14
Micellar Electrokinetic Chromatography

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14.1
Introduction

Electrophoresis was originally developed as a simple analytical method that allowed the operator to obtain the separation of charged molecules by applying an electric field to the system. While appearing of great interest, in its early stage, this technique suffered from serious drawbacks, the most important being the strong deterioration of separated zones as a consequence of the thermal convection caused by Joule heating. If the introduction of several types of supporting material (gels, films, and paper) contributed to suppress, at least partially, thermal convection, it was the development of narrow-bore silica capillaries that succeeded in overcoming this limitation (almost) totally. Due to a variety of intrinsic advantages (high resolution power, speed of analysis, and handling of very small sample volumes) associated with the use of silica capillaries, the popularity and attractiveness of capillary electrophoresis (CE) have rapidly spread to a broad user base. From among the various operational modes that can be performed in a capillary, free-zone electrophoresis, known as capillary zone electrophoresis (CZE), was developed first. By using background electrolytes (BGEs) with both neutral and alkaline pHs, most CZE procedures have been shown to be excellent analytical tools for achieving the separation of complex mixtures. Under these pH values, the double layer of positively charged buffer counterions adsorbed on the silanol groups of the inner surface of the capillary, combined with the electric field applied along the capillary axis, generate a phenomenon known as electroosmotic flow (EOF) that, being stronger than the analytes' intrinsic electrophoretic mobility, makes all of them migrate toward the cathode. If positively and negatively charged analytes are easily separated on the basis of their charge difference and/or of their charge-to-size ratio, no separation can occur for neutral compounds all of which migrate together at the same velocity as does the bulk solution. The idea that the separation of these analytes could be promoted by the addition of surfactants to BGE was proposed in the eighties by Terabe *et al.* [1]. The enrichment of the electrolyte solution with surfactants, in a concentration above their critical micelle concentration (CMC), was shown to

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induce the formation of micelles that act as a pseudo-stationary phase (PSP). The distribution of neutral analytes between the micelles and the surrounding phase, while affecting their relative velocity, will promote their separation. In fact, while the fraction of the neutral analytes that remains in the bulk solution migrates with the EOF, the velocity of analyte fraction completely incorporated in the micelles will be affected (positively or negatively) by micelle's electrophoretic migration. This approach, known as micellar electrokinetic chromatography (MEKC), is essentially based on the combination of analytes' electrokinetic migration and their partitioning mechanism between the bulk solution and the micelles. Meeting the requisites for differentiating with great efficiency among molecules with similar physicochemical properties, since its introduction, MEKC has become one of the most challenging techniques.

A scheme of how analytes distribute between a micellar (anionic) phase and the BGE, on the basis of their chemical characteristics, is shown in Figure 14.1.

After a brief description of the fundamentals of the technique (Section 14.2), the intention of the authors is to focus reader's attention on the most important parameters influencing resolution and sensitivity in MEKC. An overview of different classes of PSPs and of theoretical/practical approaches that may be explored in the routine laboratory practice to improve both resolution and sensitivity will be presented in Sections 14.3 and 14.4, respectively. The most widely used detection systems will be discussed in Section 14.5 while Section 14.6 is dedicated to novelties and trends in this area. Finally, Section 14.7 will provide the reader a variety of MEKC applications in different matrices spanning from biological fluids to pharmaceutical forms and food.

14.2 Principles of MEKC

Surfactants are compounds whose molecules contain both hydrophobic hydrocarbon chains and hydrophilic head groups that may be anionic, cationic, nonionic, or zwitterionic in character. At a concentration of the surfactant monomers higher than their CMC, they tend to aggregate. This process, which results from a balance between the hydrophobic attractive forces of the tail and the ionic repulsive forces of the head group, determines the formation of micelles. CMC is different for each surfactant and greatly depends on a variety of parameters that include the presence of a cosolvent, the effect of temperature, and the ionic strength of the BGE.

By combining features of liquid chromatography and CE, the peculiarity of MEKC makes this technique excellent for exploring mixtures of analytes with subtle differences in size, shape, hydrophobicity, and charge. The mobile phase in MEKC is typically an aqueous buffer whose properties can be altered by the addition of appropriate modifiers (organic solvents, urea, chiral additives, etc.). The stationary phase or, more appropriately, the "PSP" is made of micelles in motion inside the capillary and its properties can also be easily modified by

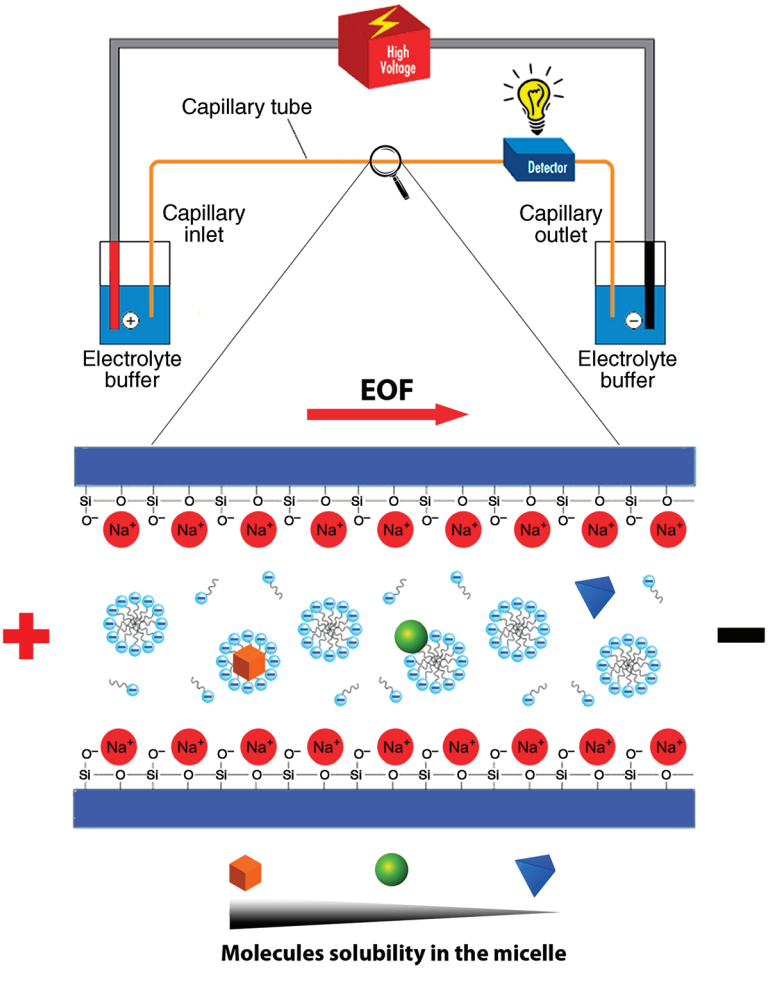


Figure 14.1 Scheme of a CE system (MEKC mode) with a magnified view of the inner surface of the capillary. Solutes characterized by different hydrophobicity and different interaction with the (anionic) micelles are represented by cubic, spherical, and cone-shaped solids.

changing the nature of surfactant. As illustrated above, a successful separation in MEKC is largely based on the differential partitioning of the components of a mixture between these two phases. Migration behavior (resolution) is influenced by both the retention factor of the solutes in the micellar phase and by the migration window, which reflects the velocity of aqueous and micellar phases involved in the separation process. Analytes that are "solubilized" inside the micelle will migrate with a velocity that is different from that of the fraction that does not interact with it. In light of this evidence, it is intuitive, and it is generally agreed, that the choice of the surfactant is the most important aspect for optimizing MEKC selectivity. In other words, while optimization of selectivity in

liquid chromatography is usually achieved by changing the composition of the mobile phase (after having selected an appropriate stationary one), in MEKC the central role in the achievement of resolution is played by the micelle. In this respect, the greatest impact in determining the extent of distribution of analytes between the two phases of this system will rely on the ability to manipulate the characteristics of a micellar phase. From among the numerous studies aimed at exploring the relationship between the surfactant structure and the separation selectivity, those listed here [2–4] will provide the reader valuable insights into the interaction mechanism of analytes. For additional information on the basic principles of MEKC the reader is also invited to refer to a recent review article in which the birth of this technique is comprehensively described [5].

14.3 Resolution

The aim of this section is to introduce the reader to the physicochemical characteristics of the most commonly employed surfactants in MEKC, grouped according to their functional properties.

14.3.1 Use of Different Surfactants and Mixed Micelles

14.3.1.1 Anionic Surfactants

Until a few years ago, the possibility to vary the selectivity in MEKC by changing the nature of surfactant was strongly limited by the restricted number and the homologous character of commercial products available. Due to the enormous technological advancements in the development of novel structures, the production of surfactants with diverse chemical nature has been dramatically accelerated over the last 10 years, thus widening the number of MEKC applications and making this technique increasingly powerful. By virtue of its numerous advantages over other compounds, sodium dodecyl sulfate (SDS) is currently one of the most popular anionic surfactants for MECK separations, its applicability ranging from charged to neutral small molecules with minute structural differences. Advantages include low cost; availability in pure form; good solubility in aqueous phase (about 10% w/w); low ultraviolet absorbance and high solubilization capability. Minor disadvantages may be represented by its relatively high CMC (8.1 mM in pure water at 25°C) and Krafft point (16°C) that causes SDS precipitation at low temperature. Due to the anionic character of the sulfate groups, the electrophoretic mobility of surfactant and micelles is toward the positive electrode. Nevertheless, as a result of the strong EOF under alkaline conditions, in analytical practice both continue to move toward the cathode, although at a lower mobility. Among anionic surfactants alternative to SDS, sodium octane sulfonate (SOS) and lithium dodecyl sulfate (LDS), two surfactants with an SDS-related structure, have been explored for their selectivity. Interestingly,

the replacement of sodium with lithium ions in the surfactant molecule resulted in an improvement of resolution with the formation of narrower peaks. This behavior was hypothesized to be promoted by a less-tight binding of Li^+ with the micelle surface, compared to that of Na^+ ions. The stronger repulsion with sulfate groups thus resulted in more "open" micelles with a higher mass transfer. To expand the applications of MEKC, anionic dimeric surfactants have also been synthesized. In particular, the selectivity of disodium1, Ω-bis(decyloxymethyl)-dioxa alkane-1 Ω disulfates, with a flexible hydrophobic spacer of different length, was compared to that of SDS. These surfactants were found to be slightly more cohesive, to interact better with polarizable compounds, and to be somewhat better hydrogen bond acceptors and worse hydrogen bond donors. Their CMC values (less than 1 mM), much lower than those of conventional surfactants used in MEKC, suggest a possible direct coupling of micellar systems employing these compounds with mass spectrometry (MS).

14.3.1.2 Cationic Surfactants

Cationic surfactants occupy a selectivity space different from other phases. The characteristics of these compounds make them likely candidates for the separation of mixtures that cannot be resolved by an anionic surfactant and provide orthogonal selectivity in a multidimensional separation system. Due to the effect of the double-layer adsorption of a cationic surfactant on the silica capillary, in fact, the inner surface gains a positive charge. As a consequence, the direction of the EOF will be reversed and negatively charged analytes migrate faster toward the anode than neutral compounds, with dramatic shortening of their analysis time. Among the cationic surfactants used in MEKC, the most frequently used include cetyltrimethyl ammonium chloride (CTAC), tetradecyltrimethylammonium bromide (TTAB) and cetyltrimethyl ammonium bromide (CTAB). The structural modification of these compounds was brought about by the synthesis of additional novel mono- and double-chain surfactants that show different/improved separation selectivity compared to the molecules of origin. This is the case for 1-cetyl-3-methylimidazolium bromide, *N*-cetyl-*N*-methylpyrrolidinium bromide, and *N*-alkyl-*N*-methylpyrrolidinium bromides, all single-chain liquid-type surfactants derived from CTAB. Although resembling the CTAB structure, these surfactants were shown to provide different separation efficiencies compared to the parent compound. Particular attention was focused on derivatives containing the cyclic pyrrolidinium head group that, compared to the molecule of origin, were shown to be more cohesive and interact more strongly with polar compounds. Further structural modifications of CTAB allowed to synthesize didodecyldimethylammonium bromide, a double-chain cationic surfactant. By virtue of its peculiar properties, it has been considered a valuable substitute of traditional single-chain CTAB. Likewise, starting from TTAB, the novel surfactant 1-tetradecyl-3-methylimidazolium bromide was synthesized that, compared to the parent molecule, exhibited superior selectivity for hydrophilic polar analytes and higher reproducibility. With the aim of exploring the structure–selectivity relationship, two series of cationic surfactants with hexadecyl

hydrocarbon tails and different head groups, have also been evaluated. The head groups of the first series contained quaternary ammonium with three linear alkyl groups (from one to four carbons in length) attached to the amine. The quaternary ammonium group in the second series was incorporated into rings from five to eight atoms in size. As expected, the replacement of the linear chain with a ring structure was shown to alter retention behavior and selectivity. In contrast, variation in the ring size did not affect steric factors or hydrophobic interactions to the same extent. Cationic surfactants containing quaternary phosphonium head groups showed electrophoretic properties that did not differ significantly from those of ammonium surfactants.

14.3.1.3 Nonionic and Zwitterionic Surfactants

The lack of electrophoretic mobility prevents the use of nonionic surfactants as the pseudostationary phase in conventional MEKC. This does not obviously mean that these surfactants are not useful in general. Their application for: (i) obtaining the separation of charged analytes and (ii) altering the selectivity of MEKC processes when they are added as the second surfactant for the formation of mixed micelles with ionic surfactants (see below) is, in fact, well-documented. Given that nonionic surfactants do not contribute to the electrical current and to the generation of Joule heating, which is a limiting factor for ionic surfactants, they permit the MEKC separation of charged analytes to be performed at voltages higher than those used with ionic surfactants. The CMC value of nonionic surfactants, in general lower than that of ionic ones, is an additional advantage offered by these components. Obviously, the other side of the picture is represented by the previously cited inability of nonionic surfactants to separate neutral compounds. This drawback may be overcome by charging *in situ* a neutral surfactant thus producing charged micelles that are now able to separate neutral analytes. An example of such a procedure is clearly shown in the work of Mechref and El Rassi [6] who charged *in situ* two neutral steroidal glycoside surfactants producing micelles with chiral selectivity. They evaluated the ability of *N*,*N*-bis-(3-D-gluconamido propyl)-cholamide (Big CHAP) and -deoxycholamide (Deoxy BigCHAP) to obtain the separation of binaphthyl and dansyl amino acid enantiomers. By charging these surfactants via borate complexation, the surface charge density of the corresponding micelles could be conveniently adjusted through variations of the borate concentration and of the running electrolyte pH. The manipulation over a certain range of the migration time window of the Big CHAP- and Deoxy BigCHAP-borate micellar system was a way for optimizing the enantiomeric resolution of the racemic mixtures investigated. Among the numerous different nonionic surfactants successfully applied in MEKC, the most common include polyoxyethylene dodecyl ether (Brij-35); polyoxyethylene sorbitan monolaurate (Tween-20); Triton X-100 or Triton X-114; bile salts (sodium cholate and deoxycholate); and cocamide monoethanolamine. Triton X-100 was used by Molina and Silva [7] to obtain the MEKC separation of phosphorus-containing amino acid herbicides and

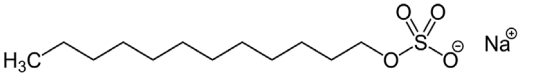
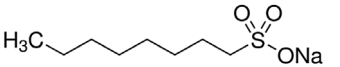
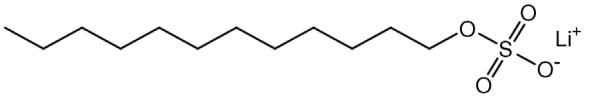
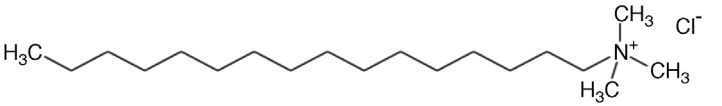
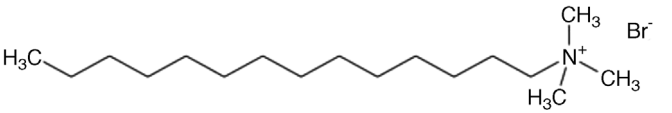
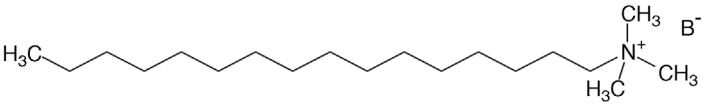
aminoethylphosphonic acids applying laser induced fluorescence (LIF) as a detection technique. The two poly(ethylene glycol)-based surfactants Triton X-114 (reduced) and Genapol X-080 have been used (in sodium borate-based running electrolyte) by Maier *et al.* [8] as micellar phases for the development of a sensitive MEKC method for the separation of six selected peroral antidiabetics widely used for the treatment of type II diabetes, and for the control of their concentration levels in biological fluids.

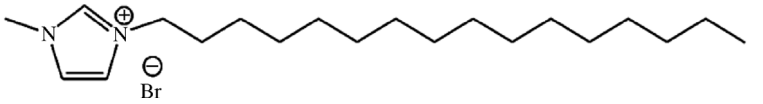
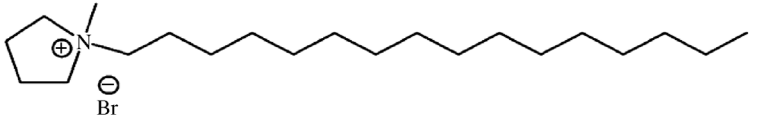
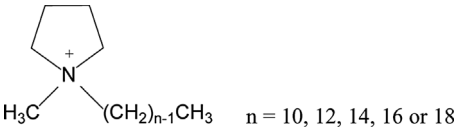
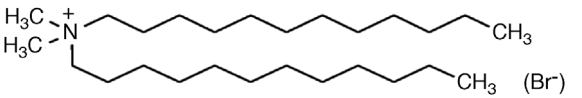
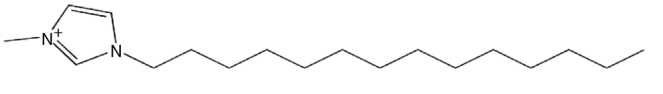
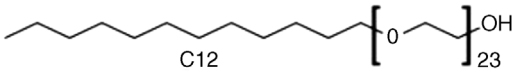
By definition, zwitterionic surfactants bear positively and negatively charged groups thus showing very low or zero electrical conductivity that results in the advantage mentioned above for nonionic surfactants. Most of them, however, show a poor aqueous solubility that indeed limits their use as a PSP in MEKC. Nevertheless, the utility of these agents as selectors for a range of structurally diverse analytes is well documented. For example, phosphocholine-type zwitterionic micelles of *N*-dodecylphosphocholine have been applied for the determination of sodium, potassium, calcium, magnesium, and ammonium ions in human saliva samples [9]. As a result of zwitterionic micelles preventing protein adsorption on the capillary wall, this could be done without deproteinization and/or dilution of the sample. 3-[(3-cholamidopropyl) dimethylammonia]-1-propanesulphonate (CHAPS) and 3-[(3-cholamidopropyl)-dimethylammonium]-2-hydroxy-1-propanesulphonate (CHAPSO) have also been employed (in a 100 mM phosphate buffer, pH 2.5) in the absence of additional coselectors or surfactants as sole micellar-forming agents for the enantiomeric resolution of 1,1′-binaphthyl-2,2′-diamine and Tröger's base [10]. A list of surfactants cited in above paragraphs with their chemical structures and their character is shown in Table 14.1.

14.3.1.4 **Mixed Micelles**

By addition of ionic or nonionic surfactants to another ionic one, ionic mixed micelles can be formed that show analytical advantages over "conventional" micelles since they involve additional interactions in the separation system. For example, mixed micelles usually show a larger radius that permits increased partitioning of hydrophobic analytes into the core and micelles made of SDS and other nonionic/zwitterionic surfactants possess a lower negative surface charge compared to "pure" SDS micelles. The decreased electrostatic repulsion shown by anionic analytes should be interpreted as a better retention that also means a higher resolution from other components of the mixture. It was stated above that manipulation of surfactant composition provides a unique opportunity to optimize selectivity. As yet, mixed micelles represent the most sensitive approach for manipulating solute–micelle interaction to obtain the desired resolution. From among all mixed micelles characterized for their selectivity, only those most frequently used will be mentioned here. These include mixed micelles made of SDS and (i) *N*-dodecyl-*N*,*N*-dymethyl-3-ammonium-1-propanesulfonate (SB-12); (ii) 1,2-hexanediol; (iii) Triton X-100; and (iv) Brij-35. As mentioned above, the latter is a nonionic surfactant that, in combination with the zwitterionic surfactant 3-(*N*,*N*-dimethylhexadeylammonium) propanesulfonate (PAPS), generates

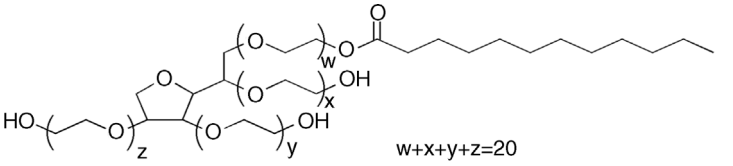
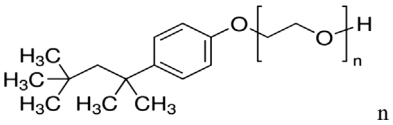
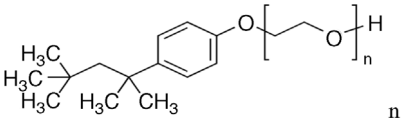
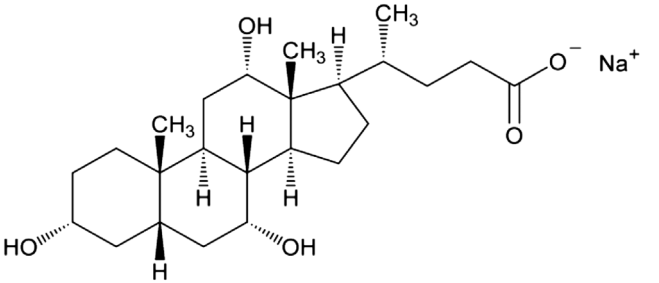
Table 14.1 List of the most widely used surfactants in MEKC with their structure and chemical character.

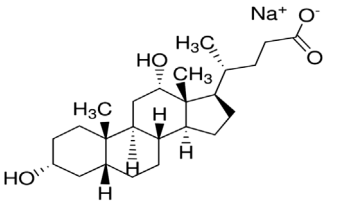
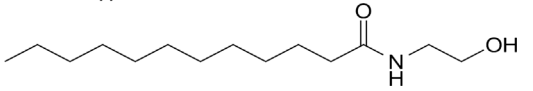
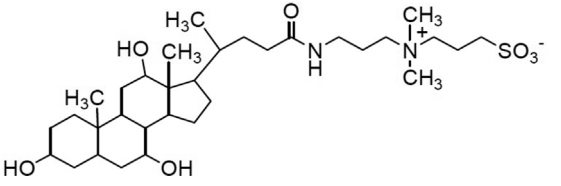
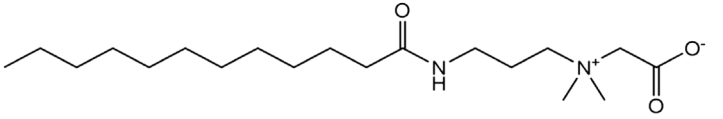
Name	Structure	Nature
Sodium dodecyl sulfate (SDS)		Anionic
Sodium octane sulfonate (SOS)		Anionic
Lithium dodecyl sulfate (LDS)		Anionic
Cetyltrimethyl ammonium chloride (CTAC)		Cationic
Tetradecyltrimethyl ammonium bromide (TTAB)		Cationic
Cetyltrimethyl ammonium bromide (CTAB)		Cationic

1-Cetyl-3-methylimidazolium bromide		Cationic
N-cetyl-*N*-methylpyrrolidinium bromide		Cationic
N-alkyl-*N*-methylpyrrolidinium bromides		Cationic
Didodecyldimethyl ammonium bromide (DDAB)		Cationic
1-Tetradecyl-3-methylimidazolium bromide		Cationic
Polyoxyethylene dodecyl ether (Brij-35)		Nonionic

(*continued*)

Table 14.1 (*Continued*)

Name	Structure	Nature
Polyoxyethylene sorbitan monolaurate (Tween-20)		Nonionic
Triton X-100		Nonionic
Triton X-114		Nonionic
Sodium cholate		Nonionic

Sodium deoxycholate		Nonionic
Cocamide monoethanolamine		Nonionic
3-[(3-Cholamidopropyl)-dimethylammonio]-2-hydroxy-1-propanesulfonate (CHAPS)		Zwitterionic
Cocamidopropyl betaine (CAPB)		Zwitterionic

highly selective mixed micelles for peptide mapping. By mixing sodium cholate (CHOL) with: (i) Triton X-100 and (ii) 3-(*N*,*N*-dimethylmyristylammonium) propanesulfonate (MAPS), nonionic and anionic micelles, respectively, may be prepared. Mixed anionic micelles made of the anionic surfactant LDS combined with lithium perfluorooctanesulfonate (LIPFOS) are also available. poly(sodium 10-undecenylsulfate) (polySUS) and poly(sodium 10-undecenyl leucinate) (polySUL) and their five molecular binary mixed micelles with varied SUS:SUL composition have also gained increased attention. "Ternary" mixed micelles have also been developed. In particular, a commercially available dishwashing soap containing anionic, zwitterionic, and nonionic agents (sodium lauryl ether sulfate, cocamidopropyl betaine, and cocamide monoethanolamine, respectively) is worthy of mention. Compared to SDS-based buffers, this system provides different selectivity and higher separation efficiency. In addition, it incorporates the advantage of being more environmentally friendly than other available systems.

Numerous applications described in the literature show that the use of mixed micelles is indeed one of the most interesting approach for tailoring the micelle environment and for exploring systematically how selectivity is affected by surfactant structure. To learn more about the variation in selectivity that should be expected from changes in the composition of the PSPs, the reader is invited to consult an interesting article [11] in which the selectivity of a great number of single, mixed, and modified PSPs is comprehensively analyzed.

14.3.1.5 Polymers as PSPs

The structural stability shown by single molecules of micelle polymers, polymeric surfactants, poly electrolyte surfactant complexes, and dendrimers allows them to form micelles characterized by a number of advantages over conventional phases. These advantages include a very low (if not zero) CMC, the use of BGEs with lower ionic strength (which minimizes Joule heating and increases reproducibility), the possibility to apply MS detection, and to separate highly hydrophobic and chiral compounds. Unfortunately, the time needed to prepare the polymeric solutions (whose composition could suffer problems of low reproducibility), may represent a disadvantage of these PSPs. Sodium polyundecenoyl sulfate is an example of a sulfated polymer; butyl acrylate-butyl methacrylate-methacrylic acid (BBMA) copolymer sodium salt and butyl methacrylate-methacryloyl-oxyethyl-trymethylammonium chloride copolymer are two examples of high molecular mass surfactants characterized by a CMC close to zero.

Also liposomes are useful PSPs in MEKC. For example, liposomes composed of zwitterionic phosphatidylcholine, anionic phosphatidylglycerol, and cholesterol, which mimic the composition of a natural cell membrane, have been applied as PSPs for the study of the interaction between cell membranes and some biomolecules and to determine the effects of pH on partitioning of basic drugs in these structures [12]. Liposomes have also been applied to study the interactions of drugs with lipid bilayers.

14.3.2 Incorporation of Additives into the Aqueous Phase

Another approach to manipulate selectivity and resolution in MEKC is the addition to the aqueous phase of modifiers that interact with the electrophoretic system. For example, organic modifiers are often added for their ability to shift the partition equilibrium toward the mobile phase, thus improving the separation of hydrophobic analytes. The most widely used organic additives are acetonitrile (ACN), ethanol, and methanol. Propanol, tetrahydrofuran, dimethylformamide, and urea may also be employed. It is important to underline that the use of these solvents at concentrations higher than 30% may lead to micelle "dissolution." To prevent such a drawback, low concentrations (10–20%) of organic phase are usually added to the BGE. Other classes of organic modifiers (i.e., formamide or the macrocycle 18-crown-6) have been observed to target the PSP through direct interaction with micelles.

An alternative to organic solvents is represented by Ionic liquids (ILs), a family of "green" solvents that, due to their properties (high conductivity, negligible vapor pressure, good thermal stability, and ability to form micelles), have attracted great attention in the last few years. Alkylimidazolium-based ILs are the most widely used IL-type surfactants in CE. They are grouped into short- (C_2–C_8) and long-chain (>C_8) classes. Both have been reported to act as cationic surfactants for the sweeping of charged or neutral compounds [13]. Although ILs have not progressed as expected, the possibility of these compounds to provide a salting-out effect and to establish electrostatic, hydrophobic, and hydrogen bonding interactions makes them even more fascinating. Information about recent applications of IL-assisted CE in the analysis of several compounds and enhancement of sensitivity, can be obtained by consulting a few interesting reports [14,15].

The ability of an analytical method to determine single enantiomers in samples of different origin makes it suitable for being applied in a variety of areas including environmental, agricultural, pharmaceutical, and related applications. MEKC may be included among these methods beyond a reasonable doubt. The addition of chiral compounds to the micellar solution allows, in fact, this technique to exhibit chiral selectivity. Among the large selection of neutral, cationic, and anionic chiral selectors available on the market, Cyclodextrins (CDs) are by far the most employed in MEKC. CDs are a family of macrocyclic oligosaccharides linked by α-1,4-glycosidic bonds, characterized by good water solubility, UV transparency, and enantiorecognition abilities. The most common members of this family are α-, β-, and γ-CDs, composed of 6, 7, and 8 D-glucose units, respectively. From a structural point of view, CDs present a truncated cone-shaped cyclic molecule with a hydrophilic external surface and a hydrophobic inner cavity capable of hosting a wide range of organic guest molecules (Figure 14.2).

The separation mechanism of enantiomers is primarily based on the formation of inclusion complexes. Since the hydroxyls in the glucose molecules that form

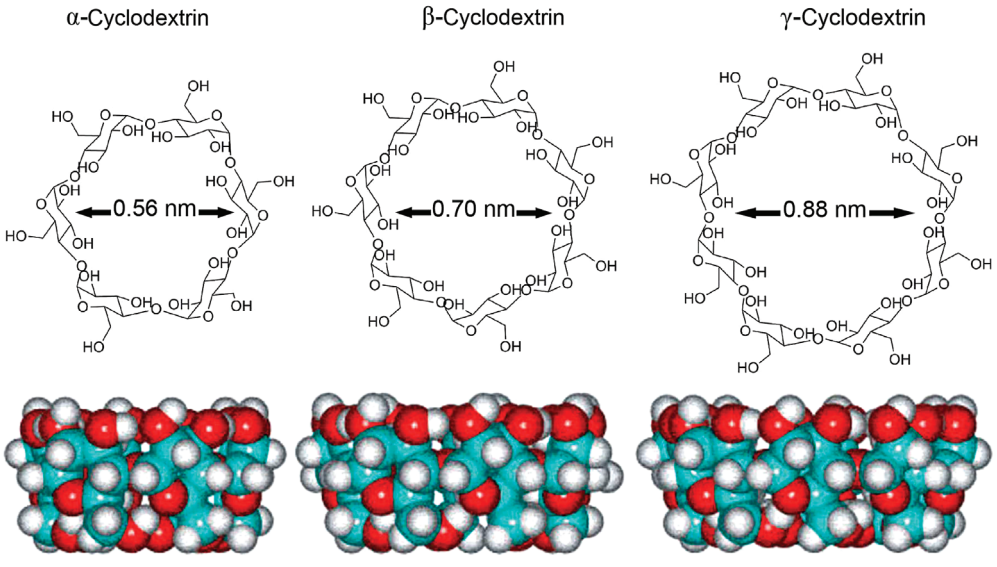


Figure 14.2 Chemical and tridimensional structure of α-, β-, and γ-cyclodextrins.

the rim of the CD cavity are chiral, inclusion is sterically selective and the inclusion complex formation will be chirally selective as well. The different affinity displayed by CD molecules for the two enantiomers determines the difference in migration velocity between the complex (CD-enantiomer) and the free enantiomer. Due to the hydrophilic nature of its outer surface, CD in fact will not interact with the micelles and, under the applied electric field, it migrates with the same velocity as the bulk solution. The use of CDs as chiral selectors for the separation of enantiomers has been comprehensively described in a recent review article by Fanali [16].

Also, modified CDs have attracted much attention for chiral separation in MEKC. Carboxymethyl-CD, sulfated-CD, sulfobutyl ether-CD, and hydroxypropyl-CD are examples of negatively charged CDs. In particular, sulphated CDs, that allow to achieve resolution by a combination of enantioselective inclusion and ion-pairing, were found to be useful in the separation of basic drugs. The high degree of enantioselectivity offered by these CDs provides such an excellent resolution that they have been applied for determining the trace-level enantiomer in single isomer drugs [17].

14.3.3
Use of 2-D CE and Chemometrics

In cases in which the mixture to be examined is very complex, two-dimensional (2-D) methods, in which MEKC is one of the separation modes, are emerging for their ability to enhance resolution and separation efficiency. 2-D CE separations

may take place in a single capillary or in two capillaries and their potential is based on the improvement of peak capacity. In single-capillary 2D-CE, assuming that the two separation mechanisms are orthogonal, the peak capacity will be given by the product of the individual separation peak capacities, thus providing a separation power that is obviously much higher than in 1D-CE. The so-called heart-cutting 2D procedure in a single capillary, is an example of multidimensional approach in which a fraction of interest, stemming from the first dimension, is selected and isolated in the capillary by evacuating other compounds of the mixture. The isolated fraction is separated in a second medium that, after being introduced into the same capillary by EOF, can reach this fraction only if solutes migrate in counter-EOF mode. This is obviously a limit of the technique. The fact that the electrolyte composition in the second dimension is restricted to electrolytes that should enter the capillary with an apparent velocity higher or identical to electroosmotic velocity is another important limit. To overcome these drawbacks it has been proposed that, instead of reversing the polarity of the voltage, the fraction from the first dimension is isolated hydrodynamically. This alternative would open up the possibility of combining electrolytes of virtually any composition.

When 2-D CE is carried out in two capillaries, provided that the mechanism of separation in each capillary may be similar (e.g., CZE at two different pH values) or different from each other (CZE and MEKC or sieving electrophoresis with a replaceable sieving matrix followed by MEKC), the design of the capillary–capillary interface used to switch the effluents from the first to the second dimension is a significant feature. In particular, it should be characterized by a low dead-volume to achieve a high-efficient transfer of analytes and prevent sample zone diffusion. A device in which CZE is online integrated with CD-modified MEKC by using an electroaccumulation focusing strategy that avoids analyte band diffusion at the interface is an example of such a multidimensional system. As an alternative, two CE methods, operating with opposite polarities and in orthogonal capillary electrophoretic separation modes, may serve the purpose. With this device, CD-MEKC performed in normal polarity is method 1 and CZE in reversed polarity is method 2. Another interesting approach in the field of 2D methods is the coupling of electromigration separation with LC, both in the online and offline modalities. While offering shorter analysis times and higher sample throughput, online connections of LC to CE have the limitation of requiring special instrument modification. Moreover, the comprehensive online approach puts strict limitations on the second-dimension separation time, which must match the collection time of the LC fractions from the first dimension. Finally, given the limited sample volumes that can be handled in the second dimension, only micro- or nano-LC columns should be considered for the first separation step. The offline LC–CE separation process uses, between the two steps, a commercial CE autosampler that collects the effluent fractionated from the LC column into a number of vials. These fractions may be stored and analyzed later in the second dimension by MEKC, without having

stringent limitations of separation time. By using this mode, it becomes evident that the volume and the number of fractions from the first LC dimension are not strictly limited and impose less restrictions to the geometry of LC columns. By contrast, this approach requires more manipulation than an online process and cannot be easily automated. A compromise between the two, which combines some advantages of the classical offline and the comprehensive online real-time coupled 2D setups, is also an interesting approach. In practice, it consists of an online LC–CE technique in which fractions collected from the first-dimension LC separation are stored in vials and subsequently submitted to MEKC analysis. The fact that this procedure can be fully automated indeed represents an additional advantage. Among the numerous reports in this field so far published, those indicated here [18–20] give the reader just a taste of the potential of 2D separations.

In the past years, many laboratories have demonstrated the potential of microfluidic devices as analytical tools useful for carrying out 2-D separations, leading to the "next generation" in separation technologies and an enormous amount of literature dealing with a variety of microchip prototypes and their applications in MEKC is available. Although being an exciting field, it will not be covered by this chapter and the reader is invited to refer to review articles specific for this area [21,22] in which current applications of these devices are carefully described.

Another growing approach for improving resolution in MEKC is represented by chemometric tools, mainly focused on the use of different algorithms for optimizing separation parameters relative to baseline resolution and migration time. From these tools, quantitative structure-retention relationship useful to explore the prediction of molecular properties can be established. Chemometrics provides much information about the effect of various factors thus allowing empirical predictions of peak resolution to be deduced with a limited number of experiments that, to prevent systematic error, are usually performed in random. This approach is particularly exciting since it allows not only to reduce the total number of experiments, but also to infer the effect of each single factor on resolution. Due to the complexity of the separation process, pitfalls with the experimental design may be encountered. Nevertheless, information from this first design is important in that it provides useful suggestions to plan a second successful in-depth study by means of response surface methodology. Other chemometric approaches, including the multivariate response surface methodology and the multivariable at-a-time approach, may also be used for this purpose. As can be expected, the choice of the specific technique is strongly influenced by the complexity of the target mixture. In other words, despite several highly powerful chemometric tools being available, this does not implicitly mean that they can be used in all cases, While an appreciable number of articles on chemometrics have been reported to date, more study is obviously needed to turn these possibilities into the realization of reliable and robust models for compounds of diverse structure. To have more details, the reader should look up an interesting article in which the state of the art of the quantitative structure–retention relationship in MEKC has been reviewed [4].

14.4 Sensitivity

This section addresses the most widespread approaches to enhance the inherent low sensitivity of MEKC. This is strictly correlated with the dimensions of the capillary that play a pivotal role in determining the limits of detection (LOD) of the technique. The total volume (μL) of the capillary and its reduced path length, while limiting the volume (nL) of the sample that can be injected, also hinder common optical detection methods (e.g., UV detection). This poses a serious problem when real samples containing low-concentrated analytes must be examined. Enhancement of detection can be achieved essentially by operating on two factors. First is the narrowing of analyte bands in the column. As the peak width of the analyte is compressed, the peak height is dramatically increased, resulting in a greater signal-to-noise ratio that improves the LOD. The second factor is the amount of sample that can be loaded onto the column. Because stacking procedures significantly reduce the peak width, much larger sample volumes may be injected without loss of separation efficiency. The greater mass of analyte in the capillary results in a greater response from the detector.

A variety of modes may be used to improve the limit of detection for the determination of different analytes in real samples. Among these, the following seem to be more consistent than others: (i) preconcentrating the sample online (the most common approach, discussed below); (ii) concentrating the sample through liquid–liquid extraction or SPE; (iii) improving the sensitivity of detection (e.g., by applying laser-induced fluorescence, LIF, see Section 14.5), and (iv) using capillaries with longer path length (e.g., Z-shape and bubble cell).

14.4.1 Online Sample Preconcentration Techniques

Sample sweeping and stacking, in which the analyte is preconcentrated within the capillary before separation and detection, are the online focusing techniques most widely used in MEKC. Both may be performed individually or in combination using different types of surfactants. The following paragraphs will provide the reader the basic knowledge on these approaches.

14.4.1.1 Sweeping

Sweeping is a technique for on-column sample concentration of nonpolar molecules based on analytes' ability to partition into the PSP. In brief, a sample prepared in a surfactant-free buffer of the same conductivity as the BGE is introduced into the capillary by pressure from the cathodic end. After replacing the inlet reservoir with a BGE containing an anionic surfactant at a concentration above its CMC, a reverse-polarity high voltage is applied. The micelles from the BGE migrate across the sample zone and incorporate the neutral analytes into a narrow stacked band. The suppression of EOF (achieved by keeping the BGE at low pH), allows anionic micelles to have a net electrophoretic migration

toward the detector. The effectiveness of this sample concentration technique is strongly dependent on the analytes' retention factor, which is the ratio between the number of solutes distributed in the micellar phase and that in the aqueous phase. While SDS is the most commonly used PSP for sweeping neutral and cationic analytes, other kinds of surfactants (cationic, nonionic, polymeric, as well as mixed micelles) have also been studied. From the point of view of electrostatic interactions, anionic analytes may be easily swept by cationic surfactants, such as TTAB. Compared with conventional MEKC injection, 80- to 5000-fold enhancement in detection sensitivity can been achieved by the sweeping method. An interesting chapter dealing with MEKC and sweeping is part of a review article by Malà *et al.* [23].

14.4.1.2 **Stacking**

Among the techniques for on-column sample concentration applied to MEKC, a derivative of field-amplified sample stacking (FASS) is probably the simplest one. This method takes advantage of the discontinuous electric field distribution across a system made of two buffers with different conductivities. Charged analytes electrophoretically migrating from the low- into the high-conductivity solution slow down dramatically and are concentrated at the conductivity boundary of the two buffers. In practice: the sample, dissolved in a low-conductivity micellar solution (usually anionic as SDS), is injected hydrodynamically in a capillary that is conditioned with a high-conductivity micellar BGE. The analytes may be stacked in either normal or reverse-polarity mode. In normal polarity mode, the anionic micelles enter the sample zone from the cathodic end, bind to neutral solutes, and carry them to the boundary with the BGE zone where their velocity is retarded and they are focused. Although electrophoretic migration of micelles is toward the anode, their velocity being lower than EOF, their net movement (e.g., that of analytes included) will be toward the cathode thus passing the detector. In the reverse-polarity mode, a negative high voltage is applied at the capillary inlet; the micelles stack toward the detector and the sample plug is backed out of the capillary. Once the current measured through the capillary returns to 90–99% of its preinjection value, the polarity is reversed and the separation proceeds in the normal polarity mode. In case cationic surfactants are used to stack neutral analytes, given their tendency to adsorb on the capillary wall making it positively charged and reversing EOF, the reversed-polarity mode should be used to allow the micelles (with the analytes) to pass the detection window. Despite sensitivity enhancements up to 1000-fold having been observed for micelle-assisted FASS, a limitation to this procedure is that the ionic strength of the sample must be significantly lower than that of the BGE. This requirement may cause problems for analysis of some physiological solutions such as dialysates. Nevertheless, FASS continues to be an important preconcentration technique in many MEKC applications.

It should also be underlined that, compared to single modes, the combination of sweeping and stacking protocols could provide better results in terms of sensitivity enhancement.

14.4.1.3 Analyte Focusing by Micelle Collapse

Analyte focusing by micelle collapse (AFMC) is another online sample preconcentration technique for MEKC that can provide hundreds fold improvement in detection sensitivity. In contrast to stacking and sweeping, the micelle sample matrix for AFMC contains anions with high electrophoretic mobility. In fact, the sample is prepared in a matrix, containing SDS micelles and high-mobility anions, whose conductivity is higher compared to that of the separation solution. Upon application of high voltage, the micelle carrying the analyte will move and reach the boundary between the sample and solution zones. On passing this latter, the micelles collapse and release analytes due to an obvious dilution of the anionic surfactant whose concentration will drop below the CMC. Continuous application of the voltage causes more micelle collapse and more analyte release at the boundary. Despite the promising results shown by this technique, it is still not consolidated, a variety of parameters (including sample injection length, sample and separation solution conductivity ratio, and surfactant micelle concentration in the sample) have been observed to affect its performance. An example of the application of this method in 2D CE separations may be found in the report by Zhang *et al.* [24].

14.5 Detection Modes

Rather than focusing on the recognized advantages of UV photometric detection (a large number of UV-absorbing analytes can be detected and compatibility with BGEs is very high), this section will deal with less "conventional" techniques, LIF and MS in particular, that, as suggested by the trend observed in the last 10 years, are going to become a solid choice for MEKC analysis. LIF is a powerful analytical tool that, compared to other techniques, provides higher sensitivity for the monitoring of analytes, whether natively fluorescent or dye-labeled. Fluorescein analogs (e.g., fluorescein isothiocyanate, FITC) or derivatives with a carboxaldehyde moiety (e.g., 3-[4-carboxybenzoyl]-2-quinoline carboxaldehyde), are the most widely used labeling reagents. While enhancing sensitivity and, in many cases providing a better quality of electrophoretic patterns compared to other detection methods, possible drawbacks related to derivatization processes (e.g., interferences that occur from the presence of excess labeling reagents and high background noise) may be detrimental to the success of LIF. The closer structural similarities after derivatization of some related solutes is an additional potential drawback that may prevent their separation. A typical example of such behavior is shown in Figure 14.3 in which UV- and LIF-electropherograms of desmosine (DES) and isodesmosine (IDES), two structurally similar isomers, are compared. The importance of quantifying these compounds in biological fluids relies on the fact that they are believed to behave as descriptors of lung elastin degradation in chronic obstructive pulmonary disease [25]. While MEKC with UV detection allowed to quantify these elastin cross-links in human

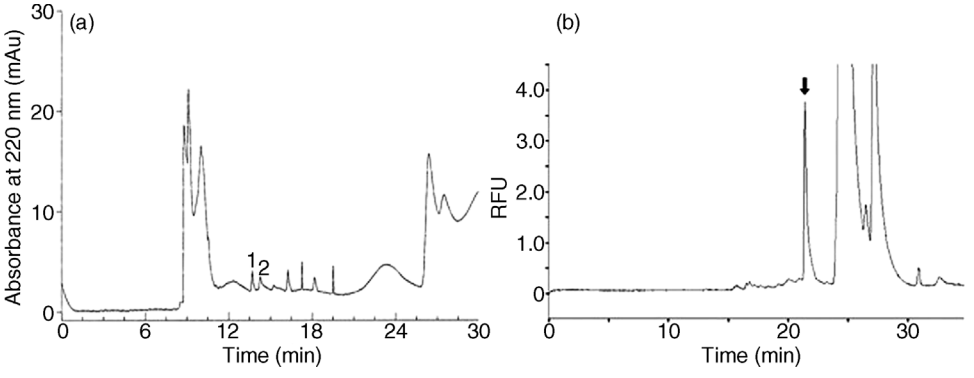


Figure 14.3 (a) MEKC with UV detection of a urine sample from a COPD patient. Peaks 1 and 2 correspond to IDES and DES, respectively. (b) MEKC with LIF detection of the same urine sample as above after derivatization with FITC. The peak indicated by an arrow corresponds to the FITC-desmosines (IDES plus DES). For details see reference [14].

urine separately (Figure 14.3a), the FITC-labeled desmosines comigrated and could be determined as the sum of only two isomers (Figure 14.3b). Although the overlapping of analytes in this case was not particularly cumbersome in view of their total quantification (the two cross-links being excreted in biological fluids in a 1:1 molar ratio), in many other cases this could be a severe complication.

The direct hyphenation of MS to MEKC, despite being still confronted with a variety of serious problems, is attracting more and more interest given the advantages (in terms of molecular weight/structural information on the analyte) given by this method over spectrophotometric ones. The main limitation for an efficient online coupling of MEKC with MS results from the BGE composition, a potential strong interference being provided by low molecular mass surfactants (such as SDS) that have low volatility, are very surface-active, and suppress the analyte signal in electro spray ionization (ESI), the most widely applied ionization interface (ESI/MS). One of the most common solutions that have been developed to circumvent this problem is partial-filling MEKC (PF-MEKC). Briefly, inside the capillary in PF-MEKC there are three plugs consisting of (i) BGE followed by (ii) a micellar solution and lastly (iii) a sample solution. When voltage is applied, the analytes migrate into the micellar region where they are separated. These analytes continue to move toward the BGE region that is free of the surfactant; elute out of the capillary, and are introduced into an ESI/MS system. The micellar plug is left behind and does not interfere with the MS analysis. While appearing a great method for avoiding the interferences indicated above, this technique shows a number of serious drawbacks spanning from alteration in selectivity (both MEKC and CZE are combined) to a decrease in efficiency given by an extra band-broadening mechanism occurring at the micelle zone buffer boundary. In addition, being the migration time window determined by the effective mobility of the surfactant monomers rather than by the effective mobility of the micelles, it is small. As a result of the relatively short surfactant

zone, the total separation volume will also be small. In contrast with PF-MEKC, other methodologies afford introduction of PSPs into the ion source. These use volatile BGEs containing MS-compatible surfactants or may be based on new interfaces, alternative to ESI, designed to be more tolerant to the presence of nonvolatile salts and surfactants. Being still under development, these "novel" techniques, which allow a better combination of MEKC with ESI/MS, will be described in more detail in the next section.

14.6 Novelties and Trends

The fast growth in the last 10 years of MEKC on microchip and the development of polymeric PSPs, demonstrate that these trends have fully met expectations. An extensive expansion in this period was shown also by microemulsion electrokinetic chromatography (MEEKC), a CE mode that utilizes microemulsion (composed of nanometer-sized oil droplets suspended in aqueous buffer that are stabilized by the presence of a surfactant and cosurfactant) as the BGE. Advances made in the development of novel MEEKC microemulsion additives sum up to the "conventional" use of this technique for sample concentration, chiral analysis, and capillary coating, and to a number of applications that include IL-in water-MEEKC and the area of multiplexed MEEKC [26]. In spite of these advancements, interesting improvements in methodological and instrumental aspects of MEKC are still being proposed and efforts have remained focus on some alternative means of analysis that allow easier interpretation of results. Based on a large number of reports, the literature seems to suggest that efforts aimed at introducing new perspectives on doing MEKC mostly emphasize the development of: (i) new PSPs, (ii) novel protocols for online preconcentration analysis, and (iii) the improvement of hyphenated techniques that combine highly efficient separation methods with very sensitive detection systems. Although the general trend of MEKC retraces, at least in part, the "track" of previous years, strengthening both theoretical principles and practical applications is expected to consolidate the continuous growing of this technique. These "novelties" are presented below.

14.6.1 Carbon Nanostructures

From among all PSPs, nanostructured materials have gradually been recognized in recent years to have the potential for playing an important role in separation sciences. Particular interest is being focused on gold nanoparticles (NPs), the components most recently introduced in MEKC. Although NPs have scarcely been used to date as PSP additives, most likely they will emerge as an important innovation in the field of separation sciences. The modification of the Au surface with appropriate chemical species results in an improvement of the separation and preconcentration efficiency, analytical selectivity, and method reliability.

However, optimization of electrophoretic systems seems to be only part of this nanorevolution, with new challenges being faced in this field. For example, with the evident increase of the role of NPs in separation science, greater control over their size, composition, and self-assembly will be required [27]. Most importantly, in any application of Au NPs, their physical and chemical properties should be accurately explored to gain information about integration of the Au NPs-based hybrid material. In this respect, given that the surface of water-soluble NPs bears the charges during dispersion in the aqueous state, good candidates for concentrating and analyzing NPs are electrophoretic methods. The MEKC-based online preconcentration strategy, the so-called "reversed electrode polarity stacking mode" (REPSM) is an excellent tool for characterizing the size of NPs. Based on the finding that differently sized NPs have different migration times, it has been shown that a linear correlation exists between electrophoretic mobility and Au NPs diameter [27]. Although additional work would be required to assess the actual potential of this nanotechnology, the advances in this field allow to hypothesize that functionalized Au NPs may contribute to improve separation and preconcentration of a variety of analytes in MEKC thus becoming the most interesting alternative for the consolidated use of SDS.

14.6.2 Novelties in Preconcentration Techniques

As mentioned in Section 14.4, since preconcentration techniques have begun to emerge in the past years as widely used approaches for improving sensitivity in MEKC, they have grown to a great extent. As for PSPs, the available literature shows that this is not a static achievement but a continuum: as instruments and procedures get better, so will method accuracy and performance. In this respect, from among the recent techniques, that called "extremely large-volume electrokinetic stacking," useful for the preconcentration of cation analytes when analyzing trace substances, is noteworthy. Briefly, the continuous introduction into the capillary of a low-pH borate buffer containing SDS (separation buffer) tends to suppress the EOF. As soon as this value has reached the same magnitude as the oppositely migrating micelles, a stagnant micelle zone and a consequent stacking state are created. Under these conditions, injection of an extremely large volume of the sample (in strong acidic phosphate buffer) results in stacking of cation analytes at the neutralized micelle zone. Rather than working on single hydrodynamic large-volume injections, another novel technique, referred to as "repetitive large volume sample injection and sweeping MEKC" (rLVSI-sweeping MEKC), allows to obtain concentration of analytes on the basis of a multiple-injection procedure. In practice, a large volume of a sample is injected and, after it stacks through sweeping, another large volume is injected again. These steps will be repeated several times. An additional approach suitable for large-volume samples, consists in increasing the difference in conductivity between BGE and the sample zone by following two different pathways. In the first case, sample stacking is obtained by establishing a conductivity gradient between the BGE

and the sample zone by inclusion of a high-conductivity zone between these two. The second pathway simply requires a low-temperature bath to reduce joule heating resulting from the use of a highly concentrated BGE for electrophoretic separation. Combination of these two pathways is expected to increase the stacking efficiency furthermore. Taken together, these reports indicate the importance of sample preconcentration as a part of the whole analytical workflow, although no universal solution nor a small set of "tools" can handle all sample types for all problems. Whether quantification of major solutes, impurity detection, or trace analysis are the final goals of the analyst, sample preconcentration definitely seems to be an issue for routine use in CE. Trace analysis in a huge number of real samples (e.g., drug impurity profiling) would be very often not possible without sample preconcentration. Obviously, for a detailed understanding of the quality and safety of these products, analysis of all components is relevant and quite necessary. Despite more research being needed, all procedures presented in this chapter allow to conclude that new concepts for sample preconcentration in MEKC have been developed and, although time will be taken before they are accepted as convention, they represent better options for minimizing sample size and solvent volumes hence leading to new avenues for facilitating the identification of analytes in very diluted mixtures.

14.6.3 Hyphenated Techniques

Hyphenated techniques in MEKC have evolved with tremendous possibilities thus becoming new analytical strategies that offer high sensitivity, accuracy, and enough reproducibility for their application in a variety of practical fields, including clinical; forensic; environmental, and food studies. Although LIF has been already consolidated as the detection system that achieves the highest sensitivity in MEKC, improvements of instrumentation may enable the sensitivity and specificity to increase further. In this respect, the introduction of quantum dot-mediated LIF detection is worthy of mention. Luminescent quantum dots (QD) consist of inorganic nanoparticle labels passivated by a layer of organic ligand. A typical example is represented by water-soluble Cadmium/Tellurium quantum dots (CdTe QDs) capped with mercapto-propyl acid (MPA). Due to their strong quantum confinement, QDs in the range of 20–30 nm exhibit many interesting optical properties that include (i) good photochemical stability (fluorescence half-life 5–40 ns) as compared to organic dyes; (ii) emission spectra over a large excitation spectral range; and (iii) resistance to photobleaching. This makes QDs suitable agents for the LIF detection system in MEKC. Their use as buffer additives to develop simple and sensitive QD-mediated LIF detection procedures is confirmed by a number of interesting reports [28,29]. Many probes have been reported to date for labeling amino compounds in MEKC-LIF analysis. The development of novel label agents, not for amino compounds, could be an innovative proposal and a possible future direction aimed at expanding the use of this technique to the study of other types of analytes.

Moving to fast becoming a very streamlined hyphenated technique, the pace of development in MEKC–MS never seems to slow. Although a number of barriers still must be overcome to fully optimize the technique and meet the expectations, several innovations have come along and acceptance of the technology has been increasing over time. As mentioned in Section 14.5, the biggest drawback for the direct connection between MEKC and MS is represented by incompatibility of the ESI interface (the most widely used ionization system), with a number of surfactants. A very recent report suggests the use of a BGE containing 100 mM perfluorooctanoic acid and 200 mM ammonium hydroxide to create a micellar phase that minimizes interferences with tandem mass spectrometry detection without significant loss of detection sensitivity. The method was demonstrated to be specific, sensitive, and reliable for the systematic toxicological analysis of new designer drugs from the group of synthetic cathinones in urine samples [30].

Also the previously cited partial-filling technique moves toward the direction of minimizing interferences in MEKC–ESI/MS. While being practically consolidated to overcome this incompatibility, it still remains a poor solution for solving the problem. In fact, when SDS is used as PSP, the partially filled micelle zone diffuses quickly and the available nonmicelle window will be narrow. Alternative ionization techniques, such as atmospheric pressure photoionization (APPI), seem to offer a solution for circumventing signal suppression and interferences by SDS and nonvolatile buffers [31]. Despite showing enhancement in both source performance and range of applicability (both polar and apolar compounds can be photoionized, thus facilitating the analysis of compounds that are not amenable to electrospray ionization), the limit of this technique is that it does not provide the sensitivity required. Also desorption electrospray ionization (DESI) is worthy of mention as an interesting example of a novel ionization source that allows chemicals on a surface to be analyzed at ambient pressure with minimal or no sample preparation [32]. According to this protocol, charged microdroplets are directed toward a surface near the inlet of the MS where species are desorbed, ionized, and transferred into the instrument. The high sensitivity (few femtomoles) and high salt tolerance (analytes can be detected at a concentration of few hundred nanograms per milliliter in the presence of 2% salt) of DESI makes this source an attractive choice for direct coupling of MEKC with MS.

Currently, novel attempts deal with the hypothesis that, rather than using different ionization techniques, the design of new approaches to interface MEKC with ESI/MS could perhaps be the best way to eliminate this compatibility drawback. In this context, the coupling of sweeping with PF-MEKC–ESI/MS is certainly an interesting novelty, with sweeping already having been proved to be an effective online preconcentration technique for neutral analytes in PF-MEKC–ESI/MS [33]. Another potential solution to overcome incompatibility in coupling MEKC with ESI/MS is the use of cleavable surfactants, also known as acid-labile surfactants (ALS) [34]. An example is sodium 4-[(2-methyl-2-undecyl-1,3-dioxolan-4-yl) methoxy]-1-propane sulfonate

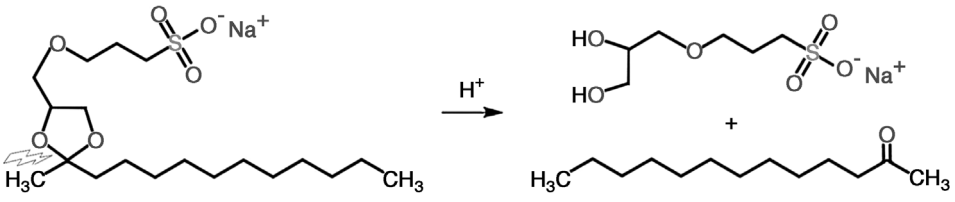


Figure 14.4 Scheme of ALS (on the left) acid hydrolysis with the formation of glycerol-propane sulfonate (top right) and tridecan-2-one (bottom right).

(shown in Figure 14.4), that has denaturing and electrophoretic properties similar to SDS. Two aspects of ALS relevant to MEKC–MS should be underlined: first, although separation efficiency is slightly lower than that achieved with SDS, the micelle mobility, and selectivity of ALS (different from that of SDS) indicate that it can be a friendly PSP for MEKC. Second, being stable for a reasonable period of time (half-life of 48 min) under acidic conditions (pH 4.0), it can be used during the duration of MEKC separation and analysis. After the period indicated, it is degraded under the same acidic conditions (according to the scheme shown in Figure 14.4) to generate less surface-active products that are more compatible than SDS with MEKC–ESI/MS. This suggests that ALS has the potential to couple MEKC with the ESI/MS detector. Unfortunately, although hydrolysis of this ALS is too slow, it is still unable to fulfill all the requirements of a MEKC–ESI/MS linked system. The synthesis of ALS structures that decompose more rapidly may help to significantly overcome this problem.

An update about the methodological and instrumental advances that improve sensitivity/resolution of MEKC and its compatibility to MS for a more fruitful application of this technique to routine analyses may be found in two interesting review articles published recently [35,36].

14.7
Fields of Application

A variety of advantages that include high versatility and efficiency, low sample volume, and short analysis time, have widened the application range of electrodriven techniques, MEKC in particular, over different analytical fields spanning from biological fluids to pharmaceuticals, environment, food, and plants. Thus, the fact that these powerful techniques have moved from academia into industry should come as no surprise making them a viable alternative to liquid and gas chromatography for the analysis of a great number of analytes in real samples. The examples described in sections below give the reader just a foretaste of the variety of MEKC applications in different fields. A general view on the selection of the MEKC procedure according to the type of analyte to be determined and as a function of the sample matrix composition is also provided.

14.7.1 Biological Fluids

Based on the numerous reports recently published, the search for efficient and sensitive methods for antibiotic analysis seems to be a topic still "hot" in analytical chemistry. The successful application of MEKC to monitor different human fluids for their content of various drugs also allowed to detect a broad number of antibiotics belonging to different groups both in biological fluids and in single cells. In most applications, SDS was the surfactant employed. Efforts devoted to the development of alternative approaches for improving resolution (in cases in which selectivity of SDS micelles was insufficient to separate highly hydrophobic compounds), eventually confirmed SDS containing a variety of additional components (i.e., ILs) to be the most effective MEKC separation medium. The separation of the widely used anticancer agents anthracycline antibiotics and taxanes represents a good example of the versatility of MEKC in this field. While being administered in combination therapy for cancer treatment, given the limitations of analytical methods available, these drugs can be detected separately only. For the first time, SDS-MEKC and high-speed MEEKC have made their simultaneous monitoring in plasma samples possible. MEKC-LIF was also applied to investigate the *in vitro* accumulation of anthracyclines enhanced by treatment of cells with inhibitors of cell membrane transporter proteins. The suitability of this technique to study the effect of specific inhibitors on the accumulation of anthracyclines in two cancer cell lines continuously exposed to subclinical concentration of these compounds was easily demonstrated by its ability to separate four anthracyclines within 15 min. For a detailed description of MEKC methods applied to the analysis of antibiotics in biological and other different samples (including food, pharmaceutical, and environmental samples) the reader is invited to refer to a recent review article by Dominguez-Vega *et al.* [37].

SDS-containing Tris buffer was the electrophoretic medium used to determine free and total levels of phenytoin, an anticonvulsant drug, in plasma of 20 patients affected by epilepsy [38]. SDS-MEKC was also applied to obtain the simultaneous determination of albumin, myoglobin, and hemoglobin in urine samples without sample pretreatment from patients with proteinuria [39]. By contrast, the concentrations of both memantine (the first in a novel class of Alzheimer's disease medications) and amantadine (an anti-Parkinsonian drug), have been monitored in human plasma of patients affected by Alzheimer's or Parkinson disease by using borate buffer containing the nonionic surfactant Brij-35. After being derivatized with 6-carboxyfluorescein *N*-hydroxysuccinimide ester, these two compounds with very similar chemical structure, were detected by LIF [40]. The achiral micellar aggregates made of SDS as the surfactant and with the addition of 2-isopropanol as the buffer modifier provided complete resolution (in urine, serum, and saliva) of antimalarian quinine from quinidine, cinchonine from cinchonidine and their

hydroderivatives, and their major metabolites diasteromers. An overview on the current status of enantiomeric and diastereomeric separations of chiral antimalarials and derivatives by CE may be found in the review article by Amin *et al.* [41].

14.7.2 Pharmaceuticals

The mentioned characteristics of high resolution and sensitivity peculiar of MEKC make this technique the ideal one for testing the quality control of several pharmaceutical forms, in different matrices. The structural and physicochemical properties of structurally related impurities of a drug and those of the main component are very similar; their separation is a challenging task. Thus, not only MEKC is a powerful tool for the separation of drug-related impurities from the main active compound, but it has been shown to also represent a valid alternative to HPLC for their exact quantitation. It has been successfully applied to the analysis of different pharmaceutical substances including Penicillins; Cephalosporins; Macrolides; Aminoglycosides; Tetracyclines; Sulfonamides; Fluoroquinolones; Barbiturates; Benzodiazepines; Tricyclic Antidepressants; Phenotiazines; and Xanthines. In terms of resolution, the best performance with these matrices was provided, in most cases, by MEKC runs carried out in sodium tetraborate/phosphate buffers containing different concentrations of SDS (with or without the addition of an organic solvent). In a limited number of cases, however, the cationic surfactants CTAB and TTAB showed acceptable resolution. Suggestions about the most appropriate experimental conditions to be applied for a successful monitoring of these substances are contained in a report by Hancu *et al.* [42]. The versatility of SDS-MEKC in the quality-control field is also underlined by the publication of a wide variety of articles dealing with: (i) the simultaneous separation/detection (in protein-containing matrices), of folic acid, hypoxanthine, mycophenolic acid, nicotinic acid, riboflavin, and xanthine, impurities of the drug product commonly present (in cell culture media) in monoclonal antibody manufacturing; (ii) the simultaneous determination in pharmaceutical formulations and creams of Valacyclovir (VCV); Acyclovir (ACV) and their major impurity, guanine. VCV and ACV are synthetic purine nucleoside analogs that show *in vitro* and *in vivo* inhibitory activity against herpes simplex virus types 1 and 2 and varicella zoster virus. Their structural similarity with endogenous compounds makes analysis of these analytes challenging; and (iii) the detection in infant formulas (*via* sweeping-MEKC) of melamine, a raw material in manufacturing some plastic wares used for serving food.

For additional information about MEKC applications in the area of pharmaceuticals and other different analytical fields, the reader is invited to refer to the review article by El Deeb *et al.* [43].

14.7.3 Food/Environment

Numerous reports have been published to date dealing with the analysis of food constituents and/or of pesticides and herbicides present in human foodstuff. The production of safe foods with the desired quality is obviously a major goal for the food industry. In light of this, enormous advancements in the development of novel, robust, efficient, sensitive, and cost-effective analytical methodologies have been observed over the last years. These tools are essential to: (i) guarantee the safety, quality, and traceability of foods and, (ii) verify compliance with food regulations. Owing to the well-known properties mentioned above, the number of MEKC applications in this area has dramatically widened thus making the role of this technique increasingly relevant among the analytical procedures commonly used for food analysis. In this context, no wonder that the number of articles dealing with the development of CE approaches to detect compounds of relevance to food science and technology has dramatically grown. These articles cover a variety of samples/matrices spanning from milk to orange juice, rice brand, wines, oils, coffee, breakfast cereals, fruit, oils, meat, animal tissues and derived foodstuffs, freshwater fish and crustaceans, water-soaked products, and others. Methods developed include the analysis of amino acids, peptides and proteins; DNA; carbohydrates; biogenic, heterocyclic, and other hazardous amines; phenols, polyphenols, and pigments; vitamins; small organic and inorganic ions; toxins, contaminants, pesticides, and residues; chiral compounds and also important compounds to investigate food interactions and food processing. An overview of these last developments and applications of CE to food analysis is provided by the review article of Garcia-Canas *et al.* [44], that also describes the recent results obtained by CE–MS in Foodomics applications.

14.7.4 Plants

That MEKC could attract great interest for the analysis of phytochemicals in herbs and their preparations is not surprising. The greatest deal of interest, however, arises from the observation of the pivotal role played by SDS in promoting separation of analytes under investigation. As underlined in previous sections, despite changes in the nature of the matrix, SDS remains the surfactant with the highest "reliability," in most cases offering the best performance in terms of resolution. As by a review of the numerous reports in this area, SDS is, in fact, in a position of undisputed leadership among the variety of surfactants that can be used to form the micellar phase. In this respect, SDS-MEKC applications span from the separation (in "conventional" sodium borate or phosphate buffers) of seven catechins and one xanthine, to that of ten conjugates of jasmonic acid with amino acids in the family of plant hormone jasmonates, to the separation of Sudan I, II, III and IV dyes in chili powder samples and of ginsenosides RG1; Re and Rb1 to assess the quality of *Panax quinquefolium* (American ginseng) and

many others. By contrast, SDC was the surfactant used to separate five coumarins in *Angelica dahurica* extracts and the nonionic surfactant Brij-35 to monitor seven aminoacids in tea leaves. The addition of TAPS to sodium tetraborate buffer was explored in the detection of four chief bioactive metabolites (phenylethanoid and iridoid glycosides) from therapeutically used *Plantago* species.

A review article by Chen *et al.* [45] emphasizes the development of CE and CEC techniques for the analysis of phytochemicals in herbal medicines.

List of Abbreviations

CE	capillary electrophoresis
CZE	capillary zone electrophoresis
BGEs	background electrolytes
EOF	electroosmotic flow
CMC	critical micelle concentration
PSP	pseudo-stationary phase
MEKC	micellar electrokinetic chromatography
SDS	sodium dodecyl sulfate
SOS	sodium octane sulfonate
LDS	lithium dodecyl sulfate
CTAC	cetyltrimethyl ammonium chloride
TTAB	tetradecyltrimethylammonium bromide
CTAB	cetyltrimethyl ammonium bromide
DDAB	Didodecyldimethyl ammonium Bromide
CHAPS	3-[(3-cholamidopropyl)-dimethylammonio]-2-hydroxy-1-propanesulfonate
CAPB	Cocamidopropyl Betaine
PAPS	3-(*N*,*N*-dimethylhexadeylammonium) propanesulfonate
CHOL	sodium cholate
MAPS	3-(*N*,*N*-dimethylmyristylammonium) propanesulfonate
LIPFOS	perfluorooctanesulfonate
polySUS	poly(sodium 10-undecenylsulfate)
polySUL	poly(sodium 10-undecenyl leucinate)
BBMA	butyl acrylate-butyl methacrylate-methacrylic acid
ACN	acetonitrile
ILs	Ionic liquids
CDs	cyclodextrins
2D	two-dimensional
LC	liquid chromatography
LOD	limits of detection
LIF	laser-induced fluorescence
FASS	field-amplified sample stacking

AFMC	analyte focusing by micelle collapse
MS	mass spectrometry
FITC	fluorescein isothiocyanate
DES	desmosine
IDES	isodesmosine
ESI	electro spray ionization
PF	partial filling
MEEKC	microemulsion electrokinetic chromatography
NPs	nanoparticles
REPSM	reversed electrode polarity stacking mode
rLVSI-sweeping MEKC	repetitive large volume sample injection and sweeping MEKC
QD	quantum dots
CdTe QDs	cadmium/tellurium quantum dots
MPA	mercaptopropyl acid
APPI	atmospheric pressure photoionization
DESI	desorption electrospray ionization
ALS	acid-labile surfactants

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