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Enantiomeric separation of local anaesthetic drugs by micellar electrokinetic capillary chromatography with taurodeoxycholate as chiral selector

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Abstract

The enantiomeric separation of local anaesthetic analogues, by means of micellar electrokinetic chromatography (MEKC), using taurodeoxycholate (TDC) both as chiral selector and as surfactant was studied. In this system the analytes are migrating with the micellar phase towards the anode. When a mixed micellar phase between TDC and Brij-35 [polyoxyethylene(23)-dodecanol] was used, the migration direction and migration order of the enantiomers were reversed due to a decreased distribution of the analytes to the micellar phase. The separation time with the mixed micelles is significantly shorter than with neat TDC as the micellar phase. The success of the chiral separation is strongly dependent on the concentration of TDC, there is an optimal concentration of the surfactant for each enantiomer pair in accordance with a proposed theory for the association between the analytes and TDC. In the mixed micellar phase optimum chiral resolution for each analyte was achieved at different ratios of TDC and Brij-35. It is further shown that the affinity of the [*S*-(+)]-enantiomers for TDC was higher than that of the [*R*-(−)]-forms.

Keywords: Enantiomer separation; Chiral selector; Micellar electrokinetic chromatography; Taurodeoxycholate

1. Introduction

It is known that stereochemistry has a significant effect on the biological activity of a drug. Racemic drugs often exhibit different pharmaceutical and/or toxicological effects as optically pure drugs [1]. The analysis of enantiomeric drugs and molecules is as a consequence an increasingly important area in separation science. It is of outmost importance to develop analytical methods which are both sensitive and selective for the determination of enantiomers. The analytical methods which are conventionally employed for enantiomeric separation are high-performance liquid chromatography (HPLC), capillary gas chromatography (GC) and thin-layer chromatography (TLC) [2]. Micellar electrokinetic chromatography (MEKC) which was introduced by Terabe et al. [3] is a variation of capillary electrophoresis (CE), where separation is a function of the distribution of the solutes between a micellar phase, working as pseudo-stationary phase, and an aqueous

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mobile phase, which are moving at different velocities within a fused-silica capillary. MEKC has the potential of separating enantiomeric compounds, when an optically active additive or surfactant, as chiral selector, is included in the running buffer [4]. The use of MEKC for optical resolution has opened an area of significant interest in analytical science, which probably will be widely applied for enantiomeric resolution of drugs in the future. A vast number of surfactants and chiral selectors, some of which have already been successfully used in conventional chromatographic procedures, are available for enantiomeric discrimination in MEKC. An important advantage of MEKC over the other types of chromatography is that the micellar phase, pseudo-stationary phase, and the chiral selector can easily be changed. The aim of the present investigation has been to evaluate MEKC performed by an anionic chiral surfactant, taurodeoxycholate (TDC) alone, and in combination with a neutral surfactant, Brij-35 [polyoxyethylene(23)-dodecanol], for enantiomeric resolution of local anaesthetics. TDC and Brij-35 were chosen as chiral selector and micellar phase on the basis of preliminary screening of different surfactants and chiral selectors. TDC, like other bile salts, forms, in aqueous solutions, small reversed micelles of helical structure, consisting of up to ten monomers [5], through interaction between the non-polar moiety of the molecules. This structure can be considered as an important reason for characteristic solubilization capability of the bile salt micelle [6,7]. Bile salts have successfully been employed as chiral selectors for resolution of various racemic amines and alcoholamines as well as amino acids [8–11]. The recognition mechanism of enantiomers is somewhat unclear and it has been suggested that the helical arrangement of TDC interacts differently with enantiomers containing a rigid and planar structure, probably owing to the rigid structure of TDC [11]. Brij-35 is a non-ionic surfactant, which will migrate with the electroosmotic flow. When it is added to an ionic surfactant it can affect the separation by forming mixed micelles. An advantage of Brij-35, as opposed to charged surfactants, is its negligible effect on Joule heating. Another advantage of Brij-35 is its low molar absorptivity in the low UV region [12].

2. Experimental

2.1. Chemicals

TDC, sodium salt, purchased from Sigma (St. Louis, MO, USA) and Brij-35 purchased from FlukaBioChemie (Buchs, Switzerland) were dissolved in phosphate buffer (pH 3.13 and $I=0.02$) at different concentrations. Prilocaine, mepivacaine and bupivacaine were kindly supplied by Astra Pain Control (Södertälje, Sweden). These compounds were dissolved, at a concentration of about 0.1 mM, in a buffer with an ionic strength ten times lower than the running buffer. The solute structures are given in Fig. 1. Water used throughout the investigation was purified through a Millipore system (Bedford, MA, USA). Phosphoric acid and sodium hydroxide utilised for preparation of buffers were of analytical grade. Micellar and other solutions used during the investigation were passed through a membrane filter, Minisart N, 0.45 μm (Sartorius, Goettingen, Germany) prior to use.

2.2. Apparatus

The capillary electrophoretic system used was a Beckman P/ACE system 2050 (Beckman Instruments, Palo Alto, CA, USA) equipped with either a 75 or 25 μm I.D. fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) of 57 cm total length and 50 cm effective length. Detection was carried out by UV detection at 214 nm and the capillary was thermostated at 25°C. Analytes were introduced in the capillary by pressure [0.5 p.s.i. (ca. $3.4\cdot10^3$ Pa)] for 5 and 10 s, depending on the

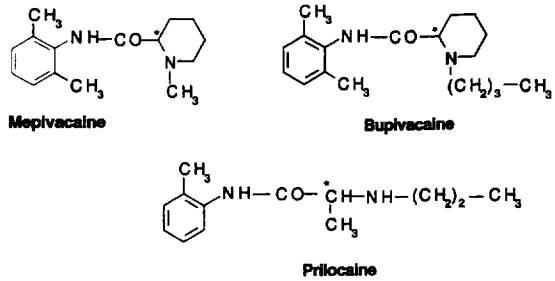

Fig. 1. Structures of prilocaine, mepivacaine and bupivacaine.

capillary I.D. Separations were performed at 10 to 30 kV either towards the anodic or the cathodic electrode. The electrophoretic mobility of the micelle was determined by injecting naphtalene, and the electroosmotic velocity of the bulk solution was evaluated from the migration of DMFA (dimethylformamide).

2.3. Capillary conditioning

A new capillary was flushed with 0.1 *M* HCl for 5 min and afterwards with water for 5 min, then it was washed with 0.1 *M* NaOH for 5 min and finally again with water for 10 min, before the running buffer was introduced into the capillary. Pre-run equilibration of the system was performed for 5 min before analysis. The capillary was stored in water overnight and rinsed with 0.1 *M* NaOH for 5 min every morning and after each change of the running buffer.

2.4. Calculation of some parameters

The number of theoretical plates (N) was calculated by the general formula $N=16(t_m/w_b)^2$, where t_m is the migration time and w_b is the width of the base of the peak. The asymmetry factor (asf) was calculated from b/a where b is the back part and a the front part of the baseline divided by a line drawn at 90° to the baseline from the peak maximum. The Kaiser factor [13], used as a measure on resolution, is defined according to f/g where f is the distance from the middle of the intersection line between the peak maxima and the valley between the peaks and g is the distance from the same point to the baseline.

3. Results and discussion

The enantiomeric separations of prilocaine, mepivacaine and bupivacaine were investigated employing TDC as chiral selector with two different types of MEKC systems under acidic conditions, where enantiomeric separations were successfully performed. Previous study [9] has, in accordance with the present investigation, shown that the best antipode discrimination was achieved at low pH, owing to the presence of electrostatic interactions between the negatively charged micellar phase and the solutes. Bupivacaine and related compounds are weak bases with pK_a values in the range of about 7.7 to 8.1, becoming increasingly charged with decreasing pH.

3.1. Neat TDC as micellar phase

The enantiomer separation of prilocaine, mepivacaine and bupivacaine were studied by utilising a TDC solution at pH 3.13. The micellar phase migrated towards the anode, as the electrophoretic mobility of TDC micelles was faster than that of electroosmotic flow of the bulk solution. Migration of the solutes occurred in the order of bupivacaine, mepivacaine and finally prilocaine, i.e., bupivacaine, due to its high hydrophobicity and hence high distribution to the micellar phase, migrated faster, towards the anode, than the other solutes. As a measure of resolution Kaiser factors are summarised in Table 1. The optimal resolution for each analyte is highly dependent on the TDC concentration, which is related to the hydrophobicity of the analyte. The bupivacaine enantiomers could not be resolved during these conditions, but tendencies for separation

Table 1
Effect of TDC concentrations on enantioresolution

[TDC]	Kaiser factor		
(m*M*)	Bupivacaine	Mepivacaine	Prilocaine
7.5	0	0	0
10	0	0	0
12.5	0	0	0
15	0.3	0	0
20	0.1	–	–
25	0	0.38	0.30
30	0	0.96[a]	0.45
35	0	0.54	0.54
40	0	0.52	0.65
45	0	0.48	0.96[a]
50	0	0.50	0.76

Conditions: TDC at different concentrations dissolved in phosphate buffer, pH 3.13 (22.5 m*M*; $I=0.02$). Running voltages, 10 and 15 kV; current, 33 μA applied over a fused-silica capillary of dimensions 57 cm×75 μm. Injection technique, pressure [0.5 p.s.i. (ca. $3.4\cdot10^3$ Pa)] for 5 s.

[a] Optimal TDC concentrations for mepivacaine and prilocaine.

were observed at rather low micellar concentrations. The optimal TDC concentrations for mepivacaine and prilocaine were higher, 30 and 45 m*M*, respectively. Wren and Rowe [14,15], using cyclodextrins as chiral selectors, showed that there is an optimum concentration of the chiral selector where the enantiomeric discrimination is maximal. Further increase of the amount of chiral selector results in an impairment of the separation. Similar relationships can be derived for micellar systems. Assuming that the enantiomer form a 1 : 1 complex with the micelle, the following equilibria and equilibrium constants (K) are valid:

$$E_R + T \rightleftarrows E_RT; \text{ and } E_S + T \rightleftarrows E_ST$$

$$K_R = [E_RT]/([E_R][T]) \quad (1)$$

and

$$K_S = [E_ST]/([E_S][T]) \quad (2)$$

where E_R, E_S and T are the two enantiomers and the micelle, respectively. The concentration of the micelles, [T], is given by $(C_{tot} - CMC)/n$, where C_{tot} is the total concentration of the micellar agent, CMC is the critical micellar concentration, and n is the number of monomers in each micelle. It can be anticipated that the mobilities of the two enantiomers (μ_E) and of the two complexes (μ_{ET}) are the same. A simple algebraic elaboration shows that the following relationship is valid for the difference in mobilities ($\Delta\mu$) between the two enantiomers:

$$\Delta\mu = [T](\mu_E - \mu_{ET})(K_S - K_R)/(1 + [T](K_R + K_S) + K_RK_S[T]^2) \quad (3)$$

The equation shows that there exists a certain concentration of the micelles where the difference in apparent mobilities of the enantiomers is maximal, corresponding to:

$$[T] = (K_R \cdot K_S)^{-1/2} \quad (4)$$

The optimal chiral selector concentration varies depending on the affinity of the solute for the selector, and a high distribution of the solute to the micellar phase is not favourable for enantiomeric discrimination. A high value of the equilibrium constants gives a lower optimal micellar concentration according to Eq. (4). However, too few experimental data were available to allow reliable calculations of the two constants for the local anaesthetics studied, but the experiments showed that there is an optimal micellar concentration for each analyte. Since Eq. (3) only contains the two equilibrium constants as unknowns it should be possible to estimate the optimal micellar concentration from two scouting experiments with two different micellar concentrations. The only requirement being that some separation between the enantiomers is obtained so that $\Delta\mu$ can be determined.

Enantiomeric resolutions of racemic mepivacaine and prilocaine at the optimal TDC concentrations are demonstrated in Fig. 2. (*S*)-Mepivacaine and (*R*)-prilocaine were spiked into the original racemic mixture in order to identify the elution order of the individual enantiomers. It was found that the (*S*)-forms migrated faster than the corresponding (*R*)-enantiomers, thus the (*S*)-enantiomers are more heavily distributed to the chiral micelles than the (*R*)-enantiomers.

3.2. Improving the chiral resolution by using a 25 μm I.D. capillary

Ineffective Joule heat dissipation in the 75-μm capillary resulting in impaired efficiencies and unstable baseline due to UV absorbance of TDC, prevented the use of a higher voltage than 15 kV. As demonstrated above bupivacaine enantiomers could not be resolved in this capillary, and the separation times for the successful chiral separations of mepivacaine and prilocaine were rather long. In order to improve the resolutions and migration times separation in a 25-μm capillary was tested. Applying 30 kV over the 25 μm I.D. capillary resulted both in better chiral resolutions and shorter migration times, as demonstrated in Table 2. Bupivacaine enantiomers were successfully separated using 15 m*M* TDC solution, the selectivity factor is only 1.02 but the relatively high efficiency (26 000–27 000 plates m^{-1}) gave baseline separation (maximal Kaiser factor, 1.0). However, the separation time is still rather long (about 90 min) as illustrated in Fig. 3a. Increasing the TDC concentration to 20 m*M* gave a much shorter separation time, 18 min, but then the peaks were not completely separated, the Kaiser factor was 0.6 (see Table 2). The enantiomeric

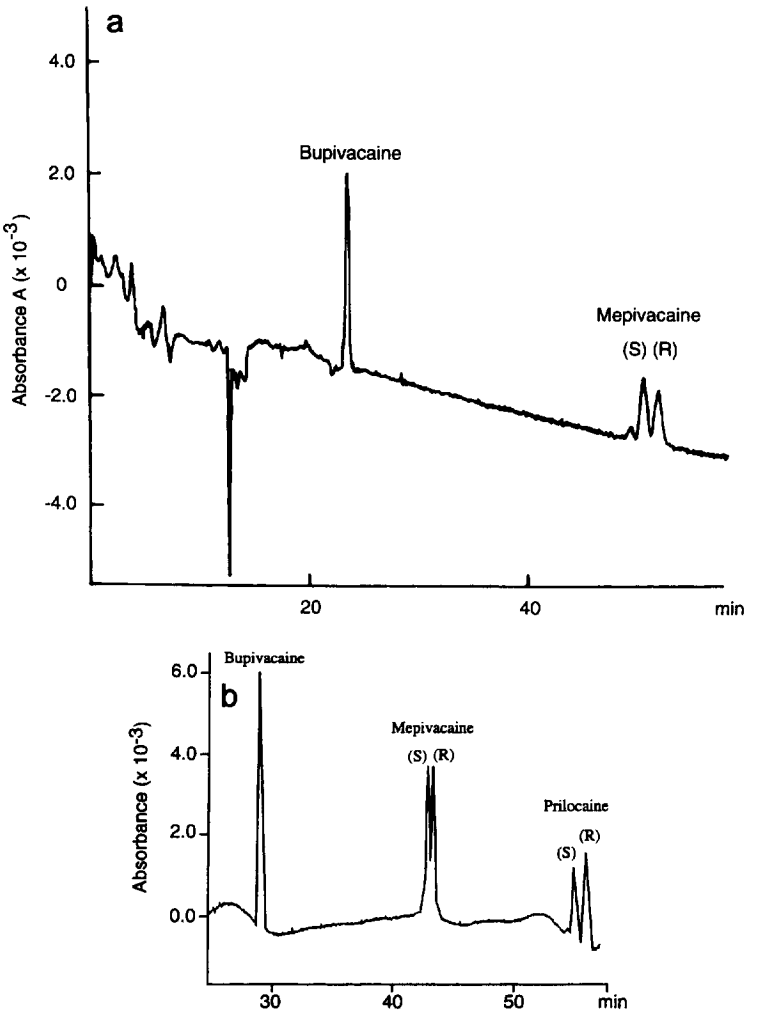

Fig. 2. Enantioseparation of bupivacaine, mepivacaine and prilocaine. Operating conditions: separation capillary, 57 cm×75 μm I.D. (effective length: 50 cm); injection technique, pressure (0.5 p.s.i.) for 5 s; TDC was dissolved in phosphate buffer, pH 3.13 (I=0.02) at different concentrations: (a) 30 mM, applied voltage was 15 kV and observed current 48 μA; (b) 45 mM, applied voltage 10 kV and observed current 32 μA.

resolutions of mepivacaine and prilocaine were also improved, see Fig. 3b. Optimum TDC concentration for the chiral separation of both compounds was 40 mM giving migration times of 20 min for mepivacaine and 34 min for prilocaine, a significant improvement compared to 50 and 55 min, respectively, with the 75-μm capillary. Mepivacaine enantiomers could also be completely separated with 30 mM TDC as shown in Table 2, however with a much longer separation time (36 min). Estimations of the equilibrium constants according to Eq. (4), requires knowledge of CMC and the number of monomers in each micelle. Such data are not known in detail for the studied systems and reliable constants can not be calculated. However, approximate values were estimated, assuming a CMC value of 1 mM [16] and an aggregation number of 10 [17]. Applying the optimal TDC concentrations for bupivacaine (15 mM) and prilocaine (40 mM), the approximate values of 710 (bupivacaine enantiomers) and 250 (prilocaine enantiomers) M^{-1}, respectively, were obtained.

Table 2
MEKC parameters with TDC micelles

Parameter	[TDC] m*M*					
	15	20	30		40	
	B	B	M	B	M	P
KF	1.0	0.6	1.0	0.0	1.0	1.0
$k'(S)$	1.24	2.30	1.70	3.92	2.28	1.47
$k'(R)$	1.22	2.28	1.67	3.92	2.24	1.44
α	1.02	1.01	1.02	1.00	1.02	1.02
$\mu_{(S)} \cdot 10^4$ cm^2 V^{-1} s^{-1}	−0.20	−0.91	−0.51	−1.34	−0.83	−0.36
$\mu_{(R)} \cdot 10^4$ cm^2 V^{-1} s^{-1}	−0.18	−0.90	−0.49	−1.34	−0.81	−0.34
$N_{(S)}$ cm^{-1}	271	–	847	–	1866	870
$N_{(R)}$ cm^{-1}	257	–	868	–	2246	1073
$\text{asf}_{(S)}$	0.9	–	0.93	–	0.16	0.37
$\text{asf}_{(R)}$	0.9	–	0.75	–	0.14	0.47
$\mu_{(\text{mc})} \cdot 10^4$ cm^2 V^{-1} s^{-1}	−2.16	−2.26	−2.25		−2.20	
$\mu_{\text{eo}} \cdot 10^4$ cm^2 V^{-1} s^{-1}	0.71	0.68	0.66		0.51	

The capacity factors (k') are the mean values from 2 to 4 experiments and were calculated by Eq. 5. Separation conditions: capillary, 57 cm×25 μm I.D. (effective length 50 cm); voltage, 30 kV; current, 9.6–14.5 μA; background electrolyte, phosphate buffer pH 3.13 (I=0.02) with different TDC concentrations. Abbreviations: B, M and P stand for bupivacaine, mepivacaine and prilocaine, respectively. KF=Kaiser factor, k'=capacity factor, α=separation factor, N=number of theoretical plates and asf=asymmetry factor. $\mu_{(S)}$ and $\mu_{(R)}$ are the observed migration mobility of the (*S*)- and (*R*)-enantiomers, respectively, and are calculated from the following equation: $\mu = (L \times l)/(V \times t_m)$, where L is the total length of the capillary and l is the effective length. A negative sign means that the migration is from negative to positive electrode.

The efficiencies in the 40 m*M* phase were rather high, about 200 000 and 100 000 plates m^{-1} for mepivacaine and prilocaine, respectively. It seems that the efficiency increases with increasing TDC concentration, i.e. increasing distribution to the micelles. One reason might then be a decreasing effective diffusion coefficient of the analyte fraction, but since a smaller percentage of the analyte will be present in the bulk electrolyte solution, another reason could be a reduced analyte–capillary surface interaction. However, as discussed above a too high micellar distribution will decrease the chiral selectivity, e.g. for bupivacaine in 30 m*M* TDC, where the k' [for definition see Eq. (5) below] is close to 4 and the enantiomer peaks are completely merged.

3.3. Capacity factors

The following equation was employed to calculate the capacity factors for the solutes [18].

$$k' = [\mu^{*}_{\text{ep(s)}} - \mu_{\text{ep(s)}}]/[\mu_{\text{ep(mc)}} - \mu^{*}_{\text{ep(s)}}] \qquad (5)$$

Where $\mu_{\text{ep(s)}}$ and $\mu_{\text{ep(mc)}}$ are electrophoretic mobilities of the solute in the absence of the micelle and electrophoretic mobility of the micelle, respectively; $\mu^{*}_{\text{ep(s)}}$ is the apparent electrophoretic mobility of the solute in the micellar solution, i.e. the difference between the observed migration mobility and the electroosmotic mobility. The mobilities used in the equation are the observed mobilities minus the electroosmotic mobilities under the appropriate conditions, i.e. with or without micelles. The equation can be rewritten by applying the observed mobilities of solute and micelle to give the expression:

$$k' = [\mu^{**}_{\text{ep(s)}} - \mu^{*}_{\text{eo}} - \mu_{\text{ep(s)}}]/[\mu_{\text{(mc)}} - \mu^{**}_{\text{ep(s)}}] \qquad (6)$$

where $\mu^{**}_{\text{ep(s)}}$ is the observed migration mobility of the solute; μ^{*}_{eo} the electroosmotic mobility in the micellar solution; and $\mu_{\text{(mc)}}$ is the observed electrophoretic mobility of the micelle.

From Eq. (6), an expression for $\mu^{**}_{\text{ep(s)}}$ is obtained

$$\mu^{**}_{\text{ep(s)}} = [k'/(k'+1)]\mu_{\text{(mc)}} + [(\mu_{\text{ep(s)}} + \mu^{*}_{\text{eo}})/(1+k')] \qquad (7)$$

There is a threshold value of $\mu^{**}_{\text{ep(s)}}$ where it is equal to zero. This corresponds to a situation in which the

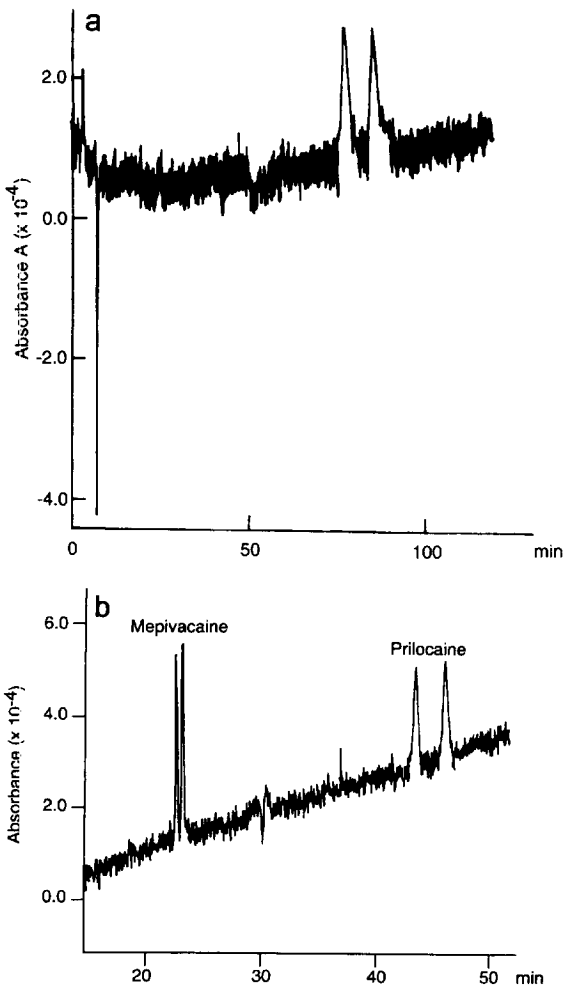

Fig. 3. Enantioseparation of bupivacaine, mepivacaine and prilocaine in a 25-μm capillary using taurodeoxycholate as micellar phase. Running conditions: separation voltage; 30 kV, capillary 57 cm×25 μm I.D. (50 cm effective length); injection technique: pressure (0.5 p.s.i.) for 10 s; background electrolyte: phosphate buffer, pH 3.13 (I=0.02), and TDC at different concentrations. (a) Resolution of racemic bupivacaine with a background electrolyte containing 15 mM TDC; observed current was 9.6 μA. (b) Resolution of racemic mepivacaine and prilocaine with a background electrolyte containing 40 mM TDC; observed current was 14.5 μA. The first peak in each case is the (S)-enantiomer.

distribution of the solute between each phase is such that its net migration velocity is zero. If $\mu^{**}_{ep(s)}$ is equal to zero then following relationship is valid:

$$k'_{lim} = -(\mu^{*}_{eo} + \mu_{ep(s)})/\mu_{(mc)} \quad (8)$$

This equation gives the threshold value of the capacity factor (k'_{lim}) when the analyte is immobile in the capillary. With higher values the analyte will migrate with the micellar phase towards the anode, while a lower value involves a migration towards the cathode. This value is given together with the measured capacity factors in Table 3. The threshold value of the capacity factor will decrease with increasing TDC concentration, i.e. for bupivacaine it is 1.03 with 15 mM TDC and 0.92 with 40 mM. From the regression equations relating the k' values with the TDC concentration it can be calculated that bupivacaine would be immobile in the capillary with about 10 mM of TDC, i.e. its experimental k' value should equal k'_{lim}.

3.4. Mixed MEKC with TDC and Brij-35 as micellar phase

The bupivacaine enantiomers were separated with rather low neat TDC concentration as described above, but the separation time was very long. The reason is a too high distribution of the rather hydrophobic compound to the micelles. Increasing the concentration in order to decrease the migration times resulted in incomplete enantioseparation. Since the strongest interaction between the analyte and the micelles are the electrostatic forces, a way to decrease the distribution of the analytes might be to decrease the density of negative charges of the TDC phase. This was accomplished by adding the non-ionic surfactant Brij-35. Earlier published studies have shown that one important approach to improve separation in MEKC is the use of mixed micellar phase [11,19]. It has also been shown that addition of Brij-35 to a solution containing an anionic surfactant, may improve the resolution [12]. Brij-35 has many advantages over other non-ionic surfactants, like low toxicity, low cost, high purity and low background absorbance [20]. The distribution of the analytes to the mixed micellar phase decreased to such a degree that the migration direction was reversed compared to the neat TDC phase, i.e. they migrated towards the cathode. The apparent mobility of the micellar phase was still towards the anode, i.e. the micellar velocity was higher than the electroosmotic velocity. This means that the migration order of the analytes was reversed, compared to the results obtained with the

Table 3
Comparison between observed capacity factors and the limiting capacity factor (k'_{lim}) with TDC as surfactant

BGE	Bupivacaine			Mepivacaine			Prilocaine		
	$k'(S)$	$k'(R)$	$k'_{(lim)}$	$k'(S)$	$k'(R)$	$k'_{(lim)}$	$k'(S)$	$k'(R)$	$k'_{(lim)}$
a	1.24	1.22	1.03	–	–	–	–	–	–
b	2.30	2.28	0.97	–	–	–	–	–	–
c	–	–	–	1.70	1.67	1.09	–	–	–
d	3.92	3.92	0.92	2.28	2.24	1.04	1.44	1.47	1.06

Separation conditions: separation voltage, 30 kV; capillary, 57 cm×25 μm I.D. (50 cm effective length); injection technique, pressure 0.5 p.s.i. for 10 s; background electrolyte (BGE), phosphate buffer pH 3.13 ($I=0.02$); a=15 m*M* TDC, 9.6 μA current; b=20 m*M* TDC, 10.8 μA current; c=30 m*M* TDC, 12.2 μA current; d=40 m*M* TDC, 14.5 μA current.

neat TDC micellar phase. Since the (*S*)-enantiomer still has the highest affinity for the micelle a reversal also of the migration order of the enantiomer pairs was obtained. The analyte with the lowest distribution degree to the micellar phase, here prilocaine, will in this system get the shortest migration time, i.e. contrary to the case with the neat TDC phase. The enantiomers of mepivacaine and prilocaine were successfully resolved by using mixed MEKC with a 75 μm I.D. capillary, see Table 4. The Brij-35 concentration was kept as low as possible in order to promote high resolution, it was varied between 1 and 3 m*M*. Using 1 m*M* Brij-35 the resolution (Kaiser factor) increased continuously for both mepivacaine and prilocaine when increasing the TDC concentration, and mepivacaine was close to baseline resolved with 12 m*M* of TDC. This separation is demonstrated in Fig. 4, a drawback is the long separation time, almost 1 h. Since the distribution of

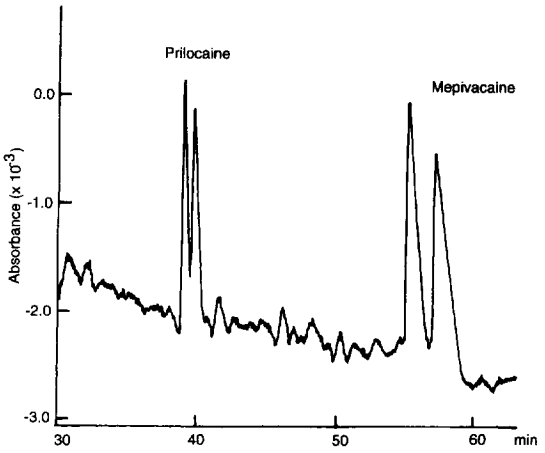

Fig. 4. Enantioseparation of mepivacaine and prilocaine with mixed micelles. Conditions: running voltage 15 kV applied over a fused-silica capillary (57 cm×75 μm), current 24–32 μA; injection technique, pressure (0.5 p.s.i.) for 5 s; background electrolyte: 1 m*M* Brij-35 and 12 m*M* TDC, were dissolved in phosphate buffer, pH 3.13 and $I=0.02$.

Table 4
Comparison of the Kaiser factors obtained with mixed micelles

[TDC] (m*M*)	[Brij-35] (m*M*)	Kaiser factor		
		Bupivacaine	Mepivacaine	Prilocaine
4	1	0.00	0.08	0
5	1	0.10	0.32	0
6	1	0.30	0.46	0.01
8	1	0.60	0.63	0.19
9	1	–[a]	0.83	0.47
12	1	–	0.97	0.77
14	2.5	–	0.85	0.96
12.5	1	–	–	0.80
12.5	3	–	–	0.50

Conditions: TDC and Brij-35 at different concentrations were dissolved in phosphate buffer, pH 3.13 ($I=0.02$). Running voltage: 15 kV applied over a fused-silica capillary of dimension 57 cm×75 μm; current 24–32 μA; injection technique: pressure (0.5 p.s.i.) for 5 s.
[a] Detection of bupivacaine in this separation system was impossible, owing to a very long migration time.

Table 5
MEKC parameters with mixed micelles

Parameter	Micellar phases												
	1 m*M* Brij-35–12 m*M* TDC			2 m*M* Brij-35–14 m*M* TDC		2.5 m*M* Brij-35–14 m*M* TDC			4 m*M* Brij-35–14 m*M* TDC		5 m*M* Brij-35–14 m*M* TDC		
	B	M	P	B	P	B	M	P	B	P	B	M	P
KF	1.0	1.0	0.9	1.0	1.0	1.0	1.0	0.9	1.0	0.8	0.9	1.0	0.7
$k'(S)$	1.49	0.75	0.59	1.45	0.68	1.46	0.81	0.65	1.46	0.60	1.47	0.74	0.56
$k'(R)$	1.47	0.74	0.58	1.43	0.66	1.44	0.79	0.64	1.44	0.59	1.45	0.72	0.55
α	1.01	1.02	1.01	1.01	1.02	1.01	1.02	1.01	1.01	1.02	1.01	1.03	1.01
$\mu_{(S)} \cdot 10^4$ cm^2 V^{-1} s^{-1}	0.17	0.89	1.13	0.14	0.97	0.22	0.85	1.07	0.42	1.26	0.58	1.19	1.41
$\mu_{(R)} \cdot 10^4$ cm^2 V^{-1} s^{-1}	0.18	0.91	1.14	0.15	0.99	0.23	0.87	1.08	0.43	1.27	0.59	1.21	1.42
$N_{(S)}$ cm^{-1}	327	1035	–	128	2125	1217	2109	–	1340	–	–	1544	–
$N_{(R)}$ cm^{-1}	219	1390	–	152	2269	1234	1541	–	1483	–	–	2112	–
$asf_{(S)}$	1.4	2.2	–	1.4	3.9	2.9	2.4	–	2.4	–	–	5.7	–
$asf_{(R)}$	1.1	1.5	–	1.4	3.3	3.1	1.6	–	2.6	–	–	7.5	–
$\mu_{(mc)} \cdot 10^4$ cm^2 V^{-1} s^{-1}		−1.18			−1.27		−1.12			−0.78		−0.50	
$\mu_{eo} \cdot 10^4$ cm^2 V^{-1} s^{-1}		0.66			0.66		0.66			0.65		0.65	

Abbreviations: B, M and P stand for bupivacaine, mepivacaine and prilocaine, respectively. Other conditions are the same as those in Fig. 7; $\mu_{(S)}$ and $\mu_{(R)}$ are migration mobility of the *S* and *R* enantiomers, respectively. KF and asf stand for Kaiser and asymmetry factor; $\mu_{(mc)}$ is the observed migration mobility of the micelle toward the anode; $k'(S)$ and $k'(R)$ are capacity factors for (S) and (R) enantiomers, respectively; α is separation factor and is equal to $k'(S)/k'(R)$; N cm^{-1} is the number of theoretical plates per cm capillary.

the analytes decreased by increasing the amount of Brij-35, the migration times decrease with increasing concentration of the non-ionic micellar agent. However, this will lead to incomplete separations. The prilocaine racemate, which was incompletely resolved with the mixture of 1:12 m*M* (Brij-35–TDC), was almost completely separated with the mixture of 2.5 m*M* Brij-35 and 14 m*M* of TDC with a Kaiser factor of 0.96. But also in this case the separation time was about 1 h using 15 kV, which is the highest voltage that could be applied with the 75 μm I.D. capillary. Also for bupivacaine the Kaiser factor increases continuously with increasing TDC concentration, however, the highest value achieved was 0.6. Applying higher amounts of TDC made the detection of bupivacaine impossible due to a very long migration time. Decreasing the migration time might be possible by applying a higher voltage but this requires a thinner capillary providing a more effective Joule heat dissipating effect. This study is described below.

3.5. Mixed MEKC resolution of bupivacaine, mepivacaine and prilocaine with a 25 μm I.D. capillary

A summary of the results obtained with different compositions of the mixed micelle is given in Table 5. Complete enantioresolution of bupivacaine was obtained with several different compositions of the micelle from 1:12 m*M* (Brij-35–TDC) to 4:14. Mepivacaine could also be completely resolved in three different compositions, while prilocaine required the composition 2:14 m*M*. The capacity ratios are of the same magnitude in all phases, especially for bupivacaine they are very close (range 1.43–1.49), while they vary somewhat more for mepivacaine (0.72–0.81) and for prilocaine (0.55–0.68). The selectivity factors are rather low, 1.01–1.03, and it is necessary to have a highly efficient separation in order to achieve the separation, and this is the case here as indicated in the table. For prilocaine the highest efficiencies, about 220 000 plates m^{-1}, were obtained with the composition 2:14, for mepivacaine about 200 000 with 2.5:14, and finally for bupivacaine about 140 000 with 4:14. However, such high efficiencies are not necessary in order to obtain a complete resolution as shown for bupivacaine in the phase 2:14, where the efficiency was only about 14 000 plates m^{-1}. However, the separation time was then very long, above 80 min. The best micellar composition of TDC and Brij-35 for baseline separation of bupivacaine was 4:14, when a reasonably low separation time (38 min) was achieved; the separation is shown in Fig. 5. The migration time of bupivacaine dropped from 90 to 28 min when the concentration of Brij-35 increased from 1 to 5 m*M*. However, at the highest surfactant concentration the separation was not complete (Kaiser factor = 0.9).

Addition of Brij-35 reduces the migration time of the solute. The effect is twofold: the distribution of the analytes to the micelles decreases, and the mobility of the micellar phase towards the anode decreases. There is a linear relationship between the micellar mobility and the concentration of Brij-35 as demonstrated in Fig. 6. The possibilities to accomplish relatively fast separations of mepivacaine, ca. 13 min, and prilocaine, ca. 16 min, are demonstrated in Fig. 7, where some of the optimal ratios of the two micellar agents according to the results shown in Table 5 were utilised. The disturbed peak shape of the (*S*)-enantiomer of mepivacaine is mainly due to an unstable baseline. A drawback with increasing concentration of the non-ionic surfactant seems to be an increasing asymmetry of the peaks (tailing). The reason is unclear, but does not seem to be related to variations of the capacity factors, since they are of about the same magnitude in all phases.

3.6. Limiting capacity factor (k'_{lim})

In analogy with the neat TDC micellar phase a limiting value of the capacity factor can be calculated according to Eq. (8), see Table 6. All the experimental k' values are lower than k'_{lim}, which means (according to the experimental observations) that the analytes are migrating towards the cathode, i.e. in opposite direction to the micellar mobility. For prilocaine it is possible to calculate, from the regression equations for the experimental and the limiting k' values, the concentration of Brij-35 required to make the experimental k' value equal to k'_{lim}, and the result is 0.9 m*M*. Such calculations were not possible neither for bupivacaine, where the experimental k' values are remarkably constant with varying con-

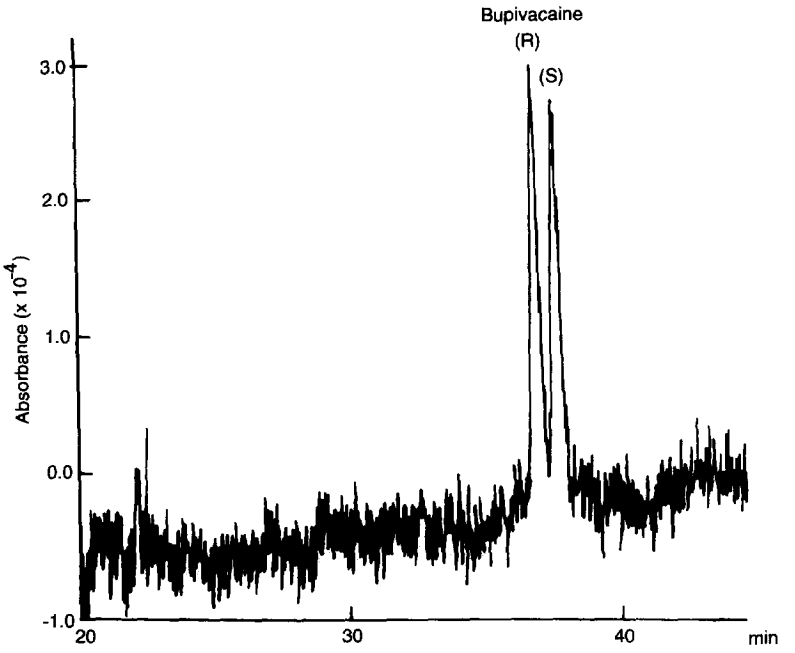

Fig. 5. Chiral separation of bupivacaine with mixed micelles. Conditions: capillary, 57 cm×25 μm I.D. (effective length 50 cm); voltage, 30 kV and current, 9 μA; injection technique, pressure (0.5 p.s.i.) for 10 s; background buffer, phosphate buffer, pH 3.13 (I=0.02); micellar mixture, 14 mM TDC and 4.0 mM Brij-35

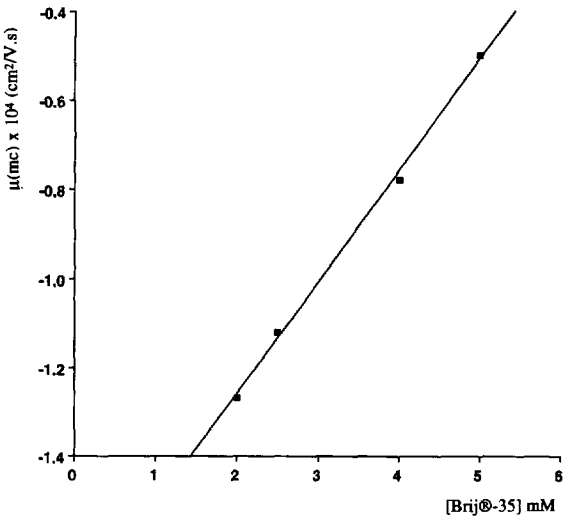

Fig. 6. Influence of Brij-35 concentrations, added to the 14 mM TDC solution, on migration mobility of the micellar phase. Conditions are the same as those given in Fig. 5.

centration of the non-ionic surfactant, nor for mepivacaine, where too few data were available.

4. Conclusions

Taurodeoxycholate (TDC) is an effective chiral selector for local anaesthetic drugs in capillary electroseparations with a background electrolyte of low pH, where the analytes are positively charged. The distribution of the analytes to the micellar phase seems to be governed by electrostatic as well as hydrophobic forces. The largest and most hydrophobic drug, bupivacaine, is the most strongly distributed to the micellar phase. With neat TDC micellar phases the analytes are distributed to such an extent that they will migrate with the micelles towards the anode. It was found that the (*S*)-enantiomers are more strongly distributed to the micelles than the (*R*)-enantiomers. The addition of Brij-35, a non-ionic surfactant, results in mixed micelles with a lower affinity than the neat TDC micelles. The reason is probably a combination of reduced charge

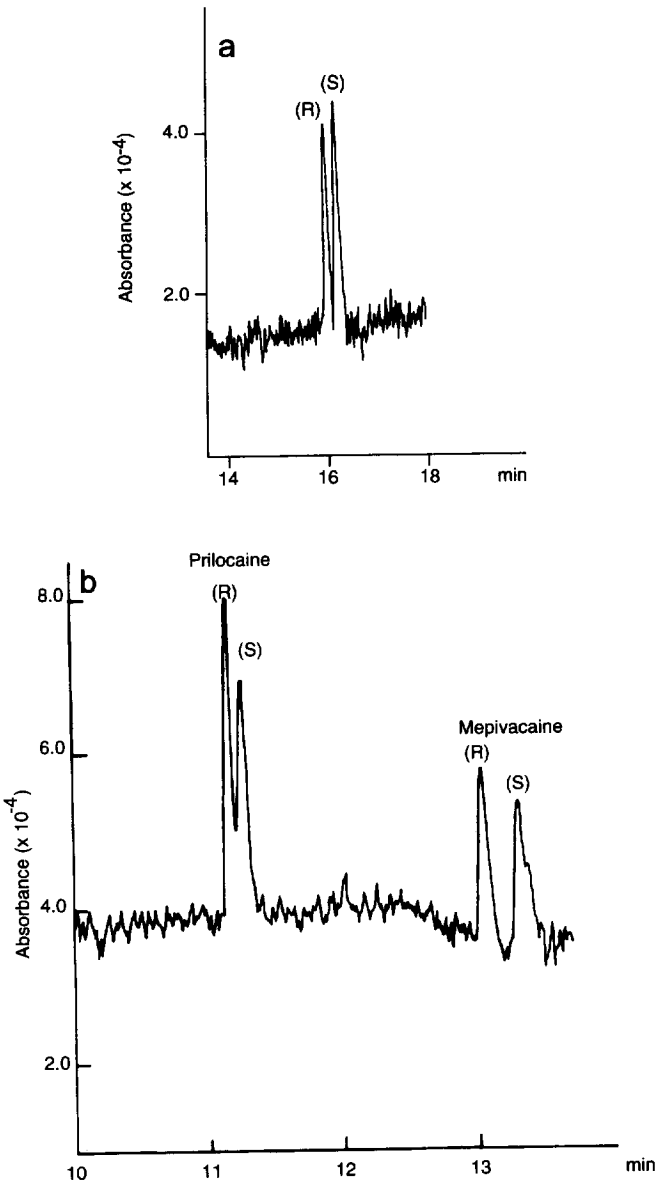

Fig. 7. Chiral separation of mepivacaine and prilocaine. Conditions: capillary, 57 cm×25 μm I.D. (the effective length of the capillary was 50 cm); separation voltage, 30 kV and current 9 μA; injection technique, pressure (0.5 p.s.i.) for 10 s; background buffer, phosphate buffer, pH 3.13 (I=0.02). (a) Prilocaine, micellar composition: 14 mM TDC and 2.0 mM Brij-35; (b) Prilocaine and mepivacaine, micellar composition: 14 mM TDC and 5.0 mM Brij-35.

density and decreased hydrophobicity of the micellar phase. With such micelles the distribution has decreased to such a degree that the analytes migrate in the opposite direction compared to the neat TDC micellar phase, i.e. towards the cathode. The migration order for both the analytes and for the enantiomers is reversed. The separation times required for complete separations are significantly shorter with

Table 6
Comparison between observed capacity factors and the limiting capacity factor (k'_{lim}) with mixed micelles

BGE	Bupivacaine			Mepivacaine			Prilocaine		
	$k'(S)$	$k'(R)$	k'_{lim}	$k'(S)$	$k'(R)$	k'_{lim}	$k'(S)$	$k'(R)$	k'_{lim}
a	1.49	1.47	1.85	0.75	0.74	2.08	0.59	0.58	2.11
b	1.45	1.43	1.72	–	–	–	0.68	0.66	1.96
c	1.46	1.44	1.72	0.81	0.79	2.19	0.65	0.64	2.22
d	1.46	1.44	2.78	–	–	–	0.60	0.59	3.18
e	1.47	1.45	4.34	0.74	0.72	4.88	0.56	0.55	4.96

Separation conditions: separation voltage, 30 kV and current 9 μA; capillary, 57 cm×25 μm I.D. (50 cm effective length); injection technique, pressure 0.5 p.s.i. for 10 s; background electrolyte (BGE), phosphate buffer pH 3.13 (I=0.02); (a) 12 m*M* TDC and 1.0 m*M* Brij-35; (b) 14 m*M* TDC and 2.0 m*M* Brij-35; (c) 14 m*M* TDC and 2.5 m*M* Brij-35; (d) 14 m*M* TDC and 4.0 m*M* Brij-35; (e) 14 m*M* TDC and 5.0 m*M* Brij-35.

the mixed micellar phase, 13 to 38 min, as compared to 24–90 min for the neat phase applying a 25 μm I.D. capillary.

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