



Fundamentals of micellar electrokinetic chromatography (MEKC)

Syed Asad Ali Rizvi^{a,*}, Duc Phuc Do^b and Ayman Mahmoud Saleh^c

^a School of Pharmacy, Lake Erie College of Osteopathic Medicine (LECOM), Erie, PA 16509, USA

^b Department of Pharmaceutical Sciences, College of Pharmacy, Chicago State University, Chicago, IL 60628, USA

^c Department of Biochemistry and Molecular Medicine, School of Medicine, Lake Erie College of Osteopathic Medicine (LECOM), Erie, PA 16509, USA

*Corresponding author at: School of Pharmacy, Lake Erie College of Osteopathic Medicine (LECOM), Erie, PA 16509, USA. Tel.: +1.814.866.8405; fax: +1.814.860.5123. E-mail address: drasad77ce@gmail.com (S. Rizvi).

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ABSTRACT

Micellar electrokinetic chromatography (MEKC) is a useful branch of capillary electrophoresis (CE) that utilizes surfactant above critical micelle concentration (CMC) as pseudo-stationary phase. MEKC can be employed to separate both charged and neutral molecules, individually or simultaneously, including chiral compounds. MEKC benefits from high peak efficiency due to electroosmotic flow (EOF) in the separation capillary, compounded with large variety of synthetic surfactants, organic modifiers, temperature and variable separation voltage has made MEKC the method of choice for separation scientists. In this review, we present the introduction of CE, fundamentals of surfactant chemistry as it relates to MEKC, separation principles in MEKC including equations involved in calculating separation parameters (capacity factor, resolution etc.).

1. Introduction

According to the International Union of Pure and Applied Chemistry (IUPAC), chromatography can be described as: "A physical method of separation in which the components to be separated are distributed between two phases, one of which is stationary (stationary phase) while the other (the mobile phase) moves in a definite direction" [1]. The mobile phase flow can be controlled by either gravity (e.g., column chromatography), by applying pressure (e.g., high pressure liquid chromatography) and by electricity (e.g., electrophoresis).

Capillary electrophoresis (CE) is an electro-driven separation techniques, it calls for low reagent consumption, high efficiency and selectivity with reasonably short analysis time. In CE, the capillary is filled with a suitable buffer and after injecting analytes from the anode side (under normal polarity conditions), a high voltage is applied at its both ends (Figure 1).

The analytes (positively or negatively charged) will move with different velocity and can be separated based on their electrophoretic mobility. However, in case of neutral molecules, since they do not bear any charge, move with the solvent front and elute as a single band and thus, cannot be separated. To solve this problem, charged surfactants above their critical micelle concentration (CMC) are added in the CE running buffer, which allows separation of uncharged molecules along with the charged ones. Surfactants in general, comprised of a hydrophobic portion, usually a long alkyl chain, attached to hydrophilic or water soluble functional groups. Before we further discuss and explore the micellar electrokinetic chromatography (MEKC), it will be fruitful to briefly mention the history and timeline of the development of this technique.

2. Electrophoresis and capillary electrophoresis

In early 1930s, Arne Tiselius developed the "moving boundary" method to separate serum proteins in solution that was later named as "zone electrophoresis" [2]. This was rightly considered as the birth of modern electrophoresis, since then, various electrophoresis modes (moving boundary electrophoresis, zone electrophoresis, isoelectric focusing, and isotachopheresis) became popular in the 1940s and 1950s. In 1981, Jorgenson and Lukas [3,4] demonstrated highly efficient electrophoresis separations by performing electrophoresis in narrow-bore capillaries filled with buffer, normally in the range from 25 to 100 μm of internal diameter (i.d). As mentioned earlier, neutral analytes cannot be separated by simple electrophoresis experiment, the charged surfactants above their CMC were used by Terabe *et al.* [5] in the CE running buffer. The micelle formed, allowed separation of uncharged molecules along with the charged ones based on hydrophobic affinity of the neutral molecules for the micelle. In the pioneering experiments by Terabe *et al.* [6], anionic micelles were used as a pseudostationary phase to separate neutral compounds.

2.1. CE Instrumentation

A simple schematic of a standard CE instrument and its components are shown in Figure 2. A typical CE instrument consists of a high-voltage power supply (up to 30 kV), fused silica capillary externally coated with polyimide (to impart flexibility) with an internal diameter (I.D) ranging from 20 to 200 μm , two buffer reservoirs that house the capillary ends, two electrodes connected to the power supply, and detector (usually ultra violet).

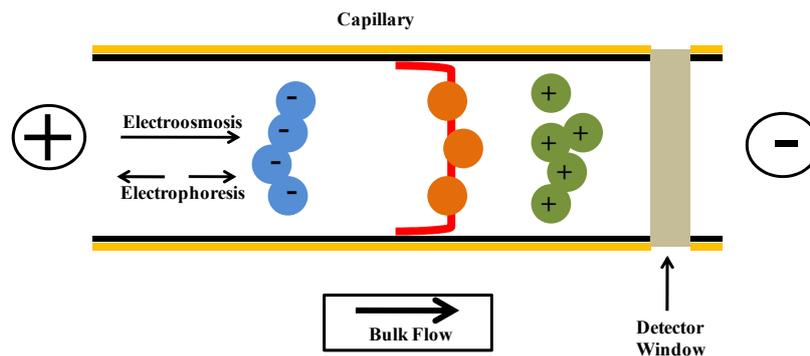


Figure 1. Separation principle of capillary electrophoresis.

To perform a CE experiment, the capillary is filled with a desired electrolyte solution (a buffer). Next, the sample is injected (from the anode side) and both ends of the capillary and the electrodes are placed into buffer reservoirs; finally voltage is applied across the capillary to start electrophoresis.

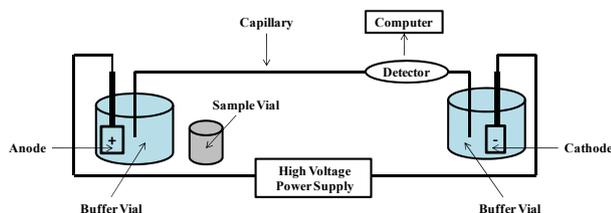


Figure 2. A simple schematic of a standard CE instrument.

2.2. Micellar electrokinetic chromatography

Micellar electrokinetic chromatography, at the beginning was sought to separate mainly neutral compounds. Currently, due to increased availability of variety of surfactants, MEKC is used to separate both neutral and charged compounds even with similar electrophoretic mobilities. What makes MEKC the most useful among the CE techniques is the fact that separation of the analytes can be obtained due to difference in electrophoretic mobilities, as well as differences in solute partitioning in the micelle [6]. Also, one instrument can be used for various modes of CE. Since MEKC involves the use of surfactants (above CMC), it is worthwhile to go over some fundamentals of surfactant chemistry as well.

3. Surface-active agents (Surfactants)

The surfactants are amphiphilic in nature and are miscible with both polar and apolar substances. A typical amphiphilic molecule itself consists of polar (hydrophilic) group (e.g., alcohol, ether, carboxylate, sulfate, sulfonate, phosphate, amine, ammonium etc) and apolar (hydrophobic) group (e.g., usually a long hydrocarbon chain) as represented in Figure 3.

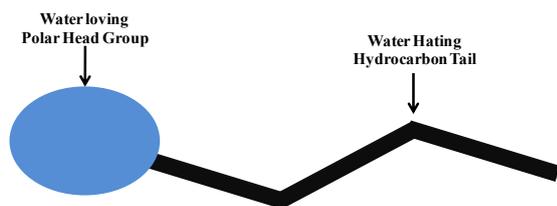


Figure 3. An amphiphilic molecule.

The hydrophilic portion exhibits a strong affinity for water, while the hydrophobic part tends to accumulate together (hydrophobic effect) due to mutual antipathy for water [7,8]. Because amphiphiles conation both water loving and repelling groups, they often tend to migrate at the interface of an aqueous solution, such that hydrophilic part is in water and hydrophobic part away from water (in the air) as represented in Figure 4. Due to accumulation at the air-water interface, the surface tension of water drops and these molecules are accordingly dubbed, surface active agents. There are many substances, such as medium- or long-chain alcohols that are surface active (e.g., *n*-hexanol, dodecanol) but they are not considered as surface-active amphiphiles (surfactants). Specifically, surfactants are distinguished by self-assembly structures (micelles, vesicles) in bulk phases [9-15] and ability to form oriented monolayers at the interface. Surfactants are also responsible for the fundamental physical effects, such as, wetting, dispersion or deflocculation and emulsification. Alternatively, surfactants interfere with the ability of the molecules of a substance to interact with one another (specially at the interface) and thereby, lower the surface tension of the substance [16].

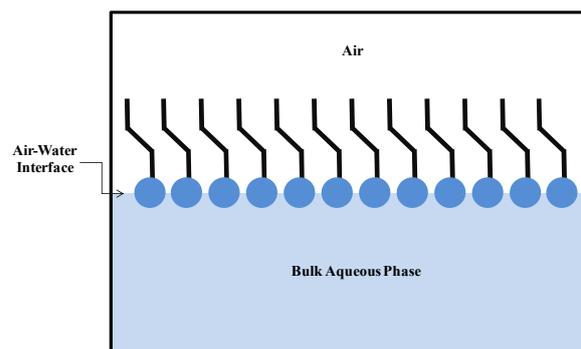


Figure 4. Surfactants at the air-water interface.

3.1. Classification of the surfactants

Surfactants are characterized based on the charge present in the hydrophilic portion of the molecule (after dissociation in aqueous solution). There are four categories of surfactant: (1) anionic, (2) cationic, (3) nonionic and (4) zwitterionic [17-19]. Anionic surfactants, when dissolve in water, dissociate into hydrocarbon chain bearing anion (e.g., -COO^- , -SO_3^- , -PO_4^{3-} , -SO_4^{2-}), and a counter cation (e.g., Na^+ , K^+) and are the most commonly used type of surfactants [20,21].

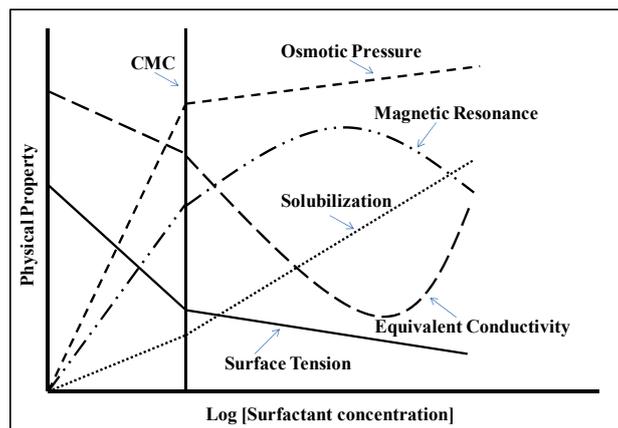


Figure 5. Measurement of CMC by plotting various solution properties against logarithm of surfactant concentration.

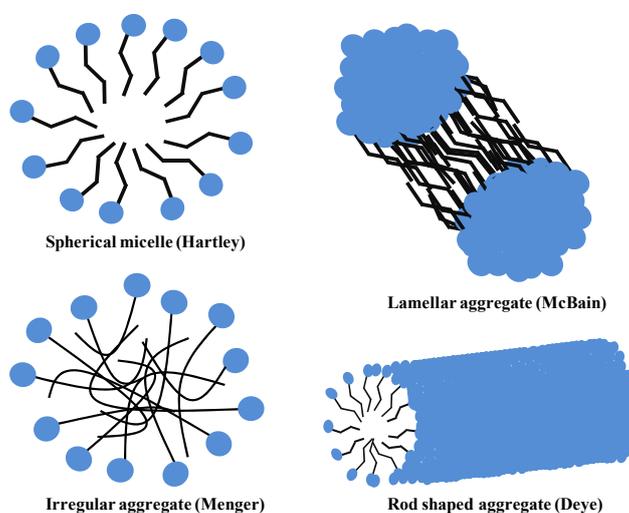


Figure 6. Various proposed structures of the micelle.

Cationic surfactants on the other hand, when dissolved in water, dissociate into hydrocarbon chain bearing cationic head group [e.g., $(R)_4N^+$, $(R)_4P^+$] and a counter anion (e.g., Cl^- , Br^-). A very large proportion of this class corresponds to fatty amine salts and quaternary ammoniums, with one or several long chain of the alkyl type, often coming from natural fatty acids. The quaternary ammonium group containing surfactants are well known for displaying emulsifying properties, antimicrobial activity, anti corrosive effects and are used in cosmetic formulations and as phase transfer catalyst in organic synthesis [21-23]. Zwitterionic surfactants contain both anionic and cationic portion within the surfactant backbone and are also known as amphoteric surfactants. Some zwitterionic surfactants stay zwitterionic at all pH, while few are cationic at low pH and anionic at high pH. They are generally quite expensive as they are not very easy to make and thus are used in special circumstances, for instance in cosmetics, due to high biological compatibility and low toxicity [24-27]. Nonionic surfactants, as name indicates, are devoid of charges. The hydrophilic group usually is alcohol, phenol, ether, ester or amide. Large proportions of these nonionic surfactants are hydrophilic by the presence of a polyethylene glycol chain and are called polyethoxylated nonionics. Sugar-derived nonionic surfactants are also in use as they exhibit very low toxicity and good have excellent biodegradability [21,28-29].

3.2. Critical micelle concentration and aggregation of surfactants

When surfactant is dissolved in water and if one of the solution properties (surface tension, osmotic pressure, electrical conductivity, solubility etc) is monitored and plotted against logarithm of concentration at a particular temperature, the resulting graph would show an abrupt change at a concentration specific for particular surfactant. This concentration usually is referred to as critical micelle concentration (Figure 5).

The hydrophobic portion of the surfactant disrupts the hydrogen-bonded structure of water and therefore increases the free energy of the system. Hence, when surfactants are dissolved in aqueous medium, they spontaneously form supramolecular aggregates of various shapes [30-33], few of them are shown in Figure 6.

McBain [34,35] was the first to study the dilute aqueous solution of the sodium salts of fatty acids and later, in a similar study by Hartley [36,37], reported the unusual behavior of the aqueous solution of these surfactants. When surfactants, after reaching a certain concentration in aqueous solution, form aggregate that can adopt a huge variety of shapes and sizes,

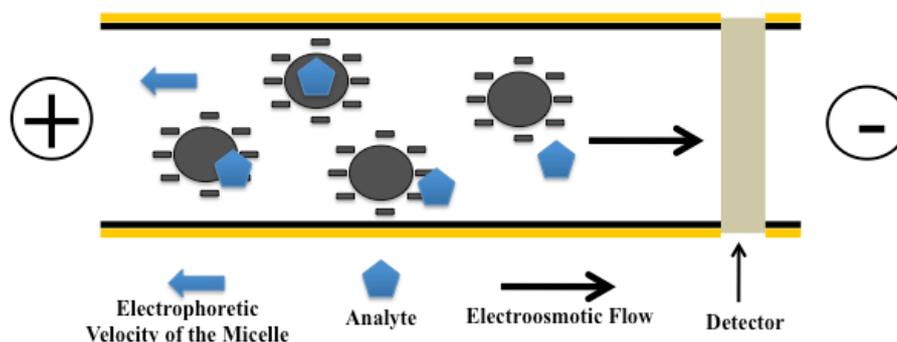


Figure 7. Separation principle of MEKC under normal polarity conditions.

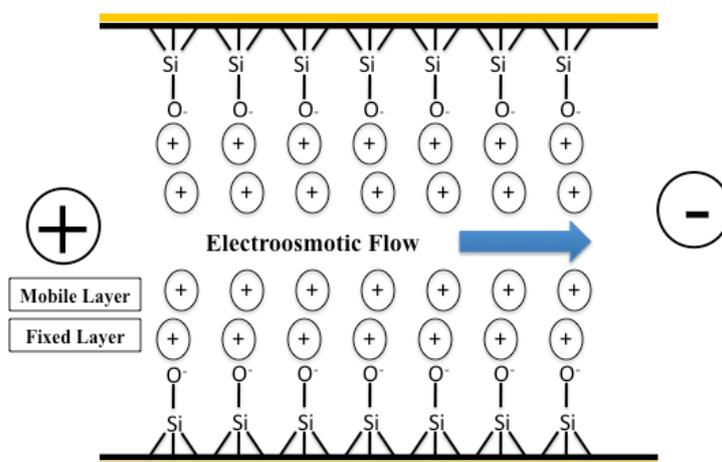


Figure 8. Generation of electroosmotic flow inside the capillary under normal conditions.

depending on the chemical properties and concentration of the surfactant molecules, co-solvents, pH, as well as temperature and pressure. However, it is important to note that these aggregates are dynamic structures (i.e., individual molecules can leave and re-join the aggregate). Several models have been put forward to explain the shape of surfactant aggregates (Figure 6). According to McBain [38], spherical and lamellar micelles coexist in the aqueous surfactant solutions. Debye and Anacker [39] proposed, micelles exist as rod rather than spherical or disk-like shape. Hartley [36,37] proposed, micelles are spherical having charged groups located at the micellar surface and hydrocarbon tail in the interior. Based on nuclear magnetic resonance (NMR) and kinetic studies, Prof. Fred Menger (Emory University) proposed a more realistic structure of a micelle, being more disorganized with nonradial distribution of chains and chain looping. Menger's NMR studies revealed that micelles have rough surface, water-filled pockets and bent chain loops with significant deviations from exact spherical shape [40].

4. Separation principles in MEKC

The MEKC techniques rely upon the differential partitioning of an analyte between a biphasic system (aqueous and micellar). Figure 7 shows a schematic representation of the separation principle of MEKC. When an anionic surfactant such as sodium dodecyl sulfate (SDS) is employed, the micelle migrates toward the anode (injection end) by electrophoresis.

The EOF transports the bulk solution toward the negative electrode due to the negative charge on the surface of fused silica. Since the EOF is usually stronger than the electrophoretic mobility of the micelle, under alkaline conditions, the anionic micelle also travels toward the cathode (detection end) with much slower velocity and hence acts like a stationary phase ("pseudo" indicates this fact). The EOF is generated by application of electricity across the buffer filled capillary (usually made of fused silica) bearing a negative charge on its interior wall. The silanol groups (Si-OH) inside the capillary acquire negative charge (Si-O⁻) after flushing with a strong base (usually NaOH) at slightly elevated temperature (usually 50 °C). When a buffer is flushed inside the capillary, cations accumulate on the surface of the capillary (Figure 8). The cations in direct contact with the capillary wall are strongly adsorbed and their layer is called fixed layer while other cationic array immediately adjacent is referred to as mobile layer. The cations in the mobile layer are pulled strongly by the cathode under applied voltage. Since these cations are solvated, the whole buffer solution moves with the mobile layer resulting in EOF.

When analyte is injected into the micellar solution, a fraction of it is incorporated into the micelle and it migrates at the velocity of the micelle (denoted by t_{mc}). The remaining fraction of the analyte remains free from the micelle and migrates either with the electroosmotic velocity (denoted by t_0) or with its own electrophoretic mobility (charged analyte). The greater the percentage of analyte that is distributed into the micelle, the slower it migrates. Other analytes are detected

between t_0 and t_{mc} . The interval between t_0 and t_{mc} is called the migration time window. The wider this window, the larger the peak capacity, which is the number of peaks that can be separated during a run. Migration time can be measured by using markers such as methanol for EOF and dodecanophenone for the micelle [41-44]. In general, three types of solute-micelle interactions are possible: (1) the solute is adsorbed on the surface of the charged micelle by electrostatic, hydrogen bonding or any other polar interactions (2) the solute is solubilized somewhere at the interface between hydrophobic/hydrophilic region of the surfactants (palisade layer); and (3) solute is penetrated deep into the core of the surfactant by strong hydrophobic interactions (Figure 9).

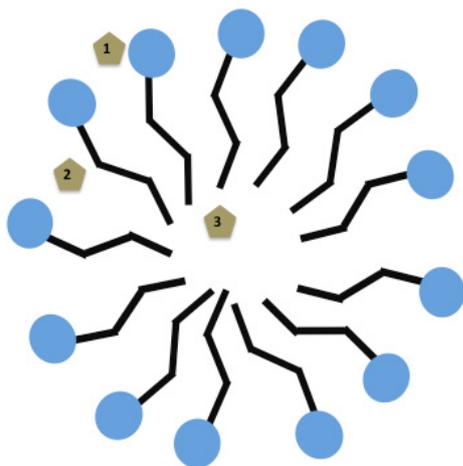


Figure 9. Interactions between micelle and an analyte: (1) on the surface, (2) in the palisade layer, and (3) in the core.

5. Polymeric surfactants

The past two decades have seen the introduction of a new class of surface active substances, referred to as polymeric surfactants or surface active polymers, resulted from the association macromolecular structures exhibiting hydrophilic and lipophilic characters, either as separated blocks or as grafts. They are now commonly used in formulating products such as cosmetics, paints, foodstuffs, and petroleum production additives. Polymeric surfactants [45-46] have gained popularity as potential pseudostationary phases for separations in MEKC in the recent years [47-52]. A considerable interest in the use of polymeric surfactants arises because of their distinct advantages over conventional micelles. First, they have zero CMC; thus, they may be used at concentrations well below the CMC of the unpolymerized surfactants. Second, molecular micelles are stable in the presence of a high content of organic solvents due to the covalent bond between surfactant monomers. Hence, organic additives do not disrupt the primary covalent structure of the micelle polymer. The use of organic solvents in combination with micelles is often required for the analysis of various compounds. Third, the fixed micellar structure prevents dissociation of surfactant molecules during the electrospray process in mass spectrometry (MS). Therefore, due to their high molecular weight, molecular micelles can be conveniently used in MEKC-MS applications without background interference from surfactant monomers of low molecular weights. Fourth, lower surface activity and low volatility of molecular micelles provide a stable electrospray and hence less suppression of analyte signal in MEKC-MS [53]. Finally, an important advantage of polymeric surfactants is the improved mass transfer of solutes in and out of the polymeric surfactant resulting in shorter analysis time and improved signal to noise (S/N) ratio [54,55].

6. Separation parameters in MEKC

General chromatography parameters can be employed to describe the migration parameters of the analyte in MEKC [41,56]. The capacity factor, k' , in MEKC is defined as the ratio of total number of moles of the analyte in the micelle (n_{mc}) and the total number of moles in bulk aqueous (n_{aq}) phase:

$$k' = \frac{n_{mc}}{n_{aq}} \quad (1)$$

The capacity factor (k') is related to the analyte and micelle migration parameters by the following relationship:

$$k' = \frac{t_R - t_0}{t_o \left(1 - \frac{t_R}{t_{mc}}\right)} \quad (2)$$

In the above equation, t_R , t_0 , t_{mc} represent the analyte migration time, EOF marker (usually methanol) and micelle marker (usually dodecanophenone), respectively. In case, if a polymeric surfactant migrates at a velocity much larger than the EOF (i.e., $t_{mc} \gg t_0$), the retention time of the most retained analytes approaches infinity ($t_{mc} \rightarrow \infty$). Hence, the term $(1 - t_R/t_{mc})$ in the denominator of the above equation is negligible and the equation reduces to:

$$k' = \frac{t_R - t_0}{t_o} \quad (3)$$

The resolution (R_s) equation in MEKC is related to selectivity (α), capacity factor (k') and efficiency (N) by the following relationship:

$$R_s = \frac{\sqrt{N}}{4} \cdot \frac{\alpha - 1}{\alpha} \cdot \frac{k'_2}{1 - k'_2} \cdot \frac{1 - \frac{t_0}{t_{mc}}}{1 + \left(\frac{t_0}{t_{mc}}\right)k'_1} \quad (4)$$

In MEKC, the analytes must migrate at a velocity between the electroosmotic velocity and the velocity of the micelle, provided the analyte is electrically neutral. In other words, the migration time of the analyte, t_R , is limited between the migration time of the bulk solution, t_0 , and that of the micelle, t_{mc} (Figure 10). This is often referred to in the literature as the *migration time window* in MEKC. As the ratio of elution window (t_{mc}/t_0) increases, the peak capacity also increases in a logarithmic fashion. Thus, increasing the elution range increases the resolving power of MEKC.

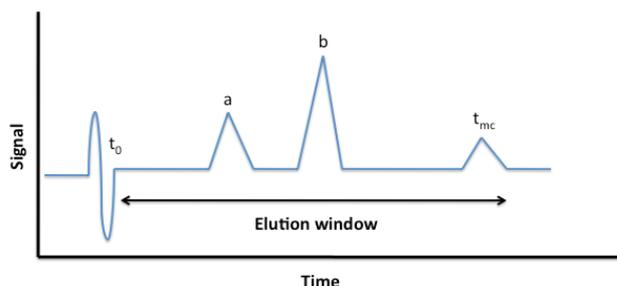


Figure 10. Schematic illustration of a hypothetical MEKC electropherogram of analytes (a and b) showing the elution window, where markers for the EOF (t_0) and micelle (t_{mc}) elution times have been added to the background electrolyte (BGE).

7. Conclusion

MEKC remains a method of choice due to its versatile applications and virtually unlimited supply of the stationary phases. The use of synthetic surfactants provides a leverage to tune the selectivity and resolution by varying structural features of the surfactants and to grasp insight into the separation mechanisms. By attaching an enantiomer in the head group, one obtains chiral surfactants, which are used for separating racemic mixtures. We believe, in the years to come, MECK will achieve its true potential and become a first line of separation technique employed.

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References

- [1]. <http://old.iupac.org/goldbook/C01075.pdf>
- [2]. Tiselius, A. The Moving Boundary Method of Studying the Electrophoresis of Proteins, Ph.D. Thesis, Nova Acta Regiae Societatis Scientiarum Upsaliensis, Ser. IV, Vol. 17, No.4, Almqvist and Wiksell, Uppsala, Sweden, 1930.
- [3]. Jorgenson, J. W.; Lukacs, K. D. *Clin. Chem.* **1981**, *27*, 1551-1553.
- [4]. Jorgenson, J. W.; Lukacs, K. D. *J. High Resolut. Chromatogr.* **1981**, *4*, 230-231.
- [5]. Terabe, S.; Otsuka, K.; Ando, T. *Anal. Chem.* **1989**, *61*, 251-260.
- [6]. Terabe, S.; Otsuka, K.; Ando, T. *Anal. Chem.* **1985**, *57*, 834-841.
- [7]. Barret, D. G.; Gellman, S. H. *J. Am. Chem. Soc.* **1993**, *115*, 9343-9344.
- [8]. Fuhrhop, J. H.; Gellman, S. H. *Acc. Chem. Res.* **1986**, *19*, 130-137.
- [9]. Clint, J. H. *Surfactant Aggregation*, Blackie & Sons, Glasgow, 1992.
- [10]. Gill, S. J.; Wadso, I. *Proc. Natl. Acad. Sci. USA.* **1976**, *73*, 2955-2958.
- [11]. Tanford, C. *The hydrophobic effect: formation of micelles and biological membranes*, John Wiley & Sons, New York, 1980.
- [12]. Lange, K. R. *Surfactants: A Practical Handbook*, Hanser Gardner Publications, Ohio, 1999.
- [13]. Rosen, M. J. *Surfactants and Interfacial Phenomena*, Wiley-Interscience, NY, 2004.
- [14]. Notter, R. H. *Lung Surfactants: Basic Science and Clinical Applications*, CRC, Boca Raton, FL, 2000.
- [15]. Kallay, N. *Interfacial Dynamics*, CRC, Boca Raton, FL, 2000.
- [16]. Tsujii, K. *Surface Activity: Principles, Phenomena, and Applications (Polymers, Interfaces and Biomaterials)*, Academic Press, San Diego, CA, 1998.
- [17]. Myers, D. *Surfactant Science and Technology*, John Wiley & Sons, NJ, 2005.
- [18]. Holmberg, K.; Jönsson, B.; Kronberg, B.; Lindman, B. *Surfactants and Polymers in Aqueous Solution*, John Wiley & Sons, NY, 2002.
- [19]. Jones, M. N.; Chapman, D. *Micelles, Monolayers, and Biomembranes*, Wiley-Liss, NY, 1994.
- [20]. Stache, H. W. *Anionic Surfactants*, CRC, Boca Raton, FL, 1995.
- [21]. Van Os, N. M.; Haak, J. R.; Rupert, L. A. M. *Physico-Chemical Properties of Selected Anionic, Cationic and Nonionic Surfactants*, Elsevier Science Pub Co, NY, 1993.
- [22]. Rubingh, D. *Cationic Surfactants*, CRC, Boca Raton, FL, 1990.
- [23]. Cross, J. *Cationic Surfactants*, CRC, Boca Raton, FL, 1994.
- [24]. Schramm, L. L. *Surfactants: Fundamentals and Applications in the Petroleum Industry*, Cambridge University Press, Cambridge, UK, 2000.
- [25]. Holmberg, K. *Novel Surfactants: Preparation, Applications, and Biodegradability*, CRC, Boca Raton, FL, 2003.
- [26]. Lomax, E. G. *Amphoteric Surfactants*, CRC, Boca Raton, FL, 1996.
- [27]. Rieger, M. *Surfactants in Cosmetics*, CRC, Boca Raton, FL, 1997.
- [28]. Schick, M. J. *Nonionic Surfactants*, CRC, Boca Raton, FL, 1987.
- [29]. Van Os, N. M. *Nonionic Surfactants*, CRC, Boca Raton, FL, 1997.
- [30]. Patist, A.; Kanicky, J. R.; Shukla, P.; Shah, D. O. *J. Colloid Interface Sci.* **2002**, *245*, 1-15.
- [31]. Hiemenz, P. C.; Rajagopalan, R. *Principles of Colloid and Surface Chemistry*, Dekker, New York, 1997.
- [32]. Hunter, R. J. *Foundations of Colloid Science*, Oxford Univ. Press, New York, 1987.
- [33]. Tanford, C. *The Hydrophobic Effect. The Formation of Micelles and Biological Membranes*, Wiley, New York, 1980.
- [34]. McBain, J. W. *Trans. Faraday Soc.* **1913**, *9*, 99-101.
- [35]. McBain, J. W.; Salmon, C. S. *J. Am. Chem. Soc.* **1920**, *42*, 426-436.
- [36]. Hartley, G. S. *Aqueous Solutions of Paraffin-Chain Salts*, Hermann, Paris, 1936.
- [37]. Hartley, G. S.; Collie, B.; Samis, C. S. *Trans. Faraday Soc.* **1936**, *32*, 795-815.
- [38]. McBain, J. W.; Martin, H. E. *J. Chem. Soc.* **1914**, *105*, 957-977.
- [39]. Debye, P.; Anacker, E. W. *J. Phys. Colloid. Chem.* **1951**, *55*, 644-655.
- [40]. Menger, F. M. *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 1086-1099.
- [41]. Terabe, S. *Anal. Chem.* **2004**, *76*, 240A-246A.
- [42]. Sandra, P.; Vindevogel, J. *Introduction to Micellar Electrokinetic Chromatography*, Huthig Pub Ltd, Verlag, Heidelberg, 1992, pp 43-67.
- [43]. Khaledi, M. G. (Ed.), *High-Performance Capillary Electrophoresis: Theory, Techniques, and Applications*, Wiley-Interscience, New York, 1998, pp 77-131.
- [44]. Lander, J. P. (Ed.), *Handbook of Capillary Electrophoresis*, Second Edition, CRC-Press, Florida, 1996, pp 49-74.
- [45]. Piirma, I. *Polymeric Surfactants*, Marcel Dekker, Inc, New York, 1992.
- [46]. Gambogi, R. J.; Blum, F. D. *J. Colloid Interface Sci.* **1990**, *140*, 525-534.
- [47]. Palmer, C. P.; Terabe, S. *Anal. Chem.* **1997**, *69*, 1852-1860.
- [48]. Palmer, C. P. *J. Chromatogr. A* **1997**, *780*, 75-92.
- [49]. Shamsi, S. A.; Warner, I. M. *Electrophoresis* **1997**, *18*, 853-872.
- [50]. Ward, T. J.; Hamburg, D. M. *Anal. Chem.* **2004**, *76*, 4635-4644.
- [51]. Palmer, C. P.; McCarney, J. P. *Electrophoresis* **2004**, *25*, 4086-4094.
- [52]. Palmer, C. P.; McCarney, J. P. *J. Chromatogr. A* **2004**, *1044*, 159-176.
- [53]. Rundlett, K. L.; Armstrong, D. W. *Anal. Chem.* **1996**, *68*, 3493-3497.
- [54]. Liu, H.; Farrell, S.; Urich, U. *J. Control. Release.* **2000**, *68*, 167-174.
- [55]. Camilleri, P. *Capillary Electrophoresis: THEORY and PRACTICE*, CRC Press, Florida, USA, 1998, pp 217-218.
- [56]. Chankvetadze, B. *Capillary Electrophoresis In Chiral Analysis*, John Wiley & Sons, Ltd, West Sussex, England, 1997.