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Selectivity in microemulsion electrokinetic chromatography

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Abstract

Microemulsion electrokinetic chromatography (MEEKC) is a most promising separation technique providing good selectivity and high separation efficiency of anionic, cationic as well as neutral solutes. In MEEKC lipophilic organic solvents dispersed as tiny droplets in an aqueous buffer by the use of surfactants provide a pseudo-stationary phase to which the solutes may have an affinity either to the surface or they may even partition into the droplets. When the droplets are charged, typically negatively, they will migrate opposite to the electroosmotic flow and hence separation of neutral solutes may take place. In the present paper focus has been set on how to change selectivity in MEEKC. Changes in the nature of surfactant as well as in pH have been shown to be powerful tools in changing the selectivity. The type of lipophilic organic phase is of less importance for the separation of fairly lipophilic solutes. Also changes in the temperature surrounding the capillary may alter the selectivity.

Keywords: Selectivity; Microemulsion electrokinetic chromatography; Pseudo-stationary phases; Surfactants

1. Introduction

Microemulsions are defined as macroscopically homogeneous, optically transparant fluids having more than one liquid phase. They are formed using water, lipophilic organic solvents and suitable surfactants. Microemulsions have been known since 1943 [1] and the high solubilising ability of these emulsions have been extensively used in industry [2,3]. Recently microemulsions have also been used in high-performance liquid chromatography [4], and in a number of papers the use of microemulsion electrokinetic chromatography (MEEKC) for separation of a wide range of compounds and among these pharmaceuticals and excipients have been described [5–7]. MEEKC has even been used for the separation of proteins [8]. MEEKC may be considered an extension of the well known principle of micellar electrokinetic chromatography (MEKC) which has been thoroughly reviewed [9].

However, in most of these papers identical or very similar MEEKC systems have been used. The reason for this is that if a stable, optically transparent emulsion is to be obtained the relationship between the amount of organic phase and the surfactant has to be within a relatively narrow range [10]. The microemulsions therefore most often consist of a mixture of 0.8% organic solvent+3.3% sodium dodecylsulfate+6.6% *n*-butanol and 89.3% aqueous buffer (10 m*M* borate pH 9.2) and the organic solvent is typically *n*-octane, *n*-heptane or *n*-octanol.

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As partition between *n*-octanol and an aqueous phase may be one of the separation mechanisms involved a number of groups have already used MEEKC for the determination of log *P* values [11,12].

A few studies on the effect of the use of different organic phases on the separation of vitamins [13], steroids [14] and sugars [15] have been performed. Also the use of other surfactants have shortly been studied [13].

In order to explore the possibilities for altering the selectivity in MEEKC by changing the components of the microemulsions the present investigations have been made.

In this study examples of how the selectivity of the separation of a mixture of cationic, anionic and neutral test solutes is influenced, when type and concentration of surfactant are changed, when the lipohilic organic phase is changed, when the pH is changed and when the temperature outside the capillary is changed, are presented. Furthermore, MEEKC is compared to the more well known MEKC.

2. Experimental

2.1. Chemicals

Sodium dodecylsulfate (SDS) was obtained from Aldrich (Gillingham, UK). Tween 21 (polyoxyethylene sorbitan monolaurate) and Brij 35 (polyoxyethylene lauryl ether) were from Sigma (St. Louis, MO, USA). *N*-Cetyl-*N,N,N*-trimethylammonium (CTMA) bromide was from Merck (Darmstadt, Germany). 3-(*N,N*-Dimethylmyristylammonium) propanesulfonate (MAPS) was obtained from Fluka (Buchs, Switzerland) and sodium dioctylsulfosuccinate (dowsate sodium, BP 93) was from UNIKEM (Copenhagen, Denmark). All organic solvents and sodium tetraborate-10-hydrate was of analytical quality and were used without further purification.

2.2. Capillary electrophoresis

The experiments were carried out on an HP^{3D} capillary electrophoresis system (Hewlett-Packard, Waldbronn, Germany) equipped with an on-column diode-array detection (DAD) system.

Detection wavelengths of 200, 214, 254 and 280 nm, 20 nm bandwidth, were used for all samples. The separations were performed in a fused-silica capillary, 48.5 cm×50 μm I.D., 40 cm to the detector (Polymicro Technologies, Phoenix, AZ, USA). The capillary was thermostated at 25°C by air circulation unless otherwise stated. Samples were kept at ambient temperature in the autosampler and injected by applying a pressure of 5 kPa (50 mbar) for 5 s.

A voltage of +20 kV was applied during analysis. The electrophoresis medium consisted of 10 m*M* sodium tetraborate pH 9.2+organic solvent+*n*-butanol+surfactant (89.3:0.8:6.6:3.3, w/w).

Equilibration of new capillaries were performed by rinsing with 1 *M* sodium hydroxide for 60 min, 0.1 *M* sodium hydroxide for 20 min, distilled water for 20 min and the electrophoresis medium for 10 min.

2.3. Sample preparation

Test solutes were dissolved in methanol–water (50:50, v/v) to a concentration of ca. 0.2 mg/ml.

2.4. Microemulsions

The component of the microemulsion were mixed and treated on an ultrasonic bath for 30 min. Before use the transparent emulsion was filtered through a 0.45 μm filter.

3. Results and discussion

Most MEEKC applications reported in the literature have been performed using a microemulsion consisting of 0.8% *n*-octane, 3.3% SDS, 6.6% *n*-butanol and 10–20 m*M* sodium borate pH 9.2. Although this system is able to provide separation of a number of solutes it would be advantageous to be able to change the selectivity in a controlled way. In order to do this a study of the possibilities of changing the selectivity is needed. Taking the system mentioned as a the starting point the effect of changing the type and concentration of surfactant, of

the lipohilic organic phase, of the pH and of the temperature outside the capillary are investigated.

3.1. The organic phase

The effect of changing the inner organic phase in the microemulsion droplets was studied using seven different organic solvents. The solvents used are given in Table 1 and all solvents were used in the same amount (0.8%). Furthermore, in Table 1 the normalized migration times of six test solutes (Fig. 1) are given using caffeine as the internal reference. To further evaluate the selectivity of the systems the relative migration (α) between some of the test solutes are given. The α-value is calculated as:

$$\alpha = \frac{\frac{\text{Test solute 2}}{\text{Caffeine}}}{\frac{\text{Test solute 1}}{\text{Caffeine}}}$$

using the migration times observed; and test solute 2 will normally be the one with the longest observed migration time. Although some changes in selectivity can be seen no dramatic changes like change in the order of migration are observed even though a number of solutes with very different functionality are used. α-values between the two anionic solutes are very similar and only minor selectivity changes between the anionic cinnamic acid and the cationic pindolol are observed. Some selectivity changes between the lipophilic steroids and the polar anionic

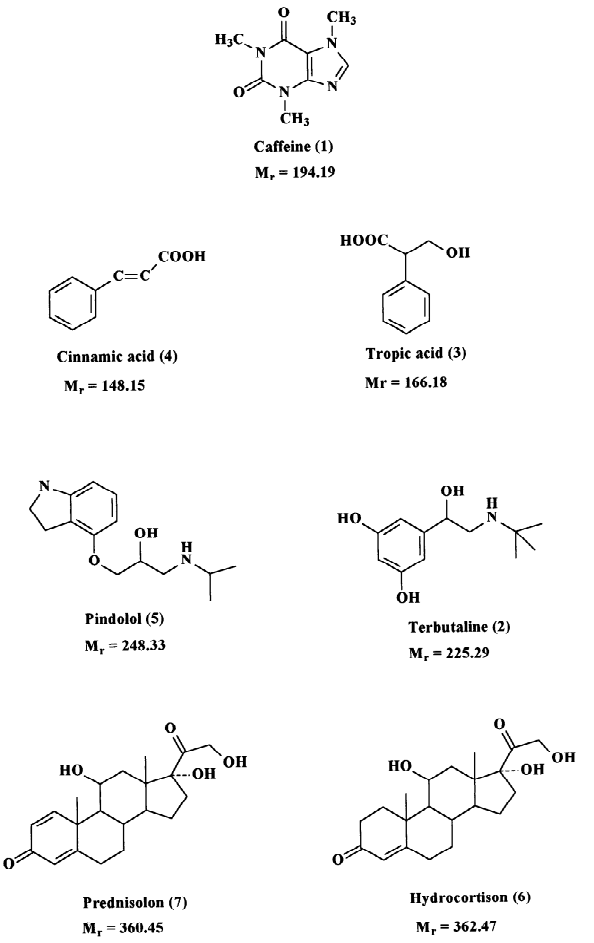

Fig. 1. Test solutes used in the present investigations.

Table 1
Observed migration times normalised using caffeine as internal reference are given. Furthermore, α values are calculated for some solute pairs

Solvent	Test solute									
	Terbutaline (2)	Tropic acid (3)	α **4/3**	Cinnamic acid (4)	α **5/4**	Pindolol (5)	α **6/5**	Hydrocortisone (6)	Prednisolon (7)	α **7/3**
Iso-octane	1.29	1.54	**1.14**	1.76	**1.49**	2.63	**1.08**	2.85	2.91	**1.85**
Toluene	1.24	1.58	**1.18**	1.87	**1.38**	2.58	**1.15**	2.96	3.01	**1.87**
1-Chloropentane	1.26	1.56	**1.21**	1.89	**1.38**	2.61	**1.15**	2.99	3.04	**1.92**
Diisopropyl ether	1.23	1.59	**1.24**	1.97	**1.35**	2.66	**1.22**	3.25	3.30	**2.04**
2-Octanone	1.21	1.56	**1.19**	1.86	**1.27**	2.37	**1.12**	2.66	2.68	**1.71**
Butyl acetate	1.31	1.67	**1.21**	2.02	**1.33**	2.69	**1.15**	3.09	3.13	**1.85**
n-Octanol	1.17	1.53	**1.18**	1.81	**1.15**	2.08	**1.15**	2.40	2.43	**1.57**
None[a]	1.85	1.08		1.14		2.16		2.09	2.16	

[a] Neither inner organic phase nor *n*-butanol added.

tropic acid are seen. The higher the affinity of the steroids for the pseudo-stationary phase is, the higher the selectivity becomes. This is to be expected as the polar anionic solutes only migrates by its electrophoretic migration. The reason for this is that the anionic test solutes have no affinity neither to the anionic surface of the droplets of the emulsion nor to the inner organic phase. The separation mechanisms for the cationic solutes are much more complex as they may exhibit their own electrophoretic migration as well as they may form ion-pairs either with the negatively charged droplets or with the monomers of SDS in the aqueous phase. This latter aspect may influence the migration as it may facilitate distribution into the pseudo-stationary phase. The cationic solutes will also exhibit a high affinity towards the anionic surface of the microemulsion droplets. The only solutes that could be expected to be influenced exclusively to the change in the organic solvent is the lipophilic neutral solutes, but only minor changes in the selectivity of these were observed probably due to high distribution to the organic phase independent of the type of lipophilic organic phase.

All the systems show very high separation efficiency expressed as the number of theoretical plates:

$$N = 5.54 \cdot \left(\frac{t_{obs}}{W_{1/2}} \right)^2$$

About 150 000–300 000 plates were obtained in the 40 cm capillary and this should be compared to the efficiency obtained in the similar MEKC system which only generated about 50 000–100 000 plates in the same capillary (Fig. 2). A similar increase in separation efficiency compared to MEKC have been reported by Boso et al. [13] in the separation of vitamins and by Vomastová et al. [14] when separating ten different steroids.

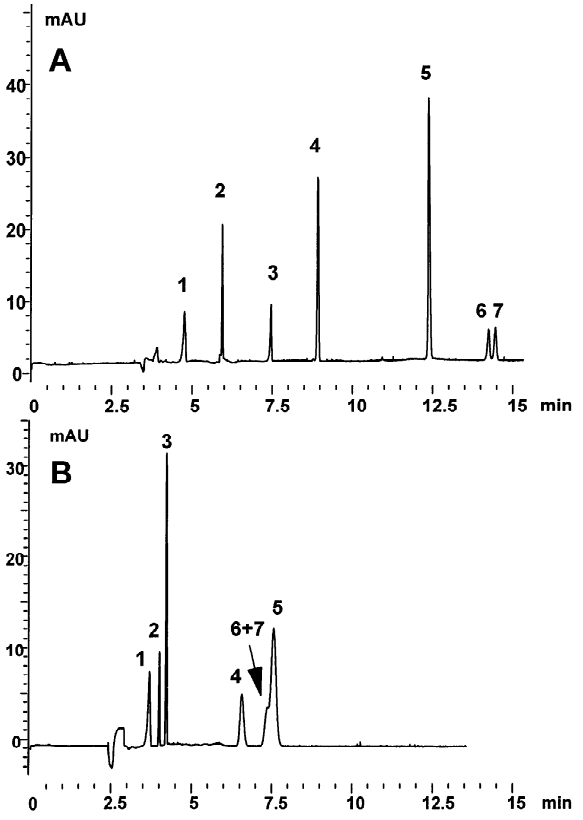

Fig. 2. Comparison of MEEKC and MEKC. Capillary: 48.5 cm (40 cm to detector)×50 μm; Voltage: 20 kV; detection: 215 nm. Electrophoresis medium: (A) 0.8% 5-chloropentane+3.3% SDS+6.6% *n*-butanol+89.3% 10 m*M* sodium tetraborate pH 9.2 and (B) 100 m*M* SDS in 10 m*M* sodium tetraborate pH 9.2. Peak identification: 1, caffeine; 2, terbutaline; 3, tropic acid; 4, cinnamic acid; 5, pindolol; 6, hydrocortisone; 7, prednisolon.

3.2. The surfactant

A cationic, a zwitterionic as well as some nonionic surfactants were substituted fully or in parts for the original surfactant SDS in order to study the influence on selectivity of the separation of the seven test solutes shown in Fig. 1. The concentration of the surfactants was kept constant at 3.3% in all these experiments. Six different microemulsions with iso-octane as the inner organic solvent were investigated and the observed as well as the normalised migration times using caffeine as internal reference are given in Table 2.

The data obtained show that selectivity may be drastically altered expressed by changes in migration order by replacing SDS partly or fully with other surfactants (Fig. 3).

Incorporation of a surfactant having a larger polar group into the microemulsion droplet results in a reduction of the separation window. This decrease in the separation window is considered to be due to a decrease in the number of negative charges relative

Table 2
Observed migration times normalised using caffeine as internal reference and isooctane as inner organic solvent in the microemulsion. Another capillary with identical dimensions to the one used in Table 1 was applied

Surfactant	Test solute									
	Terbutaline (2)	Tropic acid (3)	α **4/3**	Cinnamic acid (4)	α **5/4**	Pindolol (5)	α **6/5**	Hydrocortisone (6)	Prednisolon (7)	α **7/3**
SDS	1.31	1.62	**1.18**	1.91	**1.66**	3.18	**1.10**	3.51	3.59	**2.22**
Dioctyl sulfosuccinate	1.14	1.54	**1.15**	1.78	**0.98**	1.75	**0.95**	1.66	1.68	**1.09**
SDS/MAPS	1.10	1.84	**1.16**	2.13	**0.85**	1.80	**1.07**	1.92	1.95	**1.06**
SDS/Brij 35	1.15	2.02	**1.14**	2.30	**0.73**	1.68	**1.07**	1.80	1.83	**0.91**
SDS/Tween 21	1.09	1.67	**1.11**	1.86	**0.84**	1.56	**1.04**	1.63	1.66	**0.99**
CTMA	1.14	1.21	**1.56**	1.89	**0.88**	1.66	**1.08**	1.79	1.82	**1.50**
CTMA[a]	1.17	1.47	**1.29**	1.90	**0.92**	1.75	**1.09**	1.90	1.92	**1.31**

[a] Neither inner organic phase nor *n*-butanol added.

to the size of the droplet which will result in a slower migration of the droplets. The relative decrease in migration of the lipophilic steroids may be due partly to that they have less affinity to the more polar microemulsion droplets and partly to the decrease in the separation window. The increase in migration of the anionic solutes using mixtures of SDS and MAPS or Brij 35 respectively may be explained by some affinity to the surface of the microemulsion droplets.

When using CTMA ions as surfactant the polarity has to be reversed as the internal surface of the capillary becomes positively charged and thus reverses the electroosmotic flow (EOF). In this case it is the separation mechanism of the anionic solutes that becomes very complex due to e.g. ion-pair formation.

3.3. pH

In the present investigation pH was changed in the interval from 8.1 to 10.6 using borate buffer. At pH 8.1 a doubling of the migration times and a loss in efficiency were observed, but the selectivity remained about the same. When changing the pH from 9.2 to 10.6 only little change in the overall migration times and in efficiency were observed but the migration of pindolol decreased relatively to the other test solutes. This is probably due to a decrease in the protonation of pindolol and thus less affinity of this solute to the anionic surface of the microemulsion droplets. This also shows that the electrostatic interactions of cationic solutes with the surface of the microemulsion droplets are of relatively high importance compared to the partition into the lipophilic droplets. Terbutaline also being an amine is not affected in the same way which probably is due to an increase in the ionisation of the phenolic groups and thus a decrease in migration due to a decrease in the interaction with the microemulsion droplets is compensated by an increase in the electrophoretic migration.

3.4. Temperature

The temperature was changed between 15 and 50°C and the observed migration times as well as the normalised migration times with caffeine as internal reference are given in Table 3. It is seen that the temperature not unexpectedly also may be used as a way to control selectivity as selectivity in general decreases with increasing temperature. The decrease in migration time for terbutaline and tropic acid is primarily due to an increase in EOF resulting from the decrease in viscosity. The normalised migration times of the two solutes do not change.

However, for pindolol, hydrocortisone and prednisolon a decrease in the normalised migration times is also seen which may be due to less distribution to the droplets. The change in selectivity is most

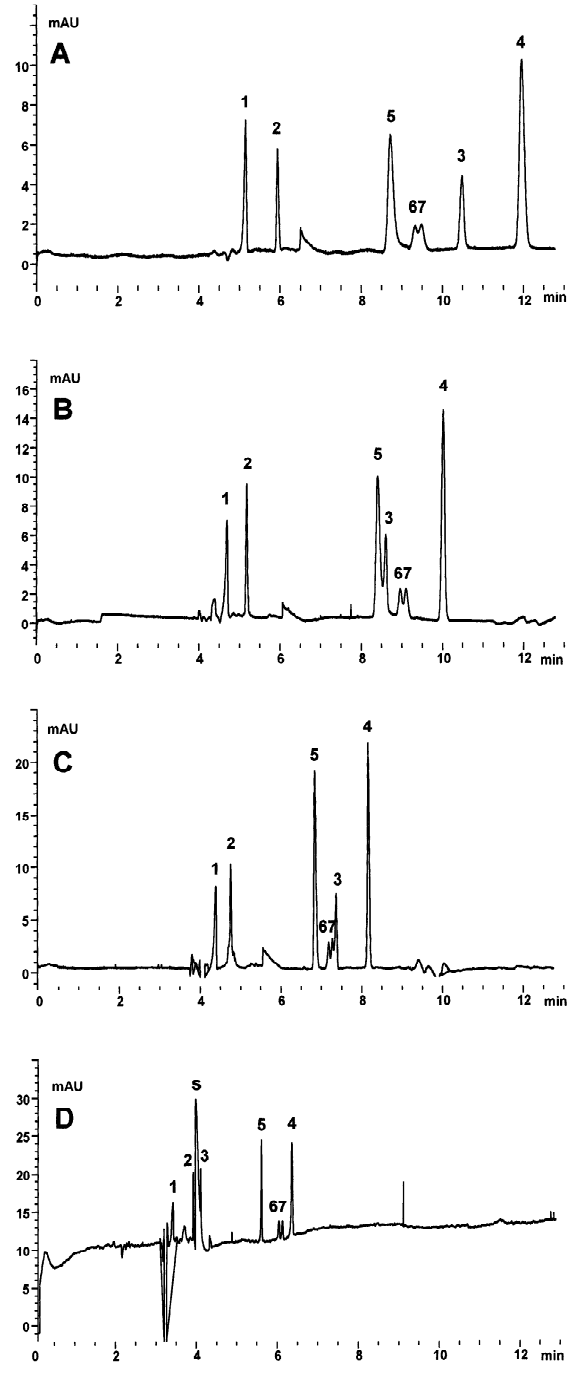

Fig. 3. Electropherograms of seven test solutes using four different surfactant systems. Conditions and peak identification as in Fig. 2A but the 3.3% SDS is replaced by (A) 1.65% SDS + 1.65% Brij 35; (B) 1.65% SDS + 1.65% MAPS; (C) 1.65% SDS + 1.65% Tween 21 and (D) 3.3% CTMA; S, system peak.

pronounced when comparing the more hydrophilic solutes like tropic acid with the lipophilic steroids, on the other hand the selectivity between the steroids and pindolol does not change.

3.5. Stability of microemulsions

The microemulsions used in these investigations was all found to be stable for at least 6 weeks but one. The microemulsion using sodium dioctylsulfosuccinate was only stable for a few days after which it separated into two phases. This is what may happen when the relative proportions of the component in the mixture are at the borderline of what may lead to a microemulsion. By changing these relative proportions it probably will be possible to obtain a stable microemulsion using sodium dioctylsulfosuccinate as well.

4. Conclusion

The present investigations confirm the earlier observations that microemulsion electrokinetic capillary chromatography provide good selectivity and high separation efficiency. The efficiency compared to MEKC is improved from about 50 000 to more than 150 000 plates. If changes in selectivity in a MEEKC system are desired the change of the surfactant may provide major changes, while changing the inner organic solvent are of only minor importance especially when analysing anionic and cationic solutes. The influence of surfactant type on neutral solutes have to be further investigated. pH will of course be of importance if the solutes have pK_a values in the pH range investigated. It is important to realise that anionic solutes will not have affinity to the microemulsion droplets based on SDS as surfactant and thus will only migrate according to a combination of EOF and their electrophoretic migration, whereas cationic solutes may form ion pairs with SDS in the buffer solution and interact with the anionic surface of the droplets. The situation is vice versa when using cationic surfactants for the microemulsion.

Table 3
Observed migration times/normalised migration times using caffeine as internal reference and isooctane as inner organic solvent in the microemulsion

Temperature	Test solute									
(°C)	Terbutaline (2)	Tropic acid (3)	α **4/3**	Cinnamic acid (4)	α **5/4**	Pindolol (5)	α **6/5**	Hydrocortisone (6)	Prednisolon (7)	α **7/3**
15	9.05/1.38	10.85/1.65	**1.25**	13.55/2.07	**2.13**	28.95/4.42	**1.13**	32.86/5.01	33.77/5.15	**3.12**
25	6.70/1.27	8.55/1.62	**1.23**	10.46/1.99	**1.49**	15.65/2.97	**1.18**	18.35/3.49	18.71/3.36	**2.19**
40	5.04/1.23	6.86/1.68	**1.20**	8.29/2.03	**1.19**	9.82/2.41	**1.20**	11.76/2.89	11.91/2.92	**1.74**
50	4.34/1.43	5.88/1.93	**1.18**	6.95/2.28	**1.10**	7.64/2.51	**1.19**	9.08/2.98	9.17/3.01	**1.56**

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