Manjula T.et al. / Vol 4 / Issue 4 / 2014 / 244-249.

International Journal of Pharmaceutical Research & Analysis

e-ISSN: 2249 – 7781
Print ISSN: 2249 – 779X

www.ijpra.com

MICELLAR ELECTROKINETIC CHROMATOGRAPHY: AN OVERVIEW

T.Manjula*, G.Nagasowjanya, A.Aitha, V.Uma maheswara Rao

Dept. of Pharmaceutical Analysis & Quality Assurance, CMR College of Pharmacy, Kandlakoya, Medchal, Hyderabad, Andhra Pradesh, Indiia.

ABSTRACT

Micellar electrokinetic chromatography (MEKC) is a useful branch of capillary electrophoresis (CE) that utilizes surfactant above critical micellar concentration (CMC) as pseudo-stationary phase. MEKC can be employed to separate both charged and neutral molecules, individually or simultaneously, including chiral compounds. MECK 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 MECK. In this review, we present the introduction of CE, fundamentals of surfactant chemistry as it relates to MEKC, separation principles in MECK including equations involved in calculating separation parameters.

Keywords: Micelles, Surfactants, Buffer additives, Optimization parameters.

INTRODUCTION

Micellar electro kinetic chromatography (MEKC) is a useful branch of capillary elctrophoresis (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. MECK benefits from high peak efficiency due to electro osmotic flow (EOF) in the separation capillary, compounded with large variety of synthetic surfactants, organic modifiers, temperature and variable separation voltage has made MECK the method of choice for separation. Fundamentals of surfactant chemistry as it relates to MEKC, separation principles in MECK including equations involved in calculating separation parameters. Electro kinetic chromatography (EKC) is a family of electrophoresis techniques named after electro kinetic phenomena, which include electro osmosis, electrophoresis, and chromatography. Micellar electro kinetic Chromatography (MEKC) is a mode of EKC in which surfactants (micelles) are added to the buffer solution. Surfactants are molecules which exhibit both hydrophobic and hydrophilic character. They have polar "head" groups that can be cationic, anionic, neutral, or zwitterionic and they have non polar hydrocarbon tails. The formation of micelles or "micellization" is a direct consequence of the "hydrophobic effect." The surfactant molecules can self-aggregate if the surfactant concentration exceeds a certain critical micelle concentration (CMC). The hydrocarbon tails will then be oriented toward the center of the aggregated molecules, whereas the polar head groups point outward. Micellar solutions may solubilise hydrophobic compounds which otherwise would be insoluble in water. The front cover picture shows an aggregated SDS molecule. In the center of the aggregate, *p*-fluorotoluene is situated depicting the partitioning of a neutral, hydrophobic solute into the micelle. Every surfactant has a characteristic CMC and aggregation number, *i.e.*, the number of surfactant molecules making up a micelle (typically in the range of 50-100).The size of the micelles is in the range of 3 to 6 nm in diameter; therefore, micellar solutions exhibit properties of homogeneous solutions. Micellar solutions have been employed in a variety of separation and spectroscopic techniques. In 1980, Armstrong and Henry pioneered the use of micellar solutions as mobile phases for reversed-phased liquid chromatography (RPLC) [1].

Corresponding Author:- T.Manjula Email:- manjula.thoomu@gmail.com

ADVANTAGES

- When compared to CE we can separate neutral molecules, chiral compounds
- High peak efficiency due to EOF(electro osmotic flow)
- Very small amounts of samples can be separated
- simplicity

PRINCIPLE

MEKC is based on the addition to the buffer solution of a micellar "pseudo stationary" phase, which interacts with the analytes according to partitioning mechanisms, just like in a chromatographic method. The "pseudo stationary" phase is composed of a surfactant added to the buffer solution in a concentration above its critical micellar concentration (CMC). In this system, EOF acts like a chromatographic "mobile phase". From a "chromatographic point of view", the EOF's "plug-like" flow profile is almost ideal as it minimizes band broadening, which can occur during the separation process.5-7. The most commonly used surfactant sodium dodecyl sulfate (SDS), an anionic surfactant. The anionic SDS micelles are electro statically attracted towards the anode. The EOF transports the bulk solution towards the negative electrode due to the negative charge on the internal surface of the silica capillaries. But the EOF is usually stronger than the electrophoretic migration of the micelles and therefore the micelles will migrate also towards migration order.9,12.Analytes which is highly retained by the micelle will have longer migration times, while analytes which have limited interactions with the micelle will have migration times close to the EOF (t0). Very hydrophobic compounds may be totally included into the micelle and will migrate with the micelles velocity (tmc). Methanol is not retained by the micelles and migrates with t0 being used as marker for the EOF, while a dye Sudan III is totally included into the micelle and can be used as a micellar marker. The period between the migration time of the bulk solution and the migration time of the micelle is often referred as migration time window [2].

A relatively recent development in MEKC has been to perform separations in the absence of EOF. This may be achieved using coated capillaries or at low pH values. This could be especially useful in the separation of acidic analytes, which would ionized at high pH values and would not interact with the negatively charged SDS micelle.15.Cationic surfactants can be used in MEKC to reverse the charge on the capillary wall, by absorption on the capillary wall surface through a mechanism involving electrostatic attraction between the positively charged ammonium moieties and the negatively charged Si-O- groups; when a reversal of the EOF takes place.

Micelles and Surfactants

Surfactants are molecules with detergent properties, which are composed of a hydrophilic water-soluble head group and a hydrophobic water-insoluble hydrocarbon chain group. Although a large number of surfactants are commercially available, a limited number are widely used in MEKC separations. The surfactants suitable for MEKC must be soluble in the buffer solution to form micelles and the micellar solution must be homogeneous, UV transparent and also have a low viscosity. There are four major classes of surfactants: anionic, cationic, zwitterionic and nonionic (Table 1). Of these, Nonionic surfactants do not posses electrophoretic mobility and cannot be used, as "pseudostationary phase" in conventional MEKC, however can be useful for the separation of charged analytes. This technique using nonionic micelles can be classified as an extension of MEKC.9,16 s Micelles are amphiphilic aggregates of surfactants. Above a specific surfactant concentration, the surfactant molecules begin to self-aggregate, forming micelles, spherical aggregates that exhibit electrophoretic migration like any other charged particle. Micelles are long chain molecules and are characterized as possessing a long hydrophobic tails and a hydrophilic head group. Generally micelles are formed in aqueous solution with the hydrophobic tails oriented towards the center of the aggregated molecules and the hydrophilic heads pointing outward into the aqueous solution. Micelle formation is a very dynamic process, as micelle disaggregate and reconstruct continuously, composing the "pseudostationary phase" which can include hydrophobic analytes. Micellar solutions can solubilize hydrophobic compounds which otherwise would be insoluble in water. Micelles have the ability to interact with the analytes at molecular level based on hydrophobic and electrostatic interactions. Even neutral analytes can bind to micelles due to the very strong solubilization power of the hydrophobic core.8,9 The micelles used in MEKC are charged on the surface, so an analyte with the opposite charge will strongly interact with the micelle through electrostatic forces while an analyte with the same charge will interact weakly due to the electrostatic repulsion. Therefore the ionic surfactants are generally used in MEKC. Every surfactant has a characteristic CMC and aggregation number (the number of surfactant molecules necessary to form a micelle). Another important parameter is the Kraft point, which represents the minimum temperature where the solubility of surfactants increases steeply due to the formation of micelles [3].Use of a cationic or an anionic surfactant will result in an entirely different result. The micellar phase can be modified by adding two different surfactants to form a mixed micelle; addition of an ionic and a nonionic surfactant can provide different selectivity in separation. A mixed micelle has a lower surface charge and a larger size; consequently its electrophoretic mobility will be lower than the one of a simple ionic micelle. Some surfactants like bile salts are chiral and can be used for enantiomers separation [4].

Buffer Additives

Since MEKC is often applied in the separation of analytes with very similar hydrophobicities and chemical characteristics, sometimes is useful to extend the concept of using a "mobile phase" and a "pseudostationary phase" to the use of buffer additives such as organic modifiers and cyclodextrines. Organic solvents (methanol, acetonitrile) are used in CZE in order to increase solubility of the analytes, but their role in MEKC is more complex and profound. Organic solvents reduce EOF, consequently increase the migration times and migration time window of the analytes. Also, organic additives reduce the hydrophobic interactions between the micelle and the analyte and can be useful in the separation of analytes which otherwise are almost completely incorporated in micelles. The addition of organic solvents will increase the migration velocity of these hydrophobic analytes, by reducing the partition coefficient between the micelle and the bulk solution. However high concentration of organic solvents may break down the micellar structure, consequently concentrations above 25-30% should be avoided [5].

OPTIMISATION PARAMETERS

1. **Plate Number**

Resolution increases in proportion to the square root of the plate number.

The higher the applied voltage, the higher the plate number, unless conditions are such that the applied voltage generates too much Joule heating. Average plate numbers for most analytes are usually in the range of 100,000 to 200,000. If the plate number is considerably lower, analytes are likely to be adsorbed on the capillary wall. In such cases, experimental conditions must be optimized to produce more efficient separations. Cleaning of the capillary is a possible procedure, as is changing the pH of the run buffer. Hydrophobic analytes, or those having longer migration times, typically yield high theoretical plate numbers because the micelle has a smaller diffusion coefficient. The plate number does not depend significantly on the capillary length. With short capillaries, however, the amount of sample volume injected must be minimized to avoid zone broadening [6].

2. Separation Factor

The separation factor, a, is the most important and most effective term to maximize resolution. The separation factor reveals the relative difference of the distribution coefficient between the two analytes and can be manipulated by chemical means. Since the distribution coefficient is a characteristic of a given separation system consisting of a micellar and an aqueous phase, we can manipulate the separation factor by changing either the type of micelle or by modifying the aqueous phase. Various factors affecting the selectivity are discussed later. Generally in MEKC it is not very difficult to separate a pair of analytes with α=1.02.

3. **Capacity Factor**

It can be calculated that the optimum value of the capacity factor is equal to $(t_{mc}/t_0)1/2$. Under conditions of pH above 6, the optimum *k*' value is close to 2 for most long alkyl chain surfactants. Under most conditions, the capacity factors must be adjusted to be between 0.5 and 10. A large capacity factor means that the major fraction of the analyte is incorporated into the micelle. It is necessary for the analyte to be distributed evenly between the micellar and the aqueous phase, *i.e.*, the analyte must not spend most of its time in one phase.

The capacity factor is related to the distribution coefficient, *K*, by

$$k' = K(V\text{mc}/ V\text{aq})$$

where *V*mc/*V*aq is the phase ratio and *V*mc and *V*aq are volumes of the micelle and the remaining aqueous phase.

4. Electro osmotic Velocity

The effect of the electro osmotic flow velocity on resolution can be discussed in terms of the migration time ratio, $t_0/t_{mc,}$ which can be expressed as

$$t_0 / t_{mc} = [1 + \mu_{ep} (mc) /\mu_{eo}] E$$

where *E* is the electrical field strength. The mobilities $_{\mu eo \text{ and}}$ $\mu_{ep}(mc)$ usually have different signs and the ratio $\mu_{ep}(mc)/\mu_{eo}$ is smaller than zero and larger than minus one. Therefore, t_0/t_{mc} is less than one. The t_0/t_{mc} is also directly related to the width of the migration time window. The smaller the value of t_0/t_{mc}, the wider the migration time window, hence the higher resolution. A longer run time is required, however. The value of the migration time ratio t_0/t_{mc} is in the range of 0.2 to 0.3 for most ionic micelles under the conditions of pH above 6 [7].

INSTRUMENTATION

- Stationary phase
- Mobile phase
- Sample preparation
- Injection
- Separation
- detection

1. Stationary phase

The "pseudo stationary" phase is composed of a surfactant added to the buffer solution in a concentration above its critical micellar concentration (CMC). The most commonly used surfactant sodium dodecyl sulfate (SDS), an anionic surfactant.

Table 1.Examples of surfactants

Surfactant	Type	CMC	N
Sodium dodecyl sulphate (SDS)	anionic	8.1×10^{-3}	62
Sodium	Anionic	2.1×10^{-3}	138

tetradecylsulphate (STS)			
Siodium dodecane sulphate	Anionic	7.2×10^{-3}	54
Sodium cholate	Anionic	15×10^{-3}	2-4
Cetyl trimethyl ammonium bromide	Cationic	0.92×10^{-3}	61
Dodecyl trimethyl ammonium bromide	Cationic	15×10^{-3}	56

2 . Mobile phase

In this system, EOF acts like a chromatographic "mobile phase". From a "chromatographic point of view", the EOF's "plug-like" flow profile is almost ideal as it minimizes band broadening, which can occur during the separation process.The EOF transports the bulk solution towards the negative electrode due to the negative charge on the internal surface of the silica capillaries. But the EOF is usually stronger than the electrophoretic migration of the micelles and therefore the micelles will migrate also toward the negative electrode with a retarded velocity. And therefore,the anionic micelle also travels toward the negative electrode at a retarded velocity.

3. Sample preparation

Chiral molecules and neutral molecules are dissolved in ionic buffer solution of surfactants like sodium dodecyl sulphate for the formation of micelle. Buffers include borate buffers, phosphate buffers at pH 9 and 7.8.The solution is directly injected into the instrument without further treatment.

4. Injection

Samples can be introduced by applying a relative low voltage for a short time interval or by applying a carefully controlled pressure for a short time interval(hydrodynamic injection). In order to maintain high efficiency, only minute volumes of samples are introduced, in the range of 0.1-50 nl. To improve the reproducibility of hydrodynamic injection , an integrated pressure-time profile with active feedback control is used to compensate for system rise time effects and variations in the applied pressure**.**

5. Separation

Separations are carried out in polyimide coated fused silica capillaries, ranging from 20 to 100cm in length and from 25 to 100µm in internal diameter. The capillary is situated in cartridge and is thermostated, using either a circulating liquid coolant or forced air stream. Both systems are equipped with a high voltage power supply that can deliver upto about 30kv.

6. **Detection**

Sample zones are monitored by programmable multi wavelength uv absorbance detector at the outlet side of the capillary. a small section of capillary serves as detection volume. To allow uv light to pass through the capillary, a small part of the polyimide coating of the fused silica is removed, thus creating a uv transparent detection window [8].

WORKING

Operating conditions

- Capillary: 25–75 mm i.d. ´ 20–75 cm length
- Run buffer: A solution of an ionic micelle in a buffer solution. The surfactant concentration must be higher than its critical micelle concentration.
- Applied voltage: 10 to 25 kV
- Current: Below 75 mA, preferably below 50 mA

The separation capillary is placed between two electrolyte reservoirs, filled with a electrolyte solution.these reservoirs also contain platinum electrodes which serve to connect the high voltage power supply. Sample injection is carried out either electro kinetically or hydrodynamically. Optical detection is carried out at the opposite end of the capillary. The instrument is fully automated computer controlled systems comprising a thermo regulated capillary cartridge, a high voltage power supply, a uv absorbance detector and 1 or 2 vial carousels containing randomly assemble sample and electrolyte reservoirs.

To perform the experiment, the capillary is filled with desired electrolyte solution (a buffer). Next sample is injected to separate the analytes at both ends of the capillary [9].

FACTORS EFFECTING MEKC

pH influence

The pH of the BGE determines the degree of ionization of individual solutes and their net charge in solution. The pH influence on the behaviour of solutes was studied in the range from 6.6 up to 8.2 in increments of 0.2 pH units. Initial separation conditions were 25 mM phosphate electrolyte, 20 mM SDS, 20 kV and 25 °C.

Voltage influence

Voltage influence was studied in the range between 18 kV up to 28 kV in increments of 2 kV by using the previously found optimum pH value of 7.8 for the phosphate electrolyte. Fast sample analysis, moderate current values of ca. 58 µA and a power consumption of ca. 1.62 W was obtained at 22 kV.

Influence of surfactant concentration

SDS was used as surfactant in order to facilitate the separation of NP9 through MEKC mechanism. The SDS influence was studied for the concentration interval ranging from 10 to 50 mM SDS in increments of 5 mM units. All other parameters were kept constant while varying the SDS concentration. As the SDS concentration increases the NP9 peak becomes very broad due to its non-

ionic oligomers starting to separate. At higher SDS concentrations problems related to capillary blockage were encountered. A concentration of 10 mM SDS was considered sufficient in order to allow for the optimum separation of NP9.

Ionic strength influence

The influence of ionic strength was studied on electrolyte solutions with phosphate concentrations varying between 10 and 50 mM. Currents ranging from 24 μA up to 102 μA were obtained. By increasing the ionic strength the buffering capacity increases with benefits for separation. Above 35 mM the Joule effect becomes noticeable through heating and consequently peak broadening, especially towards the end of the separation. However, at low BGE concentrations the TMP and NP9 peaks are overlapping [10].

APPLICATIONS

- For the analysis of bioactive naphthoquinones
- Determination of benzidines following extraction from water, soil, sediment, and chromatographic adsorbents
- The separation of nine furano naphthoquinones
- To perform separation in the absence of EOF. This may be achieved using coated capillaries or at low pH values. This could be especially useful in the separation of acidic analytes.
- Recent applications of MEKC include the analysis of uncharged pesticides, amino acids in neutraceuticals products

Figure 1. Migration time window in a MEKC separation

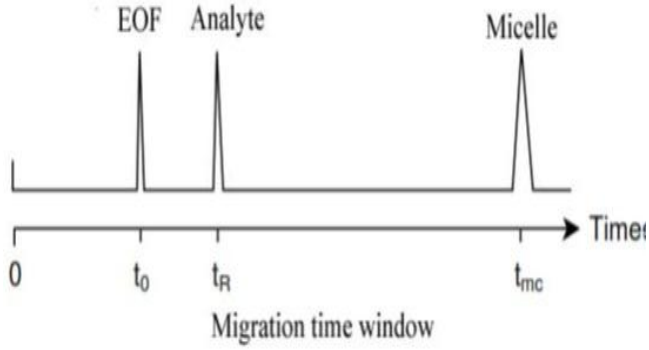


Figure 2. Separation Principle Diagram

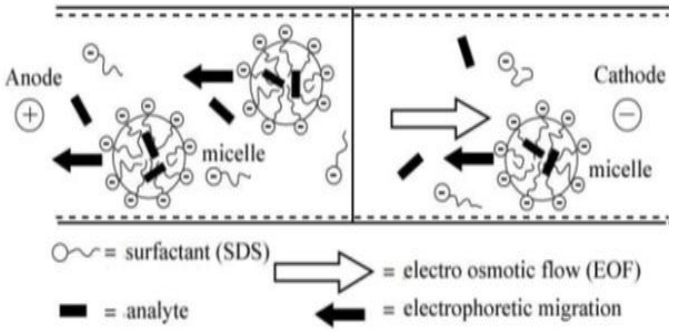
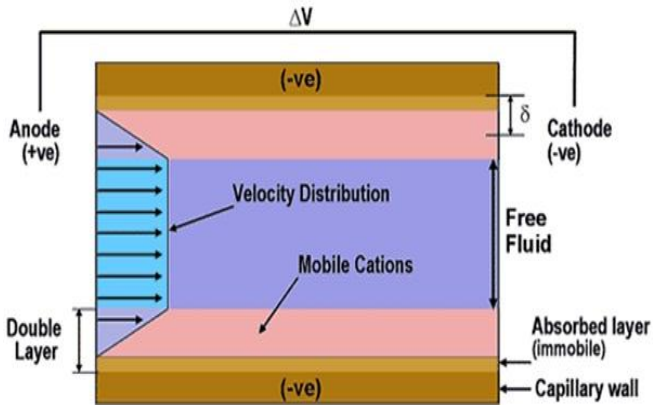
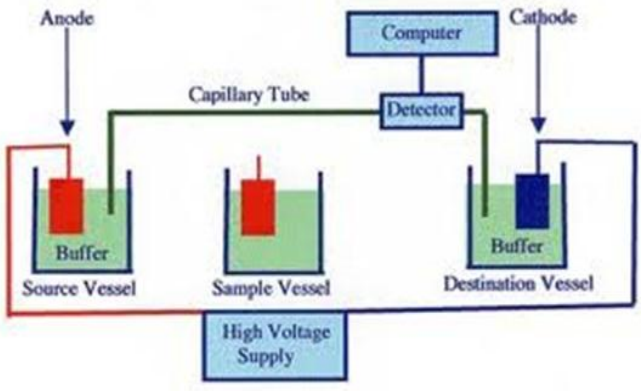


Figure 3. Schematic Representation of MEKC





CONCLUSION

In this technique surfactants are added to the buffer solution which acts as a stationary phase for the separation of neutral and chiral molecules. The advantages of the present method are derived from the complex separation mechanisms governing MEKC. Compounds that cannot be separated on the same RP-HPLC chromatographic column due to their diverse chemical nature can be separated through MEKC in the presence of a suitable background electrolyte. MEKC requires little setup time of the analytical equipment and could be suitable for the monitoring of equipment cleanliness in a routine-type working environment where fast turn-around of production is essential.

REFERENCES

1. Fan L, Liu L, Chen H, Chen X and Hu Z. *J. Chromatogr. A*, 1062, 2005, 133–137.
2. United States Pharmacopeia, 31st ed., United States Pharmacopeial Convention Inc., Rockville, MD, 2007, 3298–3301.

3. Terabe S, Otsuka K, Ando T. Band broadening in electrokinetic chromatography with micellar solutions and open-tubular capillaries. *Anal.Chem.,* 61, 251-260
4. Lux JA, Yin H, Schomburg G. Influence of polymer coating of capillary surfaces son migration behavior in micellar electrokinetic capillary chromatography. *J. High Resolut. Chromatogr*., 13, 145-147
5. Nishi H, Fukuyama T, Matsuo M. Separation and determination of aspoxicillin in human plasma by micellar electrokinetic chromatography with direct sample injection. *J. Chromatogr.*, 515, 1990, 245-255.
6. Otsuka K, Terabe S. Effects of pH on electrokinetic velocities in micellar electrokinetic chromatography. *J. Microcol.*, 1, 1989, 150-154.
7. Liu J, Cobb KA, Novotny MV. Capillary electrophoretic separations of peptides using micelle-forming compounds and cyclodextrins as additives. *J. Chromatogr.*, 1990, 519, 189-197.
8. Van Os NM, Haak JR and Rupert LAM. Physico- Chemical Properties of Selected Anionic, Cationic and Nonionic Surfactants, Elsevier, Amsterdam, 1993.
9. Gorse AT, Balchunas, Swaileand DF and Sepeniak MJ. *J.High Resolut. Chromatogr. Chromatogr. Commun*. 11, 1988, 555.
10. Rasmussen HT, Goebel AT and McNair HM. *J. High Resolut. Chromatogr.*, 14, 1991, 25.