Chapter 5
Micellar Electrokinetic Chromatography

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Abstract Since the introduction of micellar electrokinetic chromatography by Terabe, several authors have paid attention to the fundamental characteristics of this separation method. In this chapter the theoretical and practical aspects of resolution optimization, as well as the effect of different separation parameters on the migration behavior are discussed. These among others include fundamentals of separation, retention factor and resolution equation, efficiency, selectivity, and various surfactants and additives. Initial conditions for method development and instrumental approaches such as mass spectrometry detection are also mentioned covering the proposals for overcoming the difficulties arising from the coupling micellar electrokinetic chromatography with mass spectrometry detection.

5.1 Introduction

Capillary Electrophoresis (CE), described and implemented in the late 1970s of the twentieth century as a modern and highly efficient analytical technique, allowed the separation of charged analytes in small amounts quickly and successfully. Although, in comparison to HPLC, it was characterized by higher resolution obtained in a shorter period of time, it could not be applied to separate neutral analytes. The above-mentioned fact imposed a limitation to the applicability of CE until 1984 when Prof. Terabe developed micellar electrokinetic chromatography (MEKC). Professor Terabe admits that the idea of using ionic micelles in CE

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stemmed from Nakagawa's suggestion he had reached 3 years earlier [1]. A neutral analyte obtains apparent electrophoretic mobility when it interacts with an ionic micelle and migrates in electric field with the same velocity as the micelle. Because the analyte distribution equilibrium between the micelle and the surrounding aqueous phase is quickly reached, the migration velocity is determined by the distribution coefficient.

The idea of MEKC is easy to implement. Namely, a surfactant, most often sodium dodecyl sulfate (SDS), is added to a background electrolyte (BGE) used in capillary zone electrophoresis (CZE) in the amount that is sufficient to create micelles, with no need to modify an apparatus. These micelles are called the pseudo-stationary phase as they play the role of the stationary phase in chromatography. Although they migrate inside the capillary tube, they only exist in equilibrium with a solution containing surfactant molecules and cannot be separated. The separation of neutral analytes was initially based on ionic micelles (MEKC). Later, other pseudo-stationary phases (e.g. micro-emulsions) were implemented. All the electrophoretic techniques that involve the use of pseudo-stationary phases [2] belong to (Fig. 5.1) electrokinetic chromatography (EKC).

The fundamentals of MEKC were discussed in numerous papers and handbooks [3–13]. The technique was developed for neutral analytes but soon it was found that it could be practically applied in the selective separation of all substances including the charged ones. For the past 26 years (since MEKC was devised), it has been studied in as many as 3,000 papers.

5.2 Principles of Separation

Micellar EKC (MEKC) is most frequently performed with the use of anionic surfactants, the most popular of which is sodium dodecyl sulphate (SDS). These substances, when added to water in appropriate amounts, create micelles. In the micelles, internally oriented, hydrophobic parts constitute the core while externally oriented hydrophilic parts are in water medium. The MEKC system is composed of two phases: the aqueous phase and the micellar phase also called the pseudo-stationary phase. SDS micelles are of significant negative charge, and thus, exhibit adequate mobility (μ_{ep}) towards the anode in the direction opposite to the electro-osmotic flow (μ_{eo}) in the majority of buffers used in CE. As in neutral and alkaline medium EOF is higher than the electrophoretic migration of micelles, the latter will migrate to the cathode at speed lower than EOF. When migrating, the micelles interact with analytes hydrophobically and electrostatically. The stronger the interaction of the analyte with the micelle, the longer the time of its migration. It results from the fact that the micelle decelerates the analyte migration with EOF. Analytes which do not interact with micelles are carried with EOF. A separation of sample components is shown in Fig. 5.2.

Therefore, MEKC is a way to selectively separate neutral and ionic compounds and retains all the advantages offered by CZE. The migration in MEKC is

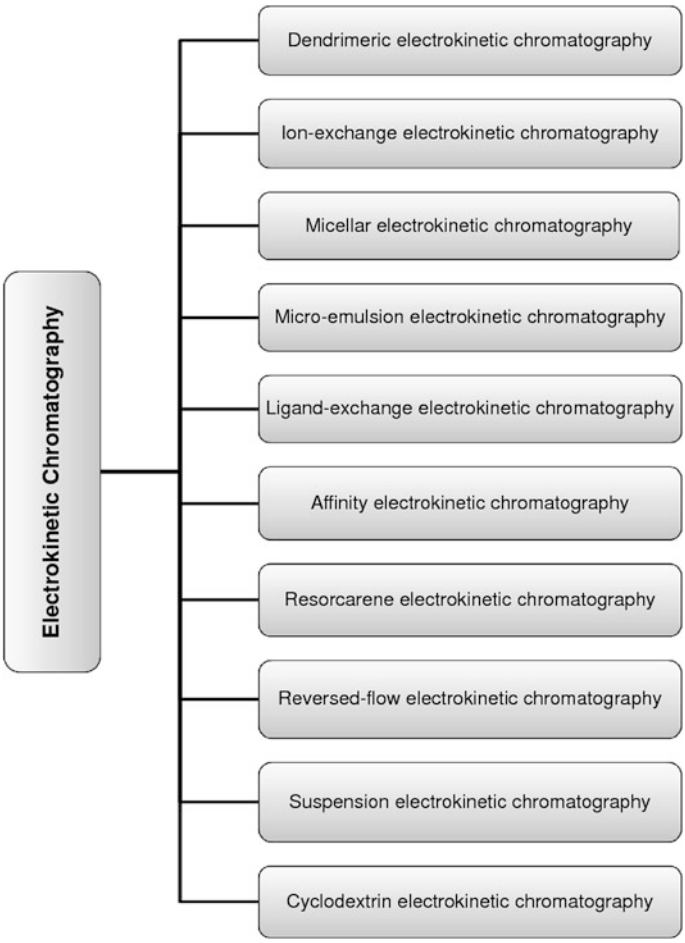

Fig. 5.1 Classification of electrokinetic chromatography based on a kind of pseudo-stationary phase

determined mainly by hydrophobicity but the final result, i.e., analytes separation, is influenced by the charge to mass ratio, hydrophobicity, and the interaction between charges.

The migration time of the analyte (t_R) which interacts with the micelle must be in the range between the migration time of the substance that interacts with the micelle only slightly or no interaction occurs (t_0), and the migration time of the substance that is fully incorporated into the micelle (t_{mc}). Times t_0 and t_{mc} are determined with the use of markers. Methanol can serve as a t_0 marker while Sudan III, Sudan IV, or quinine sulphate can be t_{mc} markers. An electropherogram presenting a so-called MEKC time window is shown in Fig. 5.3.

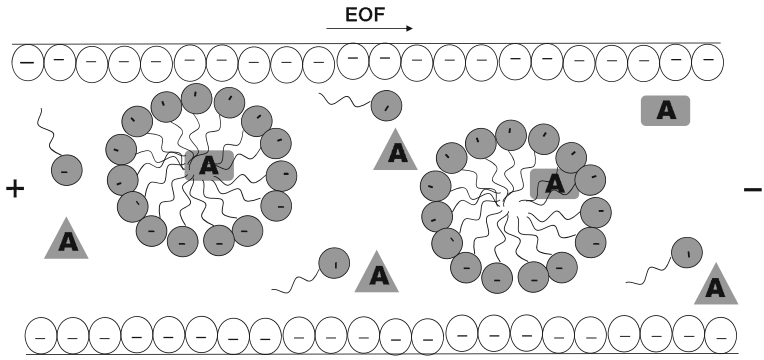

Fig. 5.2 Sample components separation by MEKC using anionic micelles where A = analyte, according to [14]

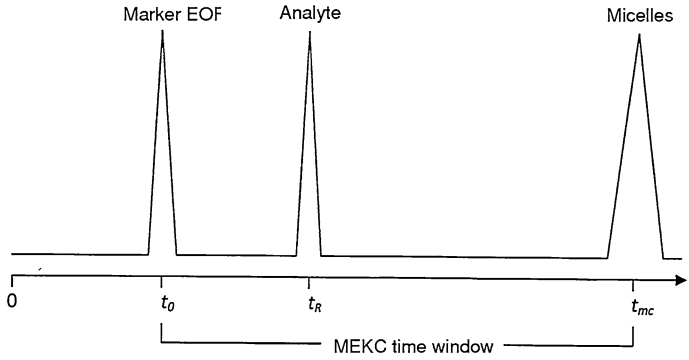

Fig. 5.3 A scheme representing the order of migrations for neutral analytes in MEKC

The necessity to elute the analyte in the time between t_0 and t_{mc} markedly differentiates MEKC from chromatography. In conventional chromatography, the retention coefficient (k) is expressed by the formula:

$$k = \frac{t_R - t_0}{t_0} \tag{5.1}$$

describes the ratio of a number of moles of a component in the stationary phase to its number of moles in the mobile phase. In MEKC, neutral molecules are divided between two moving phases. Hence, the expression of (k) requires modification. Terabe [1] proposed the equation for the retention coefficient in MEKC:

$$k = \frac{t_R - t_0}{t_0(1 - t_R/t_{mc})} \tag{5.2}$$

If t_{mc} approaches infinity, then the expression of (k) becomes identical to the expression of (k) in conventional chromatography; the solid pseudo-phase

becomes the solid phase. Consequently, the equation for separation in MEKC takes the form [15]:

$$R_s = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k_2}{1 + k_2}\right)\left(\frac{1 - (t_0/t_{mc})}{1 + (t_0/t_{mc})k_1}\right) \tag{5.3}$$

where N is number of theoretical plates; α is selectivity coefficient equal to k_2/k_1 (from the assumption >1); k_2, k_1 are retention coefficient of 1 and 2 analytes.

The last factor in the above equation is ascribed to the change in effective length of a capillary tube applied in the separation process. Inside the capillary tube, micelles move during the separation process. It results in the fact that the length of the zone in which micelles interact with analytes is shorter than the physical length of the capillary tube. The difference in the lengths is equal to the distance which micelles will pass in time t_R.

5.2.1 Efficiency

In MEKC, capillary tube efficiency is high. When expressed in a number of theoretical plates, it amounts to between 100 and 200 thousands. The diffusion along the capillary tube is the most important determinant of the theoretical plate height, according to formula [10, 16]:

$$H_l = \frac{2\left(D_{aq} + kD_{mc}\right)}{1 + (t_0/t_{mc})}\frac{1}{v_{eo}} \tag{5.4}$$

where H_l is the component of the plate height generated by longitudinal diffusion, k- retention coefficient, D_{aq} is diffusion coefficient of solute dissolved in liquid phase, D_{mc} is diffusion coefficient of solute dissolved in micellar phase, t_0 is migration time of the substance that does not interact with the micelle, t_{mc} is migration time of substance completely dissolved in micelle, v_{eo} is the velocity of electro-osmotic flow.

Because D_{mc} of a micelle is one order of magnitude smaller than the diffusion coefficient of a small molecule (analyte), higher N can be expected for the analytes of a higher retention coefficient, which means stronger interacting with micelles. In the case of CZE, the situation is reverse; the number of theoretical plates decreases together with an increase in retention coefficient. Another factor that should be taken into consideration is the length of a sample zone in a capillary tube. High efficiency is obtained if the length of a sample zone does not exceed 1 % of the capillary length unless stacking occurs in the capillary tube. The influence of temperature on efficiency is negligible on the condition that current does not exceed 50 μA. A potential adsorption of sample components to the capillary walls constitutes a threat to high efficiency.

5.2.2 Selectivity

The selectivity coefficient, analogously to HPLC, is an important parameter during separation optimization. As a result, while developing the MEKC analytical method, the choice of a surfactant should be carefully made. The initial experiments are usually carried out with the use of SDS. In the case of a failure, other anionic and cationic surfactants as well as bile acids salts can be applied. Introducing additives such as organic solvents or cyclodextrins supports selectivity optimization. Owing to the high MEKC efficiency, it is assumed that a selectivity coefficient higher than 1.02 assures good separation.

5.2.3 Retention Coefficient

The retention coefficient exists in the third and fourth elements of the above-mentioned equation for resolution. Its optimum value (k_{opt}) giving the biggest product of these components is represented by the formula [17]

$$k_{opt} = \sqrt{\frac{t_{mc}}{t_0}} \tag{5.5}$$

The t_0 value is easy to determine using methanol, dimethyl sulfoxide, or other compound that does not interact with a micelle. t_{mc} determination can be problematic which results from a difficulty in finding the micelle marker (Sudan III or IV) peak. In such a case, Terabe advises [15] to assume that t_{mc} is four times higher than $t_{0,}$ and then k_{opt} should be found in the range from 1.7 to 2.0. On the other hand, k is dependent on a surfactant concentration in the form [15]:

$$k = \frac{KV_{mc}}{V_{aq}} \cong K\bar{v}\left(C_{sf} - c_{mc}\right) \tag{5.6}$$

where K is coefficient of analyte distribution between the micellar phase and the aqueous phase, V_{mc} is volume of micelles, V_{aq} is volume of the aqueous phase, v is specific volume of micelle, C_{sf} is surfactant concentration

The equation suggests an almost proportional dependence between k and the surfactant concentration, which means that the V_{mc}/V_{aq} relation is regulated by the changes in a surfactant concentration. With such an assumption, the optimization of k is an easy task if v (specific volume of micelle) and critical micellar concentration (CMC) are known. If such data are not available, then, assuming a linear dependence between the retention coefficient and surfactant concentration, k can be optimized on the trial-and-error basis by changing the surfactant concentration.

5.3 Factors Influencing the Separation Process

A hybrid nature of micellar electrokinetic chromatography results in the fact that the quality of separation and the final results of quantitative analysis are influenced by more factors than in the case of the simplest and most often applied capillary zone electrophoresis.

5.3.1 Surfactants

Of the large number of commercially available surfactants, only some can be applied practically in MEKC. These compounds, when added to water in amounts slightly exceeding a certain concentration called critical micellar concentration (CMC), create spherical aggregates. In these aggregates, also called micelles, the core is made of hydrophobic fragments of the surfactant structure, whereas the hydrophilic fragments are externally oriented and exist in a water electrolyte. Micelles formation results in lowering the amount of free energy of a system. The micelles are dynamic formations with a lifespan shorter than 10 μs. The process of micelles formation is accompanied by a change in surface tension, viscosity, and the ability to scatter light. Surfactants, and thus, micelles created out of them can be negatively or positively charged. They can migrate according to EOF or in the opposite direction. At the neutral or alkali pH, the EOF flow is usually higher than the migration rate of micelles. Hence, the net migration occurs in the same direction as EOF, i.e., towards the cathode. Due to a number of reasons, SDS is the most popular surfactant in MEKC. Among these reasons are high stability, low absorption in the ultraviolet region, high ability to dissolve, and the commercial availability of the high quality reagent. The reagent CMC amounts to 8 mmol/L in water; for buffer solutions, it is even 3 mmol/L. In most cases, 10–50 mmol/L SDS solutions are applied. Still, higher concentrations, even up to 100 mmol/L, give good results provided current intensity does not exceed 50 μA.

Micelles exhibit the ability to order analytes by hydrophobic and electrophoretic interactions. According to Terabe [10, 18], three kinds of analyte interaction mechanisms with micelles can be differentiated, as presented in Fig. 5.4. These are introducing the analyte to the hydrophobic core, the analyte adsorption on the surface or palisade layer, and implementing the analyte as the co-surfactant. Highly hydrophobic nonpolar compounds such as aromatic hydrocarbons are introduced to the core. In such a case, a slightly positive correlation can be observed between the separation coefficient and the length of an alkyl surfactant chain. Higher selectivity in the separation of those kinds of analytes is obtained using bile acid salts to create the pseudo-stationary phase. It is supposed that the majority of analytes interact with micelles through the surface, palisade layer, and polar groups. Separation selectivity can be significantly improved by applying mixed micelles composed of ionic and non-ionic surfactants (Fig. 5.4b).

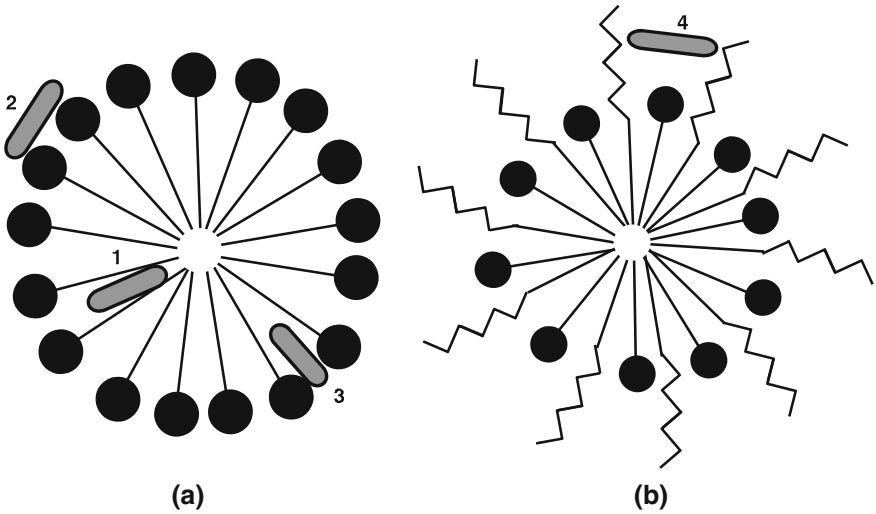

Fig. 5.4 Analytes interactions with micelles: **a** ionic; **b** mixed ionic–non-ionic. *1* Interaction with a hydrophobic core, *2* interaction with the surface, *3* interaction as a co-surfactant, and *4* interaction with the non-ionic surface, according to [16]

In addition to anionic and cationic ones, non-ionic, zwitterion, and mixed micelles are used in MEKC. Some of them are presented in Table 5.1. In practice, the applicability of a surfactant in MEKC is determined by its solubility and CMC. Those of high CMC are inappropriate owing to big amounts of generated current deteriorating separation results. Mixed micelles and additives applied broaden a variety of pseudo-stationary phases that can be applied in MEKC. Surfactants interact with analytes, but they can also adsorb on capillary walls modifying EOF. Depending on a charge, EOF can be increased, reduced, or reversed, which is depicted in Fig. 5.5. The phenomenon occurs at surfactant concentrations below CMC.

Naturally occurring bile acids salts (Fig. 5.6) are also helpful during hydrophobic analytes separation. They function as chiral selectors. A molecular structure of their micellar aggregates markedly differs from the structures of surfactants with long alkyl chains. Hydroxyl groups remaining on one plane cause the micelles to have hydrophobic and hydrophilic regions. Their internal part is less hydrophobic in comparison to the SDS micelle core. They are characterized by a smaller number of aggregation and higher tolerance towards organic modifiers. The critical micellar sodium cholate concentration changes markedly when addition of methanol exceeds 30 % while in SDS such changes occur at the concentration above 10 %.

An increase in the surfactant concentration usually prolongs the migration time of most compounds as the probability of getting in contact with micelles rises. Such a dependence is observed for hydrophobic and electrostatic interactions. Lowering the pH can enable ionic pair formation with negatively charged micelles,

Table 5.1 Some surfactants use in MEKC

Surfactant	
Anionic	
Sodium decyl sulfate	$CH_3(CH_2)_9OSO_3^-Na^+$
Sodium dodecyl sulfate (SDS)	$CH_3(CH_2)_{11}OSO_3^-Na^+$
Sodium tetradecyl sulfate (STS)	$CH_3(CH_2)_{13}OSO_3^-Na^+$
Sodium dodecyl sulfonate	$CH_3(CH_2)_{11}SO_3^-Na^+$
Cationic	
Dodecyltrimethylammonium chloride (DTAC)	$CH_3(CH_2)_{11}Na^+(CH_3)_3Cl^-$
Dodecyltrimethylammonium bromide (DTAB)	$CH_3(CH_2)_{11}Na^+(CH_3)_3Br^-$
Cetyltrimethylammonium chloride (CTAC)	$CH_3(CH_2)_{15}Na^+(CH_3)_3Cl^-$
Cetyltrimethylammonium bromide (CTAB)	$CH_3(CH_2)_{15}Na^+(CH_3)_3Br^-$
Cationic fluorosurfactant (Fluorad FC 134)	$CF_3(CF_2)_7SO_2NH(CH_2)_3N(CH_3)_3I$
Non-ionic and zwitterionic	
Octyl glucoside	
3-[3-(chloroamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS)	
Chiral	
Sodium N-dodekanoyl-L-valinate (SDVal)	
Bile salts	
Sodium cholate	
Sodium taurocholate	

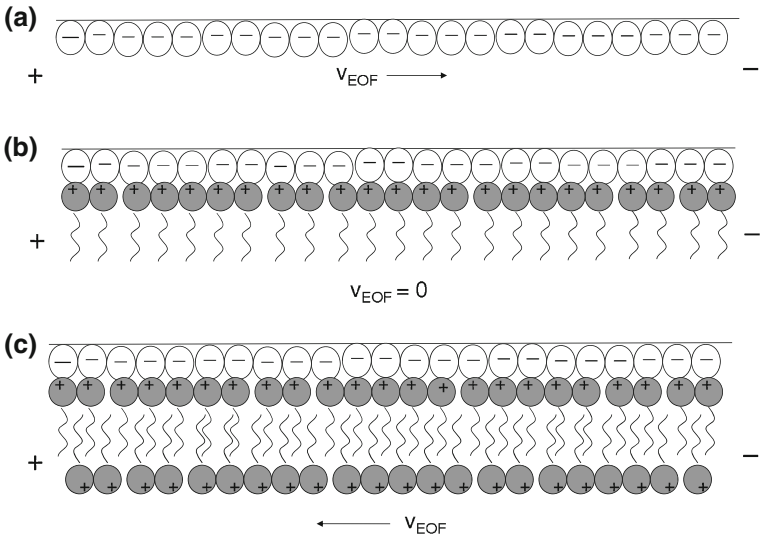

Fig. 5.5 Influence of a cationic surfactant on electroosmotic flow: **a** normal flow with no surfactant added; **b** adsorption of cationic surfactant on internal walls of a capillary which results in stopping EOF; **c** creating a double layer as a result of hydrophobic activity of aliphatic chains which brings about EOF reverse

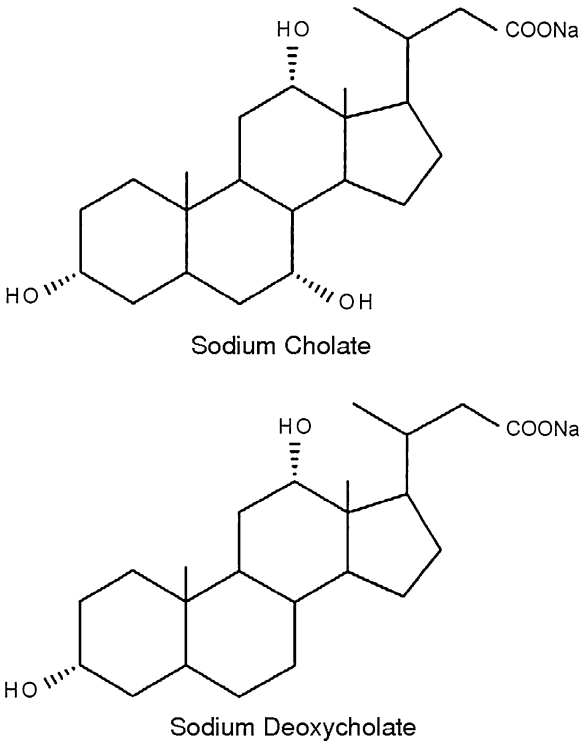

Fig. 5.6 Bile acid salts

which is meaningful during the separation of mixtures of compounds with amino groups. A retention coefficient of a neutral compound rises together with an increase in a surfactant concentration.

5.3.2 Modifiers

Cyclodextrins (CD) are macro-cyclic oligosaccharides obtained by enzymatic starch digestion. They are composed of 6, 7, or 8 glucopyranose units and are called α-, β-, and γ- CD. They are of toroidal shape with a relatively hydrophobic internal part that allows the formation of inclusive complexes with analytes that match the internal CD sizes. The external surface is hydrophilic. The complexes result from hydrogen bonds formation, van der Waals forces activity, or hydrophobic interactions. They, however, influence CD separation only when an analyte size accurately corresponds to their internal sizes. Too big analytes do not form complexes, and in the case of small analytes, the molecular contact with CDs is insufficient for influencing the separation. The phenomenon of inclusion has been applied in a range of separation methods [19, 20].

Cyclodextrins are added to the BGE in MEKC in order to weaken the interactions with micelles. Consequently, the separation of strong hydrophobic analytes is improved. Adding CDs can change selectivity mainly in relation to aromatic isomers. CDs possess an optically active hydrocarbon structure and, when added, they enable enantiomers (particularly those neutral ones) separation. In practice, the most commonly used ones are β-CD and γ-CD at concentrations ranging between 10 and 40 mmol/L. A surfactant molecule can get inside CD causing a change in an inclusive complexes formation constant between an analyte and a CD.

Organic modifiers that mix with water are useful in MEKC, similar to HPLC. Adding an organic solvent reduces EOF flow, and therefore, elution window is extended. A more important role the organic solvent plays is based on a change in an analyte distribution coefficient between the micelle and the BGE. A modifier makes the aqueous phase more "friendly" to hydrophobic analytes, and so the migration time increases.

In MEKC, a number of solvents are used. Most often methanol and acetonitrile are added in amounts between 5 and 25 %. Aprotic solvents such as tetrahydrofurane, dimethyl sulfoxane, dimethylformamide are less commonly used. The amount of a modifier added is limited by its influence on the number of aggregation as well as micelles dissociation. When a modifier concentration exceeds certain value, the separation which proceeds according to the MEKC mechanism changes into the CZE separation mechanism.

Reagents used to form ionic pairs in MEKC act in a way similar to that in reversed-phase HPLC [21]. The addition of tetraalkylammonium salts (cationic ion pair reagents) improves the ability to separate many ionic analytes by shortening cations migration times and prolonging anions migration. It suggests the possibility of forming ion pairs with anions which leads to their more efficient incorporation to the micellar phase.

Urea influences the micelles formation [22, 23] and, when added, improves separation selectivity in MEKC [24], reduces the EOF and migration time of the micelle. In 8 mmol/L urea solution, viscosity increases by 66 % and current drops by three times. Retention coefficient is reduced for most analytes.

5.3.3 pH

The pH value influences electrokinetic velocities and ionizable compounds charges. SDS charge and electrophoretic micelles flow (v_{ep}) do not change together with the change in the pH. EOF changes with the change in the pH, and consequently, micelles migration velocity (v_{mc}) is changed [25]. At pH 5, the net velocity of micelles approximates zero. At pH below 5, the direction of the micelles flow is reversed because electrophoretic micelles flow is higher than that of EOF. Analytes separation in solutions with pH $\approx$ 5 is not advised due to low reproducibility of retention times. At pH $\approx$ 3 hydrophobic analytes and ionic pairs formed reach the detector before other components of an analyzed mixture do.

The pH influence on an analyte charge is essential and should be considered when designing method selectivity. At a low pH value, amino compounds will undergo protonating, bringing about strong affinity towards micelles. Analytes of weak acids character will then undergo ionic suppression.

5.3.4 Elution Order

In MEKC analysis with the use of SDS micelles, a highly probable elution order is as follows: anions–neutral compounds–cations. Anions spend more time in the electrolyte phase mainly as a result of their repulsion by anionic micelles. The higher the negative charge of an ion, the shorter the retention time. Neutral analytes incorporated into micelles as a result of hydrophobic interactions with their cores elute in the secondary order. Cations keep their tendency to elute as the last ones mainly due to electrostatic attraction. Ionic pairs with micelles are formed which leads to a generalization: the higher the positive charge, the higher the retention.

The above rules may not apply to particular analytical tasks. Strong hydrophobic interactions may overcome electrostatic attraction or repulsion. As far as non-homologous structure analyte mixtures (e.g., drugs mixtures) are concerned, the elution order in MEKC can be significantly changed with no obvious correlation between migration rate and a charge to mass relation or hydrophobicity.

5.4 Equipment and Analytical Procedure

MEKC does not require any apparatus modification usually applied in CE. A capillary tube of dimensions typical to CE (internal diameter 50 or 75 μm), made of fused silica is filled with a buffer containing a dissolved ionic surfactant. Phosphate, borate, and Tris buffers at the concentration of 20–50 mmol/L are most commonly used. Regarding the pH, it should be remembered that electro-osmotic flow (EOF) almost completely ceases at the pH value below 2; above 7, it is fast and stable. Together with a rise in the buffer concentration, its buffer capacity also grows. Moreover, there is a risk of generating significant amounts of current and Joule heat with all the negative consequences for quality of separation. For MEKC, the risk is particularly big owing to the presence of a surfactant in the ionic buffer. It is advised to avoid using buffers with potassium because it can lead to the surfactant precipitation in the form of a potassium salt after the ionic exchange with the buffer. Current obtained during the MEKC separation can be higher than that obtained during CZE. It results in the need to refill an electrolyte in order to prevent changes in the pH. If cationic surfactants such as CTAB, which cause a change in EOF are used, the capillary regeneration has to be done with much care.

In an anodic vial, CTAB undergoes electrolysis, and bromine which contaminates the electrolyte is evolved. It should be remembered when refilling the electrolyte.

5.4.1 Detection

Detection in MEKC does not differ much from that applied in CZE. UV–Vis, fluorescence, and electrochemical detectors are used. The problems that emerge while connecting a mass detector (MS) to CZE are thus more real in the case of MEKC in which problems result from the presence of naturally non-volatile surfactants in the BGE. Surfactants and strong electrolytes pollute and lower the ionizing ability of interfaces. To omit the problem, a separation with micelles migrating in the opposite direction or volatile surfactants was carried out.

In practice, it occurred that the MEKC-MS procedure can be successfully applied if a non-volatile surfactant and the BGE concentrations do not exceed 20 and 10 mmol/L, respectively [26, 27]. Another good solution is to implement fotoionization at the atmospheric pressure, resistant to the surfactant concentration even up to 50 mmol/L [28].

5.4.2 Method Development

Terabe [3] gives initial operating conditions (Table 5.2) to start with when elaborating a separation method for neutral or slightly ionizable analytes by means of MEKC. As he claims, for the BGE, a slightly alkali borate buffer should be chosen as it enables keeping small current due to low electrophoretic borate ion mobility. The sample can be dissolved in any solvent that mixes with water. In the case of sample preconcentration in a capillary tube, the sample solution must be prepared according to the sample preconcentration technique requirements (stacking,

Table 5.2 Initial conditions of the analysis by MEKC, according to [3]

Capillary	50–70 μm × 20–50 cm, standard, uncoated
BGE	50 mmol/L SDS in 50 mmol/L borate buffer (pH 8.5–9.0) or 50 mmol/L SDS in 50 mmol/L phosphate buffer (pH 7.0) (sodium salt)
Voltage	10–25 kV (current below 50 μA)
Temperature	25 °C or ambient
Solvent of sample	Water, methanol or other solvent miscible with water
Sample concentration	0.1–1.0 mg/mL (or lower detectable concentration)
Sample introduction	Hydrodynamically on the anodic capillary side
Volume of sample introduced	Less than 1 % of the capillary length
Detection	UV–Vis

sweeping). In the first experiments, relatively high concentrations of standard analytes solutions should be used in order to make their detection easier. Migration times depend on EOF and the effective capillary tube length. When the separation is not satisfactory after the first attempt, selectivity should be manipulated by making changes in a series of parameters. The first step is to determine retention coefficients using time window parameters. If k is higher than 10, micelles concentration should be lowered, methanol or cyclodextrin added, or other surfactant applied. When k is too low, a surfactant concentration should be increased for a neutral analyte, an ionic pair reagent added, or a surfactant exchanged with a cationic one for anionic analytes.

Detailed directions concerning selectivity manipulation on the basis of migration behaviors are described in literature [8]. For the resolution optimization process in MEKC, computer modeling based on physicochemical models describing migration behaviors [29–31] can be applied; in the chemometric approach, polynomial equations can be useful [32–36].

5.5 Summary

As a method, MEKC turned out to be more universal. It also showed its higher capability of selective separation when compared to CZE. Nevertheless, it tends to exhibit low concentration sensitivity of detection, which is a common feature of all the capillary electromigration techniques. These inconveniences are minimized, however, by the development of various techniques of analyte concentration in an electrophoretic system and outside it, as well as sensitive detection methods. Preconcentration techniques and detectors used in electrophoresis are discussed in other chapters of the book. Also, comments on some papers selected from more than 2,000 research works on MEKC applications can be found. The method is employed in, e.g., pharmaceutical analysis, bio-analysis, environmental, or food analysis.

Intensive studies on the improvement of reproducibility and repeatability of migration times as well as peak heights and areas are being carried out. Providing stable EOF, hence significant in almost all CE modes, requires even more attention in the case of MEKC as its magnitude is determined by a greater number of parameters.

MEKC is comparable with HPLC in the reversed phase system, but in MEKC, there is a broader range of factors influencing separation selectivity. Green chemistry enthusiasts emphasize smaller amounts of reagents as well as organic solvents used. MEKC requires a small sample volume (a few nanolitres), however, to introduce such a volume into a capillary tube, at least a few microliters of a sample solution in a conical-shaped vial are required.

References

1. S. Terabe, K. Otsuka, K. Ichikawa, A. Tsuchiya, T. Ando, Electrokinetic separations with micellar solutions and open-tubular capillaries. Anal. Chem. **56**, 111–113 (1984)
2. S. Terabe, Electrokinetic chromatography: An interface between electrophoresis and chromatography. Trends Anal. Chem. **8**, 129–134 (1989)
3. S. Terabe, *Micellar Electrokinetic Chromatography* (Beckman, Fullerton, 1992)
4. J. Vindevogel, P. Sandra, *Introduction to Micellar Electrokinetic Chromatography* (A. Hüthig, Heidelberg, 1992)
5. U. Pyell (ed) (2006) Electrokinetic chromatography. Theory, instrumentation and applications. Wiley, Chichester
6. S. Terabe, N. Chen, K. Otsuka, Micellar electrokinetic chromatography. Adv. Electrophoresis **7**, 87–153 (1994)
7. M.G. Khaledi, Micellar electrokinetic chromatography, in *High Performance Capillary Electrophoresis*, ed. by M.G. Khaledi (Wiley-Interscience, New York, 1998)
8. S. Terabe, Selectivity manipulation in micellar electrokinetic chromatography. J. Pharm. Biomed. Anal. **10**, 705–715 (1992)
9. K. Otsuka, S. Terabe, Micellar electrokinetic chromatography. Bull. Chem. Soc. Jpn. **71**, 2465–2481 (1998)
10. S. Terabe (2008) Micellar electrokinetic chromatography, ed. by J.P. Landers handbook of capillary and microchip electrophoresis and associated microtechniques, 3rd ed., CRC Press, Taylor and Francis Group, Boca Raton, London, New York
11. J.R. Mazzeo, Micellar electrokinetic chromatography, in *Handbook of Capillary Electrophoresis*, 2nd edn., ed. by J.P. Landers (CRC Press, Boca Raton, 1996)
12. M. Silva, MEKC: An update focusing on practical aspects. Electrophoresis **28**, 174–192 (2007)
13. S.F.Y. Li (ed) (1993) Capillary electrophoresis. In: J. Chromatography Library. vol. 52, Elsevier, Amsterdam
14. Technical Bulletin in Polish/Hewlett-Packard (1997) 12-5965-5984E
15. S. Terabe, K. Otsuka, T. Ando, Electrokinetic chromatography with micellar solution and open-tubular capillary. Anal. Chem. **57**, 834–841 (1985)
16. S. Terabe, K. Otsuka, T. Ando, Band broadening in electrokinetic chromatography with micellar solutions and open-tubular capillaries. Anal. Chem. **61**, 251–260 (1989)
17. J.P. Foley, Optimization of micellar electrokinetic chromatography. Anal. Chem. **62**, 1302–1308 (1990)
18. S. Terabe, Micellar electrokinetic chromatography. Anal. Chem. **76**, 240A–246A (2004)
19. C.P. Ong, C.L. Ng, N.C. Chong, H.K. Lee, S.F.Y. Li, Analysis of priority substituted phenols by micellar electrokinetic chromatography. Environ. Monitor Assess **19**, 93–103 (1991)
20. S.K. Yeo, C.P. Ong, S.F.Y. Li, Optimization of high-performance capillary electrophoresis of plant growth regulators using the overlapping resolution mapping scheme. Anal. Chem. **63**, 2222–2225 (1991)
21. N. Nishi, N. Tsumagari, S. Terabe, Effect of tetraalkylammonium salts on micellar electrokinetic chromatography of ionic substances. Anal. Chem. **61**, 2434–2439 (1989)
22. P. Mukerjee, A. Ray, The effect of urea on micelle formation and hydrophobic bonding. J. Phys. Chem. **67**, 190–192 (1963)
23. M.J. Schick, Effect of electrolyte and urea on micelle formation. J. Phys. Chem. **68**, 3585–3592 (1964)
24. S. Terabe, Y. Ishihama, H. Nishi, T. Fukuyama, K. Otsuka, Effect of urea addition in micellar electrokinetic chromatography. J. Chromatogr. **545**, 359–368 (1991)
25. K. Otsuka, S. Terabe, Effects of pH on electrokinetic velocities in micellar electrokinetic chromatography. J. Microcol. Sep. **1**, 150–154 (1989)

26. G.W. Somsen, R. Mol, G.J. de Jong, On-line micellar electrokinetic chromatography–mass spectrometry: Feasibility of direct introduction of non-volatile buffer and surfactant into the electrospray interface. J. Chromatogr. A **1000**, 953–961 (2003)
27. R. Mol, E. Kragt, I. Jimidar, G.J. de Jong, G.W. Somsen, Micellar electrokinetic chromatography–electrospray ionization mass spectrometry for the identification of drug impurities. J. Chromatogr. B **843**, 283–288 (2006)
28. R. Mol, G.J. de Jong, G.W. Somsen, Atmospheric pressure photoionization for enhanced compatibility in on-line micellar electrokinetic chromatography–mass spectrometry. Anal. Chem. **77**, 5277–5282 (2005)
29. M.G. Khaledi, S.C. Smith, J.K. Strasters, Micellar electrokinetic capillary chromatography of acidic solutes: Migration behavior and optimization strategies. Anal. Chem. **63**, 1820–1830 (1991)
30. J.K. Strasters, M.G. Khaledi, Migration behavior of cationic solutes in micellar electrokinetic capillary chromatography. Anal. Chem. **63**, 2503–2508 (1991)
31. C. Quang, J.K. Strasters, M.G. Khaledi, Computer-assisted modeling, prediction, and multifactor optimization in micellar electrokinetic chromatography of ionizable compounds. Anal. Chem. **66**, 1646–1653 (1994)
32. J. Vindevogel, P. Sandra, Resolution optimization in micellar electrokinetic chromatography: Use of Plackett-Burman statistical design for the analysis of testosterone esters. Anal. Chem. **63**, 1530–1536 (1991)
33. Y. He, H.K. Lee, Orthogonal array design experiments for optimizing the separation of various pesticides by cyclodextrin-modified micellar electrokinetic chromatography. J. Chromatogr. A **793**, 331–340 (1998)
34. K. Persson-Stubberud, O. Åström, Separation of ibuprofen, codeine phosphate, their degradation products and impurities by capillary electrophoresis: I method development and optimization with fractional factorial design. J. Chromatogr. A **798**, 307–314 (1998)
35. S. Mikaeli, G. Thorsén, B. Karlberg, Optimisation of resolution in micellar electrokinetic chromatography by multivariate evaluation of electrolytes. J. Chromatogr. A **907**, 267–277 (2001)
36. H. Wan, M. Öhman, R.G. Blomberg, Chemometric modeling of neurotransmitter amino acid separation in normal and reversed migration micellar electrokinetic chromatography. J. Chromatogr. A **916**, 255–263 (2001)