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Research Article

Comparison of the use of anionic and cationic surfactants for the separation of steroids based on MEKC and sweeping-MEKC modes

In attempts to improve the selectivity and sensitivity of steroid separation and to determine their migration order, a comparison of the use of anionic and cationic surfactants based on the MEKC and sweeping-MEKC modes was made. A mixture of six steroids (progesterone, 17-hydroxy progesterone, 11-deoxycortisol, corticosterone, cortisone, and cortisol) could be separated and detected by means of the CE/UV-absorption method. The order of migration time for these steroids was compared under various conditions, including acidic/alkaline buffers, anionic/cationic surfactants, and positive/negative applied voltage, causing the direction of the EOF and the migration of micelles to change. The major rules for generally predicting the migration order of steroids are summarized. The detection limits were significantly improved when the sweeping-MEKC mode was applied.

Keywords: Anionic and cationic surfactants / MEKC / Progesterone / Steroids / Sweeping MEKC
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1 Introduction

Steroids, a class of lipids that contain a distinctive ring structure called the cyclopentanoperhydro-phenanthrene ring system, comprise 17 carbon atoms arranged in four rings. The determination of steroids in the body continues to be an analytical challenge not only because their chemical structures are similar but also because their levels in biological fluids are extremely low. Most studies reported to date have employed RIA [1, 2], fluorimetry [3, 4], HPLC [5–8], LC-ESI MS [9], GC/negative ion chemical ionization MS [10], and CE [11–18] for the measurement. Each of these methods has unique advantages and disadvantages with respect to sensitivity, precision, and simplicity of use. In attempts to improve the separation efficiency and increase the LOD, and realize their migration order in CE separations, six types of surfactants (Fig. 1A) were selected for use and were compared, based on the MEKC and sweeping-MEKC modes; six steroids (Fig. 1B) were selected as model compounds. SDS is the most common anionic surfactant currently used in CE. Sodium dioctyl sulfosuccinate (DOSS) is a double-chained surfactant that can form a bilayer structure [19]. Brij-S (sulfonated Brij-30, tetraethylene glycol dodecyl ether) forms a larger micelle than the others [20]. On the other hand, octyltrimethylammonium bromide (OTAB), tetradecyltrimethylammonium bromide (TTAB), and CTAB are common cationic surfactants that are also in current use [21, 22].

In this study, we report on an investigation of the optimal separation of steroids and their migration order, using MEKC and sweeping-MEKC/UV-absorption methods. The migration order of these steroids are discussed and summarized when different CE conditions are applied, including acidic/alkaline buffers, anionic/cationic surfactants, and the positive/negative applied voltage which causes the direction of the EOF to change, as well as the migration of micelles. In the case of the sweeping-MEKC mode, the optimized conditions, including the buffers used and the optimal sample injection length, respectively, are described.

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Abbreviations: DOSS, sodium dioctyl sulfosuccinate; **OTAB**, octyltrimethylammonium bromide; **TTAB**, tetradecyltrimethylammonium bromide

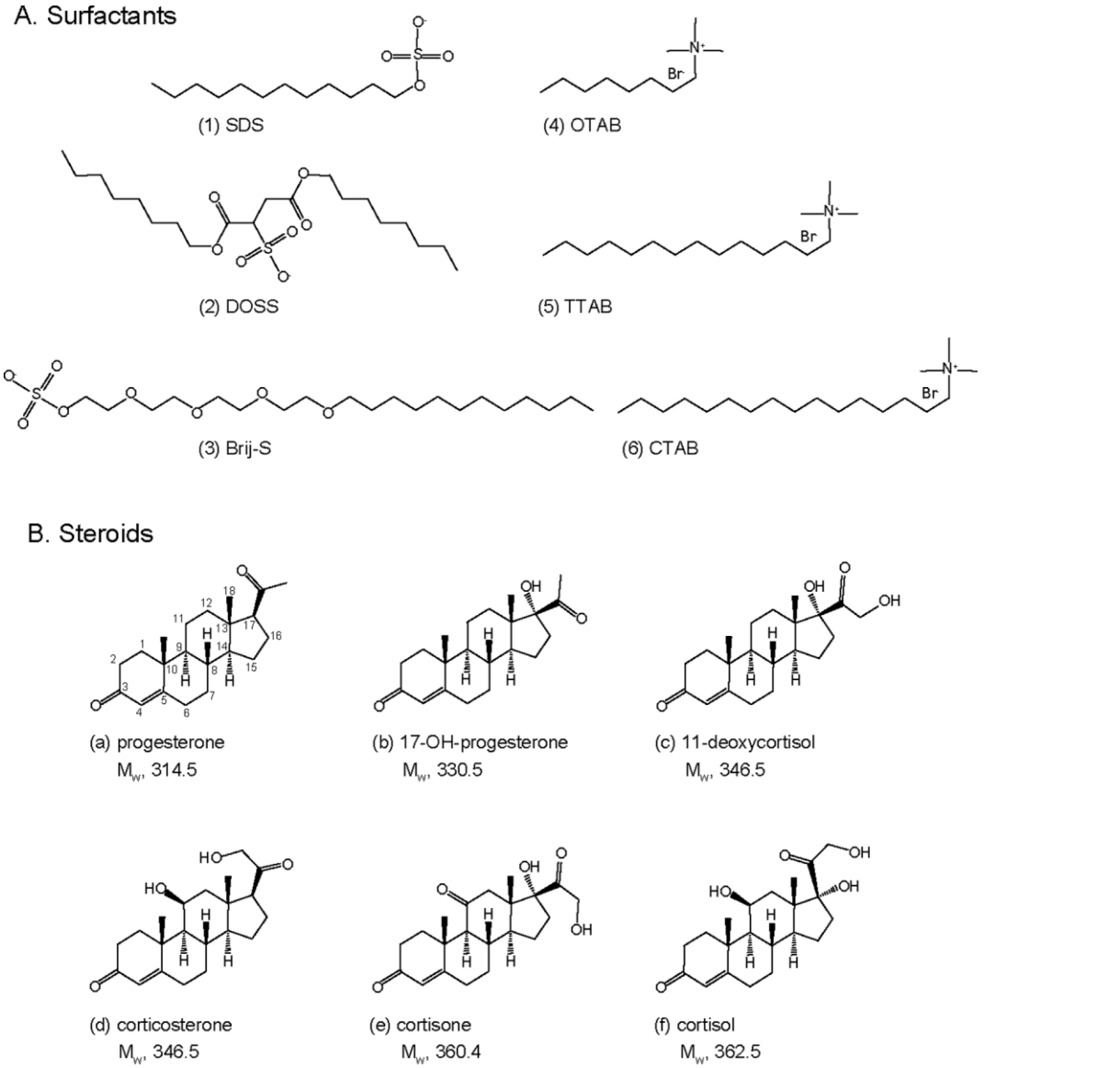

Figure 1. Chemical structures of the six types of surfactants (A) and the six steroids (B) used in this study.

2 Materials and methods

2.1 Reagents

All chemicals used were of analytical grade. Sodium tetraborate, DOSS, and Brij-30 (tetraethylene glycol dodecyl ether), progesterone (4-pregnene-3,20-dione), 17-OH-progesterone (17α-hydroxy-4-pregnene-3,20-dione), 11-deoxylcortisol (17,21-dihydroxy-4-pregnene-3,20-dione), corticosterone (4-pregene-11β,21-diol-3,20-dione), cortisol (11β,17α,21-trihydroxypregn-4-ene-3,20-dione), and cortisone (4-pregnene-17α,21-diol-3,11,20-trione) were purchased from Sigma-Aldrich (St. Louis, MO, USA). ACN and sodium hydroxide were obtained from Fisher Scientific (Fair Lawn, NJ, USA). OTAB and chlorosulfonic acid were obtained from Fluka (Buchs, Switzerland). TTAB, CTAB, SDS, methanol, ethanol, and ethyl acetate were purchased from Acros (Geel, Belgium). Brij-S (sulfonated Brij-30) was obtained as described in the literature [23].

2.2 Apparatus

The CE setup was identical with that used in our previous study [18]. Briefly, a high-voltage power supply (Model RR30-2R, Gamma, FL, USA) was used to drive the electro-

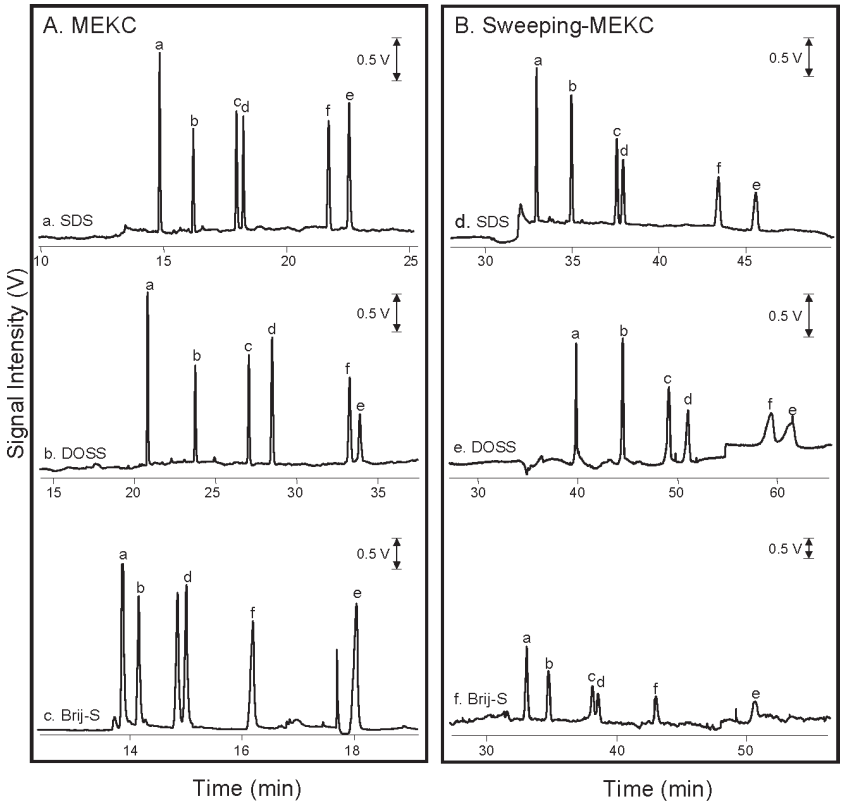

Figure 2. (A), Typical CE electropherograms of the six steroids (concentration of each, 50 ppm) obtained by the MEKC method when SDS, DOSS, and Brij-S surfactants were used, respectively. Peaks indicated as "a–f" correspond to progesterone, 17-hydroxyprogesterone, 11-deoxycorrtisol, corticosterone, cortisone, and cortisol, respectively. CE conditions for the electropherograms d–f: (d) phosphate buffer (30 mM) in a methanol–water (25:70 v/v) solution containing 100 mM SDS; (e) phosphate buffer (30 mM) in ACN–methanol–water (15:15:70 v/v/v) containing 100 mM DOSS; and (f) a phosphate buffer (45 mM) in a methanol–water (25:75 v/v) solution containing 250 mM Brij-S, respectively. Applied voltages used were −20 kV. Samples were prepared in matrices (electropherograms d–f: d–e, 20 mM H_3PO_4 aqueous solution; f, 60 mM H_3PO_4 aqueous solution). Sample injection lengths of electropherograms d–f: 40, 20, and 20 cm, respectively; total/effective length of the capillary, 90/77 cm.

phoresis, and a 50-μm ID fused-silica capillary column (J&W Scientific, CA, USA) was used for the separation (total/effective length: 90/77 cm in the case of MEKC and sweeping-MEKC modes, respectively). The sample was hydrodynamically injected by raising the reservoir relative to the exit reservoir to provide the injection length (depending on the specific situation). A UV-detector (CE-971 UV, Jasco, Japan) was used for the determination of the analytes, and the wavelength used for the detection was 247 nm.

3 Results and discussion

3.1 MEKC

3.1.1 Anionic surfactants

It would be interesting to consider the parameters that would determine the migration order of steroids when CE separation was applied. Figure 2A shows typical CE electropherograms for the six steroids (concentration of each, 50 ppm) obtained by the MEKC method when SDS, DOSS, and Brij-S surfactants were used, respectively. The complete, optimal separations of the six steroids was achieved in electropherograms a–c by using a phosphate buffer (30 mM) in an ACN–water (25:75 v/v) solution containing (a) 100 mM SDS, (b) 100 mM DOSS, and (c) an aqueous phosphate buffer (30 mM) containing 50 mM Brij-S, respectively. The pH* values of these buffers (with anionic surfactants) were adjusted to ~2.0; the applied voltages were −20 kV. It can be seen from the electropherograms, irrespective of the anionic surfactant used, that the migration order is: (a) progesterone (M_w 314.5) > (b) 17-hydroxy progesterone (M_w 330.5) > (c) 11-deoxycortisol (M_w 346.5) > (d) corticosterone (M_w 346.5) > (f) cortisol (M_w 362.5) > (e) cortisone (M_w 360.4). The migration order basically followed the mass *per* charge. However, it is difficult to comprehend the migration order of isomers (peaks c and d) and compounds which are against the order of mass *per* charge (peaks f and e). In principle, in an acidic buffer system, the EOF would be suppressed. The major driving force for the separation comes from the micelles; anionic micelles, carrying the analytes, slowly move toward the outlet when a negative charge is applied. Meanwhile, an analyte whose molecular weight is smaller and the interaction force with the micelles is stronger should move faster and, as a result, quickly pass through the detection window, leading to a shorter migration time. Furthermore, if the analyte contains charged components such as a cationic compound, the interaction force between the analyte and the anionic micelle becomes stronger and, as a result, it would pass through the detection window more quickly. However, in

an acidic buffer system (in this case; pH* ~ 2), steroids are nearly neutral compounds and, as a result, the charge effect is minor. For isomers, the migration orders are mainly determined by the interaction force between the analytes and the micelles. In this study, we found that when carbon number 11 in the steroid structure contains a polar group, such as a hydroxyl functional group (in this case, corticosterone; peak, d), the interaction force between the analyte and the micelle would be decreased, and as a result, the migration speed becomes slower than that of 11-deoxycortisol (peak, c), leading to a longer migration time. Furthermore, we also found that when the position at carbon 11 is a highly polar group, such as a ketone (in this case, cortisone; peak, e), the interaction force between the analyte and the micelle is further decreased. As a result, the migration speed of cortisone (M_w 360.4) becomes even slower. As that can be seen in Fig. 2A, the migration time for cortisone (peak e) is longer than that for cortisol (peak f). Similar migration behavior can also be found in a recent report [14] where prednisolone (with a hydroxyl group at carbon 11; M_w 360.4) and cortisone (with a ketone at carbon 11; M_w 360.4) were separated under acidic conditions (pH 2.5) with SDS. The findings show that cortisone moves more slowly than prednisolone. In fact, in the 17-carbon ring system of a steroid, various functional groups can be introduced at position 17, resulting in a variety of steroids each with its own biochemical characteristics. These functional groups have a significant effect on the migration order of the steroids, making it a difficult value to predict. Suffice it to say, if the functional group at position 17 is nonpolar, the migration speed is faster; if it is a polar group, then the migration speed is slower. Some of this can be seen in previous literature reports [15, 16]. The major rules for generally predicting the migration order of steroids are summarized in Table 1 (case I). In the other case, if an

Table 1. Comparison of the use of anionic and cationic surfactants for the separation of steroids when MEKC and sweeping-MEKC modes were applied

Buffer	Polarity	EOF	Micelle	Migration time of the observed peaks		
Anionic surfactants						
(I) Acidity	(−)	- -	⇒	Parameters	Shorter migration time	Longer migration time
				M_w	Smaller	Larger
				Charge (*z*)	Positive	Negative
				interaction	Stronger (nonpolar)	Weaker (polar)
				Functional group	Note 1	Note 2
(II) Acidity	(+)	- -	⇐	–	×	
(III) Alkalinity	(−)	←	⇒	–	×	
(IV) Alkalinity	(+)	→	⇐	M_w	Larger	Smaller
				Charge (*z*)	Negative	Positive
				Interaction	Weaker (polar)	Stronger (nonpolar)
				Functional group	Note 2	Note 1
Cationic surfactants						
(V) Acidity	(−)	- -	⇐	–	×	
(VI) Acidity	(+)	- -	⇒	Parameters	Shorter migration time	Longer migration time
				M_w	Smaller	Larger
				Charge, (*z*)	Negative	Positive
				Interaction	Stronger (nonpolar)	Weaker (polar)
				Functional group	Note 1	Note 2
(VII) Alkalinity	(−)	→	⇐	M_w	Larger	Smaller
				Charge (*z*)	Positive	Negative
				Interaction	Weaker (polar)	Stronger (nonpolar)
				Functional group	Note 2	Note 1
(VIII) Alkalinity	(+)	←	⇒	–	×	

Note 1. -deoxy on position 11; nonpolar group on position 17; Note 2. -hydroxyl group or -ketone on position 11; polar group on position 17; (−) negative charge applied at the inlet; (+) positive charge applied at the inlet; (→) direction of the EOF (toward the outlet); (←) direction of the EOF (toward the inlet); (- -) No EOF occurs; (⇒) direction of the micelle (toward the outlet); (⇐) direction of the micelle (toward the inlet); (×) no peak can be observed; interaction: interaction between the micelle and the steroid; and functional group: effects by the functional groups.

acidic buffer system is used (case II in Table 1) and a positive charge is applied at the inlet, the EOF would be suppressed and the micelles would migrate in the reverse direction. Thus, no peaks would be observed. If the buffer system is changed to alkaline and a negative charge is applied at the inlet (the case III), the EOF would move in the direction of the inlet but the micelles would migrate in the direction of the outlet. Since the driving force of the EOF is greater than the micelles, no peaks would be observed. Unless, an alkaline buffer system is used and a positive charge is applied at the inlet (the case IV), the EOF (greater driving force) and the micelles (smaller driving force) would migrate in the reverse direction, thus permitting analytes to be separated and detected. For example, if corticosterone (with a hydroxyl group at position 11) and 11-deoxycortisol (M_w 346.5) were mixed together, under these conditions (alkaline buffer/positive charge/SDS), the migration time of corticosterone would be shorter [15]. However, it should be noted that in an alkaline buffer system, if the analyte forms an anionic compound, it would be a charged compound and the migration order could be switched. In fact, under alkaline conditions when borate was used, the steroids formed chelate compounds with borate to produce anionic compounds, especially on the 11-hydroxy position. For example, if 11-deoxycortisol and 21-deoxycortisol (both M_w 346.5) were mixed together for separation, the negative charge of 11-deoxycortisol is greater than the other, and as a result, the migration time of 11-deoxycortisol would be shorter [16]. The use of DOSS seems to be better than the others for the separation of isomers (peaks c and d). The migration orders do not change when DOSS and Brij-30 are used. However, it should be noted that the migration order of the studied compounds depends on the surfactants (micelles) or micellar mixtures used and it can change if different types of anionic surfactants are used.

3.1.2 Cationic surfactants

When cationic surfactants are used, under acidic conditions/negative applied voltage, the low pH value would cause the EOF to be equal to zero, even though the micelles would migrate in the direction of the inlet. As a result, no peak would be observed (case V in Table 1). For this reason, a positive applied voltage is necessary (case VI in Table 1). Figure 3A shows typical CE electropherograms of the six steroids (concentration of each, 50 ppm) obtained by the MEKC method when the OTAB, TTAB, and CTAB surfactants were used, respectively. This is case VII in Table 1. The complete, optimal separations of the six steroids were achieved using an aqueous Tris-HCl (100 mM) buffer containing 50 mM OTAB, TTAB, and

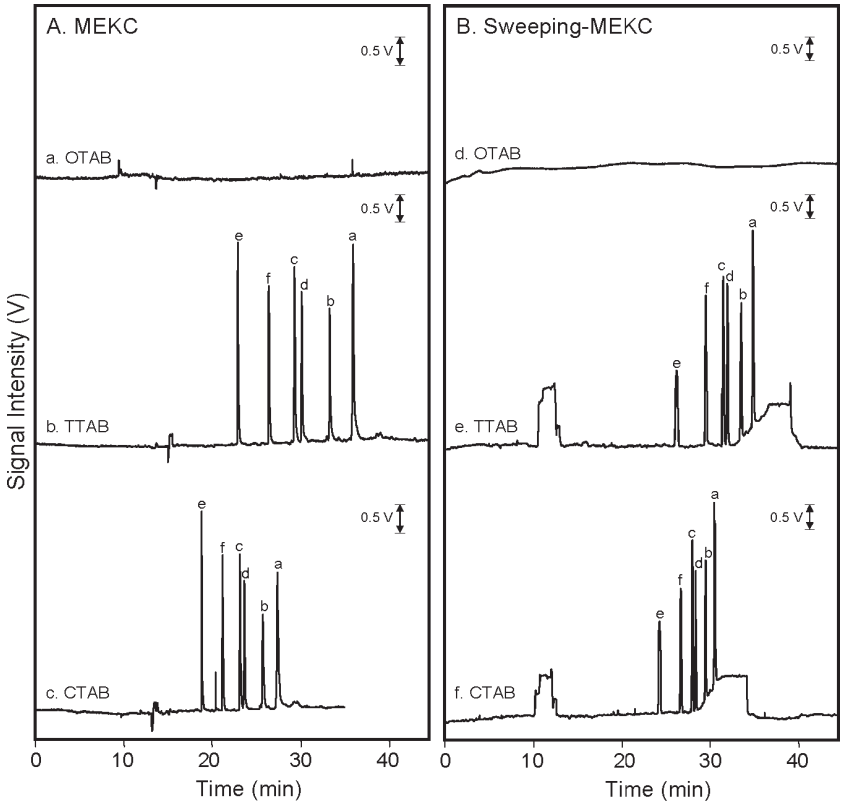

Figure 3. (A) Typical CE electropherograms of the six steroids (concentration of each, 50 ppm) obtained by the MEKC method when OTAB, TTAB, and CTAB surfactants were used, respectively. CE conditions: Tris-HCl (100 mM) aqueous buffer containing each 50 mM OTAB, TTAB, and CTAB, respectively; applied voltage, −20 kV. (B) Separation results for the six steroids (concentration of each, 1 ppm) obtained by the sweeping-MEKC method when OTAB, TTAB, and CTAB surfactants were used, respectively. CE conditions: Tris-HCl buffer (50 mM) in a methanol–water (10:90 v/v) solution containing 50 mM of OTAB, TTAB, and CTAB, respectively. Applied voltage used was −20 kV. Samples were prepared in a matrix containing of a nonmicelle solution only containing 100 mM Tris-HCl. Optimized sample injection lengths for the cases of TTAB and CTAB were 7 and 13 cm, respectively; total/effective length of the capillary, 90/77 cm.

Table 2. LOD, MEKC, ppm/sweeping-MEKC, ppb values at S/N = 3, coefficients of variation, and plate number (*N*) for the six steroids (a) progesterone; (b) 17-OH-progesterone; (c) 11-deoxycortisol; (d) corticosterone; (e) cortisone; and (f) cortisol examined by cationic and anionic surfactants, respectively, based on MEKC and sweeping-MEKC modes (UV-absorption at 247 nm)

Steroids	(a)	(b)	(c)	(d)	(e)	(f)
A. Anionic surfactants						
SDS						
MEKC						
LOD (ppm)	1.0	1.5	1.3	1.6	1.9	1.1
Plate number	2.4×10^6	2.2×10^6	1.5×10^6	1.7×10^6	1.8×10^6	2.2×10^6
Sweeping-MEKC (optimized sample injection length: 40 cm)						
LOD (ppb)	2.4	1.4	2.3	1.0	2.7	1.0
Plate number	3.0×10^6	4.5×10^5	6.3×10^6	6.2×10^6	6.8×10^5	8.6×10^5
DOSS						
MEKC						
LOD (ppm)	2.9	2.0	2.0	2.5	3.7	1.3
Plate number	5.6×10^5	5.2×10^5	3.6×10^5	4.1×10^5	3.2×10^5	2.8×10^5
Sweeping-MEKC (optimized sample injection length: 20 cm)						
LOD (ppb)	17.6	20.8	46.9	23.5	105.9	123.3
Plate number	1.1×10^5	1.4×10^5	1.0×10^5	1.4×10^5	5.0×10^4	5.0×10^4
Brij-S						
MEKC						
LOD (ppm)	3.4	2.6	2.9	3.5	1.3	1.4
Plate number	4.3×10^5	6.4×10^5	6.0×10^5	5.0×10^5	3.1×10^5	6.5×10^5
Sweeping-MEKC (optimized sample injection length: 20 cm)						
LOD (ppb)	11.5	34.9	14.0	13.9	233.5	145.3
Plate number	1.5×10^5	1.9×10^5	5.8×10^5	2.0×10^5	1.0×10^5	1.4×10^5
B. Cationic surfactants						
TTAB						
MEKC						
LOD (ppm)	5.0	3.6	3.9	2.5	2.3	2.0
Plate number	3.1×10^5	3.1×10^5	2.4×10^5	2.1×10^5	1.1×10^5	1.6×10^5
Sweeping-MEKC (optimized sample injection length: 7 cm)						
LOD (ppb)	21.7	16.9	15.5	13.0	12.5	13.1
Plate number	6.4×10^4	1.5×10^5	1.9×10^5	2.1×10^5	1.7×10^5	1.7×10^5
CTAB						
MEKC						
LOD (ppm)	2.8	1.9	2.0	1.9	2.0	6.2
Plate number	3.7×10^5	3.1×10^5	3.8×10^5	3.8×10^5	2.7×10^5	3.9×10^5
Sweeping-MEKC (optimized sample injection length: 13 cm)						
LOD, ppb	15.2	13.6	14.5	12.0	11.8	12.2
Plate number	2.3×10^5	2.0×10^5	3.1×10^5	2.8×10^5	3.4×10^5	2.8×10^5

Total/effective length of the separation capillary: 90/77 cm.

CTAB, respectively; applied voltage, −20 kV. The pH* values of these buffers (with cationic surfactants) were adjusted to ~7.0. In the case of OTAB, the EOF and micelles both migrate in the direction of the inlet, and no peaks can be observed. Unless, 2 mM CTAB (CMC ~ 1.3 mM) is added to the buffer to reverse the EOF [24], then only peaks can be observed (data not shown). In the cases of TTAB and CTAB, the direction of the EOF was "reversed" toward the direction of the outlet. Meanwhile the micelles migrate in the direction of the inlet. The driving force of the EOF is greater than the micelles and, as a result, peaks can be observed. Again, it can be seen

that the order of migration is: (e) cortisone (M_w 360.4) > (f) cortisol (M_w 362.5) > (c) 11-deoxycorrtisol (M_w 346.5) > (d) corticosterone (M_w 346.5) > (b) 17-hydroxy progesterone (M_w 330.5) > (a) progesterone (M_w 314.5). In principle, under an alkaline buffer system, the EOF is the major driving force and migrates in the direction of the outlet. In the meantime, the cationic micelles carry the analytes and gently migrate in the direction of the inlet. An analyte whose molecular weight is lower and the interaction force with the micelles is stronger should move faster (against the direction of the detection window) and, as a result, pass through the detection window slowly causing a longer migration time. Furthermore, if the analyte is an anionic compound, the interaction force between the analyte and the cationic micelle becomes stronger and, as a result, it would pass through the detection window slowly. In particular, under an alkaline buffer system, steroids that contain diols may form complexes with borate to form an anionic compound [11]. In such a case, the migration order may be reversed. This effect is stronger than that of functional groups. Finally, in the last case (case VIII) in Table 1, no peaks are observed.

3.2 Sweeping-MEKC

3.2.1 Anionic surfactants

Figure 2B shows typical CE electropherograms of the six steroids (concentration of each, 0.1 ppm) separated using the sweeping-MEKC method when SDS, DOSS, and Brij-S surfactants were used (electropherograms, d–f), respectively. The observed orders remained unchanged when the sweeping-MEKC was applied. The complete, optimal separation, as shown in electropherograms d–f, were achieved by using phosphate buffer (30 mM) in (d) a methanol–water (25:70 v/v) solution containing 100 mM SDS, (e) ACN–methanol–water (15:15:70 v/v/v) containing 100 mM DOSS, and (f) a phosphate buffer (45 mM) in a methanol–water (25:75 v/v) solution containing 250 mM Brij-S, respectively. The applied voltages were −20 kV. The samples were prepared in matrices (electropherograms d–f: d–e, 20 mM H_3PO_4 aqueous solution; f, 60 mM H_3PO_4 aqueous solution). The optimized sample injection lengths for the cases of SDS, DOSS, and Brij-S were 40, 20, and 20 cm, respectively; total/effective length of the capillary was 90/77 cm. Using the optimal conditions obtained by MEKC and the sweeping-MEKC modes, the LOD and theoretical plate numbers (*N*) are summarized in Table 2 (part A). The LODs can be improved by two to three orders when the sweeping-MEKC mode is applied. The use of SDS provides the most adequate results.

3.2.2 Cationic surfactants

Figure 3B shows some typical CE electropherograms for the six steroids (concentration of each, 1 ppm) obtained by the sweeping-MEKC method when the OTAB, TTAB, and CTAB surfactants were used (electropherograms, d–f), respectively. Their complete, optimal separation was achieved by using Tris-HCl buffer (50 mM) in a methanol–water (10:90 v/v) solution containing 50 mM of OTAB, TTAB, and CTAB, respectively. The applied voltage was −20 kV. The samples were prepared in a matrix which was a nonmicelle solution containing only 100 mM Tris-HCl. The optimized sample injection lengths for the cases of TTAB and CTAB were 7 and 13 cm, respectively; total/effective length of the capillary was, 90/77 cm. Under the optimal conditions obtained using MEKC and the sweeping-MEKC modes, the LOD and theoretical plate numbers are summarized in Table 2 (part B).

4 Concluding remarks

We demonstrate here that CE/UV-absorbance, in conjunction with MEKC, can be successfully used for the general estimation of the migration order of steroids under different CE conditions. After applying the sweeping-MEKC technique, the LOD can be dramatically improved, while preserving the migration order. Low levels of the six steroids can be separated and detected in both urine and blood samples. This study demonstrates the utility of the technique and the methods discussed herein can also regarded as a routine tool in pharmacological studies dealing with different types of steroids, whether derived from a natural source or by synthesis.

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