Chun-Min Shih
Cheng-Huang Lin

Department of Chemistry,
National Taiwan Normal
University,
Taipei, Taiwan

Low-temperature bath/coupled-capillary/ sweeping-micellar electrokinetic capillary chromatography for the separation of naphthalene-2,3-dicarboxaldehyde-derivatized dopamine and norepinephrine

The use of a low-temperature (0°C) bath-assisted coupled capillary for the separation of naphthalene-2,3-dicarboxaldehyde (NDA)-derivatized dopamine and norepinephrine using the sweeping-micellar electrokinetic capillary chromatography (MEKC) mode is described. In this technique, a capillary consisting of two portions with different inside diameters is used. Therefore, the field strength inside the capillary is different. Hence, the electrophoretic migration velocities of the analytes and the electroosmotic flow (EOF) also are different. Furthermore, when a portion of the capillary (wide portion, used for sweeping) is immersed in a low-temperature bath, the viscosity of the buffer and the retention factor of the analytes inside are increased. Thus, not only are the interactions between the SDS micelles and the analytes increased, but the SDS-analytes also move more slowly. As a result, a more complete separation can be achieved, even when the sample injection volume is large, up to ~ 2 μL. In general, when the volume of an injected sample is larger, the effects of sweeping and separation would become insufficient, especially when the retention values (*k*) of the analytes are quite different. However, this limitation can be improved when the low-temperature bath/coupled capillary/sweeping-MEKC mode is used.

Keywords: Coupled capillary / Dopamine / Micellar electrokinetic capillary chromatography / Norepinephrine / Sweeping / Violet light-emitting-diode DOI 10.1002/elps.200510409

1 Introduction

In attempts to improve the limit of detection (LOD), a series of reports on on-line sample concentration techniques have recently appeared, concerning the so-called "stacking" and "sweeping" techniques [1–14]. When such techniques are used, a dramatic increase in sensitivity can be obtained. In general, most of these techniques were developed to accommodate a large volume of sample injection, since the LOD is proportional to the amount of sample injected. Unfortunately, the LOD cannot be improved by simply increasing the length of the sample zone, because individual electrophoretic parameters, such as buffer conductivities, pH values, the magnitude and direction of electroosmotic flow (EOF), the concentration of surfactants (if needed) used, the injection length of the sample solution, and even the polarity of the electrode must be optimized. A specific buffer is frequently suitable for one of the above parameters, but it may cause problems relative to the others. This is because each analyte has its own physical and chemical characteristics (such as solute pK_a, affinity for the pseudostationary phase, hydrophobicity or hydrophilicity in aqueous, *etc.*), making it difficult to predict and rationalize the peak shape when on-line sample concentration techniques are used. On the other hand, if a larger volume of sample solution is injected, the remaining portion of the capillary becomes shorter, leading to an incomplete separation. Thus, it is difficult to decide if sensitivity is important, as opposed to selectivity, since either case has its merits and demerits. In a previous research project, we reported on the use of two types of coupled capillaries to increase the sample injection volume [15]. We were surprised to find that the accumulated SDS-analytes are still maintained as a sharper peak (theoretical plate number, $\sim 1 \times 10^6$), even when a larger sample volume (1.8 μL) is injected in the case of a coupled capillary (100–50 μm ID). In contrast, when a normal single capillary (50 μm ID) was

Correspondence: Dr. Cheng-Huang Lin, Department of Chemistry, National Taiwan Normal University, Taipei, Taiwan
E-mail: chenglin@cc.ntnu.edu.tw
Fax: +886-2-2932-4249

Abbreviations: DOSS, dioctyl sulfosccinate, **NDA**, naphthalene-2,3-dicarboxaldehyde; **NDA-Dop**, NDA-derivatized dopamine; **NDA-Nor**, NDA-derivatized norepinephrine

used for a larger sample injection, the accumulated SDS-analytes could not be maintained as a sharper zone. In this study, in a continuation of our investigations of the separation of catecholamines, naphthalene-2,3-dicarboxaldehyde (NDA)-derivatized-dopamine (NDA-Dop) and -norepinephrine (NDA-Nor) were examined, using the coupled-capillary/sweeping-MEKC mode. A low-temperature bath (LTB) was introduced to improve the separation of the two analytes. Several electrophoretic parameters, such as SDS concentration, applied voltage, and the injection length required for sample concentration and separation, were optimized and these data are also reported herein.

2 Materials and methods

2.1 Apparatus

The CE setup was fabricated in-house and is similar to that described previously [15]. Figure 1 shows schematic diagrams of a single capillary (A) and an LTB-assisted coupled capillary (B) used in the CE separations, respectively. The coupled capillary (100–50 μm ID) was prepared by directly connecting the different diameters of fused-silica capillaries (J&W Scientific, Folsom, CA, USA), modified from the original literature description [16] by means of a section of 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 appropriate size for connecting. Hydrodynamic injection was achieved by raising the sample reservoir to a height relative to the exit reservoir. The LTB was an insulated container (diameter, 12 cm; height, 15 cm), used as a temperature controller *via* mixtures of ice/rock salt [17].

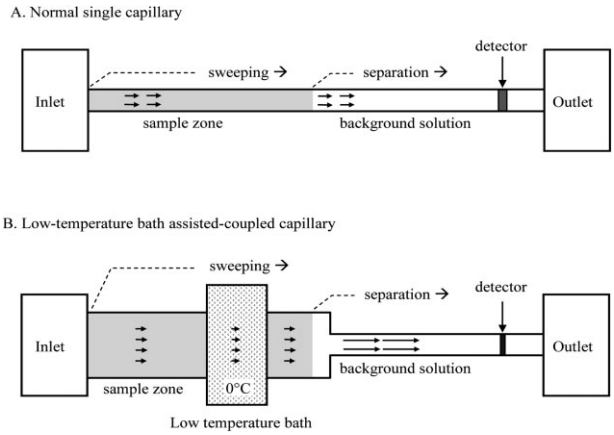

Figure 1. Schematic diagrams of (A) a single capillary (uniform size of inside diameter), and (B) an LTB/coupled capillary (connected by different diameters of capillary) used in a sweeping-MEKC separation, respectively.

2.2 Reagents

All chemicals used were of analytical grade. Dopamine, norepinephrine, and NDA were purchased from Sigma (St. Louis, MO, USA). SDS, dioctyl sulfosccinate (DOSS), sodium tetraborate, methanol, and phosphoric acid were purchased from Acros (Geel, Belgium).

2.3 Derivatization procedure of NDA-derivatized dopamine and norepinephrine

The derivatization procedure was modified from the original literature description [18]. To 1.0 mL of a 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 of 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 separations. NDA-Nor was also prepared using the same procedure.

3 Results and discussion

3.1 Peak-broadening problem in the sweeping-MEKC mode

Figure 2 shows a typical CE electropherogram of a mixture of NDA-Dop and NDA-Nor, using the normal sweeping-MEKC. Herein, the diameter and the length of the capillary were 50 μm ID and 80 cm, respectively. It was found that when the sample zone is shorter (as shown in the inset, sample zone is 15 cm), the separation appeared to be good. However, when the length of the injected sample zone was increased, the shapes of the peaks were altered. For the accumulated SDS-NDA-Dop, this alteration is minor; it is still maintained as a sharper peak (peak width, 2.3–2.4 s when the sample zone was increased from 15 to 30 cm, respectively). However, for the other sample, the change was substantial; the peak was totally disintegrated (peak width, 4.8–~20 s; sample zone, 15–30 cm).

Based on the sweeping mechanism, when two analytes are present, the swept zone with the higher retention factor (*k*) analyte is narrower than the lower one, where *k* can be expressed as:

$k = K\phi$

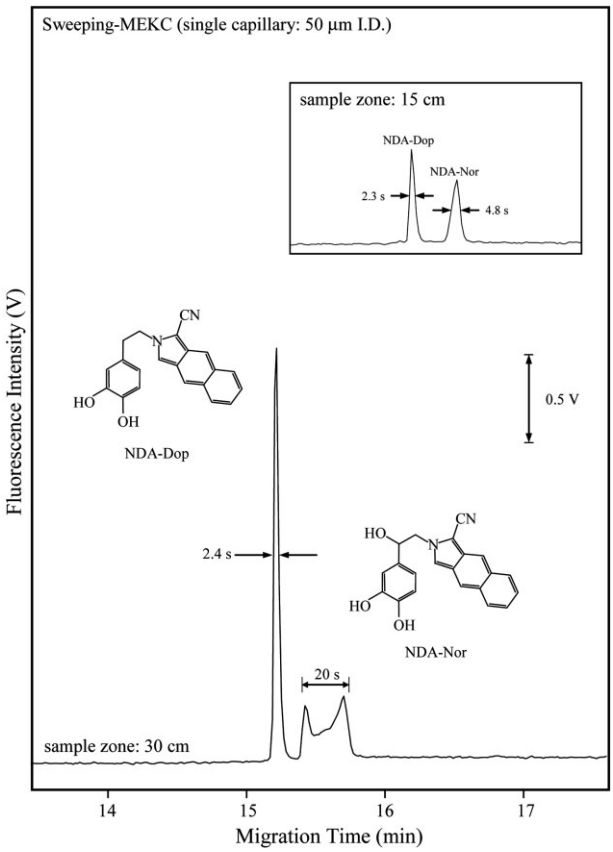

Figure 2. CE electropherogram of NDA-Dop (peak 1) and NDA-Nor (peak 2) obtained by the sweeping-MEKC mode using a normal capillary (50 μm ID; total/effective length, 80/74 cm) CE conditions: background solution, 100 mM SDS and 30 mM H_3PO_4 in a mixed acetonitrile-water solution (15:85 v/v); pH 1.5; applied voltage, −11 kV; current, −30 μA; sample injected length, 30 cm (the inset, 15 cm). Sample concentration, both 1.0×10^{-7} M.

Herein, *K* is the partition or distribution coefficient (concentration of the solute in the pseudostationary phase (PS)/concentration of the solute in the surrounding liquid phase) and ϕ is the phase ratio (volume of the PS/volume of the surrounding liquid phase) [11]. In general, the greater the affinity of the solute (in this case, the hydrophobicity of NDA-Dop is greater than NDA-Nor) for the PS (in this case, SDS) leads to a high *K*; the greater the volume of PS (for example, a higher concentration of SDS) produces a higher ϕ. Both values of *K* and ϕ would affect the *k* value and the effect of sweeping. In this case, the *k* value of NDA-Dop appears to be much greater than that of NDA-Nor in this buffer system (100 mM SDS; 15% ACN in water, pH, 1.5). This could be the reason for why NDA-Dop is swept as a sharper peak, but NDA-Nor is not. Once the analytes ($k_{NDA\text{-}Dop} \gg k_{NDA\text{-}Nor}$) combine for separation using the sweeping mode, it is clear that problems would arise. (This occurrence may not be seen when the injection volume is small, as in the case of the normal MEKC mode). This problem cannot be simply resolved by altering the pH values, the ratios of organic solvent and water, SDS concentrations, applied voltages, if an injection length larger than ~ 2 μL (in length, longer than 25–30 cm to a 50 μm ID capillary) needs to be maintained. Since the *k* values of the two analytes are different, various buffers were tested to optimize the separation efficiency, including changing the pH value, prolonging the separation time, and adding methanol. None of these approaches resolved the problem.

3.2 Methods for resolving the peak-broadening problem in the sweeping-MEKC mode

3.2.1 Use of the coupled-capillary/sweeping-MEKC mode

As we demonstrated in a previous report [15], when a capillary consisting of two portions with different inside diameters is used, the field strength inside the capillary must be different. Hence, the electrophoretic migration velocities of the analytes and EOF must also be different (wide portion: analytes move slower; narrow portion: analytes move faster). In order to measure the actual speed of the analytes inside the capillaries, the following experiment was carried out. Figure 3 shows the result obtained from a coupled capillary when it was used in the normal manner (Fig. 3A: using a 100 μm portion for the inlet) and in the reversed manner (Fig. 3B: using a 50 μm portion for the inlet), respectively. The effective length was 74 cm. The CE buffer was a solution of water-acetonitrile (85:15 v/v), which contained 120 mM SDS and 30 mM phosphate buffer (pH 1.5). The test sample was NDA-Dop (2.0×10^{-5} M). The voltage used was −11 kV (current, ~ −38 μA). When the wide-to-narrow capillary configuration was used, the analytes were suddenly flowing into a narrow portion. As a result, a sharper peak was obtained, compared to the narrow-to-wide capillary configuration.

Furthermore, assuming the apparent mobility v_a ($v_a = v_{SDS\text{-}micelles} - v_{effective\ mobility\ of\ the\ analyte} - v_{EOF}$) of the analyte is v_1 (in the wide portion) and v_2 (in the narrow portion), respectively, the individual velocities can be calculated, *i.e.*, in the case of A, the migration time is 1733 s; in the case of B, the migration time is 1576 s. Since the intra-day RSD of our system is 1.1% ($n = 5$) when the MEKC mode is used, the time shifts can be neglected. Hence, two equations can be obtained: A, 40 cm/v_1 + 34 cm/v_2 = 1733 s; B, 40 cm/v_2 + 34 cm/v_1 = 1576 s. As a result, v_1 and v_2 are equal to 0.02 cm/s and 0.67 cm/s, respectively. It is clear that the apparent mobility in the wide and narrow portions are quite different (wide portion, slow

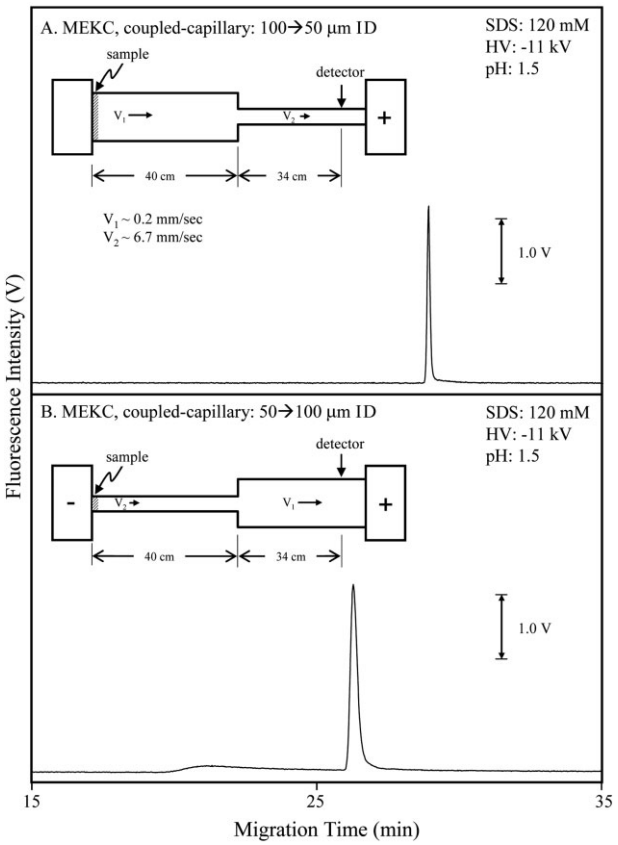

Figure 3. Normal MEKC electropherogram of NDA-Dop obtained from a coupled capillary when used in the normal configuration. (A) Using 100 μm portion for inlet; (B) in the reversed configuration, using 50 μm portion for inlet. CE buffer: solution of water-acetonitrile (85: 15 v/v), containing 30 mM phosphate buffer (pH 1.5) and 120 mM SDS. The test sample was NDA-Dop (2.0×10^{-5} M). Voltage, −11 kV, current, ~ −38 μA.

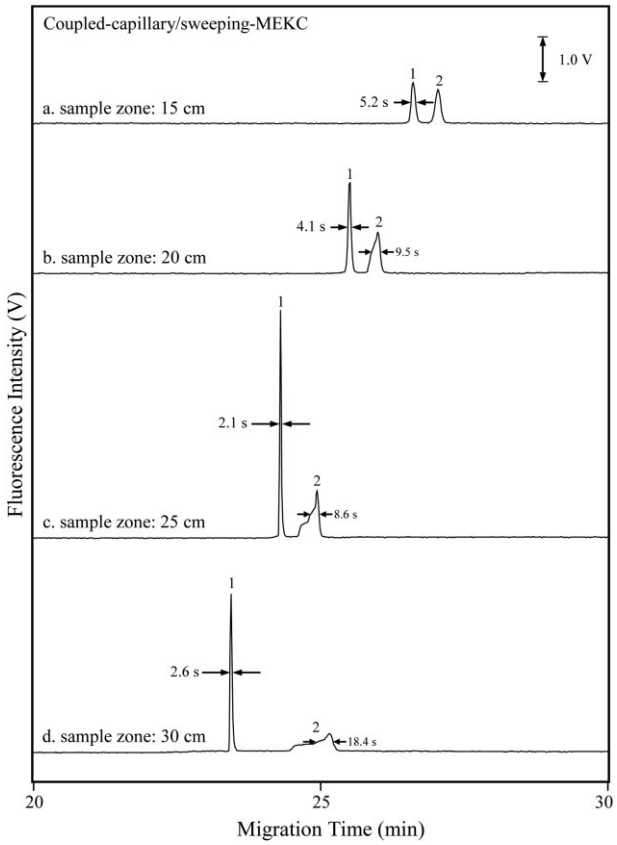

Figure 4. Sweeping-MEKC electropherograms of a mixture of NDA-Dop and NDA-Nor obtained at different sample injection lengths using a coupled capillary (100–50 μm ID). (a)–(d) Sample injected lengths, 15, 20, 25, and 30 cm in the wide portion. CE conditions as in Fig. 2.

migration; narrow portion, rapid migration), with an almost 30-fold difference. By the use of such an unequal field strength for separation, the separation efficiency could be improved (*k* values of the analytes were changed). Figure 4 shows typical CE electropherograms of a mixture of NDA-Dop and NDA-Nor when the coupled-capillary/sweeping-MEKC mode was used; electropherograms a–d show the result obtained for the different capillary lengths for sample injection (a–d; sample zone, 15, 20, 25, and 30 cm, respectively). The separations were performed under the same experimental conditions as described in Fig. 4. As shown in electropherograms a–d, the NDA-Dop peaks are maintained as a shaper zone, when the sample zones are increased from 15 to 30 cm. However, the NDA-Nor peak shows some unexpected results. The change in field strength was not useful for changing the *k* values. However, taking an optimistic view, since the field strength in the wide portion is extremely low, the NDA-Dop was gradually and slowly swept, thus resulting in a shaper peak, under the optimized injection length (found to be 25 cm). The data obtained under these conditions are summarized in the Table 1 (frame A).

3.2.2 Use of a mixed surfactant

In order to improve the separation efficiency by changing the *k* values of the two analytes, various concentration ratios of the surfactants SDS and DOSS were tested. Figure 5 shows typical CE electropherograms of a mixture of NDA-Dop and NDA-Nor when mixed surfactants were used, based on the coupled-capillary/sweeping-MEKC mode (sample zone, 25 cm). The CE conditions are the same as described in Fig. 3, beside the concentration ratios of the surfactants (electropherogram a, SDS/DOSS = 100/50 mM; electropherogram b, SDS/DOSS = 120/50 mM). The NDA-Nor peak now becomes narrower, as shown in Figs. 4c and 5b, respectively, although the NDA-Dop peak is broader (from 2.1 s to 5.1 s, as shown in

Table 1. Comparison of the use of (A) a normal coupled capillary (100–50 μm ID) and (B) the LTB-assisted (at 0°C) coupled capillary for NDA Dop and NDA-Nor by the sweeping-MEKC mode

(A) Normal coupled-capillary/sweeping-MEKC

Compound	NDA-derivatized dopamine		NDA-derivatized norepinephrine	
Concentration range	$1.0 \times 10^{-7} \sim 2.0 \times 10^{-9}$ M		n.d.	
Equation of the line	$y = 1.3371x + 0.0239$		n.d.	
Coefficient of variation	$R^2 = 1$		n.d.	
LOD (S/N = 3)	1.0×10^{-9} M		n.d.	
RSD (%); n = 3	Intra-day	Inter-day	Intra-day	Inter-day
(a) Migration time	0.6	2.3	n.d.	n.d.
(b) Peak area	11.0	10.4	n.d.	n.d.
Plate number (*N*)	$9.4 \pm 3.6 \times 10^5$		n.d.	

(B) LTB/coupled-capillary/sweeping-MEKC

Compound	NDA-derivatized dopamine		NDA-derivatized norepinephrine	
Concentration range	$1.0 \times 10^{-7} \sim 2.5 \times 10^{-9}$ M		$1.0 \times 10^{-7} \sim 2.5 \times 10^{-9}$ M	
Equation of the line	$y = 1.5966x + 0.1239$		$y = 1.5202x - 0.0577$	
Coefficient of variation	$R^2 = 0.9986$		$R^2 = 0.9997$	
LOD (S/N = 3)	7.0×10^{-10} M		1.1×10^{-9} M	
RSD (%); n = 3	Intra-day	Inter-day	Intra-day	Inter-day
(a) Migration time	1.92	3.45	1.82	3.28
(b) Peak area	3.97	8.11	2.98	3.46
Plate number (*N*)	$2.5 \pm 0.4 \times 10^6$		$1.2 \pm 0.2 \times 10^6$	

n.d., no data
The length of each piece of the capillary used was 40 cm; total length/effective length = 80 cm/74 cm
Exciting source: violet light-emitting diode (peak emission wavelength, 410 ± 7 nm; power, ~ 2 mW)
Sample zone, 25 cm (~ 1800 nL).

Figs. 4c and Fig. 5b, respectively). Thus, the use of mixed-surfactant buffers can be helpful in changing the *k* values. Electropherogram a shows that even a minor change of the ratio (SDS, 120–100 mM), led to a drastic alteration in the shape of the peaks (peak 2, in the electropherograms a and b). Optimized conditions, including the type of surfactants used and the concentration ratio, should be further investigated.

3.2.3 Use of the LTB/coupled-capillary/sweeping-MEKC mode

Figure 6 shows typical CE electropherograms of a mixture of the two analytes when the LTB/coupled-capillary/sweeping-MEKC mode was used; electropherograms a–d show the results obtained for different capillary lengths for sample injections (a–d; sample zone, 15, 20, 25, and 30 cm, respectively). The temperature of the bath was 0°C. The temperature of the outside of the capillary other than the part of the LTB was 22°C. A portion of the capillary (13 cm in length, wide portion) was immersed in the 0°C bath, which was maintained at this temperature during the entire separation time. Hence, the viscosity of the buffer inside this portion of the capillary would be changed. Under this condition, the ϕ value (phase ratio, volume of the SDS/volume of the surrounding liquid phase) is increased. As a result, not only the interaction between SDS micelles and analytes are changed, but the phase ratio is also increased (ϕ value is increased). These changes are insignificant for NDA-Dop, but are large for NDA-Nor. As shown in Fig. 6, the NDA-Nor peak is shaper now (from 13 s to 3.7 s, as shown in Figs. 5b and 6c, respectively). Thus, we conclude that when an LTB/coupled-capillary/sweeping-MEKC method is applied, the phase ratio, the partition or distribution coefficient, and the retention factor would be increased leading to better separation efficiency and a wide-capillary would be useful for a large sample injection. As a result, a dramatic improvement in separation efficiency (selective) and sensitivity can be obtained.

Figure 7 shows the results obtained under the optimal conditions described in Fig. 6c (sample zone, 25 cm; bath temperature, 0°C) at various concentrations of analytes (electropherograms, a–d; 2.5×10^{-9}, 5.0×10^{-8}, $1.25 \times$

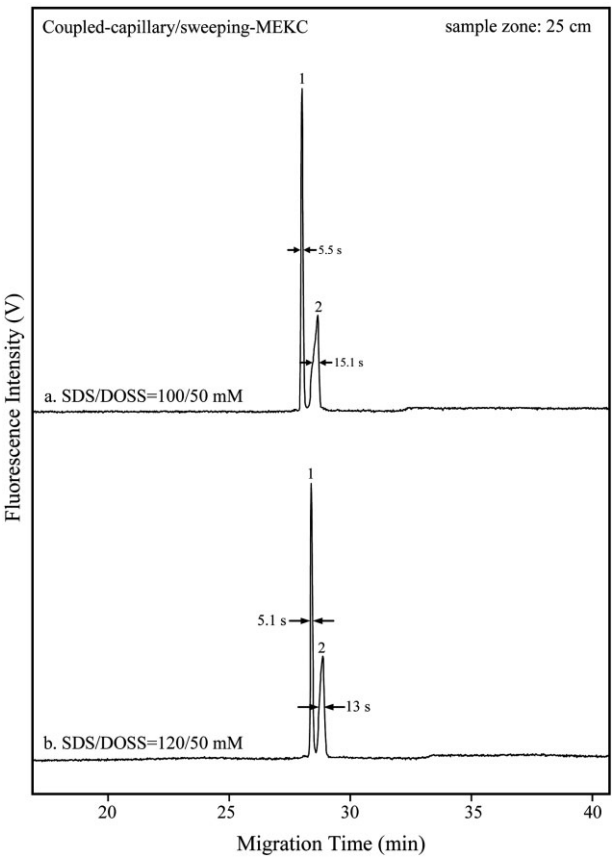

Figure 5. Sweeping-MEKC electropherograms of a mixture of NDA-Dop and NDA-Nor obtained at different ratios of SDS and DOSS (a) SDS/DOSS = 100/50 mM; (b) SDS/DOSS = 120/50 mM. Sample injection length, 25 cm. CE buffer as in Fig. 2.

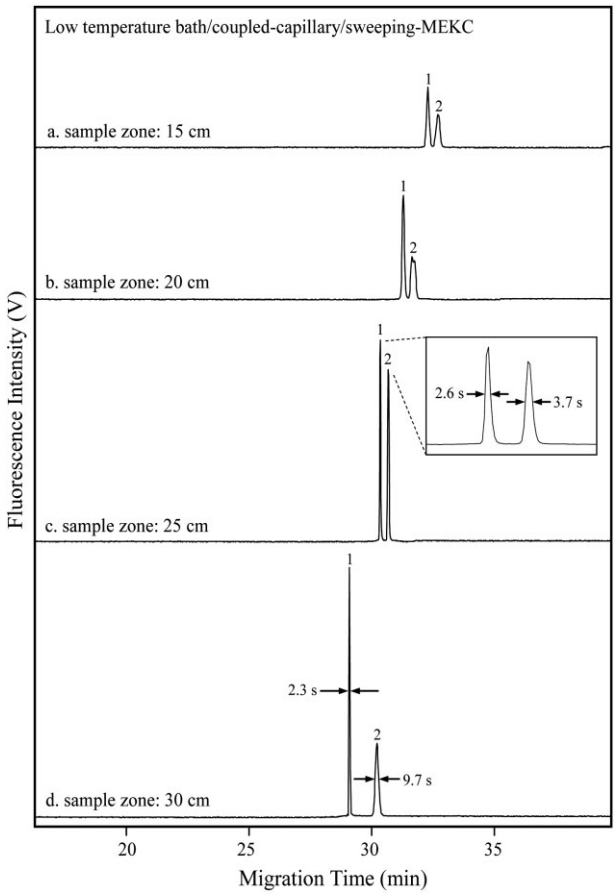

Figure 6. CE electropherograms of the mixture of NDA-Dop and NDA-Nor using the LTB/coupled-capillary/sweeping-MEKC mode. (a)–(d) Sample injected lengths, 15, 20, 25, and 30 cm in the wide portion. Bath temperature and effective region: 0°C, 13 cm in length. CE buffer as in Fig. 2.

10^{-8}, and 1.0×10^{-7} M, respectively). At a signal to ratio of 3 (S/N = 3), the LOD corresponds to 7.0×10^{-10} M (0.2 ppb). Using these conditions, the injected volume (nL), the detected concentration range, equation of the calibration line, the coefficients of variation, the LODs, the relative standard deviations (RSD%) of peak area/migration times and plate numbers (*N*) for NDA-Dop and NDA-Nor are summarized in Table 1 (frame B). By comparison of Table 1A and B, it can be seen that the separation efficiency (plate numbers) and sensitivity (LOD) are both increased. Especially, NDA-Nor can not be swept by SDS at room temperature and this can be improved when an LTB is used.

4 Concluding remarks

This work describes the successful application of an LTB/coupled-capillary/sweeping-MEKC for a large sample injection volume in CE separations. When a coupled capillary is used, a larger sample injection (compared to a single one) is possible. Furthermore, when an LTB is introduced, the phase ratio, the partition or distribution coefficient, and the retention factor would be changed, leading to better separation efficiency. Although the utility of the coupled capillary was investigated by the sweeping-MEKC mode in this study, it would be possible to extend the performance to other types of on-line sample concentration techniques, such as stacking, pH junction techniques, as well as the other related methods. Further applications can be expected.

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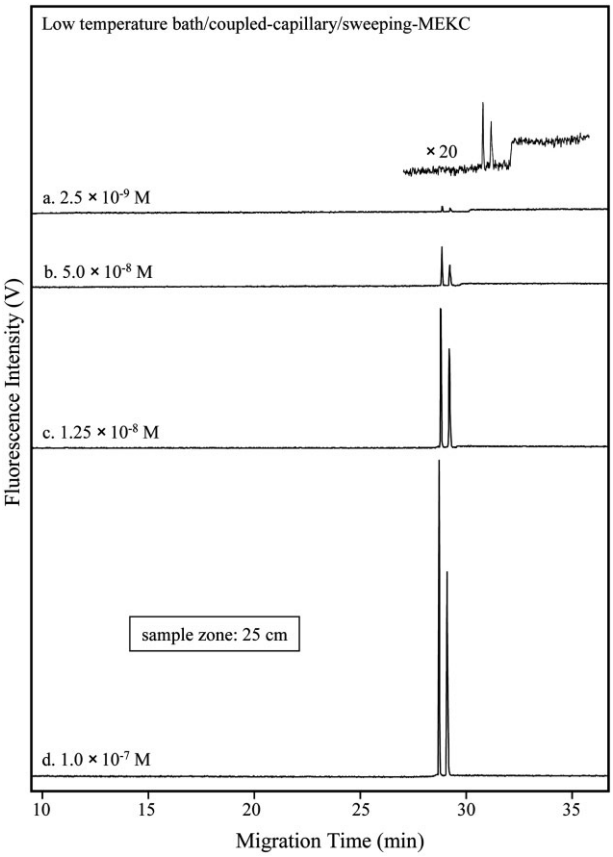

Figure 7. CE electropherograms obtained under the optimized conditions described in Fig. 6 (electropherogram c) at various concentrations of analytes (a)–(d) 2.5×10^{-9}, 5.0 10^{-8}, 1.25×10^{-8}, and 1.0×10^{-7} M.

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