15 Micellar Electrokinetic Chromatography

MORTEZA G. KHALEDI

Department of Chemistry, North Carolina State University, Raleigh, NC, USA

15.1 INTRODUCTION

In capillary electrophoresis (CE), mixtures of charged compounds migrate under the influence of an electric field in a capillary tube and are separated according to their differences in charge-to-size ratio. The capillary is typically made of fused silica with typical dimensions of internal diameter (i.d.) $<100\,\mu m$ (most commonly used are 50 and $75\,\mu m$ i.d.) and length smaller than 100 cm. The advantage of performing electrophoresis in capillary tubes is the effective dissipation of joules heating that is generated as a result of the application of the electric field. A number of key advantages of CE have been demonstrated over the past three decades, including very high resolving power due to high peak efficiency, fast analysis time, small sample size, feasibility and flexibility of manipulating chemical selectivity, broad scope of samples and applications, high throughput capabilities (especially with microfluidic platforms), and so on. There are four main CE-based techniques: capillary zone electrophoresis (CZE), capillary isotachophoresis (CITP), capillary isoelectrofocusing (CIEF), capillary gel electrophoresis (CGE), and electrokinetic chromatography (EKC). These methods differ with respect to migration and separation mechanisms and subsequently the scope of applications. Micellar electrokinetic chromatography (MEKC) is the only CE technique that is capable of separating uncharged and charged compounds [1–5].

Encyclopedia of Drug Metabolism and Interactions, 6-Volume Set, First Edition.
Edited by Alexander V. Lyubimov.

Terabe *et al*. [6] extended the scope of CZE to separate uncharged molecules by incorporating charged micelles in the aqueous buffer solution. The technique, named MEKC, uses the same instrumental setup as CE. Charged organized media such as micelles are incorporated in the buffer solution and serve as the separation medium for uncharged solutes. The charged micelles serve as pseudostationary phases as they provide sites of interactions for solutes in the sample mixture (similar to stationary phases in chromatography). For the MEKC separation of uncharged solutes, the pseudostationary phase must be charged; thus, nonionic and zwitterionic surfactants can only be used in mixed micelles along with a charged surfactant.

Hydrophobic interaction plays a predominant role in solute partitioning into micelles and consequently retention in MEKC, while hydrogen-bonding and dipolar interactions contribute to selectivity. MEKC is very similar to reversed-phase liquid chromatography (RPLC) with regard to the scope of applications. This is due to highly similar underlying types of interactions that control retention and selectivity in both techniques. MEKC can be viewed as a hybrid of RPLC and CZE as the separation process incorporates hydrophobic and polar interactions, a partitioning mechanism, and electromigration. However, MEKC offers a combination of unique features of CZE and RPLC such as high efficiencies, rapid analysis, small sample size, small solvent consumption, and versatility of incorporating chemical selectivity in the separation process. It offers higher efficiency than RPLC and capillary electrochromatography (CEC). The number of theoretical plates in an MEKC separation can be 10 times or higher than that in RPLC. Another major advantage over conventional chromatographic techniques as well as CEC is the flexibility and ease of changing chemical composition of the pseudostationary phases. For example, the type and/or composition of the micellar solution can be easily modified or replaced by simply rinsing the capillary with a new type of pseudostationary phase. The equilibration times are often very rapid. This inherent flexibility of controlling key parameters leads to enhanced separations and greatly facilitates the process of method development. MEKC is mostly suited for analysis of small molecules and is the only CE technique that is capable of separation of both charged and uncharged compounds as well as structural, positional, and chiral isomers with applications in analysis of pharmaceutical, clinical, environmental, biological, and chemical samples [1–5].

15.2 MIGRATION PATTERNS AND RESOLUTION IN MEKC

15.2.1 Uncharged Solutes

Figure 15.1 illustrates the migration pattern and separation of two hypothetical uncharged solutes in MEKC using a fused silica capillary with negatively charged walls. The mixture is introduced at the anode inlet of the capillary and migrates with the electroosmotic flow (EOF) toward the cathode, where the detector is located. The negatively charged micelles migrate toward anode in the electric field at an electrophoretic mobility (μ_{mc}) that is proportional to their charge-to-size ratios. Anionic micelles migrate in the opposite direction of the EOF in an uncoated capillary. Typically, the EOF velocity is stronger than the electrophoretic velocity of anionic micelles under "normal" conditions (e.g., an uncoated capillary and pH >6). As a result, the anionic micelles are carried toward the cathode. Using cationic micelles, the capillary wall is coated with the positively charged surfactants and often times it

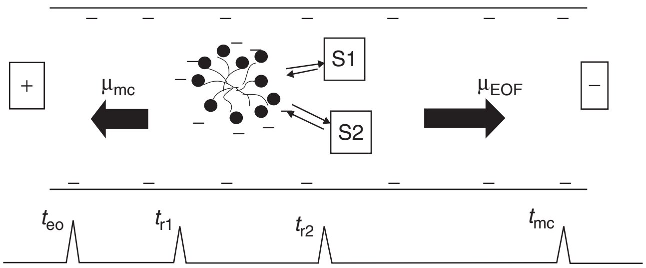

Figure 15.1 Schematic diagram of migration of uncharged compounds in MEKC with anionic pseudostationary phases. Separation of solutes S1 and S2 is achieved due to their differential partitioning into the pseudostationary phase. The uncharged solutes are eluted within an elution window ($t_{\mathrm{mc}}/t_{\mathrm{eo}}$).

leads to a reversal in the direction of the EOF. It is therefore necessary to reverse the polarity of the electrodes in the CE setup to ensure the elution of the cationic micelles and consequently the uncharged solutes through the detection window.

The two uncharged compounds, S1 and S2, are separated due to differences in their water–micelle partition coefficients, K_{mw}. Charged micelles migrate in the electric field and elute at a certain time (t_{mc}). Subsequently, MEKC has a limited migration window between the elution time of an unretained solute, t_{eo}, and the migration time of micelles, t_{mc} (Figure 15.1). The elution window is defined by two types of solutes. First are the unretained solutes that do not interact with micelles ($K_{\mathrm{mw}} \sim 0$) spend all of their migration times in the bulk aqueous phase and migrate at the electroosmotic mobility. These are typically uncharged polar molecules such as methanol or acetonitrile that are EOF markers and elute at t_{eo}. The other end is defined by the elution of analytes that interact so strongly with the micelles ($K_{\mathrm{mw}} \sim \infty$) that they spend all of their migration time with micelles. The t_{mc} markers are typically very hydrophobic compounds that are sparingly soluble in the aqueous media, reported examples being Sudan III and dodecanophenone. The elution times for these analytes coincide with the micellar migration time, t_{mc}. The existence of an elution window limits the peak capacity in MEKC as all uncharged solutes are to be separated between the migration time of an unretained solute, t_{eo}, and a fully retained solute, t_{mc}. The size of the elution window varies with the type and concentration of the charged pseudophases, the presence of organic modifiers, mixed micelles, or capillary wall modification [1–5].

Like chromatography, retention factor in MEKC is defined as the ratio of the number of moles of solute in micellar pseudostationary phase, n_{mc}, and that in bulk aqueous phase, n_{aq}. Retention factor is directly proportional to the micelle–water partition coefficient, K_{mw}, and phase ratio, ϕ as follows:

$$k = \frac{n_{\mathrm{mc}}}{n_{\mathrm{aq}}} = K_{\mathrm{mw}}\Phi \tag{15.1}$$

Retention factor in MEKC can be determined from migration time data using the following equation:

$$k = \left\{ \frac{t_{\mathrm{r}} - t_{\mathrm{eo}}}{t_{\mathrm{eo}}\left[1 - \left(\frac{t_{\mathrm{r}}}{t_{\mathrm{mc}}}\right)\right]} \right\} \tag{15.2}$$

This is very similar to the equation for retention factor in conventional chromatography with the exception of the additional term $(1 - t_r/t_{mc})$ in the denominator. This term indicates the existence of an elution window due to the fact that the "stationary phase in MEKC is actually mobile." If t_{mc} approaches infinity (i.e., stationary micelles), the extra term in the denominator is omitted and the retention factor equation becomes the same as that in conventional chromatography.

15.2.2 Ionizable Solutes

In addition to partitioning into micelles and migrating at the micellar mobility, charged compounds possess electrophoretic mobilities of their own in the bulk aqueous solvent. As a result, the observed retention time also includes the time that solute migrated electrophoretically in the bulk aqueous phase. Migration patterns for ionizable solutes (weak acidic and basic compounds) are considerably more complicated than uncharged compounds in MEKC due to existence of acid–base and ion-pairing equilibria in the aqueous solution and [7–9]. For separation of ionizable solutes in MEKC, control of buffer pH (in addition to type and concentration of micelles) would be critical in method development and optimization of separation. Quantitative models have been developed that would allow prediction of migration patterns of ionizable solutes and subsequently rapid optimization of separation conditions based on limited initial experiments [9].

15.2.3 Resolution in MEKC

Equation 15.3 shows the resolution equation for uncharged solutes in MEKC:

$$R = \left(\frac{N^{1/2}}{4}\right)\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k_2}{1 + k_2}\right)\left(\frac{1 - \left(\frac{t_{eo}}{t_{mc}}\right)}{1 + \left(\frac{t_{eo}}{t_{mc}}\right)k_1}\right) \tag{15.3}$$

As can be seen, Equation 15.3 has the same format as that for conventional chromatography as it indicates that resolution depends on three terms related to efficiency, selectivity, and retention [6]. The fourth term is unique to MEKC that represents the existence of an elution window.

Again, if micelles were truly stationary (i.e., $t_{mc} \sim \infty$), the fourth term would drop out and the equation would be identical to that in conventional chromatography. The size of the elution window has a significant impact on MEKC separations. Likewise, peak capacity, n, can also be increased with wider elution windows as follows:

$$n = 1\frac{\sqrt{N}}{4}\ln\frac{t_{mc}}{t_{eo}} \tag{15.4}$$

Like chromatography, better resolution is achieved at higher efficiency. The disadvantage of the limited elution window is mostly compensated by the large number of theoretical plates that are routinely achieved in MEKC. The concentration and type of surfactant (pseudostationary phase) should be optimized to enhance resolution due to their influence on at least three of the parameters (k, α, and t_{mc}/t_{eo}).

15.3 CONTROL AND PREDICTION OF RETENTION IN MEKC

The primary role of surfactant concentration is to adjust retention factor to within the optimum range in order to achieve better resolution. The relationship between retention factor, k, and surfactant concentration can be described as in the following equation [6]:

$$k = \frac{\upsilon(C_{\mathrm{sf}} - \mathrm{CMC})}{1 - \upsilon(C_{\mathrm{sf}} - \mathrm{CMC})} K_{\mathrm{mw}} \tag{15.5}$$

where the first term is the phase ratio, ϕ, defined as the volume of the micellar pseudostationary phase over that of the bulk aqueous phase (i.e., $V_{\mathrm{mc}}/V_{\mathrm{aq}}$) and is related to ν (surfactant molar volume), C_{sf} (total surfactant concentration), and critical micelle concentration (CMC). K_{mw} is the partition coefficient of a solute between an aqueous phase and micelles. In general, retention factor increases linearly with surfactant concentration, as Equation 15.5 can be simplified to, because the denominator approximately equals to one under typical operating surfactant concentrations as follows:

$$k = K_{\mathrm{mw}} \nu (C_{\mathrm{sf}} - \mathrm{CMC}) \tag{15.6}$$

A unique advantage of MEKC is that the phase ratio can be determined accurately (first term in Eq. 15.5) and will not change from capillary to capillary or with time as is the case for chromatography columns. The phase ratio in MEKC is related to two intrinsic properties of the surfactant: partial specific molar volume (ν) and CMC, as well as surfactant concentration. This provides a unique opportunity to predict retention factor in MEKC for solutes with known micelle–water partition coefficients without any prior experiments. Unfortunately, there is a lack of extensive and reliable K_{mw} databases.

Additivity-based approaches have been developed to calculate micelle–water partition coefficients from solute structure [10,11]. Micelle–water partition coefficients can be described as the sum of the partition coefficients of the constituent atomic/molecular fragments, measured by fragmental constant values, f_i, as well as correction factors to account for various “intramolecular effects” that cause deviations from the predicted partition coefficients as follows [10,11]:

$$\log K_{\mathrm{mw}} = \sum_{i=1}^{n} a_i{}^* f_i + \sum_{i=1}^{m} k_i{}^* C_m \tag{15.7}$$

where f_i is the fragmental constant and a_i is the frequency of occurrence of each fragment found in the solute structure, k_i is a constant that varies with the type of “intramolecular effect.” This equation is based on the Rekker’s fragmental constant approach for calculation of octanol–water partition coefficients. The fragmental constants of 41 fragments in the sodium dodecylsulfate (SDS) micelle system have been determined. Using Equation 15.7, the SDS micelle–water partition coefficients and subsequently their retention factor, and retention times for 170 aromatic solutes were successfully predicted in MEKC using SDS micelles. The linear regression of the predicted versus observed retention times resulted in $R^2 = 0.971$ and slope of 0.992.

15.4 PSEUDOSTATIONARY PHASES IN MEKC

In addition to micelles made of synthetic surfactants above their CMCs [1–6], a wide variety of pseudostationary phases have been used that has significantly extended for the applications of the technique. Some examples of pseudostationary phases are ionic polymers [12] cyclodextrins (CDs) and other chiral reagents [13], fullerenes and carbon nanotubes (CNTs) [14], vesicles [15,16], liposomes [17–20], dendrimers, and others.

15.4.1 Micellar Pseudophases

Micelles have been the most commonly used pseudostationary phase in EKC. Anionic alkyl chain surfactants, especially SDS, have been the most widely used surfactant type. The popularity of SDS can be attributed to the high aqueous solubility, low CMC, low Kraft point, low UV molar absorptivity even at low wavelengths, availability, and cost. Serendipitously, SDS might provide the “right” type of selectivity for many solute mixtures. Anionic alkyl chain surfactants, especially SDS, have been used in a variety of applications such as MEKC separations of phenols and other acidic solutes, pharmaceutical amines, organic solvents, keto compounds and carbohydrate derivatives, fungicide and phytotoxin, caffeine metabolites in human urine, DNA adducts, pharmaceuticals such as antibiotics, vitamins, sulfonamides, xanthines, peptides and proteins, organometallic and ligands, amino acids, sulfur and nitro compounds in environmental applications, flavonoids in foods, and nucleic acids constituents in body fluids [1–5].

As an alternative to SDS, the use of bile salt surfactants in MEKC separations has become popular due to their different selectivities as compared to SDS. Bile salt surfactants have very different aggregation properties and structures from SDS micelles. They can tolerate relatively higher concentration of organic modifier (30% organic solvent) than SDS micelles without a disruption of their structural integrity. Bile salts have been used in a variety of MEKC separations such as steroids, amino acids, benzodiazepines, synthetic colors, bioactive compounds, environmental analysis, proteins and peptides, bilirubin, organic acids, and hydrophobic compounds, including polycyclic aromatic hydrocarbons, chiral and diastereoisomers, and anti-HIV agents [1–5].

The use of fluorinated surfactants in MEKC separations has been very limited. This has been mostly due to the lack of availability of MEKC compatible fluorocarbon surfactants in high purity. Ye *et al*. [21] reported the first application of the flurocarbon micelles of lithium perfluoroctane sulfonate (LiPFOS) for the MEKC separations of a group of small peptides and reported very different selectivity and elution patterns as compared to SDS. LiPFOS micelles are stronger hydrogen-bond donor (HBD) than other micellar systems that have been studied thus far [22,23].

Cationic surfactants generally interact with the negatively charged silica capillary wall and reverse the direction of the EOF. As a result of the reversed EOF, the polarity of the electrodes would have to be reversed in order to elute solutes through the detector window [1–5]. Cetyltrimethylammonium bromide (CTAB) micelles are among the strongest hydrogen-bond acceptors (HBAs), thus have a very different interactive behavior than SDS or LiPFOS micelles [22,23]. The usefulness of cationic surfactants has been explored in a number of MEKC applications. A few examples are phenolic carboxylic acids, charged molecules with nearly identical electrophoretic mobilities such as bis(amidinohydrazones), nucleic acid constituents, cocaine and illicit drugs, adrenergic blocking agents, glycosylated compounds, and aromatic hydrocarbons [1–5].

Surfactants with a zero net charge such as nonionic and zwitterionic surfactants can be used along with an ionic surfactant as mixed micelles. In order to separate uncharged molecules in MEKC, charged surfactants must be used. They can also have a great influence on the MEKC separation of charged molecules. Both nonionic and zwitterionic surfactants have been used alone for the separations of charged compounds such as amino acids and polypeptides. Nonionic surfactants and mixtures of anionic/nonionic surfactants have been shown to provide different selectivities, reduced currents, and changes in elution window sizes as compared to anionic surfactant systems. Zwitterionic surfactants have been used alone and in conjunction with SDS in MEKC. Because these surfactants do not increase the conductivity of the buffer, they can be used at high concentrations, while still allowing the use of high voltages and large i.d. capillaries.

15.4.2 Polymeric and Nanoparticle Pseudostationary Phases

At least five different classes of polymeric phases have been reported, including polymer micelles where surfactant monomers are polymerized and covalently bonded together [12], cascade macromolecules such as starburst dendrimers, and ionic block copolymers [1–5], fullerenes, and nanoparticles [24,25]. Figure 15.2 illustrates the structures of some polymeric surfactants. Initially, polymeric pseudostationary phases (PSP) were used for the MEKC separation of highly hydrophobic compounds that would require use of relatively high concentrations of organic modifiers in the aqueous buffer. One of the shortcomings of the micellar aggregates is their incompatibility with the presence of higher than 20–30% organic cosolvents. In addition, the high molecular weight of the PSP makes them more compatible with MS detection, which

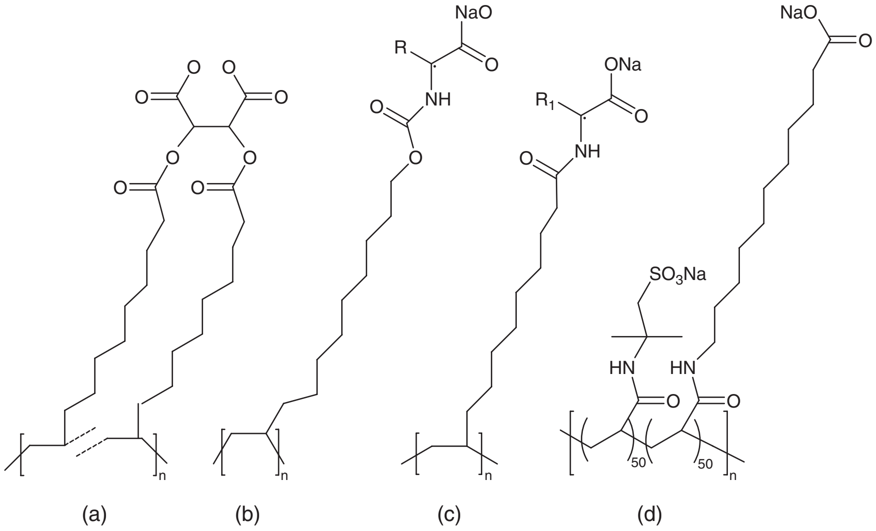

Figure 15.2 Structures of polymeric surfactants. (a) Gemini surfactant polymer poly(sodium diundecenyl tartrate), (b) poly(sodium undecenoxy carbonyl amino acidate), (c) poly(sodium undecenyl amino acidate), and (d) poly-(sodium 2-(acrylamido)-2-methylpropanesulfonate/11-(acrylamido)-undecanoic acid). *Source*: Palmer CP. Electrophoresis 2007;28:164–173; reprinted with permission.

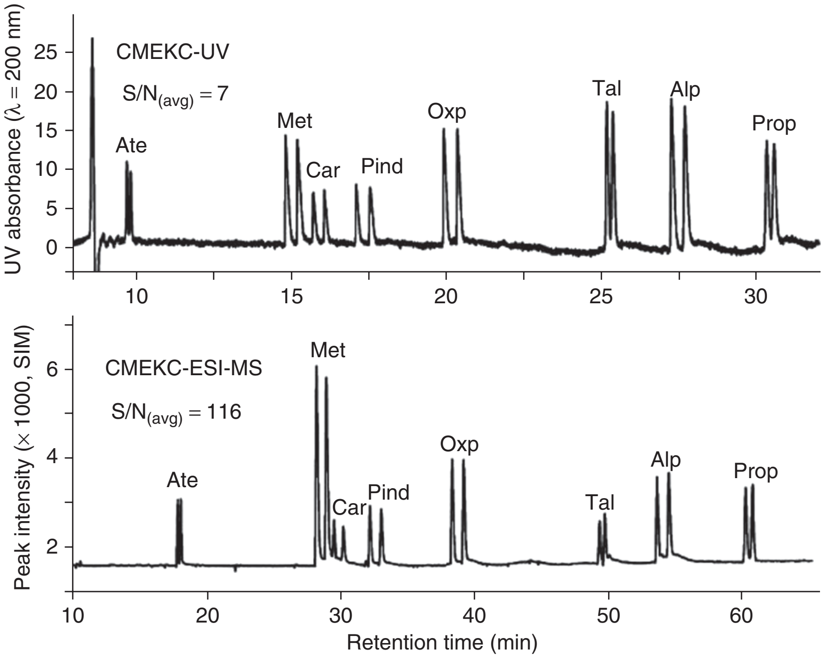

Figure 15.3 MEKC separation of β-blockers with UV and MS detection; MEKC buffer composition: polymeric pseudophase poly-l-SUCL at 15 mM in 25 mM ammonium acetate/25 mM triethylamine at pH 8.0. Peak i.d. Ate, atenolol; Met, metoprolol; Car, carteolol; Pind, pindolol; Oxp, oxprenolol; Tal, talinolol; Alp, alprenolol; and Prop, propanolol. *Source*: Akbay C, Rizvi SAA, Shamsi SA. Anal. Chem 2005;77:1672–1683; reprinted with permission.

is a significant potential advantage. Figure 15.3 illustrates the MEKC separation of an enantiomeric mixture of β-blockers with both the UV and MS detection using a chiral PSP (poly-L-SUCL) (poly(sodium N-undecenoxy carbonyl-L-leucinate)). Some PSP are not fully compatible with UV detection and lower efficiencies could be observed with certain PSP due to higher polydispersity and slow mass transfer kinetics. Another potential advantage of PSP is the possibility of extending the range of selectivity through control of the chemical composition and structure of the polymers (see below) [1–5,12].

The use of nanoparticles as PSP in MEKC has only been investigated in recent years. For examples, applications of polymeric, molecularly imprinted, silica, gold nanoparticles as well as CNTs have been reported. The CNT would have to be functionalized (e.g., with carboxylate groups) or be dispersed in SDS solutions to be used as PSP in EKC. Several recent publications have reported the applications of CNT and fullerene PSP for the separation of caffeine and theobromine, purine and pyrimidine, DNA fragments, anti-inflammatory drugs, enantiomers, and polyaromatic hydrocarbons (PAH) [26–31]. An example of the application of CNT is shown in Fig. 15.4.

Suspensions of silica nanoparticles in EKC have been reported for the separation of phenolic compounds, polyaromatic hydrocarbons, steroids, and aromatic acids [26–33]. Gold nanoparticles have been used in polymeric solutions of poly(ethylene oxide) (PEO) for the separation of double-stranded DNA fragments up to 48.5 kbp [34,35]. Note that the separation mechanism in this case is molecular sieving, which is different

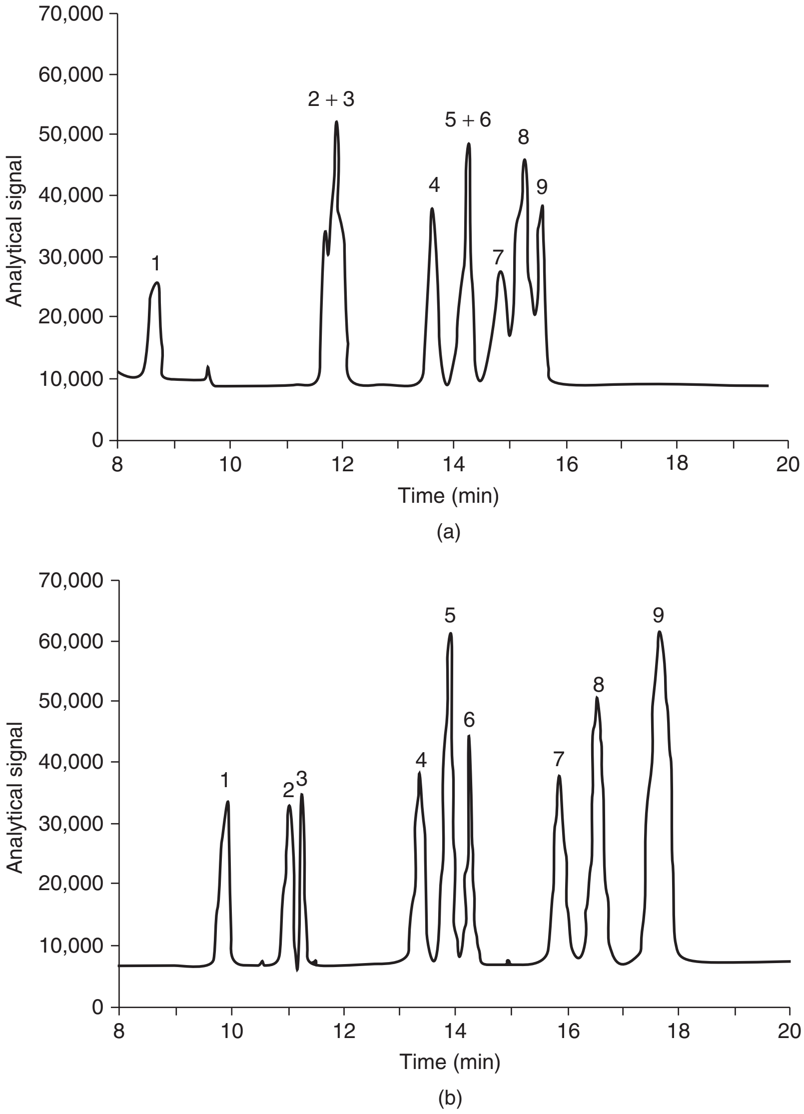

Figure 15.4 EKC separation of chlorophenols with carbon nanotubes. In (a) absence and (b) presence of surfactant-coated single-walled carbon nanotubes (SC-SWNTs) (3.2 mg/L). The background electrolyte (BGE) was 25 mM sodium hydrogen phosphate, 15 mM sodium tetraborate, 100 mM SDS, and 6% ethanol at pH 7.95. Other operating conditions: capillary 30 cm_75 mm i.d., 15 kV, 201 C. Peaks: (1) 2,6-dichlorophenol; (2) 3,5-dichlorophenol; (3) 2,3-dichlorophenol; (4) 2,5-dichlorophenol; (5) 3,4-dichlorophenol; (6) 2,3,5-trichlorophenol; (7) 2,4,5-trichlorophenol; (8) 2,3,6-trichlorophenol; and (9) pentachlorophenol. *Source*: Ref. 29; reprinted with permission.

from EKC, where differential solute partitioning into a pseudostationary phase is the main driving force for the separation process.

15.4.3 Mixed Micelles

For certain complex mixtures of structurally similar solutes, one might not be able to find a suitable surfactant type that provides adequate resolution. This is typically due to a lack of selectivity and/or narrow elution window. In these situations, the use of mixed micelles (or mixed surfactant–polymer) can lead to enhanced separations. Mixing surfactants with different interactive properties can lead to great changes in selectivity for a given mixture. Selection of optimum composition would then be crucial in improving the quality of separation. The size of the elution window for certain mixed micellar systems is often larger than that for the individual constituent surfactants. Retention factor and selectivity change with the mole fraction of surfactants in the mixed micelles. Efficiency of the mixed micellar systems is often not much different from those of the single surfactant systems; however, in a few cases, loss of efficiency has been observed. Various combinations of different surfactants such as bile salt/anionic, anionic/zwitterionic, cationic/zwitterionic, fluorocarbon/anionic, anionic/nonionic, cationic/cationic, anionic/cationic, bile salt/bile salt, and nonionic/nonionic (for charged solutes) have been used in MEKC [1–5].

15.4.4 Organic Modifiers

Modifiers such as organic solvents, CDs, and urea are typically incorporated in the aqueous buffers of MEKC in order to reduce the retention factors of strongly bound solutes to micelles. Their presence can also lead to wider elution ranges and/or higher selectivity. Their use in MEKC separations, however, has been mostly for improving the separations of hydrophobic compounds that interact strongly with micelles and elute at or near migration time of micelles. The main role of organic modifiers in MEKC has been to reduce retention factors of highly hydrophobic solutes to within or near the optimum range. Typically, inclusion of organic solvents leads to an increase in the size of the elution window. As a result, resolution in MEKC can be enhanced at the expense of longer analysis times. The concentration of organic solvents would have to be limited (typically $\leq 20\%$) in order to maintain the integrity of micelles. The use of polymeric phases will provide an opportunity to investigate the role of organic solvents over a wider range of concentrations.

In addition to typical organic solvents, the use of other modifiers such as urea and glucose has also been reported. Urea reduces the interactions of hydrophobic compounds with micelles by increasing their solubility in the aqueous solutions. Retention factors of hydrophobic compounds in micellar solutions are therefore decreased dramatically. The size of the elution window has also been shown to increase with the addition of urea to MEKC systems. Another type of modifier that has been used in MEKC is CDs. In CD-modified MEKC (CD-MEKC), the hydrophobic cavity of CDs provides an alternative site of interaction to micelles for the hydrophobic solutes. Since uncharged CDs migrate at the EOF velocity and in the opposite direction of anionic SDS micelles, the net retention factor of solutes decreases in the presence of CDs. As a result, hydrophobic solutes that would otherwise elute with micelles can be better separated. In addition, CDs introduce a shape selectivity effect that is beneficial for the separation of structural isomers [1–5].

15.5 CHARACTERIZATION AND CLASSIFICATION OF PSEUDOSTATIONARY PHASES

The first attempt for characterization and classification of the chemical selectivity of pseudostationary phases in MEKC was reported in 1990's using linear solvation energy relationships (LSERs) [36–38]. Since then, the LSER methodology has been extensively used for achieving a better understanding of the underlying interactions that control retention and selectivity of various types of micelles, block copolymers, polymerized micelles, mixed micelles, organically modified micelles, as well as vesicular and liposomes. The retention factor, k, in MEKC can be described by the general LSER model as follows:

$$\log k = c + vV + bB + aA + sS + eE \tag{15.8}$$

The LSER models quantitatively describe the contributions of hydrophobic/dispersion (vV term), type A hydrogen bonding (bB term, micellar phase as a HBD), type B hydrogen bonding (aA term, micellar phase as a HBA), dipolarity (sS term), and polarizability (eE term). Solute properties are described by the McGowan volume (V), HBA strength (B), HBD strength (A), dipolarity (S), and polarizability (E). The interactive properties of the micellar pseudophase are described by coefficient ν, a measure of cohesiveness and dispersion interactions, coefficient b that is a measure of the HBD strength, coefficient a that is proportional to the HBA strength of the micelles, s that describes dipolarity, and e that is a measure of the affinity of micelles for ν and π electrons of solutes, and c is the regression constant. The LSER analysis clearly indicates that while the hydrophobic interaction (as quantified by the vV term) is the main driving force for retention in MEKC, the main source of selectivity variations between pseudophases is their hydrogen-bonding donor strength (the b coefficient) followed by hydrogen-bonding acceptor strength and dipolarity/polarizability interactions.

Fu and Khaledi have developed a novel micellar selectivity triangle (MST) to characterize and classify the chemical selectivities of pseudophases in EKC [22,23]. The MST scheme was devised based on the LSER parameters for construction of new, normalized scales for hydrogen-bonding (donor and acceptor) strengths, dipolarity, and polarizability of pseudophases and MST plots can be used to readily visualize the relative strength and classification of pseudophases based on their interactive properties. Figure 15.5 illustrates the comparison of the selectivities of over 70 pseudophases in terms of their hydrogen-bonding basicity, hydrogen-bonding acidity, and dipolarity and shows that the MEKC pseudophases clustered into fours general groups. Group A consists of the SDS micelles and its analogs plus some mixed micelles containing SDS; group B consists of stronger HBD pseudophases such as LiPFOS micelles in single and mixed format with other surfactants and organic modifiers; group C consists of HBAs such as CTAB micelles, bile salts, microemulsions, as well as biphasic partitioning system of octanol–water; and group D, which is slightly stronger acceptor

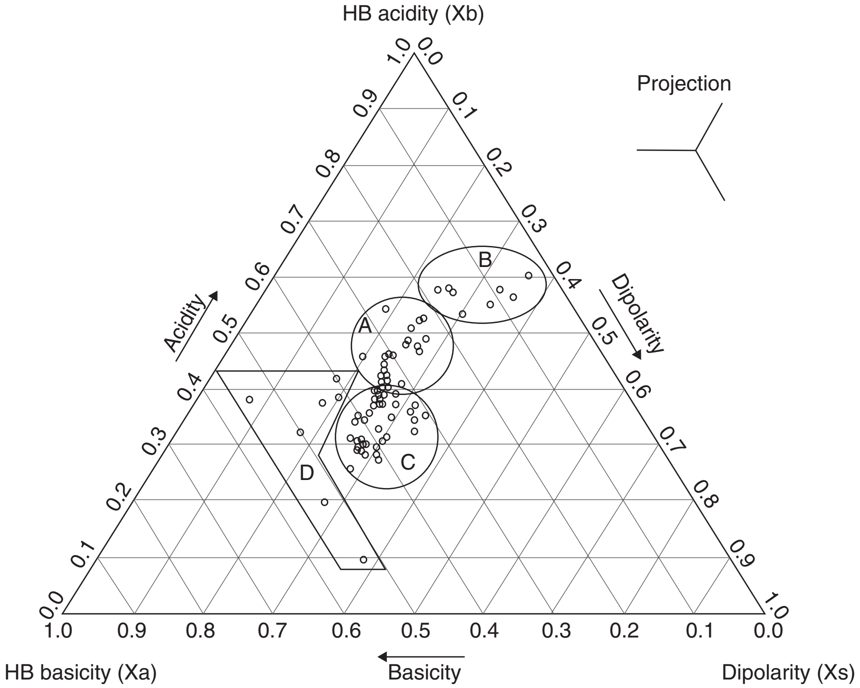

Figure 15.5 Micellar selectivity triangle for characterization and classification of various micellar, polymeric, vesicular, and mixed pseudophases. *Source*: Ref. 22; reprinted with permission.

and has wider dipolarity range, consists of polymeric micelles of AGENT as well as some CTAB-modified micelles.

An advantage of MEKC is the feasibility and flexibility of controlling selectivity through adjusting the composition of the pseudophase. The MST scheme facilitates the selection of optimum pseudophases based on their selectivities. For example, Fig. 15.6 shows that solvation properties of SDS, CTAB, and LiPFOS micellar systems could be significantly changed through addition of organic modifiers hexafluoroisopropanol (HFIP) (a strong HBD) and pentanol (HBA). The two organic modifiers, PeOH and HFIP, mainly affect the hydrogen-bonding capability of micelles; thus, it is interesting to monitor the migration behaviors of HBA and HBD solutes in the selected micellar phases. Naturally, all HBD solutes have measurable HBA capability as well. Figure 15.7a–c shows the changes in retention patterns on addition of HFIP and PeOH to SDS micelles. As illustrated, on the addition of HFIP, the SDS micelles become a stronger HBD and have stronger interaction with solutes that have HBA groups, while addition of PeOH would enhance the HBA strength of the micelles and result in stronger interactions with HBD solutes.

15.6 DETECTION AND SENSITIVITY ENHANCEMENT

The detection systems for MEKC (and CE in general) are very similar to those used in HPLC. Absorbance detection is the most commonly used due to convenience, capability of detecting a wide range of compounds, feasibility of operation for on-column

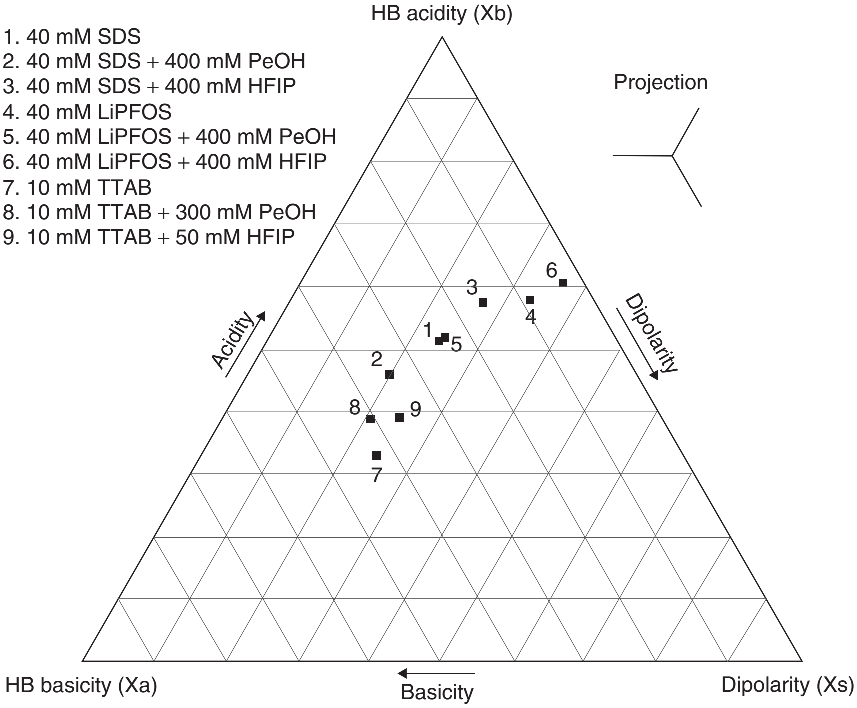

Figure 15.6 MST for three micelles and six modified micellar systems. *Source*: Ref. 22; reprinted with permission.

detection, robustness, and good linearity. However, due to the exceedingly small peak volume (in nanoliter range), peaks must be detected in the capillary (i.e., on-column detection). Subsequently, the detection sensitivity of absorbance detection is modest (in micro–molar range) due to small light path length (which is the capillary diameter, typically 50–75 μm) and very small injection size. Detection path length (thus sensitivity) could be enhanced through the use of bubble cell [1–5]. Laser-induced fluorescence (LIF) has significantly better detection sensitivity (in subnanomolar range), albeit narrower scope of application as fewer classes of compounds are inherently fluorescent. Attachment of various fluorophore moieties to the parent compound would extend the range of applications of LIF detection of wider classes of compounds. Electrochemical detection is readily compatible with the CE techniques and offer great sensitivity for certain groups of compounds such as neurotransmitters. Unfortunately, MEKC has limited compatibility with MS detection due to the presence of high concentrations of surfactants in the buffer solution. The use of partial filling method, fluorinated surfactants, or polymeric pseudophases has been reported to improve the situation [1–5].

Owing to poor concentration sensitivity (LOD) in MEKC (and CE in general), on-line concentration of samples before the separation process is necessary for trace analysis. The two widely reported methods for sample preconcentration are based on stacking and sweeping mechanisms [1–5,39].

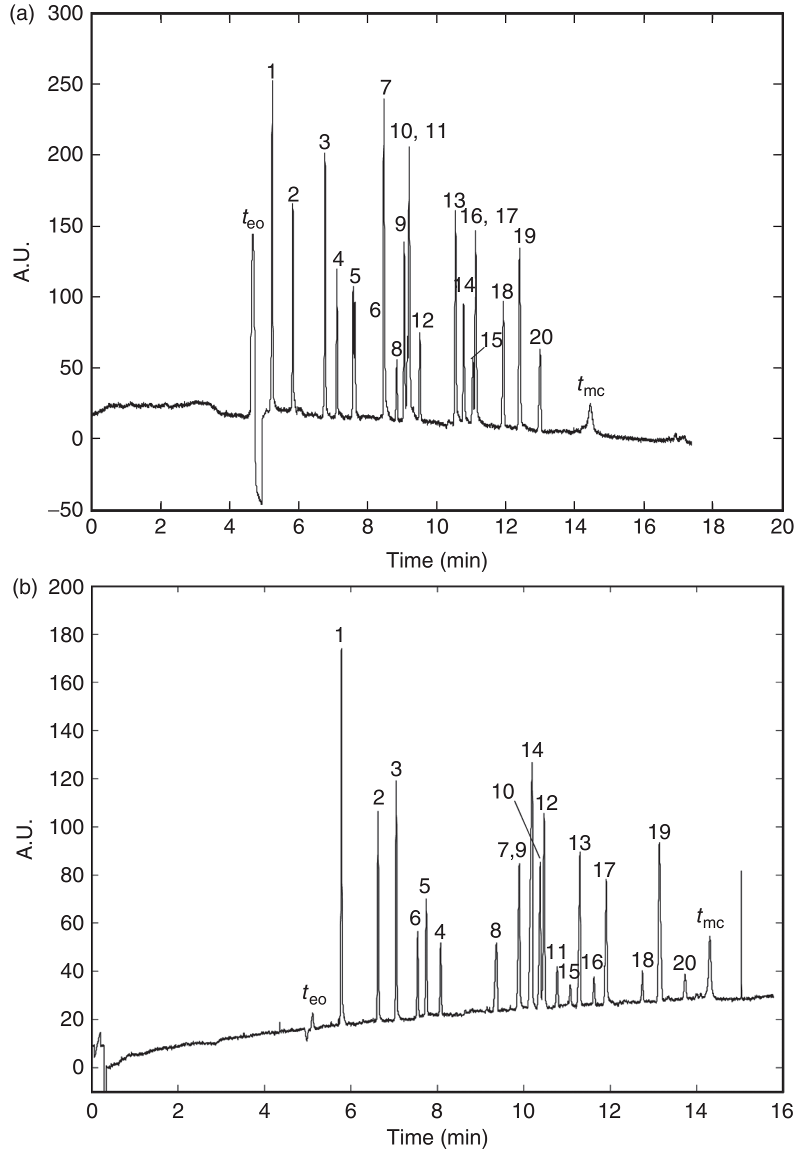

Figure 15.7 Chromatograms of the separation of 20 aromatic solutes composed of hydrogen-bond donors (HBDs): (1) resorcinol; (2) phenol; (7) 3-chlorophenol; (10) 4-ethylphenol; (12) 3,5-dimethyl phenol; and (15) 4-iodophenol. Hydrogen-bond acceptors (HBAs): (3) phenethyl alcohol; (5) nitrobenzene; (6) acetophenone; (8) methylbenzoate; (9) benzyl chloride; (14) 4-chloroacetophenone; and (16) methyl-2-methyl benzoate. Nonhydrogen-bond donors (NHBDs): (4) benzene; (11) toluene; (13) chlorobenzene; (17) ethylbenzene; (18) 4-chlorotoluene; (19) naphthalene; (20) propylbenzene. Micellar phases IDs: (a) 40 mM SDS, (b) 40 mM SDS/400 mMPeOH (4.34% v/v), and (c) 40 mM SDS/400 mM HFIP (4.16% v/v). *Source*: Ref. 22; reprinted with permission.

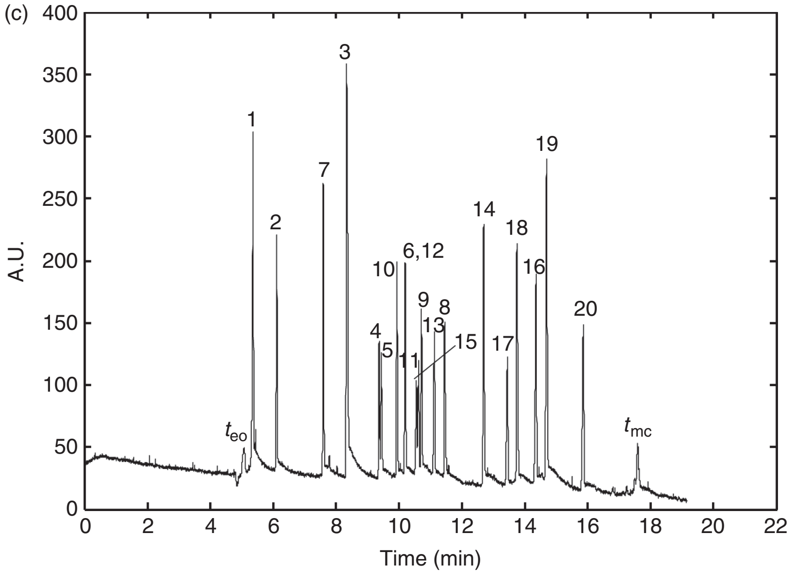

Figure 15.7 (*continued*)

15.7 MICROFLUIDIC MEKC

Microfluidic platform offers unique advantages over capillary tubes in MEKC (and CE) separations. The exceedingly smaller dimensions of separation channels in microchips as compared to capillary tubes are much more effective in dissipation of joules heating, thus allowing the use of much higher electric field strength and require significant reduction in the sample injection size. This results in higher separation efficiencies as well as significant reduction in analysis times from minutes to seconds. In addition, the microchip platform greatly facilitates designing parallel analysis (or separation) for increasing sample throughput, integration of sample manipulation and separation, as well as combining orthogonal separation techniques for two- or multidimensional separations for enhancement of peak capacity for resolving complex samples [40].

Ramsey's group first reported MEKC separations on microchips and investigated the effects of geometry and dimensions of separation channels. Using the straight channel design would allow a short separation length where high field separations can be performed. For example, a mixture of three coumarins were separated in <3 s using an electric field strength of 500 V/cm over a distance of 1.3 cm [41–44]. While the shorter separation channels and higher electric field strengths are desirable for achieving very fast separations of relatively simple mixtures, longer separation channel lengths are needed to generate larger absolute efficiencies for more complex and challenging mixtures. Increasing channel lengths on microchips can be achieved by incorporating turns in channel designs such as the use of serpentine and spiral geometries. However, it was determined that the turns in the separation channels could introduce additional band dispersion through "racetrack effect" as the molecules on the inside wall travel a shorter distance than those on the outer walls. New changes in microchip design

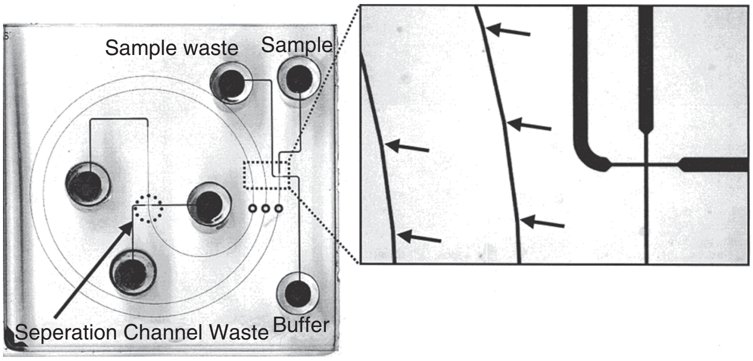

Figure 15.8 Image of a microchip with a spiral separation channel. The reservoirs on the interior of the spiral were replaced by a single reservoir located at the injection cross (dashed circle) for the experiments reported here. The small circles are the locations of the detection points from outside to inside 0.25, 11.9, and 22.2 cm. The inset is a magnification of the injection cross and parts of the spiral channel. The buffer, sample, and sample waste channels are 219-mm wide (half-depth) for all their length except for the last 500 μm before the injection cross where they narrow to only 40 μm (half-depth). The separation channel was 24.9-cm long and 40-μm wide (half-depth). The black arrows on the inset indicate the vertexes of the polygonally shaped channels. *Source*: Ref. 43; reprinted with permission.

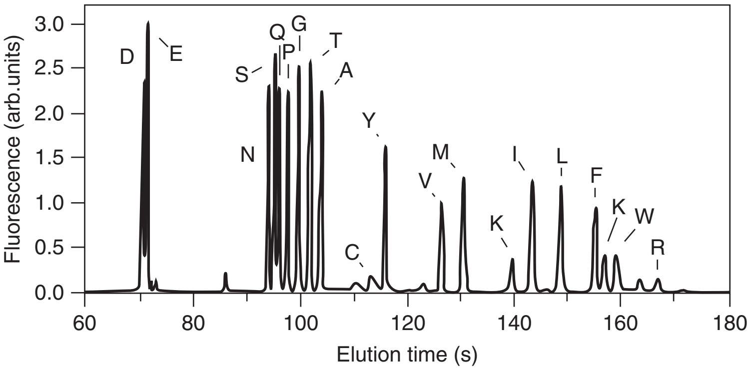

Figure 15.9 MEKC separation of 19 TRITC-labeled amino acids in a 10 mM sodium tetraborate/50 mM SDS buffer with 10% (v/v) 2-propanol. The field strength was 770 V/cm, and the detection point was 11.87 cm from the injection cross. The peak locations of the amino acids are indicated by their standard one-letter abbreviations. *Source*: Ref. 43; reprinted with permission.

can be incorporated to minimize this geometrical band broadening effect; for example, by reducing the width of the channel in the turn or increasing the radius of the turn. Figure 15.8 illustrates the microchip spiral design with a separation length of about 25 cm. Figure 15.9 shows the MEKC separation of 19 tetramethylrhodamine isothiocyanate (TRITC) amino acids in <3 min using the spiral microchip with a field strength of 770 V/cm and channel length of 11.87 cm between the injection and detection. The separation efficiency is over 280,000 with a minimum resolution of 1.2 between adjacent peaks.

15.8 TWO-DIMENSIONAL MEKC SEPARATIONS

In multidimensional separations, different techniques with orthogonal separation mechanisms are combined to enhance peak capacity and achieve the necessary resolving power for complex mixtures as in proteomics applications. Theory predicts that the peak capacity of a multidimensional separation method equals the product of the individual peak capacities of the constituent orthogonal methods [45–51]. There are three main orthogonal mechanisms based on solute hydrophobicity (using RPLC or MEKC), charge (ion exchange LC or CZE), and size (size exclusion LC or capillary sieving electrophoresis). The widely used conventional two-dimensional (2D) gel electrophoresis is a powerful separation methodology with high resolving power where solutes are

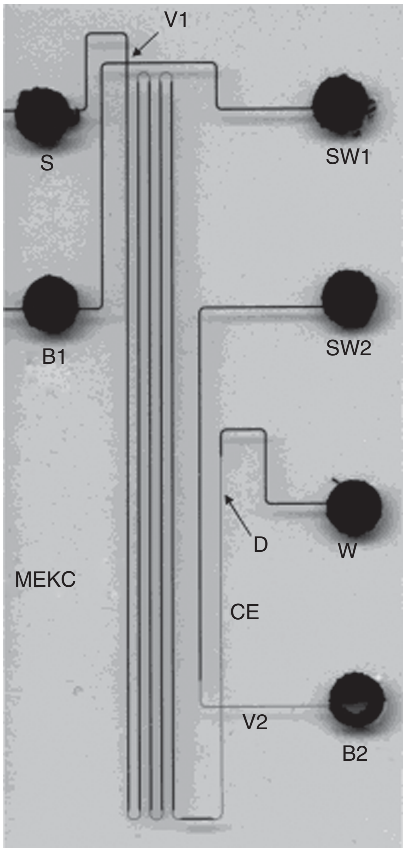

Figure 15.10 Image of a microchip with a serpentine channel for two-dimensional separations. Injections were made at valve 1 (V1) for the first-dimension MEKC separation and at valve 2 (V2) for the second-dimension CE separation. The sample was detected 1-cm downstream from V2 at point D using laser-induced fluorescence. The reservoirs are labeled sample (S), buffer 1 (B1), sample waste 1 (SW1), buffer 2 (B2), sample waste (SW2), and waste (W). The channels and reservoirs were filled with black ink for contrast. *Source*: Ref. 49; reprinted with permission.

separated based on charge and size in the two dimensions. However, the technique has the disadvantages of being slow, labor intensive, and not quantitative. Over the past two decades, researchers have investigated 2D methods by combining a variety of HPLC and/or CE techniques in series, where the sample bands from the first dimension are transferred sequentially into a second dimension. A great majority of the 2D separations have involved the combination of different LC methods or LC in one dimension and an electrophoretic technique (IEF or CE) in the other dimension. There are only a few 2D separation reports where both dimensions are CE methods. In a handful of reports, MEKC has been used as one of the dimensions where solutes are separated based on their hydrophobicity.

Sheng and Pawliszyn [46] designed a 2D system by combining MEKC as the first dimension and CIEF as the second dimension using a 10-port valve as an interface for the separation of protein digests [46]. Hu *et al*. [47] analyzed protein expression in single mammalian cell using a 2D system with capillary sieving electrophoresis in the first dimension for size-based separation and MEKC in the second dimension and LIF for sensitive detection [47].

A major challenge in performing 2D capillary-based CE separations is interfacing the capillaries and independently controlling the electric fields in each dimension. On the other hand, microchip platform is much more suitable for performing two (and multi)-dimensional electrophoretic separations, as different channels can be readily connected and voltages at various locations can be easily monitored to control the flow of solutions and sample zones between different channels. Ramsey and coworkers reported 2D separation of protein digests using microfluidic devices where peptides were separated by MEKC in the first dimension followed by zone electrophoresis (CE) in the second dimension [48,49]. The microchip design for 2D MEKC–CZE separation of protein digests is illustrated in Fig. 15.10, where a 19.6-cm-long serpentine channel is used for MEKC separation followed by rapid CE separation in the second dimension using a short 1.3-cm-long straight channel. A peak capacity of 4200 was observed for the separation of the bovine serum albumin (BSA) tryptic digest mixture (shown in Fig. 15.11). Note that the timescale for the MEKC dimension is <15 min, while it is only around 1 s in the CE dimension. The electric field strength for MEKC was 200 and 2400 V/cm for the second CE dimension. The combination of short channel length and high electric field strength ensures rapid separation to avoid remixing of separated zones in the second dimension.

Soper's group reported 2D separations of proteins on microchips made of poly(methyl methacrylate) (PMMA) with SDS-PAGE for size-based separation in the first dimension and MEKC separation in the second dimension [50]. The proteins were labeled with a fluorescent tag and LIF was used for high sensitivity detection. Initially, they used the 2D system to separate a sample of 10 proteins with a molecular weight range of 38–110 kDa. The separation was completed in 12 min and the method provided a peak capacity of 1000. More recently, they reported comprehensive 2D SDS-CGE × MEKC separation of a complex protein mixture in fetal calf serum on a cross-design PMMA microchip (Fig. 15.12). The separation was completed in <30 min with an average peak capacity of 2600. The results were compared with that using conventional 2D gel electrophoresis, which is a combination of IEF and SDS-PAGE. The 2D microchip separation was 60× faster with a peak capacity three times of the conventional method.

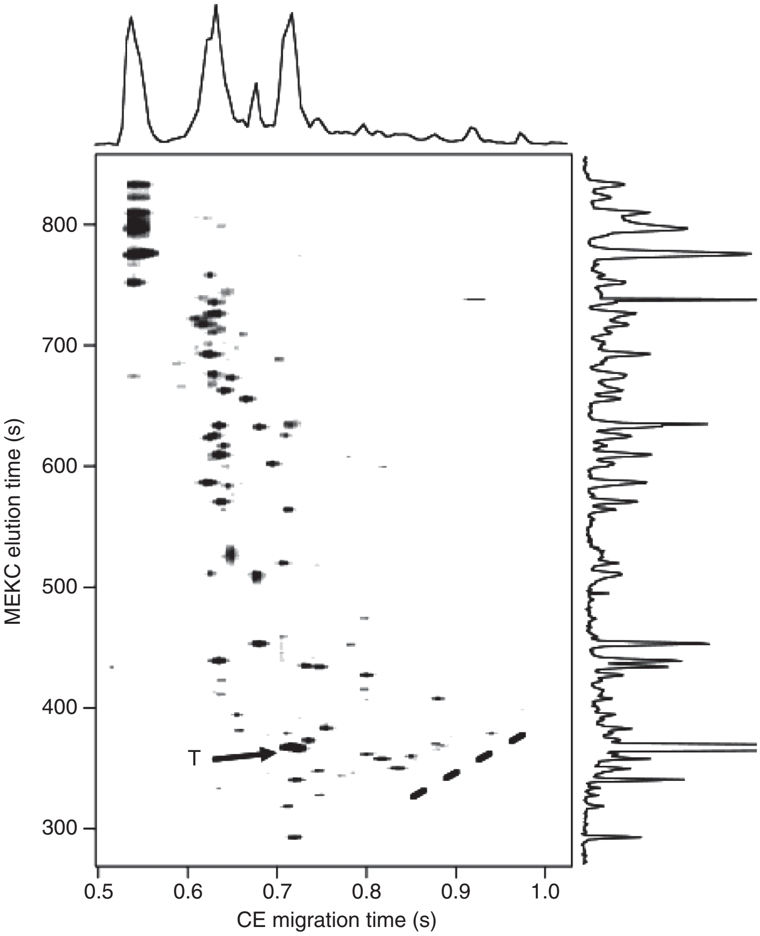

Figure 15.11 Two-dimensional separation of a bovine serum albumin tryptic digest. The projections of the two-dimensional data onto each axis are shown. Spots marked with a "T" represent unreacted 5-TAMRA dye. The dotted line illustrates correlation in the separation mechanisms. *Source*: Ref. 49; reprinted with permission.

15.9 CONCLUSIONS

EKC has matured into a microscale separation technique since its first introduction in 1984. The technique offers unique capabilities such as high resolving power, fast analysis time, availability of a wide range of pseudophases, and flexibility of rapidly adjusting the pseudophase composition for optimizing separation selectivity and enhancing resolution. In addition, the possibility of calculating micelle–water partition coefficients from solute structure and subsequently retention factor in MEKC is quite powerful for rapid optimization and enhancement of separation of complex mixtures. Significant progress has been made in synthesizing new pseudophases (especially polymeric and nanoparticles). and this trend will likely continue in the coming years. The wide range of selectivities in MEKC would be a great asset in 2D separations.

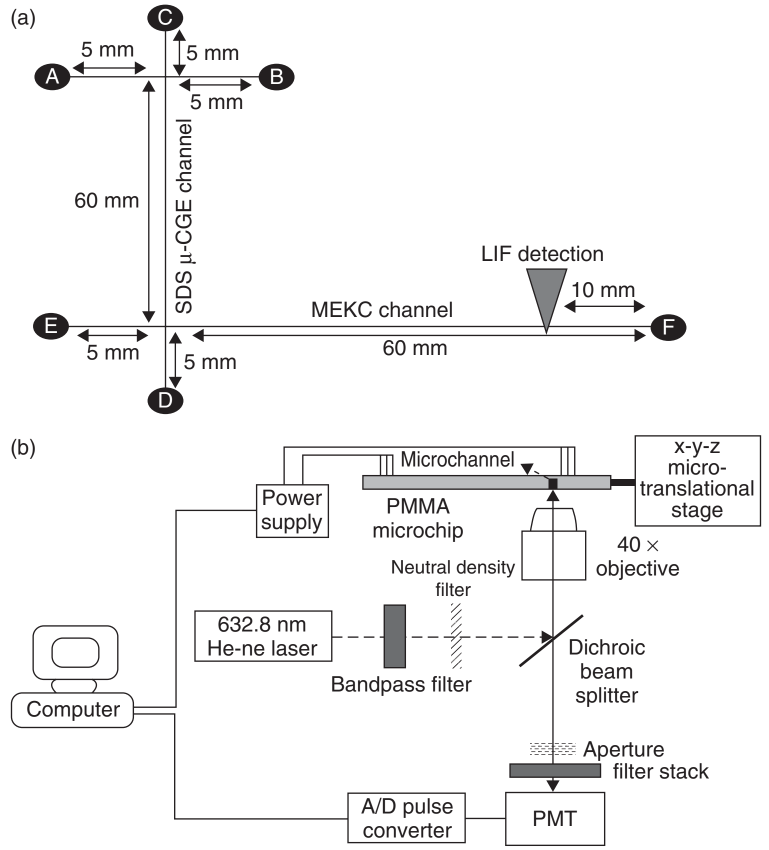

Figure 15.12 (a) Topography of the 2D microchip used for these studies. The channels were 50-µm deep and 20-µm wide in all cases. The first- and second-dimension channels were 7 cm (filled with gel media) and 6 cm (filled with MEKC buffer), respectively, in terms of their total column lengths. The effective column lengths for the first and second dimensions were 6 and 5 cm, respectively. (b) Diagram of the in-house constructed LIF system used for the separation. *Source*: Ref. 51; reprinted with permission.

The microfluidic platforms are ideal for interfacing two or more orthogonal electromigration techniques. Finally, much progress has been made in interfacing MS detection with MEKC. However, more work is needed before MEKC-MS comes to maturation for use in routine analytical applications.

REFERENCES

1. Khaledi MG. Micellar electrokinetic chromatography. In: Khaledi MG, editor. High performance capillary electrophoresis: theory and applications. New York: Wiley and Sons, Inc; 1998. Chapter 3.
2. Pappas TJ, Gayton-Ely M, Holland LA. Recent advances in micellar electrokinetic chromatography. Electrophoresis 2005;26:719–734.
3. Silva M. MEKC: an update focusing on practical aspects. Electrophoresis 2007;28: 174–192.

4. Silva M. Micellar electrokinetic chromatography: methodological and instrumental advances focused on practical aspects. Electrophoresis 2009;30:50–64.
5. Terabe S. Capillary separation: micellar electrokinetic chromatography. Annu Rev Anal Chem 2009;2:99–120.
6. Terabe S, Otsuka K, Ichikawa K, *et al*. Electrokinetic separations with micellar solutions and open-tubular capillaries. Anal Chem 1984;56:111–132.
7. Khaledi MG, Smith SC, Strasters JK. Micellar electrokinetic capillary chromatography of acidic solutes: migration behavior and optimization strategies. Anal Chem 1991;63: 1820–1830.
8. Strasters JK, Khaledi MG. Migration behavior of cationic solutes in micellar electrokinetic capillary chromatography. Anal Chem 1991;63:2503–2508.
9. Quang C, Starsters JK, Khaledi MG. Computer-assisted modeling, prediction, and multifactor optimization in micellar electrokinetic chromatography of ionizable compounds. Anal Chem 1994;66:1646–1653.
10. Kelly K, Burns ST, Khaledi MG. Prediction of retention in micellar electrokinetic chromatography from solute structure. 1. Sodium dodecyl sulfate micelles. Anal Chem 2001;73: 6057–6062.
11. Burns ST, Khaledi MG. Predictions of micelle-water partition coefficients and retention in micellar electrokinetic chromatography from solute structure. 2. Fragmental constant approach. Anal Chem 2004;76:5451–5458.
12. Palmer CP. Recent progress in the use of ionic polymers as pseudostationary phases for electrokinetic chromatography. Electrophoresis 2009;30:163–168.
13. Preinerstorfer B, Lammerhofer M, Linder W. Advances in enantioselective separations using electromigration capillary techniques. Electrophoresis 2009;30:100–132.
14. Moliner-Martinez Y, Cardenas S, Simonet BM, *et al*. Recent developments in capillary EKC based on carbon nanoparticles. Electrophoresis 2009;30:169–175.
15. Agbodjan AA, Bui HH, Khaledi MG. Study of solute partitioning in biomembrane-mimetic pseudophases by electrokinetic chromatography: dihexadecyl phosphate small unilamellar vesicles. Langmuir 2001;17:2893–2899.
16. Pascoe RJ, Foley JP. Characterization of surfactant and phospholipid vesicles for use as pseudostationary phases in electrokinetic chromatography. Electrophoresis 2003;24: 4227–4240.
17. Burns ST, Khaledi MG. Rapid determination of liposome–water partition coefficients (Klw) using liposome electrokinetic chromatography (LEKC). J Pharm Sci 2002;91:1601–1612.
18. Burns ST, Agbodjan AA, Khaledi MG. Characterization of solvation properties of lipid bilayer membranes in liposome electrokinetic chromatography. J Chromatogr A 2002;973:167.
19. Holland LA, Leigh AM. Bilayered phospholipid micelles and capillary electrophoresis: a new additive for electrokinetic chromatography. Electrophoresis 2003;24:2935.
20. Wiedmer SK, Jussila MS, Riekkola ML. Phospholipids and liposomes in liquid chromatographic and capillary electromigration techniques. TRAC 2004;23:562–582.
21. Ye B, Hadjmohammadi M, Khaledi MG. Selectivity control in micellar electrokinetic chromatography of small peptides using mixed fluorocarbon-hydrocarbon anionic surfactants. J Chromatogr A 1995;692:291–300.
22. Fu C, Khaledi MG. Micellar selectivity triangle for classification of chemical selectivity in electrokinetic chromatography. J Chromatogr A 2009;1216:1891–1900.
23. Fu C, Khaledi MG. Selectivity patterns in micellar electrokinetic chromatography. Characterization of fluorinated and aliphatic alcohol modifiers by micellar selectivity triangle. J Chromatogr A 2009;1216:1901–1907.
24. Nilsson C, Nilsson S. Nanoparticle-based pseudostationary phases in capillary electrochromatography. Electrophoresis 2006;27:76–83.

25. Nilsson C, Birnbaum S, Nilsson S. Use of nanoparticles in capillary and microchip electrochromatography. J Chromatogr A 2007;1168:212–224.
26. Wang Z, Luo G, Chen J, *et al*. Carbon nanotubes as separation carrier in capillary electrophoresis. Electrophoresis 2003;24:4181–4188.
27. Xiong X, Ouyang J, Baeyens WRG, *et al*. Enhanced separation of purine and pyrimidine bases using carboxylic multiwalled carbon nanotubes as additive in capillary zone electrophoresis. Electrophoresis 2006;27:3243–3253.
28. Xu Y, Li SFY. Carbon nanotube-enhanced separation of DNA fragments by a portable capillary electrophoresis system with contactless conductivity detection. Electrophoresis 2006;27:4025–4028.
29. Suarez B, Simonet BM, Cardenas S, *et al*. Surfactant-coated single-walled carbon nanotubes as a novel pseudostationary phase in capillary EKC. Electrophoresis 2007;28:1714–1722.
30. Moliner-Martínez Y, Cárdenas S, Valcárcel M. Evaluation of carbon nanostructures as chiral selectors for direct enantiomeric separation of ephedrines by EKC. Electrophoresis 2007;28:2573–2579.
31. Treubig JM, Brown PR. Novel approach to the analysis and use of fullerenes in capillary electrophoresis. J Chromatogr A 2000;873:257–267.
32. Bächmann K, Göttlicher B, Haag I, *et al*. Capillary electrokinetic chromatography with a suspension of chromatographic particles. J Chromatogr A 1994;688:283–292.
33. Neiman B, Grushka E, Gun J, *et al*. Organically modified silica sol-mediated capillary electrophoresis. Anal Chem 2002;74:3484–3491.
34. Huang M, Huang C, Chang H. Improved separation of double stranded DNA fragments by capillary electrophoresis using poly(ethylene oxide) solution containing colloids. Electrophoresis 2003;24:2896–2902.
35. Huang M, Kuo Y, Huang C, *et al*. Separation of long double-stranded DNA by nanoparticle-filled capillary electrophoresis. Anal Chem 2004;76:192–196
36. Yang Y, Khaledi MG. Chemical selectivity in micellar electrokinetic chromatography: characterization of solute-micelle interactions for classification of surfactants. Anal Chem 1995;67:499–510.
37. Trone MD, Khaledi MG. Characterization of chemical selectivity in micellar electrokinetic chromatography. 4. Effect of surfactant headgroup. Anal Chem 1999;71:1270.
38. Poole SK, Poole CF. Quantitative structure–retention (property) relationships in micellar electrokinetic chromatography. J Chromatogr A 2008;1182:1–24.
39. Sera Y, Matsubara N, Otsuka K, *et al*. Sweeping on a microchip: concentration profiles of the focused zone in micellar electrokinetic chromatography. Electrophoresis 2001;22:3509–3513.
40. Kitagawa F, Otsuka K. Micellar electrokinetic chromatography on microchips. J Sep Sci 2008;31:794–802.
41. Moore AW, Jacobson SC, Ramsey JM. Microchip separations of neutral species via micellar electrokinetic chromatography. Anal Chem 1995;67:4184–4189.
42. Kutter JP, Jacobson SC, Ramsey JM. Integrated microchip device with electrokinetically controlled solvent mixing for isocratic and gradient elution in micellar electrokinetic chromatography. Anal Chem 1997;69:5165–5171.
43. Culbertson CT, Jacobson SC, Ramsey JM. Microchip devices for high-efficiency separations. Anal Chem 2000;72:5814–5819.
44. Ramsey JD, Collins GE. Integrated microfluidic device for solid-phase extraction coupled to micellar electrokinetic chromatography separation. Anal Chem 2005;77:6664–6670.
45. Cortes HJ. Multidimensional chromatography. New York: Marcel Dekker; 1990.
46. Sheng L, Pawliszyn J. Comprehensive two dimensional separation based on coupling micellar electrokinetic chromatography with capillary isoelectric focusing. Analyst 2002;127:1159–1163.

47. Hu S, Michels DA, Abul Fazal Md, *et al*. Capillary sieving electrophoresis/micellar electrokinetic capillary chromatography for two-dimensional protein fingerprinting of single mammalian cells. Anal Chem 2004;76:4044–4049.
48. Rocklin RD, Ramsey RS, Ramsey JM. A microfabricated fluidic device for performing two-dimensional liquid phase separations. Anal Chem 2000;72:5244–5249.
49. Ramsey JD, Jacobson SC, Culbertson CT, *et al*. High-efficiency, two dimensional separations of protein digest on microfluidic devices. Anal Chem 2003;75:3758–3764.
50. Shadpour H, Soper SA. Two-dimensional electrophoretic separations of proteins using poly(methyl methacrylate) microchips. Anal Chem 2006;78:3519–3527.
51. Osiri JK, Shadpour H, Park S, *et al*. Generating high peak capacity 2-D maps of complex proteomes using PMMA microchip electrophoresis. Electrophoresis 2008;29:4984–4992.