Chapter 6

Microemulsion Electrokinetic Chromatography

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Abstract

Microemulsion electrokinetic chromatography (MEEKC) is a special mode of capillary electrophoresis employing a microemulsion as carrier electrolyte. Analytes may partition between the aqueous phase of the microemulsion and its oil droplets which act as a pseudostationary phase. The technique is well suited for the separation of neutral species, in which case charged oil droplets (obtained by addition of an anionic or cationic surfactant) are present. A single set of separation parameters may be sufficient for separation of a wide range of analytes belonging to quite different chemical classes. Fine-tuning of resolution and analysis time may be achieved by addition of organic solvents, by changes in the nature of the surfactants (and cosurfactants) used to stabilize the microemulsion, or by various additives that may undergo some additional interactions with the analytes. Besides the separation of neutral analytes (which may be the most important application area of MEEKC), it can also be employed for cationic and/or anionic species. In this chapter, MEEKC conditions are summarized that have proven their reliability for routine analysis. Furthermore, the mechanisms encountered in MEEKC allow an efficient on-capillary preconcentration of analytes, so that the problem of poor concentration sensitivity of ultraviolet absorbance detection is circumvented.

Key words Microemulsion, Electrokinetic chromatography, Capillary electrophoresis, Pseudostationary phase, Hydrophobic interaction

1 Introduction

Microemulsion electrokinetic chromatography (MEEKC) covers variants of capillary electrophoresis (CE) employing a microemulsion as carrier electrolyte. Contrary to other CE techniques, MEEKC allows the separation of neutral analytes. In addition, this technique is also suited for separation of charged species, whereby separation selectivities may be achieved which are significantly different from those obtained by commonly used CE techniques for separation of ionic analytes. Microemulsions have been discovered more than 70 years ago by Hoar and Schulman [1] and have been introduced for CE separation techniques in 1991 by Watarai [2]. Since then the numbers of applications of MEEKC have increased steadily, which has been documented in review papers that have been published regularly within the last few years [3–8].

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1.1 Fundamentals

Microemulsions are dispersions of two immiscible liquids and may consist either of oil droplets suspended in water (oil-in-water [o/w] microemulsions) or of water droplets suspended in an oil phase (water-in-oil [w/o] microemulsions). MEEKC separations are mostly carried out in oil-in-water microemulsions. Typically, they consist of octane droplets dispersed in an aqueous buffer containing surfactants to coat the octane droplets and lower the surface tension between the two liquids. Furthermore, a short-chain alcohol like *n*-butanol (called a cosurfactant) is added which also lowers the surface tension. Under such conditions, a stable microemulsion is generated with droplet sizes below 10 nm. It is optically transparent and looks like a single-phase solvent although it is a two-phase system. As mentioned earlier, o/w microemulsions are the most common form of microemulsions used in MEEKC. Therefore, the following discussions will mostly focus on this type, and w/o microemulsions will be treated only shortly in part 1.4.

Sodium dodecyl sulfate (SDS) is commonly used as surfactant for stabilization of the microemulsion droplets. At the interface between the aqueous phase and the oil phase, the dodecyl chain is oriented toward the inner of the oil droplet, whereas the negatively charged sulfate group is oriented toward the aqueous phase. The cosurfactant such as *n*-butanol will also attach to the surface of the oil droplet with the butyl group toward the oil phase and the alcohol group toward the aqueous phase. As a result of the presence of the anionic surfactant, the oil droplets will acquire a negative charge and will exhibit an electrophoretic mobility in the direction of the anode. The aqueous phase is generally buffered at an alkaline pH. In fused-silica capillaries, alkaline buffers generate an electroosmotic flow (EOF) toward the cathode. Provided that the pH is high enough, the magnitude of the EOF exceeds the electrophoretic mobility of the oil droplets (which is directed against the EOF). Therefore, the EOF will sweep the oil droplets to the cathode. The apparent mobility of the oil droplets is directed to the cathode and has a magnitude that is lower than that of the EOF.

Highly hydrophilic neutral analytes injected at the anodic side of the capillary will reside predominantly in the aqueous phase so that they will be transported to a detector positioned at the cathodic side of the separation capillary by the EOF according to the electroosmotic mobility. The time at which they reach the detector after injection may be called t_{EOF}. Conversely, highly hydrophobic analytes will reside predominantly in the oil droplets, will be transported to the cathodic detection side according to the apparent mobility of the droplets, and will reach the detector after the time t_{ME}. Analytes of medium polarity will undergo partitioning equilibria between the aqueous phase and the oil phase, and will reach the detector at a time t, which is between t_{EOF} and t_{ME}. Obviously, MEEKC separates neutral analytes according to their hydrophobicities. The technique offers a limited separation time window

governed by t_{EOF} and t_{ME}. These two parameters may be determined by injection of methanol as EOF marker (t_{EOF}) and octanophenone or dodecyl benzene as microemulsion marker (t_{ME}).

The partitioning equilibria of analytes established between the aqueous phase and the oil droplets indicate that chromatographic principles are involved in the separation (justifying the word "chromatography" in MEEKC). Therefore, the oil droplets may be called a pseudostationary phase. In analogy to chromatography, one can define retention factors k for the analytes:

$$k = \frac{t_r - t_{EOF}}{t_{EOF} - \left(\frac{1 - t_r}{t_{ME}} \right)}$$

In case of a true stationary phase as encountered in liquid chromatography, t_{ME} would become infinite and t_{EOF} would be the dead time. The equation given earlier would turn into the well-known definition of k being the ratio of net retention time to dead time.

A schematic presentation of the MEEKC separation process is given in Fig. 1. Additional details can be found in recently published review papers (see for example [9]). It should be pointed out that the separation mechanisms encountered in MEEKC are similar to those in micellar electrokinetic chromatography (MEKC), which uses micelles (aggregates of surfactant molecules) as pseudostationary phase. Advantages of MEEKC over MEKC may include the fact that oil droplets exhibit a reduced rigidity compared to micelles so that hydrophobic analytes can more easily penetrate the surface and enter the core of the pseudostationary phase. Furthermore, MEEKC may offer a somewhat larger separation time window, because the total charge of the droplets (and thereby t_{ME}) can be manipulated by employing mixed surfactants composed of charged and neutral species in different compositions.

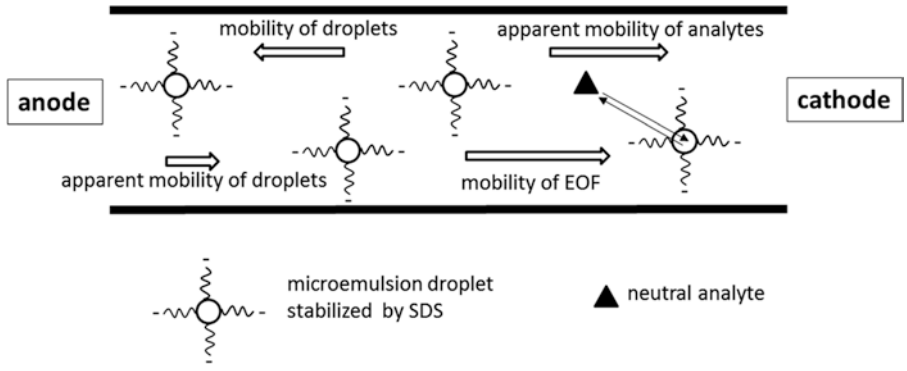

Fig. 1 Principle of the separation process in microemulsion electrokinetic chromatography for a neutral analyte in an alkaline microemulsion stabilized by an anionic surfactant like sodium dodecyl sulfate

Instead of alkaline buffers, acidic buffers are used occasionally. In such a case the EOF is very low and is no longer able to transport an anionic pseudostationary phase (droplets stabilized by SDS) to the cathodic detection side. Therefore, one has to switch the polarity and the detection must be at the anodic side of the capillary.

Besides anionic surfactants like SDS, also cationic surfactants like cetyltrimethylammonium bromide (CTAB) may be employed for stabilization of the oil droplets. Such surfactants will not only lead to positively charged droplets, but they also act as EOF modifiers due to the generation of a positively charged inner surface of the fused-silica capillary, resulting in a reversed direction of the EOF. Therefore, the detector must be positioned at the anodic end of the capillary when working with such cationic surfactants.

MEEKC separations of ionic analytes involve somewhat more complex mechanisms, because the apparent mobility of the analytes is governed by both their electrophoretic mobilities and their interactions with the pseudostationary phase. Generally, nonionic surfactants can be used leading to a neutral pseudostationary phase, but cationic or anionic pseudostationary phases may be suited as well. In the latter case, one has to take into account a possible repulsion of the charged analyte from the charged pseudostationary phase if both are anionic (or if both are cationic). In case of analytes with a charge opposite to the pseudostationary phase, additional ion-pairing equilibria at the surface of the droplets may have an impact on the separation. In addition, ion pairing between the charged analyte and excess of surfactant may occur in the aqueous phase, which may favor the partitioning reaction into the oil droplet.

Last, but not least, one should keep in mind that depending on the pH of the microemulsion the analytes may be in a neutral form or in a protonated/deprotonated form. Therefore, different types of microemulsions (neutral or charged) may be recommendable, and different separation selectivities can be expected. This is demonstrated in Fig. 2, which shows the separation of closely related methyl derivatives of quinoline that are used as raw materials for industrial production of agrochemicals and pharmaceuticals [10]. Chromatogram A presents the separation of methylquinolines at pH 9.4 (neutral analytes) using a negatively charged oil phase, and B presents the separation of the same set of analytes at pH 4.0 (protonated analytes) using a neutral oil phase [10]. In case A, the separation selectivity is solely governed by the partitioning between the aqueous phase and the oil droplets, whereas in case B separation selectivity is significantly different because it is affected by both partitioning and electrophoretic behavior of the analytes. It is worth mentioning that a buffer of pH 4 without oil droplets (corresponding to a pure capillary zone electrophoretic mode) would not lead to any satisfactory separation.

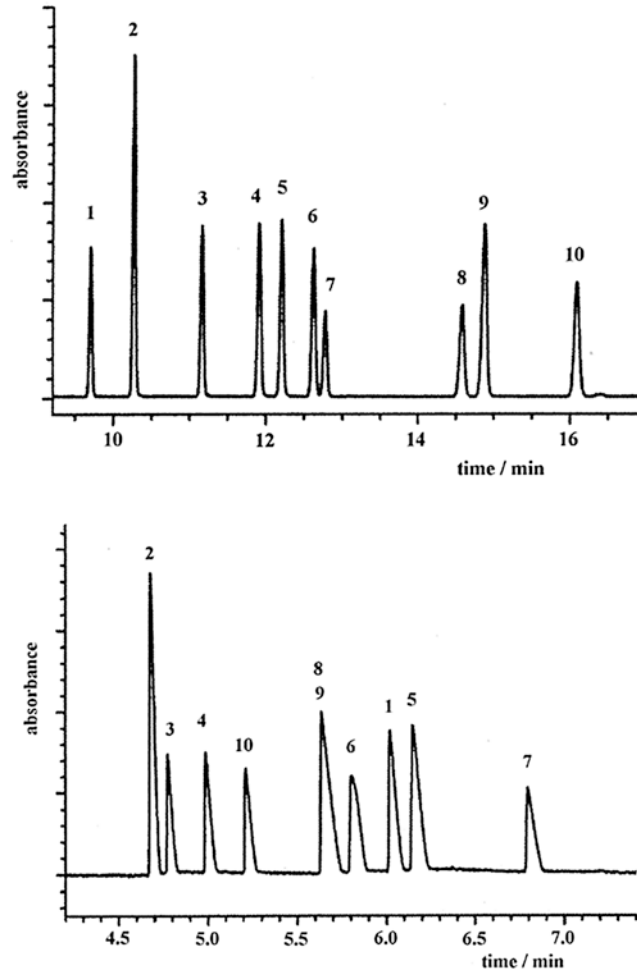

Fig. 2 MEEKC separation of methylquinolines at pH 9.4 using a negatively charged oil-in-water microemulsion consisting of SDS, *n*-butanol, *n*-octane, and borate buffer (**a**), and at pH 4 using a neutral oil-in-water microemulsion consisting of Brij35, *n*-butanol, *n*-heptane, and acetate buffer (**b**). Peaks: 1 = quinoline, 2 = isoquinoline, 3 = 2-methylquinoline, 4 = 4-methylquinoline, 5 = 3-methylquinoline, 6 = 6-methylquinoline, 7 = 8-methylquinoline, 8 = 4,8-dimethylquinoline, 9 = 2,8-dimethylquinoline, 10 = 2,4,8-trimethylquinoline. UV detection at 214 nm. Adapted from ref. [10]

1.2 Optimization of the Separation of Neutral Analytes

Variables to be optimized with respect to manipulation of migration order and optimization of separation selectivity of neutral analytes include the kind of oil phase, the kind of surfactant and cosurfactant, the addition of water-miscible solvents, and the use of specific additives such as cyclodextrins, carbon nanotubes, and others, that introduce extra effects for the separation of certain analytes.

1.2.1 Oil Phase

The concentration of the oil phase in the carrier electrolyte is typically around 1 % or less. Frequently, *n*-alkanes like hexane, heptane, or octane are employed as oil phase, with octane often being

preferred. As an alternative, ethyl acetate has been selected because of its lower surface tension which allows lower concentrations of surfactant for stabilization. Other compounds occasionally reported for preparation of microemulsions include cyclohexane, toluene, 1-chloropentane, alcohols of medium chain length like 1-hexanol or 1-octanol, and propylene glycol monomethylester acetate. More recently, ionic liquids have been investigated as oil phase, whereby 1-butyl-3-methylimidazolium hexafluorophosphate may be promising [11–13]. Even vegetable oils and artificial oils made of alkane and alcohol may have some potential [14]. Different partitioning coefficients provided by the different oil phases may lead to somewhat different separations, but major changes in migration order are not likely. Unfortunately, it is often still a matter of trial and error to find the best oil phase. In any case, octane may be a good start.

For separation of enantiomers, a chiral oil phase may be used. Chiral alkyl tartrates have been investigated for this purpose [15, 16]. Resolution between enantiomers was obtained if borate buffers were employed, whereas phosphate or Tris buffers did not lead to any enantioseparation. The authors attributed this phenomenon to the formation of a complex between borate and the alkyltartrate.

1.2.2 Surfactants

Surfactants are a key component in the microemulsion. They have a direct impact on stability of the oil droplets by lowering the surface tension, and they affect size and charge of the droplets, magnitude, and direction of the EOF. Anionic or cationic surfactants as well as mixtures of them with nonionic surfactants have been employed for separation of neutral analytes. One should keep in mind that the addition of ionic surfactants can lead to a significant increase of electric conductivity of the carrier electrolyte, which may limit the applied voltage in order to avoid excessive Joule heating.

The most common surfactant for MEEKC is sodium dodecyl sulfate (SDS), which is typically used at concentrations around 3%. Alternative anionic surfactants include lithium dodecyl sulfate (which leads to somewhat lower electric currents), bile salts like sodium cholate, or sulfosuccinates like sodium bis(2-ethylhexyl) sulfosuccinate.

Cationic surfactants reported for use in MEEKC are based on quaternary ammonium salts like dodecyltrimethyl ammonium chloride, tetradecyltrimethyl ammonium bromide, or cetyltrimethyl ammonium chloride/bromide. As mentioned in the part on fundamentals, the behavior of these salts as EOF modifiers must be taken into account.

The use of mixtures of surfactants may provide various benefits. The combination of SDS and Brij-35 (a nonionic surfactant) allows the manipulation of the charge of the droplets and thereby manipulation of the separation time window.

Chiral surfactants have been introduced for MEEKC separations of enantiomers, such as R- and S-dodecoxycarbonylvaline

(DDCV) [17–19]. This approach can also be combined with the use of a chiral oil phase (see previous part) which has been demonstrated for the use of DDCV together with dibutyltartrate or diethyltartrate [20, 21].

1.2.3 Cosurfactant

The variations of nature and concentration of the cosurfactant may be exploited for fine-tuning of the separation (see for example [22]). Short chain alcohols are frequently used as cosurfactants, with 1-butanol at a concentration of around 6% being the most common one. It has been suggested that such solvents do not only act as cosurfactants, but that a significant portion of it can partition into the oil droplet, especially as the amount of cosurfactant present in the microemulsion exceeds that of the actual oil phase [23]. Thereby, the chromatographic properties of the pseudostationary phase are modified and with it the k values of the analytes affected. General rules for selection of appropriate cosurfactants are still difficult to establish.

Chiral separations may benefit from the use of chiral 2-alkanols like R(−)-2-pentanol, R(−)-2-hexanol or R(−)-2-heptanol as cosurfactants [24]. A synergistic effect has been observed when (S)-2-hexanol was employed together with a chiral surfactant [25]. In addition, even three-chiral-component microemulsions (R- or S-DDCV, S-2-hexanol, and R- or S-diethyltartate) have been investigated and compared with one- and two-chiral-component microemulsions [26].

Interestingly, it has also been claimed that a stable microemulsion prepared by hexane and SDS in an ammonium acetate solution can be generated without the use of any cosurfactant [27], but such an approach has not made its way to a wider range of applications.

1.2.4 Water-Miscible Solvents

For certain applications, water-miscible organic solvents may be added to the microemulsion [23]. In this way, the partitioning equilibria of the analytes between the aqueous phase and the oil phase may be manipulated. This aspect is of major significance when analytes with very poor solubility in water are separated. Such analytes would not partition at all into a purely aqueous phase and would therefore reach the detector after the time t_{ME}. A typical example for the benefits of water-miscible solvents is the analysis of highly hydrophobic polymer stabilizers [28]. Depending on the type of water-miscible solvent, there are upper limits for its use in MEEKC. Exceeding these limits will result in a disintegration of the microemulsion. It has been reported that methanol may be used up to 8% (v/v), acetonitrile up to 12%, whereas 2-propanol may be used at considerably higher concentrations [29]. One should not forget the well-known side effect of organic solvents on the magnitude of the EOF which depends on the dielectric constant of the liquid phase, on the viscosity, and on the zeta potential of the capillary wall (all these parameters are directly affected by amount and type of an organic solvent in the aqueous phase of the microemulsion).

1.2.5 Other Additives

The addition of cyclodextrins to the carrier electrolyte is a well-established approach for chiral separations in capillary zone electrophoresis. The formation of transient diasteromeric complexes with cyclodextrins can also be exploited in MEEKC as an interaction in addition to the partition equilibrium between aqueous and oil phase, whereby either neutral cyclodextrins or cyclodextrins modified by charged groups (sulfated cyclodextrin) may be suited [30–32].

More recently, carbon nanotubes dispersed in the microemulsion have been investigated in order to establish additional interactions that might improve the separation selectivity in MEEKC [33–35].

Some experiments have been done with water-soluble ionic liquids as additives. In case of an anionic surfactant, the cation of the ionic liquid may interact and may partly neutralize the negative charge, thereby changing the properties of the pseudostationary phase [36].

1.3 Optimization of the Separation of Ionic Analytes

In the simplest case, ionic analytes are separated by using an oil phase stabilized by nonionic surfactants. In this case, the principles for optimization of the separation are similar to those mentioned earlier for separation of neutral analytes in a charged microemulsion. Nonionic surfactants most often employed are Brij-35, Tween-20, or Triton X-100. In addition, a less common nonionic surfactant, Pluronic F-127 has seen suggested [37] (which is an amphiphilic block copolymer consisting of ethylene oxide and propylene oxide), although so far only in combination with SDS for separation of neutral analytes. Most recently, zwitterionic surfactants like *N*-dodecyl-*N*,*N*-dimethyl-3-ammonio-1-propanesulfonate (DAPS) have been studied [38].

As mentioned in the Introduction, the use of charged microemulsions may lead to additional attraction or repulsion of ionic analytes to/from the droplets. This attraction/repulsion may be manipulated by using a mixture of a cationic and an anionic surfactant (see for example [39]). Oppositely charged analytes/droplets or analytes/excess surfactant in aqueous phase may also undergo interactions by ion-pair formation. A systematic treatment of such complex additional interactions is somewhat difficult so that generally valid strategies for optimization of separation selectivity are still limited.

1.4 Water-in-Oil Microemulsions

Although MEEKC is almost exclusively done in oil-in-water microemulsions, a few attempts have been made to apply water-in-oil microemulsions. Altria et al.[40, 41] introduced w/o microemulsions typically composed of 10% SDS, 80% butanol (or 78% butanol and 2% octane), and 10% aqueous buffer (or slight modifications of this composition). Similar compositions have been used later by other groups [42–45], but up to now the number of applications of w/o microemulsions in MEEKC is quite limited.

1.5 Sample Preconcentration by Sweeping

Spectroscopic detection techniques generally suffer from poor detection limits due to the short detection path length provided by the inner diameter of the separation capillary. Preconcentration effects occurring under proper injection conditions may help to improve detection limits. CE separation techniques based on pseudostationary phases may allow a preconcentration step called sweeping. It is generally defined as the picking and accumulating of analytes by a charged pseudostationary phase that penetrates the sample zone during application of a voltage. Most work on sweeping was done using micelles as pseudostationary phase (see, for example, the review in [46]), and the same principles work for microemulsions as well. Therefore, this chapter will not go into details regarding the theory of sweeping. In the simplest case, efficient preconcentration can be achieved with an microemulsion consisting of an oil phase stabilized by a negatively charged surfactant and an aqueous phase of low pH. The sample solution that does not contain the pseudostationary phase is injected hydrodynamically at the cathodic end of the capillary. After injection, the anionic pseudostationary phase will migrate from the cathodic carrier electrolyte vial into the capillary and through the sample zone (because of the low pH, the EOF can be neglected). In the sample zone, neutral analytes undergo partitioning and are focused into a narrow zone. As a result of the focusing effect, quite high volumes of sample may be injected without peak broadening (making possible a more than 1000-fold increase in sensitivity). Nevertheless, too long injection zones (without pseudostationary phases) may lead to instabilities of the system after applying voltage. Therefore, electrokinetic injection techniques have been used instead of hydrodynamic injection (which allows the selective injection of anions or cations without generating an excessively long zone of sample) followed by the sweeping step. Details of quite sophisticated combinations of injection techniques and sweeping would go beyond the scope of this chapter but can be found in recent review papers [3, 4, 7].

1.6 Detection

In common with other CE modes, the most widely used detection technique for MEEKC is UV–visible absorbance detection. Besides, fluorescence detection (with a xenon lamp or a laser as light source) may be the alternative for analytes that show native fluorescence or can be transformed into fluorescent derivatives prior to injection. A typical example for the latter approach is the separation of amino acids after derivatization with fluorescein isothiocyanate (FITC) [47].

Mass spectrometric (MS) detection may be most attractive as it provides the confirmation of peaks for target analytes or the structure elucidation of unknown peaks. Capillary zone electrophoresis can be hyphenated with MS via an electrospray ionization (ESI) source using a sheath–liquid interface which allows the realization of a makeup flow of a few μL/min to make flow

rates better compatible with commercial ESI sources and at the same time allows the application of the high voltage of the CE separation [48]. Unfortunately, the high concentrations of surfactants used in MEEKC make the technique hardly suited for coupling with ESI which would suffer from severe ionization suppression. Instead of ESI, atmospheric pressure photoionization (APPI) was found to tolerate components of a microemulsion much better [49–51]. A microemulsion consisting of 0.8 % octane, 2 % SDS, 6.6 % butanol, and 90.6 % of 20 mM ammonium hydrogencarbonate buffer (pH 9.5) allowed the quantitative analysis by APPI-MS of various pharmaceuticals down to the sub-µg/ml range without dedicated sample preconcentration during injection [49].

More recently, MEEKC has also been hyphenated with MS detection by an inductively coupled plasma interface, thereby allowing element-selective detection. This approach has been used for the analysis of anticancer platinum complexes [52].

1.7 Applications

The following discussion cannot give an exhaustive compilation of applications reported so far, but intends to give an idea of the broad variety of classes than can be separated. In Table 1 the focus is put on those applications that demonstrate a separation of a larger number of analytes, whereas applications dealing with just a single analyte are not included. The separations done by MEEKC range from pharmaceutical drugs to vitamins, agrochemicals, polycyclic hydrocarbons, natural products, derivatized sugars, derivatized amino acids, proteins, fatty acids, nucleosides, and chiral compounds. Actually, it is possible to use a single set of operating conditions for different applications. A microemulsion consisting of 0.8 % (w/w) octane, 6.6 % (w/w) 1-butanol, 3.3 % SDS, and 89.3 % (w/w) 10 mM sodium tetraborate buffer may be successful for a large number of different analytes and is often a quite successful starting point. In cases where this composition does not lead to satisfactory results, fine-tuning is possible by variation of the components of the microemulsion according to the principles discussed earlier.

Besides its benefits for analytical chemistry, MEEKC has frequently been employed as a simple tool for assessment of hydrophobicity (expressed as octanol–water partition coefficient $P_{o/w}$) [75–77]. The following linear relationship exists between $P_{o/w}$ and log k (k being the retention factor as mentioned earlier):

$$\log P_{o/w} = a \log k + b$$

Slope and intercept of this line can be obtained from experiments with solutes of known octanol–water partition coefficients.

Table 1
Selected applications of microemulsion electrokinetic chromatography

Analytes	Carrier electrolyte	Ref.
Fat-soluble vitamins	0.8% *n*-octane/6.6% 1-butanol/6.0% SDS/20.0% 2-propanol/66.6% 25 mM phosphate buffer pH 2.5	[53, 54]
Water- and fat-soluble vitamins	20 mM borate buffer pH 8.7 containing 1.2% SDS, 21% *n*-butanol, 18% acetonitrile, 0.8% hexane	[55]
Water- and fat-soluble vitamins	0.81% *n*-octane/6.61% 1-butanol/3.31% SDS/89.27% 10 mM sodium tetraborate	[56]
Derivatized amino acids	87.24% 30 mM phosphate buffer pH 6, 2.16% SDS, 6% 1-butanol, 0.6% cyclohexane, 4% acetonitrile	[47]
Derivatized sugars	0.81% *n*-octanol/6.61% 1-butanol/3.31% SDS/89.27% 5 mM borate buffer pH 8	[57]
Derivatized fatty acids	0.66% *n*-heptane/6.55% 1-butanol/4.87% cholate/87.93% 10 mM borate buffer pH 10.2	[58]
5-Lipoxygenase metabolites	20 mM borate buffer pH 9 containing 3% SDS, 0.5% octane, 5% 1-butanol and 15 mM α-cyclodextrin	[59]
Green tea catechins	1.13% *n*-heptane/7.66% cyclohexanol/2.89% SDS/88.09% 50 mM sodium phosphate pH 2.5	[22]
Rhubarb anthraquinones and bianthrones	0.5% di-*n*-butyl tartrate/1.2% *n*-butanol/0.6% SDS/97.7% 10 mM borate buffer pH 9.2	[60]
Plant hormones	97.2% 10 mM borate buffer pH 9.2, 1.0% ethyl acetate, 0.6% SDS, 1.2% *n*-butanol	[61]
Food-grade antioxidants	0.6 g octane, 6.6 g 1-butanol, 3.3 g SDS, 69.3 g 25 mM phosphate buffer pH 3, 20 g 2-propanol	[62]
Preservatives in food	0.8% *n*-octane/6.6% 1-butanol/3.3% SDS/89.3% borate buffer pH 9.5	[63]
Food colorants	0.81% *n*-octane/6.61% 1-butanol/3.31% SDS/10% acetonitrile/79.27% 50 mM phosphate buffer pH 2.0	[64]
Lignin degradation products	0.91% *n*-heptane/6.61% *n*-butanol/1.66% SDS/90.92% 20 mM sodium tetraborate	[65]
Sun protection agents	0.8% *n*-octane/6.6% 1-butanol/2.25% SDS/ 0.75%/ Brij35/17.5% 2-propanol/72.1% 10 mM borate buffer pH 9.2	[66]
Anticancer platinum complexes	0.82% heptane/6.48% 1-butanol/1.44% SDS, 91.26% 20 mM phosphate buffer pH 7.4	[52]
Nitrofuran antibiotics	10 mM borate buffer pH 9.7 containing 0.82% octane, 3.48% SDS, 6.48% *n*-butanol	[67]
Fluoroquinolone antibiotics	8 mM phosphate/borate buffer pH 7.3 containing 1% heptane, 100 mM SDS, 10% *n*-butanol	[68]

(continued)

Table 1
(continued)

Analytes	Carrier electrolyte	Ref.
Nonsteroidal anti-inflammatory drugs	0.8% ethyl acetate, 6.6% *n*-butanol, 6% acetonitrile, 1.0% SDS, 85.6% 10 mM borate buffer pH 9,2	[69]
Endocrine disrupting compounds	25 mM phosphate buffer pH 2, 80 mM octane, 900 mM butanol, 200 mM SDS, and 20% propanol	[70]
Phthalate esters	60 mM borate buffer pH 9 containing 0.5% *n*-octane, 100 mM sodium cholate, 5% 1-butanol	[71]
Triazine herbicides	10 mM borate buffer pH 9.5 containing 2.5% SDS, 0.8% ethyl acetate, 6% *n*-butanol	[72]
Aromatic carboxylic acids	50 mM phosphate buffer pH 2 containing 3.7% SDS, 0.975% octane, 5% cyclohexanol	[73]
Polycyclic aromatic hydrocarbons	90% of 0.81% *n*-octane/6.61% *n*-butanol/3.31% SDS/89.27% 10 mM sodium tetraborate; 10% ethanol	[74]

2 Materials

1. *Microemulsion for general applications using a negatively charged oil phase*: mix 3.3 g SDS and 6.6 g 1-butanol, and then add 0.8 g *n*-octane and 89.3 g 10 mM borate buffer pH 9.4 (prepared from a 10 mM boric acid adjusted to pH 9.4 with NaOH). The mixture is placed in an ultrasonic bath for 30 min to obtain a clear solution. Afterward, the microemulsion is filtered through a 0.45 μm membrane filter.
2. *Microemulsion for highly hydrophobic analytes using a negatively charged oil phase*: mix 2.25 g SDS, 0.75 g Brij 35 (*see* **Note 1**), and 6.6 g 1-butanol, and then add 0.8 g *n*-octane, 25 g 2-propanol, and 64.6 g 10 mM borate buffer pH 9.4 (prepared from a 10 mM boric acid adjusted to pH 9.4 with NaOH). The mixture is placed in an ultrasonic bath for 30 min to obtain a clear solution. Afterward, the microemulsion is filtered through a 0.45 μm membrane filter.
3. *Microemulsion for general applications using a neutral oil phase*: mix 3.32 g Brij 35 and 6.62 g 1-butanol, and then add 0.82 g *n*-heptane and 89.2 g 25 mM acetate buffer pH 4.0 (prepared from a 25 mM acetic acid adjusted to pH 4.0 with NaOH). The mixture is placed in an ultrasonic bath for 30 min to obtain a clear solution. Afterward, the microemulsion is filtered through a 0.45 μm membrane filter (*see* **Note 2**).
4. *Microemulsion for on-capillary preconcentration by sweeping using a negatively charged oil phase*: mix 3.3 g SDS and 6.6 g

1-butanol, and then add 0.8 g *n*-octane and 89.3 g 50 mM phosphoric acid pH 2.0. The mixture is placed in an ultrasonic bath for 30 min to obtain a clear solution. Afterward, the microemulsion is filtered through a 0.45 μm membrane filter.

5. CE instrument "7100 CE System" (Agilent, Waldbronn, Germany), or equivalent, equipped with an ultraviolet (UV) absorbance detector, high voltage supply up to +/−30 kV, and autosampler for both hydrodynamic and electrokinetic injection.
6. Fused-silica capillaries (Polymicro Technologies, Phoenic, AZ) with inner diameter and outer diameter of 50 and 360 μm, respectively, a length from inlet to detector of 51.5 cm, and a length from inlet to outlet of 60 cm (*see* **Note 3**).
7. Sample vials for autosampler of CE instrument.

3 Methods

3.1 General Procedure for Conditioning New Fused-Silica Capillaries

1. Four vials are filled with 1 M NaOH, water, 0.1 M NaOH, and 0.2 M HCl, respectively.
2. The vials are placed into appropriate positions of the autosampler for rinsing the capillary.
3. The capillary is rinsed with 1 M NaOH for 10 min, with water for 5 min, with 0.2 M HCl for 10 min, with water for 1 min, with 0.1 M NaOH for 10 min, and with water for 10 min.

3.2 Separation of Neutral Analytes Using a Negatively Charged Oil Phase

1. Two vials are filled with 0.1 M NaOH and microemulsion, respectively, for rinsing the capillary (the microemulsion is prepared according to the procedure given in Subheading 2, **item 1**.).
2. Two carrier electrolyte vials (for inlet and outlet side) are filled with the microemulsion.
3. Sample solutions and calibration solutions are filled into vials (*see* **Note 4**).
4. All vials are put into appropriate positions of the autosampler.
5. The capillary is rinsed with 0.1 M NaOH for 5 min and with microemulsion for 5 min.
6. The first sample or calibration solution is injected using hydrodynamic injection at a pressure of 50 mbar for 5 s (*see* **Note 5**), and the separation is started by applying a voltage of +25 kV (*see* **Note 6**).
7. The capillary is rinsed with 0.1 M NaOH for 1 min and with microemulsion for 1 min.
8. **Steps 6** and **7** are repeated for the next sample or calibration solution.

3.3 Separation of Highly Hydrophobic Analytes Using a Negatively Charged Oil Phase

1. Two vials are filled with 0.1 M NaOH and microemulsion, respectively, for rinsing the capillary (the microemulsion is prepared according to the procedure given under Subheading 2, **item 2**).
2. Two carrier electrolyte vials (for inlet and outlet side) are filled with the microemulsion.
3. Fill vials with sample solutions and calibration solutions, prepared in the microemulsion as solvent.
4. All vials are put into appropriate positions of the autosampler.
5. The capillary is rinsed with 0.1 M NaOH for 5 min and with microemulsion for 5 min.
6. The first sample or calibration solution is injected using hydrodynamic injection at a pressure of 50 mbar for 3 s, and the separation is started by applying a voltage of +30 kV.
7. The capillary is rinsed with 0.1 M NaOH for 1 min and with microemulsion for 1 min.
8. **Steps 6** and 7 are repeated for the next sample or calibration solution.

3.4 Separation of Positively Charged Analytes Using a Neutral Oil Phase

1. Two vials are filled with 0.1 M NaOH and microemulsion, respectively, for rinsing the capillary (the microemulsion is prepared according to the procedure given under Subheading 2, **item 3**).
2. Two carrier electrolyte vials (for inlet and outlet side) are filled with the microemulsion.
3. Vials are filled with sample solutions and calibration solutions.
4. All vials are put into appropriate positions of the autosampler.
5. The capillary is rinsed with 0.1 M NaOH for 5 min and with microemulsion for 5 min.
6. The first sample or calibration solution is injected using hydrodynamic injection at a pressure of 50 mbar for 5 s (*see* **Note 5**), and the separation is started by applying a voltage of +25 kV (*see* **Notes 6** and 7).
7. The capillary is rinsed with 0.1 M NaOH for 1 min and with microemulsion for 1 min.
8. **Steps 6** and 7 are repeated for the next sample or calibration solution.

3.5 Separation of Neutral Analytes with On-Capillary Preconcentration by Sweeping

1. Two vials are filled with 0.1 M NaOH and microemulsion, respectively, for rinsing the capillary (the microemulsion is prepared according to the procedure given in Subheading 2, **item 4**).
2. Two carrier electrolyte vials (for inlet and outlet side) are filled with the microemulsion.

3. Vials are filled with sample solutions and spiked sample solutions.
4. All vials are put into appropriate positions of the autosampler.
5. The capillary is rinsed with 0.1 M NaOH for 5 min and with microemulsion for 5 min.
6. The first sample solution is injected using hydrodynamic injection at a pressure of 100 mbar for 150 s (*see* **Note 8**), and the separation is started by applying a voltage of –20 kV (*see* **Note 6**).
7. The capillary is rinsed with 0.1 M NaOH for 1 min and with microemulsion for 1 min.
8. **Steps 6** and **7** are repeated for the next sample or spiked sample solution (*see* **Note 9**).

4 Notes

1. The partial substitution of SDS by Brij 35 results in lower charge of the oil droplet and thereby in a lower velocity. This leads to a decrease of the analysis time. For a specific separation, one can try to vary the ratio of SDS/Brij 35 to achieve optimal analysis time.
2. This microemulsion prepared in a buffer of pH 4.0 is suited for the separation of analytes that undergo protonation or deprotonation reactions at this pH, so that positively or negatively charged compounds are formed to some extent. The pH can be changed if necessary.
3. Shorter or longer capillaries can be used if necessary to optimize resolution and analysis time.
4. If the analytes are not easily soluble in water, the sample and calibration solutions can be prepared in the microemulsion as solvent. One should avoid pure organic solvents for the samples and the calibration solutions because these can disrupt the microemulsion adjacent to the zone of injected sample, leading to distorted peak shapes. It is recommended that an internal standard be added to both the sample and the calibration solutions.
5. Somewhat longer injection times can be used to achieve lower detection limits. Peak distortion will occur at too long injection times.
6. It may be advantageous to use somewhat lower or higher separation voltages depending on the length of the capillary.
7. The positive voltage applied is suited for cationic analytes. In the case of anionic analytes, it may be necessary to use a negative voltage (depending on the electrophoretic mobility of the analyte in relation to the electroosmotic mobility).

8. Depending on the analytes, this injection time may need to be decreased in order to avoid deterioration of peak shapes.
9. The incorporation of the online preconcentration effect makes quantitation by standard addition instead of external standards preferable.

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