 mW) light-emitting-diode (LED) was used as the light source. When a single capillary (50 μm ID) was used, the detection limit for NDA-derivatized dopamine was determined to be 2.0×10^{-7} M (Signal-to-nose ratio S/N = 3) based on the MEKC mode. This was improved to 4.0×10^{-9} M when the sweeping-MEKC mode was applied. In addition, this can be further improved to 1.0×10^{-9} M and 5.6×10^{-10} M when 100–50 and 75–25 μm ID coupled capillaries are used. The use of the coupled capillary is also helpful for improving the separation efficiency. Based on the sweeping-MEKC mode, the number of theoretical plates (*N*) for the detected peaks were determined to be $6.3 \pm 2.7 \times 10^5$ by means of a single capillary (50 μm ID). This can be improved to $9.4 \pm 3.6 \times 10^5$ and $9.4 \pm 0.9 \times 10^6$ when the 100–50 and 75–25 μm ID coupled capillaries were applied.

Keywords: Capillary electrophoresis / Coupled capillary / Dopamine / Micellar electrokinetic chromatography / Sweeping / Violet light-emitting-diode DOI 10.1002/elps.200410189

1 Introduction

Capillary electrophoresis (CE) has developed into a versatile and powerful tool in the field of separation science, based on the pioneering work of Jorgenson in 1981 [1]. In general, a capillary with larger inside diameter provides better sensitivity in detection but poorer separation efficiency, whereas a capillary with narrower inside diameter gives higher separation efficiency but has a poorer detection limit. Although the selection of a suitable capillary depends on the experimental conditions, as well as analyzer requirements, it leaves something to be desired: a highly sensitive method by injecting large sample and a highly efficient method for separation. For this reason, several instrumental approaches have been examined to achieve a large sample injection volume, since in the Seventies and Eighties coupled plastic capillaries and capillary troughs with rectangular cross sections were used [2–5]. In capillary isotachophoresis, the column-coupling approach also continues to be employed for trace analysis [3, 6–8]. With the same attempt to improve the limit of detection (LOD), a series of reports on on-line sample concentration techniques recently appeared, concerning the so-called “stacking” and “sweeping” technique [9–21]. Using these techniques, a dramatic increase in sensitivity can be obtained.

In this study, we investigated the use of coupled capillaries of different diameters applying the MEKC as well as sweeping-MEKC modes, respectively. Naphthalene-2,3-dicarboxaldehyde (NDA)-derivatized dopamine (NDA) was selected as the model compound because it represents a continuation of our previous research [22]. An inexpensive violet light-emitting diode (LED) was used as light source (instead of a laser). The results obtained with two types of single capillaries (25 and 50 μm ID) and coupled capillaries (100–50 and 75–25 μm ID) are reported and compared. Several electrophoretic parameters, such as SDS concentration, applied voltage, and injection length required for the sample concentration and separation, were optimized and reported herein.

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Abbreviations: LED, light emitting diode; **NDA**, naphthalene-2,3-dicarboxaldehyde

2 Materials and methods

2.1 Apparatus

Figure 1 shows schematic diagrams of a single capillary (A) and a coupled capillary (B) used in the CE separations, respectively. Two coupled capillaries (100–50 μm ID and 75–25 μm ID) were made by directly connecting the different diameters of fused-silica capillaries (J&W Scientific, Folsom, CA, USA), modified from the original literature description [8], by means of a polyethylene tubing. The polyethylene tubing, which was cut from the insulation part of a BNC (Bayonet Neill Concelman) coaxial cable, was heated to melting and pulled to an adequate size for connecting. Hydrodynamic injection was achieved by raising the sample reservoir to a height relative to the exit reservoir; for a 75–25 μm ID coupled capillary additional pressure is necessary. Since the flow rate can be used for the calculation of the injected volume, two additional "flow-rate measuring windows" were made, as shown in Fig. 1. The CE setup was fabricated in-house and is similar to that described previously [22, 23]. Briefly, a high-voltage power supply (Model RR30-2R, 0-30 kV, 0-2 mA; Gamma, Ormond Beach, FL, USA) was used to drive the electrophoresis. A violet LED (InGaN; Type No. M053UVC; Monarchal Electronics, Taiwan; operating current, 20 mA; viewing angle, 2 $\theta_{1/2}$ = 30°; peak emission wavelength, 410 nm; spectral half width, 15 nm; luminous intensity, 300 mcd) was used as the light source not only for flow-rate measuring when various capillaries were used, but also for the fluorescence detection of NDA-derivatized dopamine. 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 cut filter and slit, focused by a second microscope eyepiece (10 ×), and then detected by a photomultiplier tube (Hamamatsu-R928, Bridgewater, NJ, USA). The analog signal was converted to a digital signal by an A/D converter (ADAM-4012 module; Advantech, Taipei, Taiwan). Electropherograms were collected with a data acquisition system connected to a personal computer.

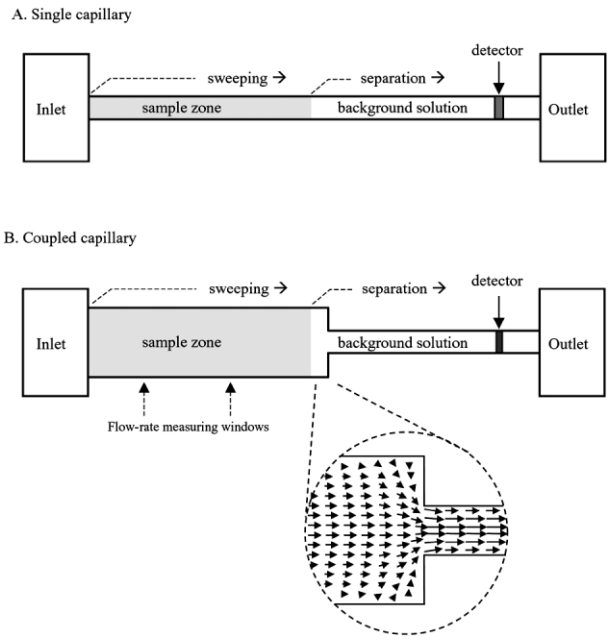

Figure 1. Schematic diagrams of (A) a single capillary (uniform size for inside diameter), and (B) a coupled capillary (connecting by different diameter of capillary) used in the CE separation, respectively.

2.2 Reagents

All chemicals used were of analytical grade. Dopamine ($C_8H_{11}NO_2$) and NDA were purchased from Sigma (St. Louis, MO, USA). SDS, sodium tetraborate, methanol, and phosphoric acid were purchased from Acros (Geel, Belgium).

2.3 Derivatization procedure of NDA-derivatized dopamine

The derivatization procedure was modified from the original literature description [24]. To 1.0 mL solution containing 0.7 mL aqueous sodium tetraborate buffer (0.1 M, pH 9) was added 0.1 mL dopamine (10^{-3} M in MeOH) and the same volume of KCN (10^{-3} M in a tetraborate aqueous buffer). The reaction was initiated by the addition of 0.1 mL 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 for the subsequent CE separation.

3 Results and discussion

Figure 2 shows typical CE electropherograms of the NDA-derivatized dopamine standard obtained by the sweeping-MEKC mode using different capillaries (electropherogram a: single capillary, 50 μm ID; electropherogram b: coupled capillary, 100–50 μm ID). In the case of a single capillary (electropherogram a), the background solution (BGS) consisted of 100 mM SDS and 30 mM H_3PO_4 in a mixed acetonitrile-water solution (15:85 v/v), pH 1.5. NDA-derivatized dopamine (1.0×10^{-7} M) was dissolved in the same solution (without SDS) resulting in a nonmicelle buffer. When the injection was completed, a negative charge (high voltage, −15 kV; currents, −21 to −30 μA) power

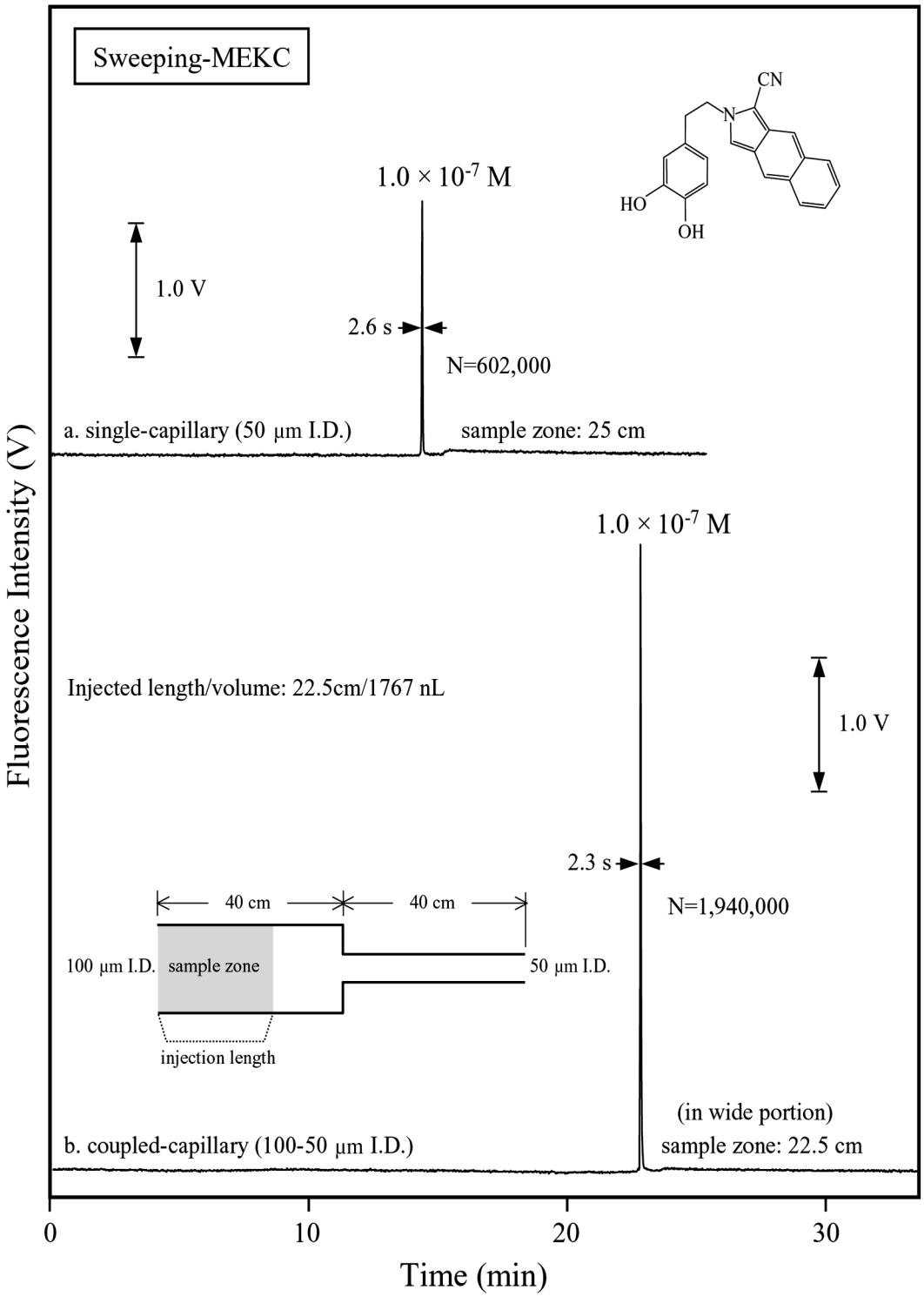

Figure 2. CE electropherograms of the NDA-derivatized dopamine standard obtained by the sweeping-MEKC mode using different capillaries. (a) Single capillary, 50 μm ID; (b) coupled capillary, 100–50 μm ID. CE conditions: (a) BGS, 100 mM SDS and 30 mM H_3PO_4 in a mixed acetonitrile-water solution (15:85 v/v); pH 1.5; applied voltage, −15 kV; current, −21 to −30 μA; sample injection length, 25 cm. (b) BGS, 120 mM SDS and 30 mM H_3PO_4 in a mixed acetonitrile-water solution (15:85 v/v); pH 1.5; applied voltage, −11 kV; current, −38 to −39 μA; sample injection length, 22.5 cm. Sample concentration, both 1.0×10^{-7} M.

supply was used for the CE separation. This procedure permits the SDS-anionic surfactant micelles (in the inlet reservoir) to enter the sample zone. Thus, along the capillary axis, the samples were swept and concentrated near the junction between the sample solution and the BGS. As in the following step, the samples were separated by the MEKC mode. In the case of the coupled capillary (electropherogram b), the procedure used was similar to that of the single capillary, but the applied voltage and SDS concentration were changed to −11 kV (resulting current: −38 to −39 μA) and 120 mM, respectively. As a result, the detected peak by means of the coupled capillary was much better than that of a single capillary. The limit of detection and theoretical plate number (*N*) were both improved from 4.0×10^{-9} M to 1.0×10^{-9} M (S/N = 3) and from $6.3 \pm 2.7 \times 10^5$ to $9.4 \pm 3.6 \times 10^5$, respectively.

It should be noted that, when a capillary consisting of two portions (wide portion: lower field strength; narrow portion: higher field strength) with 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 a strong acidic condition was employed, the EOF should be extremely small (if it exists at all, the EOF should migrate in the reverse direction, toward the inlet); the SDS micelles were migrating 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 were suddenly flowing into a narrow portion of the narrow capillary (from 100 to 50 μm ID), this process permits the SDS analytes to further collect around the boundary (as described in Fig. 1). As a result, a sharper peak can be obtained.

In order to investigate the effects of sample injection length and the corresponding signal intensity when the coupled capillary (100–50 μm ID) and sweeping-MEKC method were applied simultaneously under exactly the same experimental conditions as described in Fig. 2 (electropherogram b), several different sample injection lengths (25, 22.5, 20, 15, and 10 cm in the wide portion) were examined (Fig. 3a–e). The signal intensity (V)/the theoretical plate number (*N*) of these detected peaks correspond to 2.26, 4.50, 2.50, 0.75, and 0.34 V (background signal, ~0.45 V)/6.5×10^5, 1.5×10^6, 1.4×10^6, 3.8×10^5, and 4.0×10^5, respectively. In comparison with electropherograms a and b (25 and 22.5 cm sample injection), the longer injection length did not provide a larger peak and this would cause problems when a quantitative analysis is needed. The reason for this could be interactions between the sample molecules and the SDS micelles; if the number of sample molecules were beyond the ability of SDS micelles to collect them, then sample concentration would not be complete. In this experiment, the optimal sample injection length was found to be 22.5 cm.

Frame I in the inset of Fig. 3 shows the relationship between the sample injection length (cm) and the corresponding peak height (intensity, in voltage) when the sweeping-MEKC mode was applied. Basically the intensities of the peaks increase nonlinearly with the injection length (from 10 to 22.5 cm in injection length in the wide portion). The reason for this could be due to the fact that, when a large volume is injected, the micelle would be able to associate with more analyte molecules. As a result, the improvement was nonlinear. Another possibility is that a "stacking" phenomenon occurred as the sweeping progressed, since the EOF is weak but not equal to zero. Frame II in the inset of Fig. 3, shows the relationship between the injection length (cm) and the theoretical plate number (*N*). The plate numbers increase significantly when the sample injection length is over 15 cm, but this is decreased when the length is over 22.5 cm. Thus, we selected a 22.5 cm injection length for the investigation of the limit of detection.

Figure 4 shows the results obtained under the optimal conditions described in Fig. 3 (electropherogram b, injection length: 22.5 cm in the wide portion) at various concentrations of analytes (electropherograms a–d; 1.0×10^{-7}, 5.0×10^{-8}, 1.0×10^{-8}, and 2.0×10^{-9} M, respectively). The inset shows a calibration curve (concentration range, 1.0×10^{-7} –2.0×10^{-9} M). At an S/N = 3, the limit of detection corresponds to 1.0×10^{-9} M (0.32 ppb). Using these conditions, the injected volume (nL), detected concentration range, equation of the calibration line, coefficients of variation, limit of detection (LOD) values, relative standard deviations (RSDs%) of peak area/migration times and plate numbers (*N*) for NDA-derivatized dopamine by the MEKC and sweeping-MEKC modes, respectively, are summarized in Table 1. For comparison to the use of the coupled capillary (100–50 μm ID), the results obtained by a single capillary (50 μm ID), under MEKC and sweeping-MEKC modes are summarized in column A of Table 1. It is clear that the use of a coupled capillary not only improves the LOD but is also useful for achieving a high separation efficiency (high theoretical plate number), even when a large sample volume is injected.

In order to investigate the effect when a narrower coupled capillary is used, a similar experiment was performed to compare a single capillary (25 μm ID) and a coupled cap-

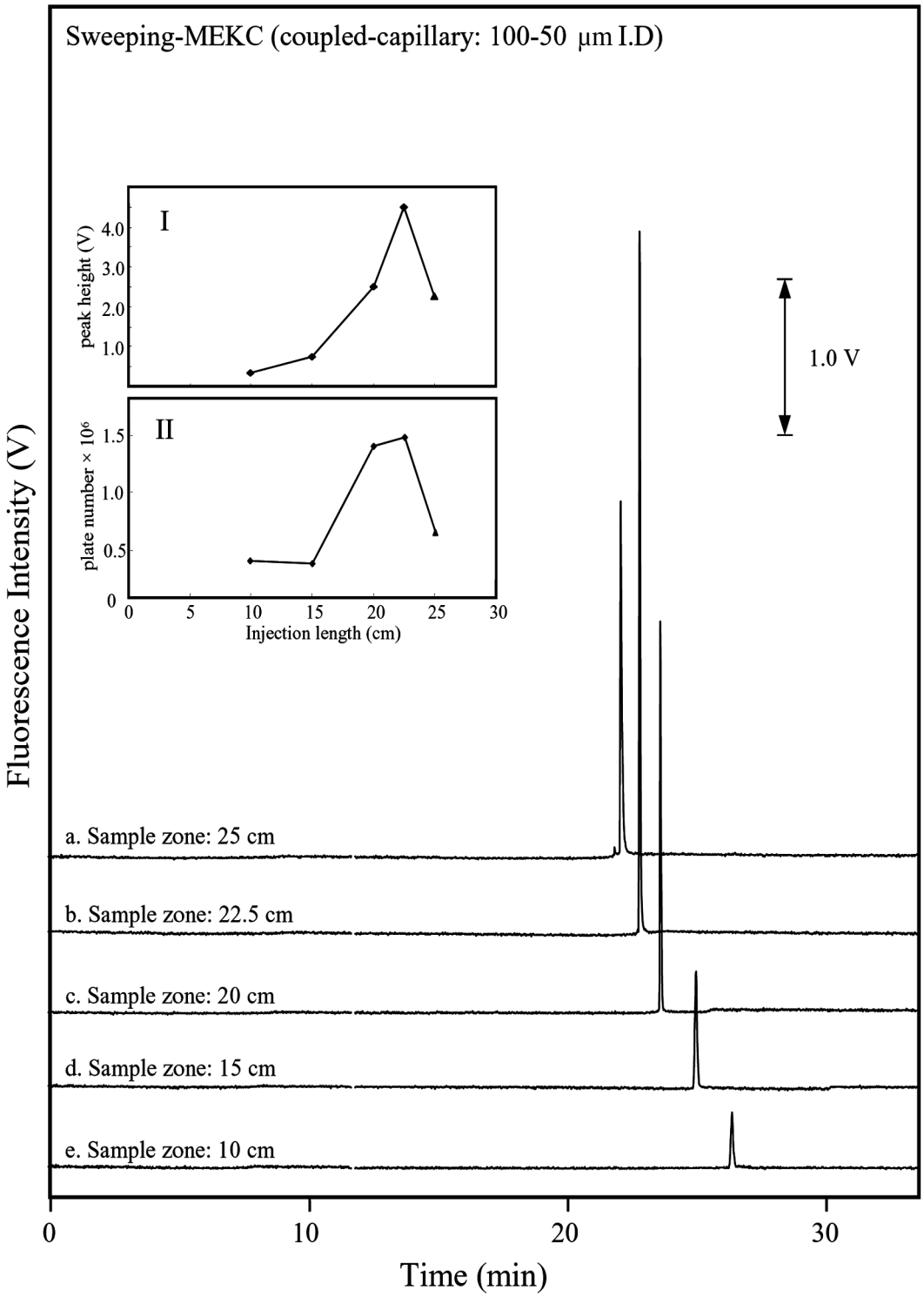

Figure 3. CE electropherograms obtained at different sample injection lengths when a coupled capillary (100–50 μm ID) was used. (a)–(e) Sample injection lengths, 25, 22.5, 20, 15, and 10 cm in the wide portion. Signal intensities (V)/theoretical plate numbers (*N*) of these detected peaks, 2.26, 4.50, 2.50, 0.75, and 0.34 V; background signal, ~0.45 V)/6.5 × 10^5, 1.5 × 10^6, 1.4 × 10^6, 3.8 × 10^5, and 4.0 × 10^5. Frames I and II show the relationship between the sample injection length (cm) and height (intensity in voltage) of the detected peaks (I), as well as the related theoretical plate numbers (II), respectively.

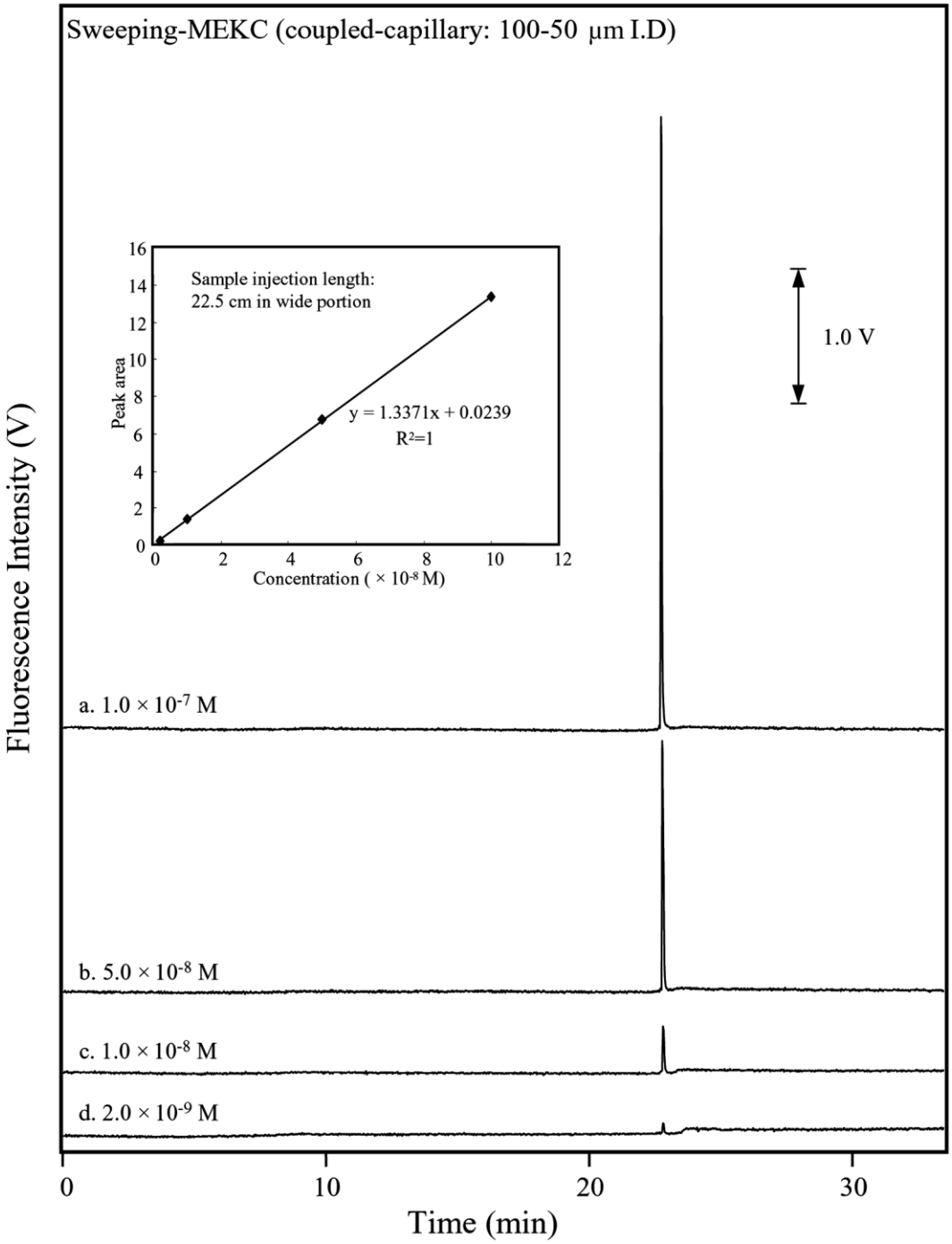

Figure 4. CE electropherograms obtained under the optimal conditions described in Fig. 3b at various concentrations of the analytes. (a)–(d) 1.0 × 10^{-7}, 5.0 × 10^{-8}, 1.0 × 10^{-8}, 2. 0 × 10^{-9} M. The inset shows a calibration curve, constructed at these concentrations.

illary (75–25 μm ID). The CE conditions were the same as described in Fig. 2. In the case of a single capillary (25 μm ID), a very high separation efficiency was obtained (1.2 ± 0.3 × 10^6). However, this can be further improved to 9.4 ± 0.9 × 10^6 when a 75–25 μm ID coupled capillary is used. In addition, since the detected peak was extremely sharp (only 1.3 s was needed for it to pass through the detection window), the detection limit (S/N = 3) was found to be 5.6 × 10^{-10} M (0.2 ppb). These data obtained by the 75–25 μm ID coupled capillary and the

Table 1. Parameters of the new technique

I. Coupled capillary

Column	A		B	
Coupled capillary	100–50 μm ID (40–40 cm)		75–25 μm ID (40–40 cm)	
Method	MEKC	Sweeping-MEKC	MEKC	Sweeping-MEKC
Injected volume	6 nL	1767 nL	1.2 nL	884 nL
Concentration range	2.0×10^{-5}–4.0×10^{-7} M	1.0×10^{-7}–2.0×10^{-9} M	1.0×10^{-4}–5.0×10^{-6} M	1.0×10^{-7}–1.0×10^{-8} M
Equation of the line	$y = 1.0141x - 0.2691$	$y = 1.3371x + 0.0239$	$y = 1.2857x + 0.3127$	$y = 0.7777x + 0.6254$
Coefficient of variation	$R^2 = 0.9999$	$R^2 = 1$	$R^2 = 0.9934$	$R^2 = 0.9789$
LOD (S/N = 3)	5.0×10^{-7} M	1.0×10^{-9} M	2.7×10^{-6} M	5.6×10^{-10} M
RSD (%); $n \geqq 2$	Intra-day Inter-day	Intra-day Inter-day	Intra-day Inter-day	Intra-day Inter-day
(a) migration time	0.9 2.9	0.6 2.3	2.6 4.2	8.6 9.7
(b) peak area	2.9 1.4	11.0 10.4	4.2 15.3	3.9 6.4
Plate number (*N*)	$4.0 \pm 0.1 \times 10^5$	$9.4 \pm 3.6 \times 10^5$	$2.0 \pm 0.3 \times 10^5$	$9.4 \pm 0.9 \times 10^6$

II. Single capillary

Column	A		B	
Single capillary	50 μm ID (80 cm)		25 μm ID (80 cm)	
Method	MEKC	Sweeping-MEKC	MEKC	Sweeping-MEKC
Injected volume	3 nL	490 nL	0.2 nL	150 nL
Concentration range	2.0×10^{-5}–4.0×10^{-7} M	2.0×10^{-7}–4.0×10^{-9} M	1.0×10^{-4}–2.0×10^{6} M	5.0×10^{-7}–1.0×10^{-8} M
Equation of the line	$y = 0.6917x - 0.0513$	$y = 0.4445x + 0.0379$	$y = 0.0841x - 0.092$	$y = 0.2405x + 0.1224$
Coefficient of variation	$R^2 = 0.9995$	$R^2 = 0.9979$	$R^2 = 1$	$R^2 = 0.9998$
LOD (S/N = 3)	2.0×10^{-7} M	4.0×10^{-9} M	2.0×10^{-6} M	5.0×10^{-9} M
RSD (%); $n \geqq 3$	Intra-day Inter-day	Intra-day Inter-day	Intra-day Inter-day	Intra-day Inter-day
(a) migration time	1.1 2.0	0.5 2.3	0.8 4.1	0.4 0.9
(b) peak area	4.7 9.0	13.4 6.4	1.4 6.8	4.8 7.0
Plate number (*N*)	$4.5 \pm 0.4 \times 10^5$	$6.3 \pm 2.7 \times 10^5$	$1.2 \pm 0.3 \times 10^6$	$1.3 \pm 0.2 \times 10^6$

Length of each capillary, 40 cm; total length/effective length, 80 cm/74 cm; exciting source, violet LED (peak emission wavelength, 410 ± 7 nm; power, ~2 mW)

single capillary (25 μm ID) are summarized in column B of Table 1, when MEKC and sweeping-MEKC modes were applied. These data again show that a coupled capillary can be very useful both for improving the LOD as well as the separation efficiency.

4 Concluding remarks

This work represents the successful application of a coupled capillary (100–50 and 75–25 μm ID) for use in large volume sample injections in CE separations. When a coupled column was used, a larger sample injection (compared to a single one) is possible. Using the sweeping-MEKC mode, the accumulated SDS analytes are still maintained as a sharper peak, passing the detection window and increasing the signal intensity. As a result, the LOD can be improved. In contrast, when a normal single capillary was used and a larger sample was injected, the accumulated SDS analytes can not be maintained as a sharp zone. Although the utility of the coupled capillary was investigated by the sweeping-MEKC method, it would be possible to extend the performance to any type of on-line sample concentration technique, such as stacking, pH junction techniques, as well as other related methods. The method is a sensitive, rapid, simple, reproducible, and economic technique, and it is also suggested that such type of capillary has a great potential for

use as a new type of capillary in the field of CE, not only for on-line sample concentration but also for other purposes, such as direct on-column derivatization or on-column complexation. Further applications could be expected.

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