1

CRITICAL EVALUATION OF THE USE OF SURFACTANTS IN CAPILLARY ELECTROPHORESIS

Jessica L. Felhofer, Karin Y. Chumbimuni-Torres, Maria F. Mora, Gabrielle G. Haby, and Carlos D. García

1.1 INTRODUCTION

Surfactants are amphiphilic molecules consisting of a hydrophobic tail and a polar head group (Figure 1.1a) [1]. This unique structure imparts to surfactants a dual affinity for polar and nonpolar solvents, therefore decreasing the surface tension of the medium in which they are dissolved and/or interfacial tension with other phases.

One of the most interesting properties of surfactants is their ability to form aggregates in solution. The concentration at which this aggregation process starts is called the critical micellar concentration (CMC) [2,3]. Because the aggregation process encompasses a delicate balance between hydrophobic attraction and electrostatic repulsion, the CMC is dependent on the structure of the surfactant, the composition of the solution, and the temperature. Probably the most common of these aggregates are spherical structures known as micelles (Figure 1.1b). In some cases, surfactants can also aggregate in solution to form vesicles (Figure 1.1c), which are spherical or ellipsoidal particles formed by a bilayer wrapping around to enclose a volume of solution [1]. Single-chain surfactants tend to form micelles and double-chained surfactants vesicles. The aggregation behavior can be predicted by calculating the packing factor (P) using Equation 1.1:

$$P = \frac{V_c}{l_c a_h} \tag{1.1}$$

where V_c and l_c are the volume and length of the hydrophobic region of the surfactant, respectively, and a_h is the electrostatic cross-sectional area of the head group [1]. When the packing factor is lower than one-third, the surfactant molecule is cone-shaped, and tends to form spherical micelles. When the packing factor is between one-third and one-half, the molecules have a truncated cone shape and tend to form either spherical or cylindrical micelles [4]. When the packing factor is between one-half and one, the surfactant is cylindrical in shape and the formation of a bilayer is favored. In all cases, the shape and number of molecules in the aggregates are affected by factors such as pH, ionic strength, temperature, presence of organic solvents, and salts [5].

Surfactants can also spontaneously aggregate at interfaces, such as the solid–liquid interface that exists between solution and the capillary wall in capillary electrophoresis (CE). In this case, the aggregation/adsorption can occur even at concentrations below the CMC [6–10]. In general, the adsorption of surfactant to solid surfaces comprises the transport of the surfactant molecule toward the interface, followed by interaction with the sorbent surface [11]. Surfactants could then attach at, or detach from, the sorbent surface producing two fluxes—one forward and one backward. The relative contribution of each of these fluxes to the overall adsorption process depends on both the attraction exerted by the surfactant to the surface and the solvent–sorbent surface interactions.

The aggregates of surfactants that form on surfaces are similar to those observed in solution, and include spheres/hemispheres, cylinders/hemicylinders, bilayers (which can be interdigitated), and others such as multilayers (Figure 1.2) [12–14]. Just as in solution, aggregation

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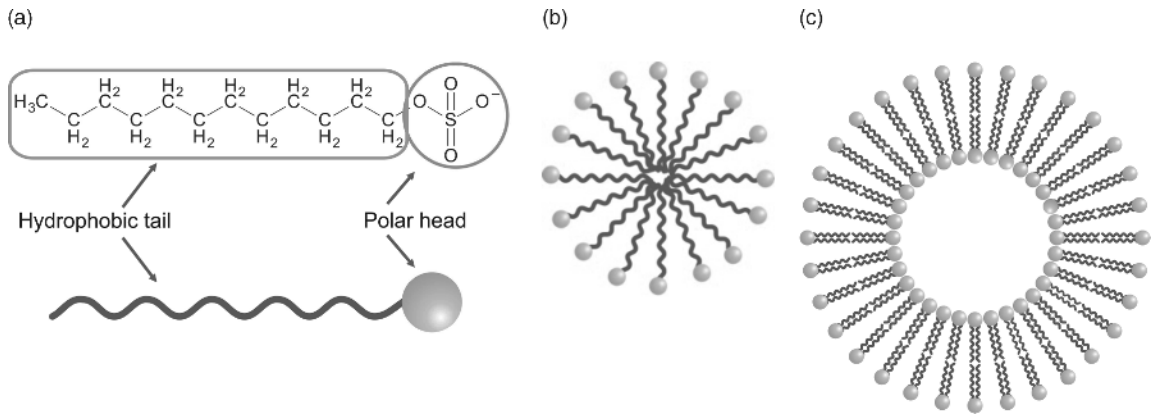

FIGURE 1.1 (a) Structure of a typical surfactant, outlining polar and nonpolar parts, and representations of (b) a micelle, and (c) a vesicle.

tendencies at interfaces are dependent on the packing factor and solution conditions. Additionally, hydrophobic and electrostatic interactions between the surface and the surfactant, as well as lateral surfactant–surfactant interactions, can influence the balance of forces defining the adsorption and aggregation [9,15].

Based on the nature of the head group, surfactants are classified as anionic, cationic, neutral, and zwitterionic. Examples of each class are shown in Figure 1.3. As a compromise between solubility and aggregation properties, the most commonly used anionic surfactants in CE contain linear chains (of 10–14 carbons) and highly polar head groups such as sulfate, carboxylate, sulfonate, or phosphate. Among them, sodium dodecyl sulfate (SDS) is probably the most used surfactant and has been successfully applied to improve the separation of a wide range of analytes [16–22]. Several cationic surfactants have also been used in CE. Like cetyltrimethylammonium bromide (CTAB) and tetradecyltrimethylammonium bromide (TDAB), the most common cationic surfactants used in CE are quaternary ammonium salts. Later described in more detail, neutral surfactants such as Tween 20, Brij 35, and Triton X-100 have also been used in CE. Zwitterionic surfactants contain both anionic and cationic groups (typically an ammonium salt) and the overall charge and effect can be regulated by solution pH. Palmityl sulfobetaine and 3-[(3-cholamidopropyl)dimethylammonio]-2-hydroxy-1-propanesulfonate (CHAPSO) are two examples of zwitterionic surfactants commonly used in CE.

Although outside the aforementioned classification based on charge, other surfactants used in CE include gemini

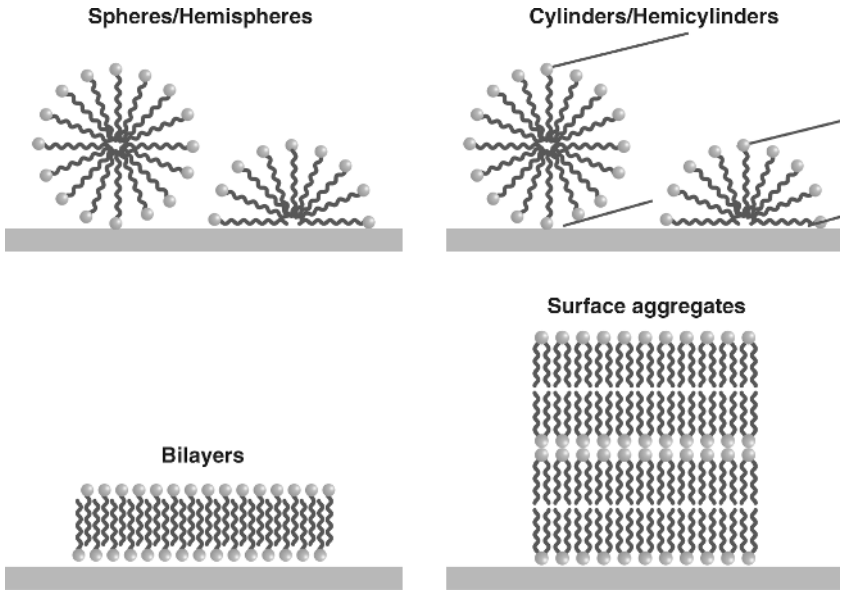

FIGURE 1.2 Various types of surfactant aggregates at the solid–liquid interface. Adapted from References 12 and 14.

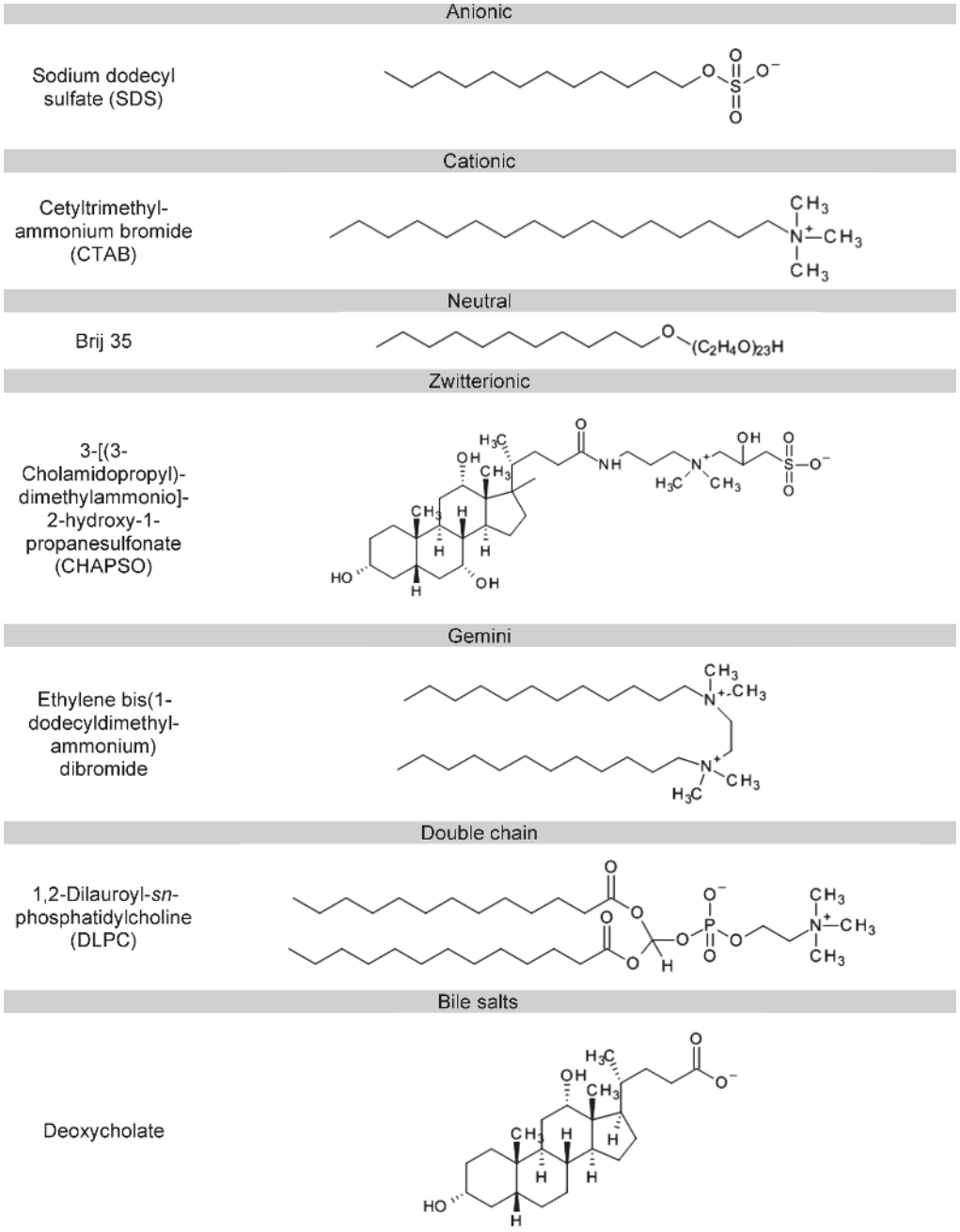

FIGURE 1.3 Examples of various classes of surfactants.

surfactants, double-chain surfactants, bile salts, and polymeric surfactants (Figure 1.3). Gemini surfactants are a relatively new class of amphiphilic compounds, consisting of two identical surfactant moieties connected by an alkyl spacer [23–25]. These molecules have attracted attention because of their high surface activity in addition to their particular aggregation structures, formed as a consequence of their unusual geometries, derived from the nature of the spacer group [26,27]. Advantageously, the CMC of gemini surfactants can be up to 100 times lower than the corresponding monomeric surfactant units [27]. Double-chain surfactants, such as 1,2-dilauroyl-*sn*-phosphatidylcholine (DLPC), are mainly derived from phospholipids. Bile salts are biological surfactants consisting of a large, rigid, and planar hydrophobic steroid moiety with two or three hydroxyl groups and a carboxylate [28]. Examples of bile salts include sodium cholate and sodium deoxycholate. Polymeric surfactants are structurally similar to other surfactants except that the hydrophobic tails in the micelles are covalently linked [29,30]. As a result, there is no dynamic

equilibrium of the polymer with the surfactant monomers, and the CMC is effectively zero.

1.2 SURFACTANTS FOR WALL COATINGS

Coating the walls of a capillary with surfactants can improve the performance of CE in two major ways: first, the electroosmotic flow (EOF) can be controlled and stabilized, leading to efficient separations and reproducible migration times; second, adsorption of sample components (proteins, analytes, and the sample matrix) onto the capillary walls can be significantly decreased, yielding good separation efficiency (approaching 1–2 million plates m^{-1}) and excellent recovery values (in some cases reaching 100%) [11,31–34]. These improvements are especially relevant for microchannels used in microchip-CE, which are often fabricated using polymeric substrates, such as poly(dimethylsiloxane) (PDMS) [35–38]. In most cases, the addition of surfactants can help minimize the negative impact of hydrophobic interactions between the sample and the capillary walls, while enabling facile generation and regeneration of the coating, low cost, applicability in a wide range of buffer conditions, and minimal interference with the detection system [31,34]. In addition, surfactants are particularly convenient as dynamic wall coatings for several practical reasons. First, surfactants spontaneously adsorb to most surfaces, requiring no instrumentation or specific skills to prepare the coating; second, the surfactant concentration can easily be controlled; third, the coating, in equilibrium with excess surfactant in the buffer, is continuously replenished; fourth, most surfactants can be removed by simply rinsing the surface with water or the separation electrolyte [39–42]; and lastly, numerous surfactants with different structures are commercially available, simplifying the experimental design [43,44].

As mentioned earlier and further described in Chapter 6, coating the walls of a capillary with surfactants can improve the performance of CE by enabling control of the EOF and by minimizing the adsorption of sample components to the capillary walls. Although these goals are often not mutually exclusive, each of these is herein described separately.

1.2.1 Controlling the Electroosmotic Flow

The EOF is a key parameter in CE because it has a crucial effect on both the efficiency and reproducibility of the separations. The EOF is generated as a consequence of the surface charge of the capillary wall and the applied separation potential. In capillaries made with glass, the presence of ionized silanol groups ($pK_a \sim 4$) provides negative charges on the surface [45]. When in contact with an electrolyte, the deprotonated silanol groups induce a build-up of counterions, followed by a diffuse double layer of anions and cations. The potential spanning the diffuse layer to the capillary wall is called the zeta potential, ζ, and is dependent on the surface charge density of the capillary wall. Upon application of normal polarity potential, solvated cations in the diffuse layer migrate toward the cathode, dragging the bulk of the solution through the capillary, toward the detector. Equation 1.2 shows the relationship between parameters that define the velocity of the EOF (v_{EOF}): the dielectric constant (ε), zeta potential (ζ), the viscosity of the solution (η), and the applied electric field (E) [15,46].

$$v_{EOF} = \frac{\varepsilon\zeta}{\eta}E \tag{1.2}$$

Since the zeta potential depends on the charge density of the capillary surface, modifications of the capillary walls by surfactants, polymers, and other charged compounds have the potential to affect (increase, decrease, suppress, or reverse) the EOF [19,47–52]. Most often, a precise control of the EOF can aid in achieving an adequate balance between separation time and efficiency.

Stabilization of the EOF is an important target in CE, especially in microchips fabricated from PDMS. Monomers from the bulk of the polymer can migrate to the surface of the channel, changing the surface charge density and resulting in EOF drift. To stabilize the EOF, the charge of the microchannel can be stabilized by including surfactants in the running buffer, providing a dynamic coating. Surfactants spontaneously adsorb to PDMS, via hydrophobic interactions between the surface and the tail of the surfactant, exposing the charged head group to the solution. In this regard, Mora et al. investigated the change in the EOF of PDMS films as a function of the structure and concentration of anionic alkyl surfactants. The selected alkyl tails ranged from eight to fourteen carbon atoms long, and the head groups included sulfates, phosphates, and carboxylates. This allowed a systematic evaluation of the hydrophobic contribution of the surfactant's tail and the electrostatic contribution of the head group to the adsorption behavior. It was shown that all surfactants produced a significant increase in the EOF and that the affinity of each surfactant for the PDMS surface correlates to the corresponding CMC value [15]. Other groups have also demonstrated the role of SDS for effective modification and stabilization of EOF in PDMS microchannels [53–55]. Anionic surfactants are not effective coatings for silica mainly due to unfavorable electrostatic interactions [56,57]. On the other hand, electrostatic interactions with cationic surfactants are widely used to manipulate surface chemistry of silica capillaries [16,17]. When cationic surfactants are adsorbed (head-to-surface), silanol groups become neutralized, resulting in reduction or complete elimination of the EOF (for concentrations leading to the formation of a monolayer of surfactant). If the

concentration is further increased, a bilayer is formed (positive head groups interact with the solution) and the EOF is reversed. Thus, the proper choice of substrate as well as surfactant type and concentration allows a simple way to control the magnitude and the direction of the EOF [58,59]. Among other examples of this approach, it is worth mentioning the work from Liu et al. who reversed the EOF on PDMS by using a didodecyldimethylammonium bromide (DDAB) dynamic coating, enabling the analysis of ATP [60].

Mixtures of cationic and anionic surfactants have been used to improve the stabilities of the coatings. For example, a mixture of CTAB with SDS formed more stable coatings in fused-silica capillaries than CTAB alone. This mixture created a reversed EOF that remained stable for over 80 min, even after the removal of the surfactants from the buffer [40].

In cases where the presence of a significant EOF is not desirable, zwitterionic surfactants, such as CHAPSO and palmityl sulfobetaine, can be used to effectively neutralize the charge of the capillary surface [61–64]. The EOF of silica has been suppressed up to 90% with this approach, while providing high-efficiency separations of basic proteins [65].

Gemini surfactants such as hexyl-α,ω-bis(dodecyldimethylammonium bromide) are also used to control the EOF. This surfactant is able to adopt a wide variety of structures and gives surface coverage of up to 92% at 5.0 mM concentration [66]. Dynamic coatings of the cationic gemini surfactant ethylene bis (1-dodecyldimethylammonium) dibromide have also been used to control the EOF. Although this surfactant can reverse the EOF even at concentrations lower than 0.01 mM, the magnitude of the resulting EOF is affected by the surfactant concentration, pH, ionic strength, and secondary electrolytes added to the buffer [67].

Lipid vesicles have been employed as coating precursors to obtain semipermanent lipid bilayers on silica capillaries. For example, after acid/base preconditioning of the capillary, a positively charged film can be formed by exposing the capillary to a solution containing 1 mM 1,2-dioleoyl 1-3-trymethylammonium propane solution for 30 min. In this case, a reverse and stable EOF (measured in 40 mM acetate, pH = 5.0) can be achieved [68]. Anionic phospholipids such as 1,2-dimyristoyl-*sn*-glycero-3-[phosphor-*rac*-(1-glycerol)] (DMPG), on the other hand, can be used to generate a weakly cathodic EOF. At neutral pH, this coating is difficult to obtain due to charge repulsion with the negatively charged surface of the capillary; however, at pH < 4, a 5:95 DMPG:1,2-dimyristoyl-*sn*-glycero-3-phosphocholine mixture can be used to suppress the EOF [69,70].

1.2.2 Preventing Adsorption to the Capillary

One of the major problems encountered in CE is the adsorption of sample matrix components, including proteins and analytes, to the capillary wall. In general, the main consequences of this adsorption process are increased peak broadening, poor reproducibility, and low recovery. Adsorption can occur by electrostatic interactions between sample components and the capillary surface, as in the case of positively charged proteins or amines. In other cases, hydrophobic interactions are the main driving force of the adsorption, as in the case of the analysis of proteins [71,72]. Dynamic coatings of anionic surfactants, mostly SDS, and neutral surfactants, such as Tween 20, Brij 35, and Triton X-100, have been widely used to reduce hydrophobicity and prevent unwanted adsorption to capillary walls and microchannels, especially those made of PDMS [19–22,53–55]. Cationic and zwitterionic surfactants have also been used for CE coatings [18,61–64]. The surfactant *n*-dodecyl-β-D-maltosine, an alkyl polyglucoside that belongs to a family of mild nonionic surfactants, adsorbs to PDMS surfaces forming an uncharged hydrophilic monolayer [73] that reduces hydrophobic and electrostatic interactions between protein and the surface. Since alkyl polyglucosides do not affect the native structure of many proteins, they can be added to the separation buffer without significantly affecting other variables. Alternatively, semipermanent coatings by surfactants can also be used [74–76]. Among others, gemini surfactants, double-chain surfactants, bile salts, and polymeric surfactants form stable semipermanent coatings [77] that remain adsorbed even after rinsing a capillary with buffer, thereby avoiding undesired interactions between surfactant and analytes. Examples of semipermanent coatings include cationic polymers such as polybrene [78] and neutral polymers such as hydroxyethylcellulose and poly(vinyl alcohol) [79]. Pluronic, a triblock copolymer of poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide), can be spontaneously adsorbed to a variety of hydrophobic polymeric materials via the hydrophobic poly(propylene oxide) moiety [80]. Hellmich et al. [81] investigated the coating of PDMS with Pluronic, which resulted in 85% reduction of serum protein adsorption relative to native PDMS. In one study, nonionic surfactants such as Triton X-100 and Tween 20 proved less effective than zwitterionic surfactants, such as palmityl sulfobetaine. A solution of >10% nonionic Triton X-100 was required to reduce protein adsorption by 90%, versus only 0.3% of the zwitterionic palmityl sulfobetaine. For zwitterionic surfactants, the inhibition of protein adsorption increases as the length of the carbon chain increases and the CMC decreases [32]. To further improve the stability of semipermanent surfactant coatings, a layer-by-layer adsorption of oppositely charged surfactants has shown to be effective. For example, DDAB and a gemini surfactant (18-6-18) were used for coating along with SDS. With an increase in the concentration of SDS in the coating, the stability dramatically increases due to the enhanced packing capabilities. These coatings showed excellent stability, efficiency,

reproducibility, and high recovery of proteins, indicating powerful suppression of adsorption of sample components to the capillary walls [82].

For a more efficient prevention of adsorption, the geometry of the surfactant monomer should be cylindrical so that aggregates form a bilayer [39]. If the surfactant geometry is conical, such as with single-chained surfactants such as CTAB, the surfactants tend to aggregate on the capillary wall as spherical hemi-micelles, which can provide incomplete surface coverage, therefore decreasing the efficiency of the coating [83]. Two-tailed surfactants such as DDAB and dioctadecyldimethylammonium bromide possess the cylindrical geometry to support a bilayer on flat surfaces [39,84,85]. A mixture of these two surfactants allowed protein recoveries of 85–100% in comparison to 0–81% for CTAB [4]. Melanson et al. produced a semi-permanent coating with the double-chained surfactant DDAB by rinsing a capillary for less than 20 min at a concentration of 0.1 mM [34,39]. After that, the separation of four basic proteins, ribonuclease A, cytochrome C, α-chymotrypsinogen, and lysozyme, was achieved with recoveries ranging from 92 to 100%. DDAB has also been employed to reduce the adsorption of fluorescent dyes on PDMS microchannels [85].

To further improve the stability of surfactant coatings to inhibit protein adsorption, polymerized surfactants have been used [86]. The surfactant 1,2-dioleoyl-*sn*-glycero-3-phosphocholine was used to coat a glass capillary, which was then heated in a solution containing the free radical initiator 2,2′-azobis(2-methylpropionitrile) for oligomerization. With this modified capillary, a separation of cationic and anionic proteins was carried out at pH 7.4 with good efficiencies. Protein recoveries for cationic proteins were improved ($97 \pm 6\%$) with respect to a capillary dynamically coated with DLPC ($75 \pm 6\%$) [87]. Highly stable cross-linked phospholipid bilayers have also been used as coatings on silica to improve protein and peptide separations. Recently, 1,2′-bis[10-(2′,4′-hexadienoyloxy)decanoyl]-*sn*-glycero-3-phosphocholine was used to prepare planar, self-assembled phosphorylcholine phospholipid bilayers with radical polymerization [88]. Protein separations for cationic and anionic mixtures were carried out, and as a result of increased resolution, more protein peaks were observed, including one for α-chymotrypsinogenin, which could not be detected after separation in a bare capillary. These films allowed continuous protein separations for up to 10 h per day with no reduction in the separation performance.

1.3 SURFACTANTS AS BUFFER ADDITIVES

The components of the running buffer directly influence the quality of separations by CE. The buffer type and concentration, pH, ionic strength, organic solvent concentration, and type and concentration of any additives affect the zeta potential and, thus, can be used to control the EOF. Additionally, these factors can be adjusted to tune the resolution. In this section, we describe methods that use surfactant additives to impart selectivity in CE, namely, micellar electrokinetic chromatography, microemulsion electrokinetic chromatography, and nonaqueous CE with added surfactants.

1.3.1 Micellar Electrokinetic Chromatography

The basis of CE separations is the difference in the charge to size ratio of analytes. The introduction of micellar electrokinetic chromatography (MEKC) in 1984 by Terabe et al. [89] expanded the versatility of CE by enabling the separation of neutral analytes in addition to charged ones. To perform MEKC, surfactants must be included in the running buffer at a concentration higher than the CMC. Micelles form a charged pseudostationary phase in solution, creating a hybrid system of electrophoresis and chromatography upon application of the separation potential. In the presence of an electric field, the micelles move with or against the EOF, depending on the charge of the head groups of the surfactants (described later). During electromigration of the micelles, analytes in solution can interact with the micelles through hydrophobic and electrostatic interactions with the core of the micelle and the surface of the micelle, respectively. Thus, the differential analyte/micelle interactions/partitions affect the migration time of the analytes and determine the resolution/selectivity of MEKC. (As a side note, analytes can also associate with surfactant monomers that are in equilibrium with micelles. These analyte–monomer associates have different partitioning behavior into micelles than free analytes have [90].) Neutral compounds are separated mainly by hydrophobicity. Less hydrophobic compounds partition to a lesser extent into the hydrophobic core of the micelles and consequently have a relatively fast migration time. In contrast, more hydrophobic compounds spend more time within the pseudostationary phase and thus have longer migration times. Charged analytes are separated based on a combination of electromigration and chromatography. The analyte charge and size influence its electrophoretic mobility. Additionally, any repulsion between the analyte and the micelles (when both have like charges) or attraction/ion-pairing effects (when both have opposite charges) influence separation [91]. Other factors such as shape, dipolar moment, and hydrogen bond interactions between the analytes and the surfactant may play fundamental roles in the separation [92]. One of the most important advantages of MEKC is that the selectivity can be manipulated by using different types of surfactants—anionic, cationic, zwitterionic, nonionic, and chiral—or even mixtures of surfactants. Also, the chain length and

branching of the surfactants tails can be varied to change the physical nature of the micelles. Organic solvents can also be used to adjust the resolution in MEKC, although the amount has to be controlled as the solvents may compromise the integrity of the micelles [93–95]. It is also worth mentioning that the fundamental and theoretical aspects of MEKC, such as calculations of retention factors and resolution, have been described extensively in many books, chapters, and reviews [96–105]. Applications of MEKC for the analysis of pharmaceutical, forensic, food, environmental, and clinical samples abound in the literature, so the reader is referred to recent reviews for further information [30,106–116].

The first micelles used for MEKC comprised the anionic surfactant SDS [89]. To date, it still remains one of the most widely used surfactants for MEKC separations due to high stability, relatively low Krafft point, low UV absorbance, high solubilizing capability, and the availability of high-quality reagent [102]. Upon application of the normal polarity electric field (anode at inlet to cathode at outlet), the anionic pseudostationary phase migrates toward the anode/inlet (Figure 1.4). However, under neutral or basic conditions, the capillary walls are negatively charged and thus the direction of the EOF is toward the detector/cathode. Although the pseudostationary phase is attracted toward the inlet (allowing separation of incorporated analytes), the EOF drives the bulk solution toward the detector. Very hydrophobic analytes such as aromatic compounds and steroids can be difficult to resolve with SDS-based MEKC because rather than having differential partitioning in and out of the micelle, the analytes are believed to completely reside in the hydrophobic core. To remedy this problem, less hydrophobic surfactants, such as the bile salts sodium cholate, sodium deoxycholate, sodium taurocholate, and sodium taurodeoxycholate, can be added, resulting in significant improvements in resolution [115]. Other nonconventional pseudostationary phases can be used to optimize MEKC separations as well. Cationic surfactants such as CTAB provide different selectivity than anionic surfactants, although a downfall is a smaller migration window (relative to SDS) [115]. Zwitterionic surfactants are not commonly used in MEKC, but they have been used to improve the separation of proteins and peptides [30,92,115]. Nonionic surfactants, such as Brij 35, Tween, and alkoglucosides, are useful for improving the separation of charged analytes [92]. They are not effective in separating neutral analytes because they have no mobility, although it is possible to form *in situ* charged micelles by complexation with ions such as borate [30]. A major advantage of nonionic surfactants is that they do not contribute to the ionic strength of the running buffer, and therefore they do not contribute to the electrical current [117]. For this reason, nonionic surfactants are used to form mixed micelles with ionic surfactants to improve resolution and optimize selectivity while avoiding increases in Joule heating [92,115]. Mixing surfactants offers powerful optimization capabilities as mixed micelles have tunable hydrophobicity and surface charge density, which alters analyte–micelle interactions [92]. Another advantage of including nonionic surfactants in mixed micelles is that the electrophoretic mobility of the micelles and incorporated analytes can be controlled without significantly increasing the electroosmotic velocity, which can be useful for extending the elution window of a separation [118]. Ahuja et al. achieved an infinite elution range in MEKC using a mixture of Brij 35 and SDS for the micelles [119]. Although countless combinations of surfactant types have been used, mixed micelles typically consist of a nonionic surfactant and an anionic surfactant, usually SDS. Chiral separations, important for pharmaceutical and biomedical fields, can be achieved by using chiral surfactants for MEKC. These surfactants include naturally occurring bile salts, saponins, digitonin, and semisynthetic surfactants derived from naturally occurring sugars and amino acids [28,120–122]. These surfactants achieve enantioseparations of analytes by stereoselective recognition interactions such as hydrophobic, steric, and electrostatic interactions [123]. Chiral surfactants have also

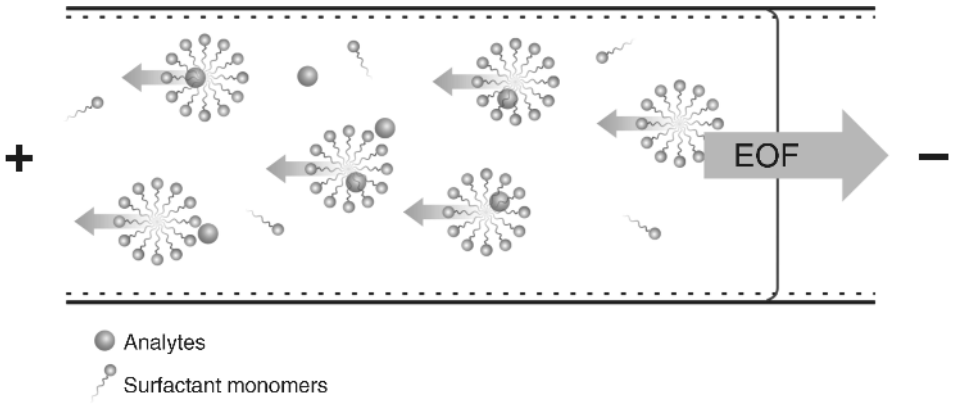

FIGURE 1.4 MEKC separation by anionic surfactants under neutral or basic conditions.

been combined with other types of surfactants to form mixed micelles, allowing for tunable selectivity in enantioseparations [30,124].

1.3.2 Microemulsion Electrokinetic Chromatography

Another surfactant-based CE mode is microemulsion electrokinetic chromatography (MEEKC), which was first reported in 1991 by Watarai [125]. In MEEKC, the separation is carried out in a microemulsion: an optically transparent mixture of immiscible liquids, stabilized by a monolayer of surfactants. Microemulsions can consist of nanometer-scale oil droplets dispersed in a bulk phase of water (o/w) or water droplets dispersed in a bulk phase of oil, the most commonly used type for CE being o/w. In that case, the charged head groups of the surfactants (usually anionic) face outside of the oil droplet toward the aqueous phase while the lipophilic tails are buried inside (Figure 1.5), reducing the surface tension between the two phases. To further stabilize the nanodroplets, a nonionic, amphiphilic co-surfactant (butan-1-ol, in Figure 1.5) can also be added to the solution. The co-surfactant has a polar, but uncharged head group, which decreases the repulsion between the primary surfactant head groups by decreasing the charge density.

Unique ratios of water, oil, surfactant, and co-surfactant are required to form a stable microemulsion [126]. As an example, 0.8% organic solvent, 3.3% SDS, 6.6% cosurfactant, and 89.3% aqueous buffer were used to form an o/w microemulsion for MEEKC [127]. The aqueous phase typically comprises borate or phosphate buffer. Common oils for emulsions include octane or heptane. Others oils that have been evaluated for performance in MEEKC include diethyl ether, cyclohexane, chloroform, methylene chloride, and amyl alcohol. Each type slightly affects selectivity and migration times of analytes. Again, the most frequently used surfactant is SDS, although sodium cholate (bile salts) and CTAB have been used. Separation with the neutral surfactant Triton X-100 has been demonstrated, although its use for separation of uncharged analytes is very limited.

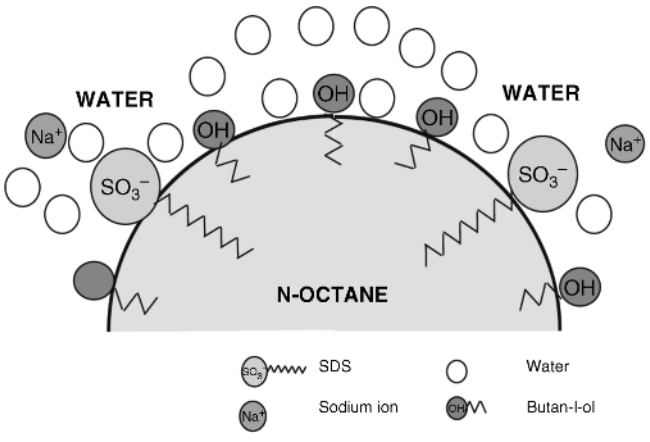

FIGURE 1.5 Schematic representation of an o/w microemulsion droplet. Extracted from Ref. 128.

The type of surfactant can be selected to tune the size and charge of the emulsion droplets, the magnitude and direction of the EOF, and ion-pairing or repulsion effects between analytes and the pseudophase. A longer length of hydrophobic tail can increase the stability of the microemulsion by reducing the polydispersity of the emulsion. The concentration of surfactant used should greatly exceed the CMC to stabilize the emulsions to a greater extent (e.g., 110 mM SDS produces an emulsion that is stable for over several months). The concentration of nonionic co-surfactants (typically a short-chain alcohol, of which the most commonly used is butan-1-ol) can affect migration times due to changes in viscosity, and consequently EOF. Also, the amount of co-surfactant used can influence the size of the droplets by changing the charge density on the surface of the droplets [91,128]. Altria et al. [129] and Hansen [127,130] have investigated the effects of type and concentration of surfactants, pH, and ionic strength of aqueous phase, type of oil used, method of emulsion preparation, as well as many other factors, including the addition of organic solvents, urea, cyclodextrins, and ion-pairing reagents. The background and principles of MEEKC methodology and descriptions of operating parameters have been discussed in several reviews [91,126,128,129,131–134].

The separation principles of MEEKC are the same as those of MEKC (described earlier), and some groups have shown that selectivity and efficiency are essentially the same in MEEKC as in solvent-modified MEKC [135,136]. However, some researchers claim that MEEKC has several advantages. Among them, authors highlight that oil droplets are larger and less rigid than standard micelles, allowing compounds with extremely high hydrophobicity to partition into the droplet. The high solubilizing characteristics of microemulsions allow dissolution of a wider variety of compounds and complex sample matrices. Thus, less sample preparation is required before analysis. The solubilizing power of microemulsions also prevents matrix precipitation during analysis, avoiding capillary deterioration. Another potential advantage of MEEKC over MEKC is the widened migration time window, due to the presence of organic solvents in solution. Since its inception in 1991, MEEKC has been demonstrated for numerous analyses of water-soluble, water-insoluble, neutral, and charged analytes, including proteins, agrochemicals, pharmaceuticals, natural products, dyes, cosmetics, foods, and biological and environmental samples. Chiral separations have been accomplished with MEEKC by including an enantioselective component either as a primary surfactant, co-surfactant alcohol, or oil (e.g., (*R*)- and (*S*)-dodecoxycarbonylvaline, (*R*)- and (*S*)-2-hexanol, or D- and L-diethyl tartrate, respectively) in the microemulsion [128,137]. Another important application of MEEKC

is the determination of partition coefficients (log P values) of hydrophobic pharmaceutical compounds. Several groups have thoroughly reviewed these applications of MEEKC [91,113,128,132,133,138–141]. As a side note, emulsions have a variety of other applications aside from CE, including oil recovery, delivery of pharmaceuticals and cosmetics, nanoparticle preparation, liquid–liquid extraction, and high-performance liquid chromatography [91,134,140].

1.3.3 Nonaqueous Capillary Electrophoresis with Added Surfactants

In 1984, Walbroehl and Jorgenson demonstrated that CE can actually be carried out in pure organic solvents [142], a separation mode termed nonaqueous capillary electrophoresis (NACE). NACE is particularly advantageous for separations of hydrophobic analytes. The increase in solubility and the decrease in aggregation of analytes achieved under nonaqueous conditions lead to a better resolution and higher throughput [143]. NACE has several other advantages. Compared to water, organic solvents have a wider range of physical and chemical characteristics such as dielectric constants, polarity, viscosity, and density [144]. Thus, the physicochemical differences between analytes can be maximized, improving the separation of closely related compounds [145]. The wide range of dielectric constants and autoprotolytic behavior in organic solvents permits a wide range of compounds to be ionized through versatile acid/base chemistry [146–148]. Organic solvents can increase separation time by decreasing the EOF through compression of the electrical double layer and a consequent decrease in the zeta potential [143]. Organic solvents reduce adsorption of analytes to the capillary surface, avoiding changes in EOF [143]. Because lower currents are generated in nonaqueous media (compared to aqueous solutions with the same ionic strength), a higher separation potential can be applied, further amplifying the physicochemical differences between analytes in the sample [147,149,150]. Thus, by selecting the type of organic solvent (and ratio, if a mixture of organic solvents is used), one can tune the separation resolution, selectivity, efficiency, and analysis time [144]. Finally, the use of volatile solvents for NACE supports the compatibility of CE and mass spectrometry. Many reviews [145,151–158] are also available detailing the fundamental physicochemical aspects, advantages, and applications of NACE, as well as critical evaluations [159] of the claims of NACE proponents.

Just as in aqueous CE, NACE separates analytes based on charge to size ratio, and therefore cannot separate nonionized species. However, surfactants have been used as additives in NACE (Table 1.1) to accomplish separations of neutral compounds. Analytes can be associated with charged monomers of surfactants for separation, as Lu et al. [143] and Li and Fritz [144] demonstrated for the separation of tamoxifen metabolites and polycyclic aromatic hydrocarbons, respectively. The majority of NACE separations have included a high enough concentration of surfactants to form aggregates (as a pseudophase) in a separation mode called nonaqueous micellar electrokinetic chromatography (NAMEKC). Typically, micelles do not form in organic solvents because the hydrophobic interactions that cause aggregation are weaker than in water and also CMCs of surfactants are higher than in water [156]. However, there are organic solvents compatible with micelle formation, particularly those with high dielectric constants, such as formamide [149,160–162]. Table 1.1 lists other organic solvents and the corresponding surfactants that are compatible with NAMEKC. The analyte–surfactant interactions in NACE include solvophobic interactions, electrostatic interactions (ion–ion, ion–dipole, and dipole–dipole interactions), and donor–acceptor interactions [147,162]. The chemical nature and concentration of the organic solvent and surfactant can be adjusted to tune such interactions. Nonpolar solvents with low dielectric constants support ion–pair interactions that can be used for chiral separations [163]. NAMEKC is particularly useful for highly lipophilic racemates with limited solubility in water. Although cyclodextrins are widely used for chiral separations, Table 1.1 also shows examples where surfactant chiral selectors have been used.

1.4 SURFACTANTS FOR ANALYTE PRECONCENTRATION

The limited dimensions of a capillary constrain the volume of sample that can be injected and also provide only a short path length for UV absorption detection (the most standard detection method for CE). Consequently, sensitivity can be poor and limits of detection relatively high. Electrochemical or fluorescence detection is a viable option with superior sensitivity; however, they sometimes require time-consuming derivatization steps. A convenient and popular alternative to overcome such difficulties is to develop online preconcentration strategies. The goal is to compress the width of analyte bands in the capillary so that a larger volume of sample can be injected without sacrificing separation efficiency. Two main approaches have been used that take advantage of the partitioning of analytes into stationary or pseudostationary phases: stacking, which manipulates the electrophoretic mobility of analytes, and extraction [176,177]. The myriad of strategies in both classes has been thoroughly discussed in many reviews [176–196]. Stacking techniques include field-amplified sample stacking/normal stacking mode, large-volume sample stacking/-stacking with matrix removal, field-enhanced sample injection, transient isotachophoresis, electrokinetic supercharging, and dynamic pH junction. Membrane filtration and

TABLE 1.1 NACE Separations Utilizing Surfactants

Organic Solvents	Surfactants	Analytes	References
Acetonitrile	Camphorsulfonic acid, Tween 20	Basic chiral drugs: atenolol, bisoprolol, bunitrolol, metroprolol, pindolol, propanolol, salbutamol, ephedrine, epinephrine, cisapride, and synthetic impurities	164
	Tetrahexylammonium perchlorate	Polycyclic aromatic hydrocarbons	146
	Planar organic cations, for example, tropylium tetrafluoroborate or 2,4,6-triphenylpyrylium tetrafluoroborate	Polycyclic aromatic hydrocarbons	147
Acetonitrile and methanol	Sodium cholate	*Trans*- and *cis*-resveratrol	165,166
	Sodium cholate, SDS	Linoleic acid oxidation products	167
	Trimethyloctadecylammonium bromide	Aromatic compounds	168
	Ammonium acetate, tetrabutylammonium bromide, tetrabutylammonium hydrogen sulfate, and tetrapentylammonium bromide	Tamoxifen and four phase I metabolites	169
Methanol	Brij 35	Porphyrin acids	170, 171
	Sodium cholate	Cryptotanshinone, tanshinone IIA, and tanshinone I	172
	SDS	Tamoxifen metabolites	143
	Sodium tetradecyl sulfate	Polycyclic aromatic hydrocarbons	144
Methanol and formamide	Sodium cholate	3,4-Methylenedioxymethamphetamine	173
Formamide	SDS	Dimethyl phthalate, diethyl phthalate, dibutyl phthalate	174
	SDS, diethylhexyl sodium sulfosuccinate, taurodeoxycholic acid sodium salt	*p*-Arylacetophones	175
Dimethyl formamide, dimethyl acetamide	Sodium caprylate, sodium laurate, sodium palmitate	Four tetracyclines	149
Propylene carbonate	Tetraalkylammonium ions, long-chain trimethylammonium ions	Phenanthrene, β-naphthol, methylparaben, ethylparaben, propylparaben, and vitamin K_1	93

solid-phase extraction are also used for preconcentration. The aim of this section is to focus on the preconcentration strategies that rely on the use of surfactants, namely sweeping, transient trapping, analyte focusing by micelle collapse, micelle to solvent stacking, combinations of methods, and offline cloud point extractions.

1.4.1 Sweeping

Sweeping is a powerful technique that was first demonstrated in 1998, yielding a 5000-fold preconcentration factor [197]. The theoretical aspects of the technique and experimental corroboration were discussed in detail soon after [198]. Briefly, sweeping is carried out by including surfactants above the CMC as a pseudostationary phase in the separation buffer, but not in the sample solution. After conditioning the capillary by rinsing with the separation buffer, the sample plug, devoid of micelles, is injected. Preconcentration occurs as analytes partition into the pseudostationary phase as it penetrates the sample plug upon electrophoresis [197]. Then, the analytes, which are typically preconcentrated up to a factor of several thousand [178], can be separated by MEKC. Figure 1.6 gives a schematic drawing of this process. Sweeping can be used for the preconcentration of neutral as well as charged analytes due to hydrophobic interactions between the neutral analytes and the surfactant tails and also through electrostatic interactions between oppositely charged analytes and surfactants [195]. In fact, the degree of preconcentration is proportional to the strength of the interactions between analytes and the micelles [198]. Sweeping can be carried out using anionic, cationic, nonionic, zwitterionic, and mixed micelles. Excellent reviews detailing sweeping with micelles and providing pertinent applications of the technique (e.g., to determine pesticides, phenols, illicit drugs, pharmaceuticals, and herbal medicines) have been published [178,185,191]. Sweeping has also been carried out as a preconcentration method hyphenated to MEEKC for

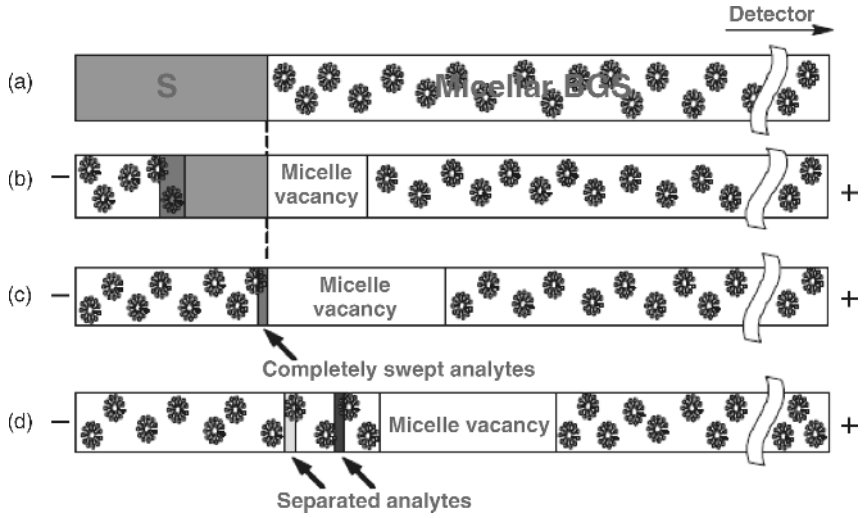

FIGURE 1.6 Mechanism of sweeping preconcentration using negatively charged surfactants and zero EOF conditions. (a) A sample plug (S), devoid of micelles, is pressure injected into a capillary that has been conditioned with a background solution (BGS) that contains micelles as a pseudostationary phase. (b) On application of the separation potential, the BGS with micelles penetrates the sample plug and begins to sweep/preconcentrate the analytes. (c) The sample plug is completely swept and the analyte zone has been compressed. (d) Preconcentrated analytes are then separated by MEKC. Extracted from Ref. 178.

analytes such as tobacco alkaloids [199], catechins [200], and phenolic compounds [201], with up to 238,000-fold increases in detection sensitivity [201].

1.4.2 Transient Trapping

Sueyoshi et al. aimed to perform mass spectrometry (MS) detection after MEKC separation on a microchip. As described later, surfactants can decrease the sensitivity of MS detection. To avoid introducing surfactants to the detector, and considering the short separation channel length on a microchip, the group used a partial filling technique in which plugs of a micellar solution and the sample solution were injected into a channel filled with separation buffer devoid of micelles [202]. As shown in Figure 1.7, upon application of separation potential, the sample plug migrates through the micellar plug and separates by MEKC. When the separated analytes reach the end of the micellar plug, they are released into the separation buffer devoid of surfactants, where they continue to migrate to the detector. The micellar plug never reaches the detector, avoiding incompatibility issues with MS. Along with allowing the compatibility of MEKC with MS, this partial filling technique was observed to actually preconcentrate the sample plug. In 2008, Otsuka's group investigated the effect further and proposed that the preconcentration occurs due to a transient trapping mechanism [203]. When a short micellar plug is injected into the separation channel before the sample plug, analytes are trapped and released in a concentrated sample zone at the interface between the two plugs, similar to the mechanism in sweeping. In sweeping, a large volume of sample can be injected and preconcentrated into a narrow zone to improve the detection limits. However, in microchips, the short channel length limits the injection volume. Thus, the transient trapping mechanism is a convenient preconcentration option for microchips [203].

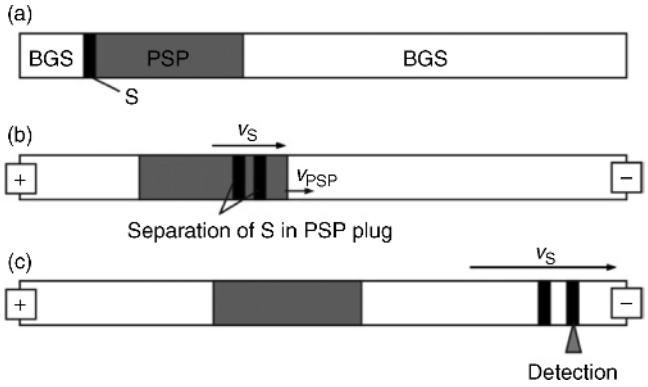

FIGURE 1.7 Schematic of transient trapping preconcentration that occurs during the partial-filling technique. (a) In a capillary filled with background solution (BGS) devoid of micelles, a short pseudostationary phase micellar plug (PSP) is injected, followed by a short plug of sample (S). (b) On application of separation potential, the sample plug is preconcentrated by transient trapping and then separated into analyte bands, which migrate faster (ν_S) than the PSP plug (ν_{PSP}). (c) The analyte bands are introduced into the detector without interference from the PSP plug. Extracted from Ref. 202.

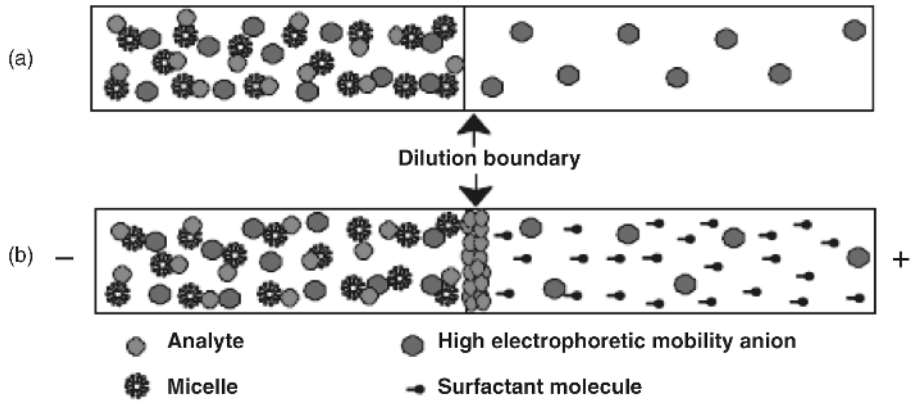

FIGURE 1.8 The basis of preconcentration in AFMC. (a) The sample plug contains analytes, anionic micelles, and an additional anion with high electrophoretic mobility. (b) On application of separation potential, the micelles in the sample plug, containing the analytes, migrate toward the separation buffer and on reaching the dilution boundary, collapse into monomers, preconcentrating and releasing analytes. Extracted from Ref. 178.

1.4.3 Analyte Focusing by Micelle Collapse

Quirino and Haddad introduced analyte focusing by micelle collapse (AFMC) in 2008 for the online preconcentration of neutral analytes [204]. As shown in Figure 1.8, to perform AFMC, a solution containing anionic micelles and an additional anion that has high electrophoretic mobility is used to transport the analyte molecules via the hydrophobic core of the micelles. As the micelles migrate due to electrophoresis, they encounter the separation buffer, which contains no micelles and has a lower conductivity than the sample solution. The micelles become diluted and collapse into monomers, releasing and accumulating their contents into a micellar dilution zone. By manipulating solution conditions in the capillary, the sample enrichment of AFMC can be combined with the separation capabilities of MEKC to preconcentrate and then separate neutral analytes. The initial proof of concept of AFMC MEKC resulted in a preconcentration factor of 160–200 for the analysis of the steroids cortisone, hydrocortisone, and prednisolone [204]. The optimal conditions for producing the best peak height enhancements were shown to be a concentration of surfactant in the sample solution just above the CMC and a minimum conductivity ratio (separation solution to sample solution) needed to collapse the micelles [205]. AFMC was demonstrated to be compatible with MS detection since the preconcentrated and separated analyte zones contain surfactant monomers rather than micelles, which do not reduce the sensitivity of MS to such a high degree [206].

1.4.4 Micelle to Solvent Stacking

Micelle to solvent stacking was recently developed for the online preconcentration of small organic cations [207]. In this technique, a capillary is conditioned with a separation buffer that contains an organic solvent. An anionic micellar sample solution, which carries the organic cations, is then injected and the separation potential applied. The electrophoretic mobility of the cations when inside the micelles is directed toward the detector. At the interface between the sample solution and the separation buffer, the cations have less affinity for the micelles due to the presence of the organic modifier, and are released. The cations then show reversed electrophoretic mobility (with respect to the micelles) directed toward the capillary inlet, causing the cations to accumulate at the boundary. After all of the micelles have passed through this interface, the analytes are focused into a narrow zone. Guidote and Quirino [208] developed a model for micelle to solvent stacking and also demonstrated the preconcentration of organic anions by using cationic micelles, for which 10-fold peak height enhancement was achieved.

1.4.5 Combinations of Preconcentration Methods

To exploit sample preconcentration to maximum capabilities, some groups have combined complementary preconcentration steps before analysis. Table 1.2 gives examples of combinations of preconcentration methods that use surfactants in at least one step. As can be seen from the table, sweeping is compatible with a wide variety of other preconcentration methods.

1.4.6 Cloud Point Extraction

Although cloud point extraction (CPE) is not a technique that has been integrated online, it is worth mentioning as a preconcentration method amenable to CE. By using the temperature-dependent phase separation behavior of aqueous surfactant solutions, cloud point preconcentration is a

TABLE 1.2 Hyphenated Preconcentration Methods that Require the Use of Surfactants

Techniques	Analytes	Preconcentration Factor	References
Dynamic pH junction + sweeping	Flavin derivatives	1,200	164
	Androgens, corticosteroids, estrogens	30	209
	Pyrrolizidine alkaloids	24–90	210
Field-amplified sample stacking + sweeping	Hypolipidemic drugs	80	211
Full capillary sample stacking + sweeping	Derivatized amino acids	400	212
Hollow fiber-based liquid-phase microextraction + sweeping	*Strychnos* alkaloids	35–50	213
Homogeneous liquid–liquid extraction + sweeping	Polycyclic aromatic hydrocarbons	100,000	214
Micelle to solvent stacking + sweeping	Beta blocker and tricyclic antidepressant drugs	20–50	215
Selective electrokinetic injection with a water plug + sweeping	Phenols	96,000–238,000	201
	Flavonoids	45–194	216
Selective exhaustive injection + sweeping	Laudanosine, 1-napthylamine	550,000–900,000	217
	Aromatic amines	39,000–146,000	218
	Carboxylic acids, dansyl amino acids, naphthalenedisulfonic acids	1,000–6,000	219
	Environmental pollutants	100	220
	Lysergic acid diethylamine, *iso*-lysergic acid diethylamide, lysergic acid *N*,*N*-methylpropylamide	100,000	221
	Lysergic acid diethylamine, *iso*-lysergic acid diethylamide, lysergic acid *N*,*N*-methylpropylamide	100,000	222
	Herbicides (paraquat, diquat, difenzoquat)	50,000	223
	Phenoxy acid herbicides	100,000	224
	Lysergic acid diethylamide	100,000	225
	Reserpine	2,500–3,800	226
	Ephedra alkaloids in herbal extracts	10,000	227
	Corticosterone, 17-hydroxycorticosterone	1,500	228
	Methamphetamine, ketamine, morphine, codeine	6,000 for methamphetamine	229
	Amphetamine, methamphetamine, *p*-hydroxymethamphetamine	Not given; LOD 15–20 ng mL^{-1}	230
	Amphetamine, methamphetamine, methylenedioxymethamphetamine	1,000	231
	Fluorescein, 5-carboxyfluorescein	4,000–4,500	232
	Morphine, codeine, ketamine, methamphetamine	1,000	233
	Amphetamine, methamphetamine, methylenedioxymethamphetamine	2,500–10,000	234
	Tobacco alkaloids	180–540	199
	Morphine and its metabolites	2,500	235
	Serotonin reuptake inhibitors	57,000–120,000	236
	Cocaine and its metabolites	1,750–39,600	237
Single-drop microextraction + sweeping	Fluorescein, 6-carboxyfluorescein	28,000–32,000	238
Solid-phase extraction + sweeping	Testosterone, progesterone, testosterone propionate	700–1,100	239

green alternative to liquid–liquid extractions that use organic solvents. When an aqueous solution of certain nonionic or zwitterionic surfactants is brought to a temperature called the cloud point (specific to the surfactant type and solution conditions), the solution separates into two phases—one layer is clear, and the other is turbid. The clear, surfactant-dilute layer is composed of micelles, and the turbid surfactant-rich layer is composed of larger aggregates that are able to scatter light. The difference in density between each phase drives the separation. Analytes that are solubilized by the micelles in the total volume of solution become extracted into the surfactant-rich layer upon reaching the cloud point,

and are effectively preconcentrated since the volume of the turbid layer is much smaller than that of the clear layer. The surfactant-rich layer with preconcentrated analytes can then be separated from the clear layer by filtration or centrifugation and then analyzed. Carabias-Martinez et al. and Quina and Hinze have reviewed the properties, behavior, and experimental considerations for the cloud point extraction method [240,241]. Some applications of this preconcentration method before CE separation include the extraction and analysis of triazine herbicides [242], Cu(II) and Co(II) [243], Pt and Pd [244], lead [245], mercury species [246], phenol and nitrophenol [247], and auxins [248]. In most of these cases, preconcentration factors of 1 to 2 orders of magnitude were achieved.

1.5 SURFACTANTS AND DETECTION IN CE

The use of surfactants is compatible with most optical detection systems, including UV–Vis absorbance, fluorescence, and thermo-optical absorbance (thermal lensing microscopy) [249,250], provided that the optical properties of the surfactants do not interfere with those of the analytes. With fluorescence detection, the separation efficiency is particularly important to maximize as labels can decrease the structural differentials of analytes, and excess labels and their impurities must be separated from analytes [115]. As described next, in MS and electrochemical detection, surfactants have larger impacts—they can either diminish or improve detection capabilities.

1.5.1 Mass Spectrometry

Coupling CE with MS yields a powerful analytical tool with excellent separation capabilities combined with identification and characterization of analytes. This is a relatively straightforward integration because, in comparison with traditional separation techniques such as gas chromatography and liquid chromatography, CE has the advantage of handling very low sample volumes and extremely low flow rates, which simplifies the interface. Typically, electrospray ionization (ESI) is the interface used to couple CE with MS because it efficiently creates gas-phase ions from nonvolatile solutes. NACE is optimal for hyphenation with MS because the high volatility and relatively low current generated by organic solvents are ideal for ESI [145]. The ionization efficiency and, consequently, detection sensitivity are higher in organic solvents versus aqueous solutions due to lower surface tension of the electrospray droplet and more rapid solvent evaporation. Also, the lower surface tension of organic solvents allows for using lower electrospray potentials, which decreases the likelihood of electric discharges, and thus stabilizes the electrospray [143].

As described throughout the chapter, surfactants are widely used to enhance CE performance. However, their presence in the background electrolyte can greatly impair compatibility with MS detection. Surfactants are nonvolatile, have high surface tension, and decrease ESI efficiency. Rundlett and Armstrong explained that the high concentration of nonvolatile surfactants at the liquid–vapor interface (at the Taylor cone) inhibits the amount and efficiency of droplet formation [251]. Over time, the ion source can become fouled by a build-up of surfactants. Another problem with integration of CE and MS is that surfactants suppress analyte ion signals and increase noise, reducing the sensitivity of the system [252]. Rundlett and Armstrong also stated that Coulombic interactions between oppositely charged surfactant and solute ions suppress analyte ionization by inhibiting the charged analyte ions from transferring from the liquid to the gas phase [251]. The high ionic strength of the background electrolyte resulting from the inclusion of surfactants can also lead to high currents during separation, which then leads to unstable electrospray conditions [253].

Overcoming the compatibility issues of surfactants with MS has been an aim since the 1990s [254], and strategies continue to be sought after today [255]. The compatibility of surfactant-based separations with MS can be increased by using more ESI-friendly surfactants such as high-molecular-weight/polymeric surfactants or semivolatile surfactants, by avoiding the entrance of surfactants into the ESI interface, or by using alternate ionization strategies before MS. The basis of each strategy will be described, but for more specific examples, the reader is referred to a review that has thoroughly tabulated applications [255].

As mentioned earlier, polymeric or high-molecular-weight surfactants are large molecules that aggregate into micelles at an effective CMC of zero, while still retaining the ability to solubilize analytes and provide a pseudostationary phase for MEKC. Because very low concentrations are required for separation, noise generated by ionized surfactants is minimized. The ions that do form from high-molecular-weight surfactants fall out of the mass range covered by the spectrometer, leading to increased signal to noise ratios (compared to low-molecular-weight surfactants) [255]. Again, Rundlett and Armstrong suggested that high-molecular-weight surfactants are not very surface active and hence do not inhibit electrospray efficiency to the same degree as lower-molecular-weight surfactants [251]. An additional advantage of polymeric surfactants is that they can be used in conjunction with high amounts of organic modifier to tune chromatographic selectivity without disintegration of the micellar structure [256]. Some examples of such surfactants include butyl acrylate–butyl methacrylate–methacrylic acid copolymer [257], poly(sodium undecylenic sulfate) [258] and a suite of chiral molecular micelles—poly(sodium *N*-undecanoyl-L-valinate) [259], poly(sodium *N*-undecenoxy

carbonyl-L-leucinate) [260–262], poly(sodium *N*-undecenoxy carbonyl-L,L-leucyl-valinate) [263], poly(sodium *N*-undecenoyl-L,L-leucyl-valinate) [264], poly(sodium *N*-undecenoyl-L-leucine sulfate), poly(sodium *N*-undecenoyl-L-valine sulfate), and poly(sodium *N*-undecenoyl-L-isoleucine sulfate) [265]—investigated by the Shamsi group for enantioseparations.

Using semivolatile fluorinated surfactants has been shown to decrease the build-up of surfactant concentration at the liquid–gas interface, avoiding decreases in electrospray efficiency. Ishihama et al. demonstrated that decreases in sensitivity caused by more commonly used MEKC surfactants are not experienced when using perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid [266]. Petersson et al. demonstrated that signal suppression was not a problem even when using 100 mM PFOA. This group also showed that the separation performance of PFOA was comparable to that of SDS, and that only the selectivity was different [267]. Van Biesen and Bottaro also demonstrated a successful MEKC–ESI–MS analysis using ammonium perfluorooctanoate as a surfactant [268]. Though promising, semivolatile surfactants have surprisingly not been thoroughly explored as a simple solution to the surfactant–ESI compatibility issue.

Much focus has been directed toward methods in which surfactant introduction into the ESI interface is totally avoided, namely partial-filling and reverse-migration MEKC. As described previously, in partial-filling MEKC, the separation capillary is filled with background electrolyte. Then, a small plug of micellar solution is injected, followed by the sample plug. On application of the separation potential, analytes migrate through the micellar zone and separate based on MEKC principles. After passing through this zone, the analytes then sequentially migrate through the micelle-free background electrolyte to the detector. The separation potential is terminated promptly after detection of the analytes to prevent surfactants from entering the ESI–MS system [269]. Drawbacks of the partial filling technique include lower separation efficiencies, lower resolution, and lower peak capacity, when compared to traditional MEKC [270]. The differences in electric field strength and in viscosity between the micellar plug and the background electrolyte cause laminar flows, leading to band broadening. Additionally, the reproducibility of the method is questionable.

In reverse-migration MEKC, the capillary is filled with a micellar background electrolyte. The pH is adjusted so that there is a low EOF toward the detector on application of the separation potential. Meanwhile, during the separation, the micelles move toward the capillary inlet, avoiding introduction into the ESI–MS interface. Typically, negatively charged surfactants are used in a low pH buffer. In these cases, the applicability of the technique is limited to positively charged analytes since negatively charged analytes will not have an overall mobility toward the detector. With a high enough EOF and a relatively low affinity for the micelles, neutral analytes can also be detected [255]. As mentioned, specific applications of both the reverse-migration and the partial-filling techniques have thoroughly been reviewed [255].

Alternatives to ESI, atmospheric pressure chemical ionization (APCI) and atmospheric pressure photoionization (APPI) are not inhibited by the presence of nonvolatile buffer constituents, including surfactants [271–275]. This is because in both the methods, the sample is vaporized in a heated nebulizer before being ionized; therefore, nonvolatile buffer constituents are left behind and do not decrease ionization efficiency as in ESI [274,276]. Mol et al. showed that even up to 60 mM SDS does not cause suppression of analyte signals, excess background noise, nor affects the stability of the APPI interface performance [277]. Himmelsbach et al. [278,279] and Schappler et al. [280] carried out MEEKC separations, which use even higher concentrations of surfactants than MEKC, with no impact on APPI-MS performance. APCI and APPI have the disadvantage of lower sensitivity [272,273,275]; however, coupling these ionization techniques with CE has only recently been attempted and further research will likely lead to improved interfaces.

1.5.2 Electrochemical Detection

Electrochemical detection (ECD) techniques such as amperometry and conductivity are commonly coupled to CE and microchip-CE. An integrated electrode offers a liquid–solid interface for surfactants in solution to interact with, as described in Section 1.2. An important characteristic of an electrode surface in particular is that the potential applied to the electrode determines the surface charge. This, in turn, can induce changes in the structure of the aggregations adsorbed to the surface [13,281–283]. For example, Burgess et al. showed that with small-to-moderate absolute charge density, SDS surfactant monomers aggregate into hemicylindrical stripes on the electrode surface. If the applied potential is increased to equal or superequivalent to the charge of the surfactants, these aggregations undergo a phase transition into a more condensed state (the surface concentration of SDS doubles), probably in an interdigitated bilayer structure (Figure 1.2) [283]. The transition between the hemicylindrical aggregations and the condensed state was shown to be reversible [281]. In contrast to the adverse effects on MS detection, surfactants actually have been shown to enhance the performance of electrodes [284].

One mechanism of enhancement occurs when adsorbed surfactants electrostatically preconcentrate analytes onto the surface of the electrode. For instance, when the positively charged surfactant CTAB was adsorbed to a glassy carbon electrode, the concentration of negatively charged dinitrophenols on the electrode was increased via

electrostatic attraction and the reduction peak currents were enhanced, increasing detection sensitivity [285]. Similarly, redox peak currents were increased due to enhanced accumulation of sodium nitroprusside on a CTAB-coated electrode [286]. With a negatively charged SDS layer hydrophobically adsorbed to the surface of a carbon paste electrode, the response of dopamine was enhanced while at the same time, a negatively charged interferant (ascorbic acid) was repelled [287]. Many other instances of this electrostatic preconcentration onto electrodes can be found in the literature [286,288–298]. Hu et al. found that a Nafion-modified electrode, which contains $-SO_3^-$ groups, adsorbed positively charged CTAB through ion exchange. This surfactant allowed for preconcentration of estradiol, estrone, and estriol by hydrophobic interactions, improving the electrochemical reaction between analyte and electrode and increasing sensitivity [299]. Other hydrophobic analytes such as diethylstilbestrol have been detected with improved sensitivity as a result of surfactants [12,300]. To systematically study the effects of surfactants on signal enhancement ECD, Ding et al. analyzed six phenolic compounds in the presence of four different surfactants. The hypothesis presented was that the electrochemical method used—pulsed amperometric detection (PAD)—promotes the formation of hemimicellar aggregates on electrode surfaces [282,283]. After computationally calculating the partial charges and dipole moments, it was determined that the analytes that have a surfactant-like structure have enhanced analytical signals, due to ease of incorporation into the hemimicelles [301]. In all of these examples, the aggregation of surfactants on the electrode surface resulted in enhanced redox peak currents of analytes by facilitating electron exchange. The high concentration of the analyte and proximity to the electrode surface increases the electron transfer rate, decreasing the overvoltage of the electrode [287,288]. Additionally, the surfactant microstructures may increase the stability of electrogenerated radicals, intermediates, and products [12,284,302–305].

In conjunction with the use of surfactants, PAD has also exhibited improvements in the electrochemical response of carbohydrates [54,306], biomarkers [307], metabolites and biomolecules (glucose, penicillin, phenol, homovanillic acid) [38,306], and antioxidants [308]. PAD shows a greater enhancement by the presence of surfactants than DC amperometry. The higher electrode potentials likely allow a greater amount of surfactant to adsorb to the electrode surface. These effects can be seen even when the concentration of surfactant is below the CMC value [282,283]. PAD may also show better response because of the repeated cycling of applied potentials that can induce repeated adsorption and desorption of surfactant aggregates. This allows enhanced response at the detection by providing a clean electrode surface with no oxidation products as well as fresh surfactant aggregates capable of preconcentration [309–311]. Surfactants have been demonstrated to stabilize electrodes during the analysis of highly selfpassivating analytes such as phenol, tyramine, tyrosine, serotonin, tryptophan, and 3,4-dihydroxyphenyl acetic acid [312,313]. Surfactants are believed to prevent electrode fouling by competitive adsorption with excessive oxidation products in addition to electrostatic repulsion of these products from the electrode surface. Similarly, surfactants have been shown to suppress protein interference in ECD by competitive adsorption. This has allowed the analysis of cadmium and lead in the presence of albumin and lysozyme and lead and copper in milk powder [314–316]. In summary, the combination of surfactant-modified CE separations with surfactant-enhanced ECD is simple, yet highly advantageous. This has proven particularly important in microchip-CE–PAD as surfactants can control/stabilize the EOF of the polymeric substrates of the microchips, while at the same time improving the detection.

1.6 CONCLUSIONS

This chapter summarizes different approaches that take advantage of the unique structure and properties of surfactants to improve analysis in CE. As described, surfactants can be used to coat the capillary surface, allowing a simple way to control EOF and minimize adsorption of analytes and sample matrix to the capillary wall. When used as buffer additives, surfactants can improve the separation of hydrophobic analytes, allowing the development of a wide variety of separation modes including MEKC, MEEKC, and NAMEKC. Surfactants can also be used to improve the sensitivity of CE by enabling preconcentration of analytes by sweeping, transient trapping, focusing by micelle collapse, micelle-to-solvent stacking, and cloud point extraction. Last but not least, the performance and selectivity of some detection modes can be improved by the addition of surfactants. The addition of surfactants is one of the most versatile alternatives to further improve the performance of CE. Theoretical aspects, advantages, and guidelines to rationally design CE methods that employ various surfactants have been herein described. The use of surfactants in CE, however, seems to be mostly limited by cost and commercial availability. Consequently, most researchers seem inclined to develop applications based on the more traditional surfactants (such as SDS or CTAB). It is also interesting to see that in most cases, reports describe the use of surfactants only for specific tasks in the analytical procedure without necessarily considering the effects on multiple steps of the analysis. In summary, we believe that the use of surfactants in CE will continue to grow, despite the aforementioned limitations. As new types of surfactants become available to researchers, innovative applications will continue to push the limits of CE in terms of performance and ability to handle real samples.

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