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Full-capillary sample stacking/sweeping-MEKC for the separation of naphthalene-2,3-dicarboxaldehyde-derivatized tryptophan and isoleucine

In an attempt to improve the sensitivity of detection in capillary electrophoresis (CE), a novel online sample-concentration method, full-capillary sample stacking (FCSS)/sweeping-micellar electrokinetic chromatography (sweeping-MEKC) mode, is proposed. Naphthalene-2,3-dicarboxaldehyde (NDA)-derivatized tryptophan and isoleucine were selected as model compounds. In the initial step, the weakly acidic compounds, dissolved in a low-conductivity buffer (35.1 μS/cm; apparent ph (pH*) in a mixed solution of acetonitrile/methanol/water, 4.6), fill the entire capillary, two vials of a high-conductivity buffer (2.06 mS/cm; pH* 2.0) are placed on each end, and a negative polarity is then applied. Under these conditions, the direction of the electroosmotic flow (EOF) is toward the inlet. Meanwhile, the anionic analytes move in the reverse direction and are neutralized and stacked at the boundary of a dynamic pH-junction (between the sample matrix and the nonmicellar background solution (BGS)). When the sample concentration is completed, the BGS is quickly changed to solutions containing SDS-BGS for the subsequent separation. Since the mobility of SDS-analytes is then greater than the EOF, the following steps occur by the sweeping (for focusing) and MEKC (for separation) mode. Using these steps, a full-capillary sample injection/separation can be achieved.

Keywords: Full-capillary sample stacking; Isoleucine; Sweeping-MEKC; Tryptophan
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1 Introduction

Most online sample-concentration techniques were developed to accommodate a large volume of sample injection, since the limit of detection (LOD) is proportional to the amount of sample injected. However, in many cases, such as the sweeping [1–3] and stacking [4–11] modes, an optimal sample-injection length needs to be determined because if a longer capillary column is used for sample-concentration strategies, the subsequent CE separation becomes insufficient. The large-volume sample stacking (LVSS) method [5] is a generally accepted and well-known technique and can be used for a large sample injection. The reason for this is that, in this method during the initial steps (before switching the electrode polarity), more analytes can be accommodated, due to the large sample that is injected into the capillary [5, 6]. However, in this method, the electric current must be monitored very carefully until it reaches an adequate value, and the electrode polarity is then quickly switched, leading to a reversed EOF for the subsequent separation. In fact, He and Lee [7] reported on a modified LVSS method (intermediate polarity switching was not necessary) for the separation of anions, where an acidic buffer was used. As a result, a maximum peak height was observed when 87% (53 cm) of the capillary was filled with the sample. Thus far, only a few attempts to use a whole capillary for sample injection have been reported [5, 12–15]. This is because achieving a full-capillary sample injection (100%) for separation is still difficult, since the physical/chemical characteristics of each analyte (solute pK_a, affinity for the pseudostationary phase, hydrophobicity or hydrophilicity in aqueous, *etc.*) and all of the electrophoretic parameters (buffer conductivities, pH values, organic solvent, the magnitude and direction of the EOF, the surfactant concentration used, and the polarity of the electrode) must be optimized. In this study, a mixed mode, four of the well-known techniques, including the dynamic pH-junction [16–18], LVSS [5, 7–10], sweeping [1–3], and MEKC mode, were combined

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Abbreviations: BGS, nonmicellar background solution; **FCSS**, full-capillary sample stacking; **LED**, light-emitting diode; **LVSS**, large-volume sample stacking; **NDA**, naphthalene-2,3-dicarboxaldehyde; **NDA-Ile**, NDA-derivatized isoleucine; **NDA-Trp**, NDA-derivatized tryptophan

into a novel technique; full-capillary sample stacking (FCSS)/sweeping-MEKC method is demonstrated. Naphthalene-2,3-dicarboxaldehyde (NDA)-derivatized tryptophan and isoleucine were selected as model compounds. An inexpensive violet light-emitting diode (LED) was used for the light source (instead of a laser). As a result, a full-capillary sample injection (100%) for separation was achieved for the first time. The basic principles of the FCSS/sweeping-MEKC are described in detail. Several electrophoretic parameters for online sample concentration and separation were optimized and these data are also reported herein.

2 Materials and methods

2.1 Apparatus

The violet LED light source (~2 mW), CE setup, and data acquisition system used were similar to that described previously [19] and are abbreviated herein. The total and effective length of the capillary (75 μm ID/360 μm OD; J&W Scientific, Folsom, CA, USA) were 60 and 54 cm, respectively. The separation capillary was equilibrated with 0.1 N NaOH and without further treatment before use.

2.2 Reagents

All chemicals used were of analytical grade. Isoleucine, NDA, and NaH_2PO_4 were purchased from Sigma (St. Louis, MO, USA). Sodium dodecyl sulfate (SDS), tryptophan, sodium tetraborate, methanol, and phosphoric acid were purchased from Acros (Geel, Belgium).

2.3 Derivatization procedure of NDA-derivatized tryptophan (NDA-Trp) and isoleucine (NDA-Ile)

The derivatization procedure was modified from the original literature description [20], and is abbreviated herein.

2.4 Sample and buffer preparation

2.4.1 Sample matrix

Various concentrations (MEKC, 1×10^{-6} M; FCSS/sweeping-MEKC, 1×10^{-7} M, respectively) of samples (NDA-Trp and NDA-Ile) were dissolved in the matrix. The matrix consisted of 0.4 mM NaH_2PO_4, 0.06 mM H_3PO_4 in a mixed solution (ACN/methanol/water 15/10/75% v/v/v), the pH* of which was 4.6. The conductivity of the sample matrix was 35.1 μS/cm.

2.4.2 SDS-micelle background

The solution consisted of 100 mM SDS, 30 mM H_3PO_4 in a mixed solution (ACN/methanol/water 15/10/75% v/v/v), the pH* of which was 1.5.

2.4.3 BGS

By removing the SDS surfactant, the remaining constituents of the BGS were the same as the SDS-BGS; the pH* and conductivity were 2 and 2.06 mS/cm, respectively.

2.5 Methodology

Figure 1 shows schematic diagrams of the FCSS/sweeping-MEKC mode. In the first step, the weakly acidic analytes are dissolved in a low-conductivity buffer (in this case, 35.1 μS/cm; pH*, 4.6), and the entire capillary is filled with this solution by pressure. The pK_a values (in water) for the NDA-Trp and NDA-Ile are 2.38 and 2.36, respectively [21]. Since their pK_a values are lower than that of the sample matrix, the analytes are present in the form of weakly anionic compounds. It should be noted that, if the sample matrix is weakly alkaline (pH > ~7), the analytes will form stronger anionic compounds, but the EOF moves faster than the anionic analytes. As a result, the "stacking" process would not be successful. When the sample injection is completed, two vials of high-conductivity BGS (in this case, 2.06 mS/cm; pH*, 2.0) were placed on each end, and a negative polarity is then

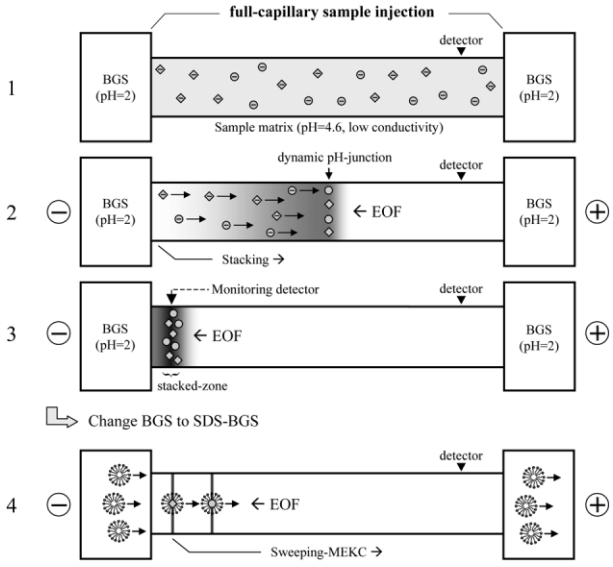

Figure 1. Schematic diagrams of the FCSS/sweeping-MEKC mode.

applied (step 2). At this moment, the direction of the EOF is toward the inlet, whereas the anionic analytes move in the reverse direction (outlet). When the anionic analytes reach the boundary (a dynamic pH-junction between the sample matrix and the BGS; pH*, 4.6/2.0), they are neutralized and stacked around the boundary. Following this, the stacked zone is then more concentrated, and is simultaneously driven by the EOF toward the outlet (step 3). When the sample-concentration process is almost completed, the BGSs should be quickly changed to an SDS-BGSs solution (a background solution containing 100 mM SDS, in this case) for the subsequent separation (steps 3 and 4). If this is not done quickly, the stacked zone would be pushed out of the capillary, since the EOF is the major driving force. If necessary, the timing for switching the buffers can be monitored by a monitoring detector (as indicated in Fig. 1, step 3). This also permits the length of the stacked zone to be observed. Once the BGS vials are replaced by the SDS-BGS vials, the SDS micelles will move faster than the EOF and become the major driving force. The analytes in the stacked zone are then swept by the micelles, and subsequent separation occurs by the MEKC mode (step 4).

3 Results and discussion

In order to measure the length of the stacked zone when the FCSS/sweeping-MEKC mode is applied, a monitoring detector (dashed-arrow) is located on the opposite side (as shown in Fig. 1, step 3), and the result is shown in Fig. 2. The CE electropherogram is obtained for a mixture of NDA-Ile and NDA-Trp. Herein, the injected length of the sample matrix was 60 cm; the concentration of both the test samples was 1×10^{-7} M. The diameter and the length of the capillary used were 75 $\times$ m ID and 60 cm (outlet to detection window, 54 cm), respectively; the applied voltage was -20 kV. The constituents in the BGS and sample matrix are described in Section 2.4. As can be seen, the full width at half maximum (FWHM) of the detected peak is broad (migration time, ~21.1 s; corresponding to ~7.6 mm of stacked sample zone). The inset shows the actual base width of the peak (migration time, ~215 s; corresponding to ~77.4 mm of the stacked sample zone). The stacked zone is obviously broad, when a large volume of sample is injected. Since the migration time of the peak is 25 min, this timing was selected for switching BGS to SDS-BGS. In fact, the timing (for switching the BGS to SDS-BGS) can also be monitored by altering the electric current until it reaches an adequate value. In this case, the current was changed from ~-3 to -39 μA (maximum value). The point at which the current was decreased to -30 μA also can be selected as the timing for switching. Both the switching methods provide similar

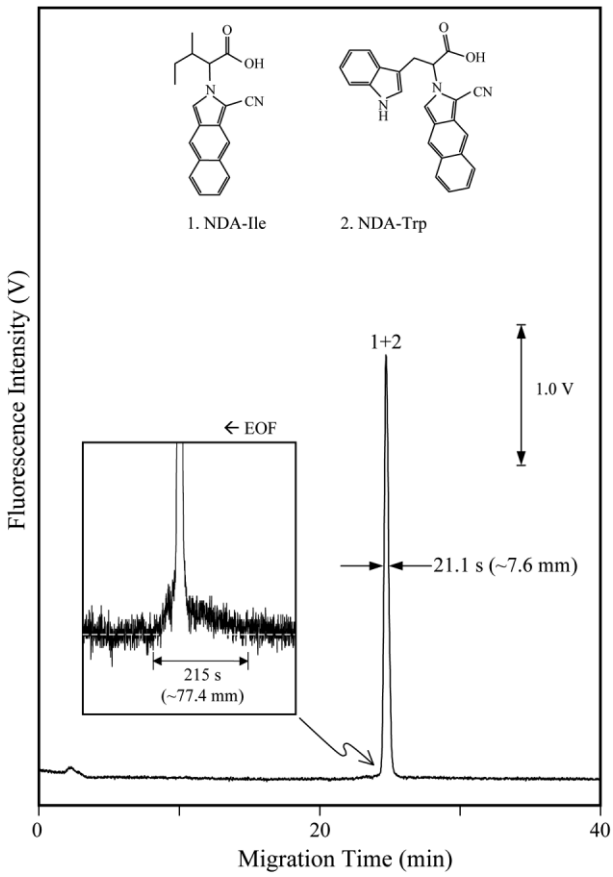

Figure 2. CE electropherogram of NDA-Ile and NDA-Trp, using the FCSS/sweeping-MEKC mode, when the detector was located on the opposite side (to measure the width of the stacked zone), as shown in step 3 (dashed-arrow) in Fig. 1 (capillary, 75 μm ID; total/effective length, 60/54 cm; applied voltage, -20 kV; samples: both 1×10^{-7} M). Step 4, applied voltage: -13 kV.

relative standard deviation (RSD) values. For convenience, we selected a time of 25 min for switching the buffers; the data obtained using this protocol are summarized in Table 1.

Figure 3 shows a typical CE electropherogram of the same mixture of NDA-Ile and NDA-Trp (both 1×10^{-7} M), when the FCSS/sweeping-MEKC mode (following steps 1–4 in Fig. 1) is used. It can be seen that the two analytes are perfectly separated. The signal intensities (V) of the detected peaks are 4.72 (peak 1, NDA-Ile) and 4.21 V (peak 2, NDA-Trp), respectively; background signal ~0.49 V. At a signal-to-noise ratio (S/N) of 3, the limits of detection (LOD) for NDA-Ile and NDA-Trp correspond to 1.1×10^{-9} M (337 ppt) and 1.4×10^{-9} M (531 ppt), respectively. In order to compare the sensitivity and separation efficiency when the FCSS/sweeping-MEKC mode was applied, a normal MEKC separation was also

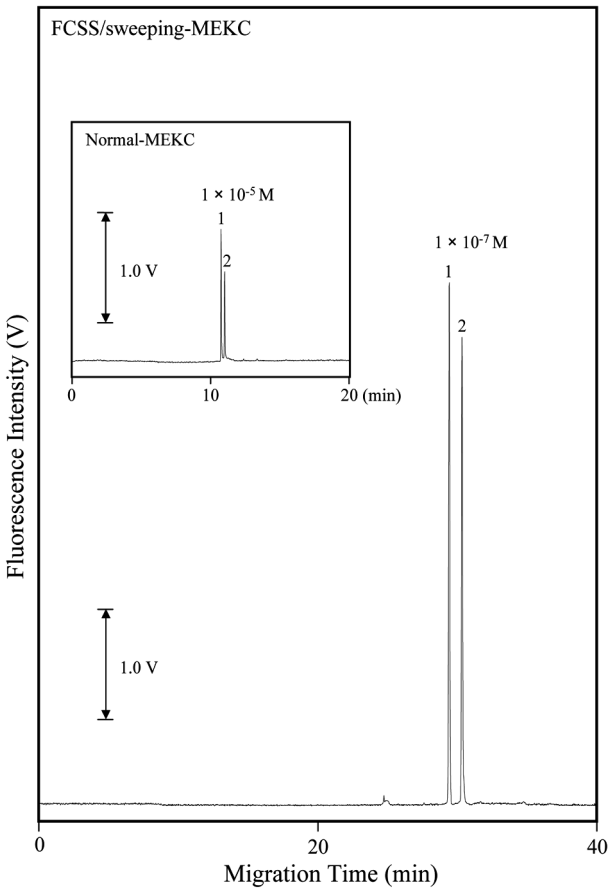

Figure 3. FCSS/sweeping-MEKC electropherograms of a mixture of NDA-Ile and NDA-Trp (peaks 1 and 2, respectively); samples, both 1×10^{-7} M. CE conditions as described in Fig. 2; the detector was located at the normal position. Inset, results obtained from the normal MEKC mode (samples: both 1×10^{-5} M).

carried out, and the result is shown in the inset of Fig. 3 (both sample concentrations, 1×10^{-5} M). The signal intensities (V) of the detected peaks are 1.2 (peak 1) and 0.8 V (peak 2), with the same background signal. The LODs (S/N = 3) for the two analytes correspond to 3.8×10^{-7} M (116 ppb) and 5.4×10^{-7} M (205 ppb), respectively. The results indicate that, when the sample matrix fills the entire capillary (in this case, 600 mm) and by applying the FCSS/MEKC-sweeping mode, almost a ~400-fold improvement in sensitivity can be achieved, compared to a normal MECK separation (as shown in the inset). However, various experimental conditions would affect the final result, including the timing for switching the BGS to the SDS-BGS vials, the characteristics of the analytes (pK_a, affinity for the pseudostationary phase, hydrophobicity, or hydrophilicity, *etc.*), experimental conditions (such as the degree of dissociation in such a buffer, the ability of a pH-junction to stop the neutralized analytes, EOF mobility, capillary wall conditions, and the concentration of SDS, *etc.*), making a linear improvement difficult to achieve. However, it is interesting to note that, when the FCSS/MEKC-sweeping mode was used, the peaks became sharper than that obtained by the normal MEKC mode. In the former case, the FWHM of the peaks are 5.5 and 5.1 s (corresponding to 1.7 and 1.5 mm in capillary length). In contrast to this, the FWHM of the peaks obtained by the normal MEKC mode are 2.6 and 2.9 s (corresponding to 2.2 and 2.4 mm in capillary length), respectively. Hence, the use of the FCSS/sweeping-MEKC mode not only results in an enhanced sensitivity, but the separation efficiency is also improved. Compared to the normal MEKC mode, the peak resolution (α) and theoretical plate numbers (N) for FCSS/sweeping-MEKC mode are improved (α from 1.2 to 5.4;

Table 1. Comparison of the use of the normal MEKC and the FCSS/sweeping-MEKC method for the separation of NDA-Ile and NDA-Trp

Method	Normal MEKC		FCSS/sweeping MEKC	
Analyte	NDA-Ile	NDA-Trp	NDA-Ile	NDA-Trp
Test concentration, M	1×10^{-5}		1×10^{-7}	
Injected length, mm	~0.84		600	
RSD (%); $n = 3$				
(a) Migration time	0.99	1.03	10.67	10.19
(b) Peak area	5.12	2.38	18.85	14.49
LOD (S/N = 3)	$(3.8 \pm 0.5) \times 10^{-7}$ M	$(5.4 \pm 0.8) \times 10^{-7}$ M	$(1.1 \pm 0.1) \times 10^{-9}$ M	$(1.4 \pm 0.2) \times 10^{-9}$ M
Plate number (N)	$(2.6 \pm 0.2) \times 10^4$	$(2.4 \pm 0.2) \times 10^4$	$(1.2 \pm 0.4) \times 10^5$	$(1.1 \pm 0.4) \times 10^5$

Total and effective length of the capillary: 60/54 cm; ID, 75 μm. Exciting source: violet LED (peak emission wavelength, 410 ± 7 nm; power, ~2 mW).

N from ~10^4 to ~10^5). These data (in addition to the RSD for peak area/migration times) are also summarized in Table 1. However, for achieving a whole capillary sample injection by this method, the pK_a values of the analytes should be lower (within a range of 1–4 would be desirable). This is because the pH* value of the sample matrix would be controlled below ~5 to maintain a weak EOF (in this situation, the anionic analytes moving faster than EOF), during the secondary and third steps (as shown in Fig. 1). If the pK_a values of the analytes are large; they cannot be present in the form of weakly anionic compounds and the "stacking" process could not be achieved. Furthermore, it should be noted that the statements concerning their degree of dissociation and electric charges in the mixed hydroorganic solvent solutions are not quite correct, since the pK_a values are related to the water solutions, but the real behavior of the analytes concerns the organic solvents/water mixture, in which the pK_a values (and consequently the charges of the analytes) can be significantly different. This should also be considered for achieving a successful whole capillary sample injection. On the other hand, surfactant (in this case, SDS) would be used in the last process (sweeping and MEKC), so the affinity of the analytes to the micellar agent should be adequately selected.

4 Concluding remarks

A novel method, FCSS/sweeping-MEKC mode, which can be used for acquiring a large volume for sample injection in CE separations, even with full-capillary injection, is described. Similar to the traditional "reversed-mode" by changing the electrode polarity leading to a reversed EOF for the subsequent separation, in this method the running buffer is changed, that is, the BGS is changed to an SDS-BGS solution when the stacked zone is nearly completely pushed out the capillary. As a result, a full-capillary sample injection can be achieved. Under optimized conditions, compared to the normal sweeping-MEKC mode, a dramatic improvement in sensitivity (~400-fold) and separation efficiency (resolution α 1.2–5.4) was obtained. Further applications can be expected.

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5 References

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