



A Review on Recent Advancement of Capillary Electrophoresis: Micellar Electrokinetic Chromatography and Its Applications

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ABSTRACT

Micellar Electrokinetic Chromatography (MEKC) is a one of the modes of capillary electrophoresis (CE) which has both the combined features of electrophoresis and chromatography principle. It is a technique used for separations of neutral, charged and chiral compound as an individual or simultaneously by using surfactant about its critical micellar concentration (CMC) as a pseudo stationary phase. This mode of separation depends on distribution of analytes between micellar and aqueous phases and due to electro osmotic flow MEKC which gives high peak efficiency. In this present review, we represent the introduction, principle, instrumentation, factor affecting and various application of MEKC.

Keywords: CE, Critical Micelle Concentration, Electrophoresis, MEKC, Surfactant.

INTRODUCTION

Micellar Electrokinetic Chromatography (MEKC) was first induced by Shigeru Terabe and co-worker in 1984 and it help to extend the use of capillary electrokinetic technique for the separation of neutral analyte. MEKC is a one of the mode of capillary electrophoresis (CE) which has both the combined features of electrophoresis and chromatography principle. When due to electro kinetic transporter the aqueous and micellar phase moves with different velocity it is an electrophoresis principle and when separation mechanism of neutral species is based on difference in the partitioning between aqueous and micellar phases is a chromatography principle. MEKC has been useful technique in pharmaceutical field and biomedical field. This mode of separation depends on distribution of analytes between micellar and aqueous phases and due to the electro osmotic flow MEKC gives high peak efficiency. It is a technique used for separations of neutral, charged molecules and chiral compound as an individual or simultaneously by using surfactant about its critical micellar concentration (CMC) as a pseudo stationary phase. About its critical micelle concentration (CMC) the micelle are forms due to the aggregation of the surfactant monomer, when this monomer is added to an electrolyte solution. This micelle form is smaller in size as compared to solute, their large number and small size have high surface area to volume area ratio. The separation mechanism is based on the electrophoretic movement of analytes. The analytes moves from one end to another end of capillary by the electro osmotic flow and electric potential applied and due to difference in charge size ratio the analytes have different migration velocity. The more the charge size ratio the higher is the mobility and lower is the retention time and due to the different size of the analyte there is different migration of analyte.¹

MEKC was established to exploit the benefits of capillary electrophoretic techniques which were able to separates very small quantities of substance in comparatively short time with high resolution. With the help of this separation method the application of CE was expand from ionic species to neutral. It has the ability to analyze a nanolitre (10^{-9} L) of sample with over 1 million theoretical plate and an injection components detection sensitivity at the attomole (10^{-18} mol) level or less, with this fast analysis time and high efficiency the separation of neutral solutes of closely similar structure is also done, and it is also applicable to the separation of charged compounds.²

Electrokinetic chromatography (EKC) is a type of electrophoresis techniques which is named after electrokinetic phenomena, which have electroosmosis, electrophoresis, and chromatography principle.

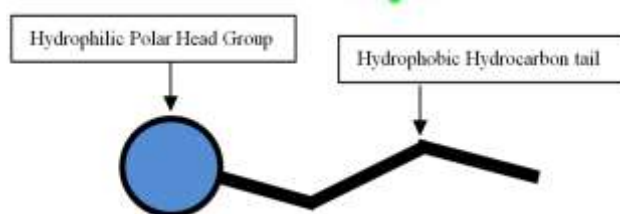


Figure 1: An Amphiphilic Molecule

Surfactant are the molecules which have both hydrophilic and hydrophobic property, so they have the polar head which consist of anionic, cationic, zwitterionic or neutral and hydrocarbon non polar tail.

The process of formation of micelles or is a direct result of the "hydrophobic effect". The surfactant molecules will self-aggregate if the surfactant concentration exceeds the value of certain critical micelle concentration (CMC). The hydrocarbon tails will get oriented toward the centre of the aggregated particles, whereas the polar head groups

will face outward. Micellar solutions will solubilize hydrophobic compounds which would then be insoluble in water. Surfactant has a characteristic value of CMC and aggregation number, which is the number of surfactant molecules making up a micelle which is typically in the range of 50-100. The size of the micelles is in the range of 3-6 nm in diameter hence, micellar solutions always exhibit the properties of homogeneous solutions. These micellar solutions have been used for variety of separation and spectroscopic techniques.^{3,4}

PRINCIPLE

The separation principle of MEKC is as shown in figure 2. When an anionic surfactant such as sodium dodecyl sulfate (SDS) is present, then the micelle migrates toward the positive electrode by electrophoresis. And due to electro osmotic flow the migration of the bulk solution toward the negative electrode due to the negative charge on the surface of fused silica. Under the influence of neutral or alkaline condition the electro-osmotic flow is stronger than that of electrophoresis flow and so micelle at anode also migrate toward the negative electrode at particular velocity.

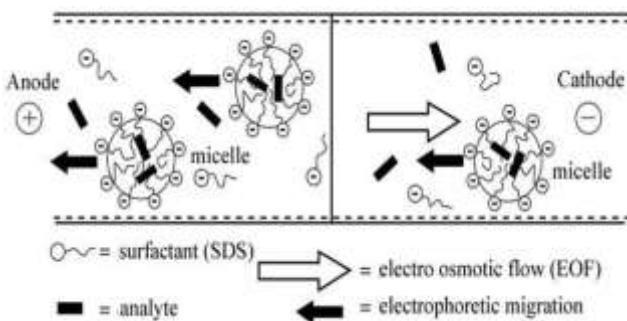


Figure 2: Schematic Representation of the Separation Principle of MEKC.

When the neutral analyte is injected into a micellar solution, a fraction of it gets trapped into the micelle and remaining parts of the analytes go away from the micelles and travelled at the electroosmotic velocity and so its migration velocity depends on the distribution of analytes between micellar and aqueous phase. The larger the percentage of analytes the more is its distribution in a micellar and slower is the migration.

The analyte must migrate at a velocity between the velocity of the micelle and the electroosmotic velocity (Figure 3A), provided the analyte is electrically neutral. The migration time of the analyte is t_R and it is limited between the migration time of the bulk solution, t_0 , and that of the micelle is t_{mc} (Figure 3B). This is often referred to in the literature as the migration time window in MEKC.⁵

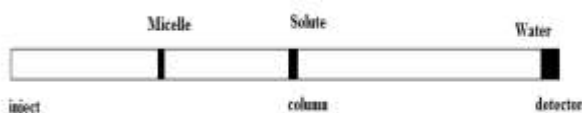


Figure 3A: Schematic of the Zone Separation in MEKC

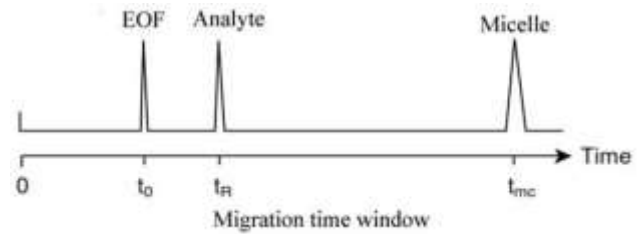


Figure 3B: Schematic of the Electropherogram in MEKC

Composition of the Micellar Solution

Ionic surfactants are essential for MEKC. Various ionic surfactants are available. The surfactants suitable for MEKC should have the following criteria:

1. To form the micelles, the surfactant should have optimum solubility in buffer solution
2. The micellar solution which is formed should be UV transparent and homogeneous.
3. The micellar solution should possess a low viscosity.

Table 1: Critical Micelle Concentration (CMC), Aggregation Number (N), And Karft Point (Kp) of Selected Ionic Surfactants.

Name of surfactant	CMC	N	Kp
Anionic			
Sodium dodecyl sulfate (SDS)	8.1	6.5	16
Sodium tetra decyl sulfate (STS)	2.1(50°C)	138	32
Sodium decyl sulfate	11.4	9.5	25
Sodium decane sulfonate	40	40	-
Sodium dodecane sulfate	7.2	54	37.5
Sodium N-lauroyl-N-methyl taurate	8.7	-	<0
Sodium N-dodecanoyl-L-valinate (SDVal)	5.7 (40°C)	-	-
Bile salt surfactant			
Sodium cholate	13-15	2-4	-
Sodium deoxy cholate	4-6	4-10	-
Sodium tauro cholate	10-15	5	-
Sodium tauro deoxycholate	2-6	-	-
Cationic			
Dodecyl trimethyl ammonium chloride (DTAC)	16 (30°C)	-	-
Dodecyl rimethyl ammonium bromide (DTAB)	15	56	-
Tetradecyl trimethyl ammonium bromide (TTAB)	3.5	75	-
Cetyltrimethyl ammonium bromide (CTAB)	0.92	61	-
Nonionic and zwitter ionic			
Octylglucoside (OGLU)	25 (25°C)	-	-
Polyoxyethylene dodecanol	23	-	-
Polyoxy ethylene sorbitane mono laurate	20	-	-

Table 1 list of CMC, aggregation number, and Kraft point of some selected ionic surfactants available for MEKC are shown. The Kraft point is usually the temperature above which the solubility of surfactant will increase rapidly due to the formation of micelles. To obtain a micellar solution, the concentration of the surfactant should be higher in comparison to its CMC. The Kraft point is the point at which the surfactant has solubility to form micelles. Kraft point is not affected by the counter ion of the ionic surfactant.

Classification of Surfactants

Surfactants are characterized based on the charge present in the hydrophilic portion of the molecule. There are four categories of surfactant

- Anionic,
- Cationic,
- Nonionic and
- Zwitterionic.

Anionic surfactants, when dissolve in water it gets dissociate into hydrocarbon chain bearing anion (e.g., $-\text{SO}_3^-$, $-\text{COO}^-$, $-\text{SO}_4^{2-}$, $-\text{PO}_4^{3-}$), and a counter cation (e.g., K^+ , Na^+), and are the most commonly used type of surfactants. Cationic surfactants, when dissolved in water, dissociate into hydrocarbon chain bearing cationic head group [e.g., $(\text{R})_4\text{P}^+$, $(\text{R})_4\text{N}^+$], and a counter anion (e.g., Br^- , Cl^-). Huge amounts of this class have to corresponds to fatty amine salts and quaternary ammonium with long chain of alkyl type and often have natural fatty acid with one or several attachments. The quaternary group consists of surfactants which are well known for its emulsifying, antimicrobial, anticorrosive and cosmetic formulation. Zwitterionic surfactant consists of both anionic and cationic properties within its surfactants and they also known as amphoteric surfactant and this zwitterionic surfactant live zwitterionic at all the pH condition where at lower pH are few cationic and higher pH are anionic. Nonionic surfactant means it does not consist of any charges. The hydrophilic group is present in, amide phenol, alcohol ester or ether.⁶

INSTRUMENTATION

- Stationary phase
- Mobile phase
- Sample preparation
- Injection
- Separation
- Detection
- Working

Stationary phase

The surfactant is added to the buffer solution which acts as a pseudo stationary phase which is above its critical

micellar concentration (CMC). The most commonly used surfactant sodium dodecyl sulfate (SDS) and Cetyl trimethyl ammonium bromide etc surfactant.

Mobile phase

The chromatographic mobile phase is the electro osmotic flows (EOF), here the EOF possess a plug like flow profile which is use to minimizes the band broadening which can be occurred during the separation mechanism. The bulk solution is migrating toward the negative electrode by the EOF. The EOF is mostly stronger than that of the electrophoretic migration due to the negative charge present on the inner wall of the silica capillary and so the anionic micellar also migrate towards negative electrode.

Sample preparation

In the ionic buffer solution containing surfactants like sodium dodecyl sulfate, the chiral molecules and neutral molecules are dissolved for the formation of micelle. The buffer solution including borate buffer and phosphate buffer at pH 9 and 7.8 respectively. Further the solution is directly injected into the instrument without any treatment

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 ml. 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.

Separation

Separations are carried out in polyimide coated fused silica capillaries, ranging from 20 to 100cm in length and from 25 to 100 μm in internal diameter. The capillary is situated in cartridge and is thermostatic, 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.

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.

Working

Operating conditions

- Capillary: 25–75 mm, interior diameter: 20–75 μm length

- Run buffer: A buffer solution containing an ionic micelle 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 an 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 hydro dynamically. 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). Then the next sample is inserted to separate the analytes at both ends of the capillary. Then the detector detects the analytes and electropherogram is obtained into computer system.

OPTIMISATION PARAMETERS

Resolution

The resolution equation for MEKC is

$$R_s = \frac{\sqrt{N}}{4} \left(\frac{\alpha-1}{\alpha} \right) \left(\frac{k_2'}{1+k_2'} \right) \left(\frac{1 - \left(\frac{t_0}{t_{mc}} \right)}{1 + \left(\frac{t_0}{t_{mc}} \right) k_1'} \right)$$

Where N = theoretical plate number

α = the separation factor

k_1' and k_2' = capacity factor of analytes 1 and 2 respectively.

This equation give the effect of N, α , k, and t_0/t_{mc} on resolution. The separation factor (α) is determined by the process of micellar solubilization and is effect by the chemical nature of both the micellar and aqueous phase. Different surfactant systems can be used and many mixed micelles which posses different solubilization characteristics are also been used in order to control migration effect of the analytes and optimize selectivity.

Plate Number

With the increases in resolution the proportion of the square root of the plate number increases. The more the applied voltage, the more is the plate number, unless in the conditions where the applied voltage produces too much Joule heating. Average plate numbers for analytes are in the range of 100,000 to 200,000. If the plate number is less than the analytes, then they are mostly to be adsorbed on the capillary wall. During such cases, the experimental conditions must be optimized to give more efficient separations.

Separation Factor

The separation factor (α) is the important and effective to increases the resolution. The separation factor shows the difference of the distribution of coefficient between the two analytes and it can be manipulated by chemical means. Since, the distribution coefficient is a characteristic of an 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.

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 longest 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

$$k' = K (V_{mc}/V_{aq})$$

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.

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 $\mu_{ep}(mc)$ and μ_{eo} 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} , 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.

Factors Effecting MEKC

pH influence

The pH of the Back ground electrolyte determines the degree of ionization of individual solutes and the net charges present in solution. The pH influence on the solutes was studied in the range from 6.6 up to 8.2 in increases of 0.2 pH units. In starting the separation conditions were 25 mM phosphate electrolyte, 20 mM SDS, 20 kV and 25 °C.

Table 2: Application of MEKC in the Analysis of Many Pharmaceutical classes

Pharmaceutical class	Drug Substance	Electrophoretic condition					References	
		Surfactant and buffer	pH	Voltage (kv)	Temp. (°C)	Detector		Wave-length (nm)
Antiemetic	Dexamethasone, Aprepitant, ondansetron	77.5mmol of Sodium dodecyl sulfate (SDS) + 62.5 mmol borate buffer	8.7	+25	25	Photodiode array (PDA) detector	240	9
Antibiotics	Ampicillin, nafcillin, penicillin G, penicillin V, cloxacillin, dicloxacillin, oxacillin, piperacillin, amoxicillin.	100mM SDS +26mM borate buffer+	8.5	+20	30	Diode array detector	220	10
Antiviral	Brivudin, acyclovir	10mM SDS + 5mM dodecyl trimethyl ammonium bromide+ 2ml of borate buffer	10	+30	25	Diode array detector (DAD)	200	11
Retinoids	Retinol, retinal, retinyl acetate, retinyl palmitate, retinoic acid	10mM SDS + sodium phosphate	7	+20	25	UV detector	325	12
Steroid	Estrone, α -estradiol, β -estradiol, and rostenedione, epitestosterone, testosterone	80mM SDS, 10mM phosphate buffer	7	+25	22	UV detector	220	13
Non-steroidal anti-inflammatory drug (NSAID)	Alcolfenac, buprofen, acetaminophen, naproxen, tenoxicam, niflumic acid, tolmetin, tiaprofenic acid, flurbiprofen, ketoprofen.	40mM SDS+ 50mM of borate buffer	9	+25	22	UV detector	214	14
Phenolic acid	Caffeic acid, leontopodium acid, flavanoids	25mM SDS + 60mM borate buffer	6.75	+30	40	Diode array detector (DAD)	254	15
Antifungal	Clotrimazole	50mM SDS + 10mM sodium phosphate salt	7.2	+30	30	UV detector	254	16
Phenolic whitening agent	Hydroquinone, phenol, kojic acid, resorcinol, salicylic acid, Arbutin	10mM SDS + 40 mM borax buffer	9	+16	25	Diode array detector (DAD)	254	17
Diuretic	Furosemide	1mM SDS + sodium diphosphate	3	+25	25	UV detector	274	18
Atropine	Tropic acid, apatropine and atropic acid	10mM SDS + 10mM sodium borate buffer	8	+12	30	Diode array detector (DAD)	195	19
Benzodiazepine	Diazepam, nitrazepam, nitrazepam	18mM SDS + 50mM sodium borate	9.5	+20	25	UV detector	214	20
Antibacterial	Pipemidic, naldixic, oxolinic	15mM SDS + 50mM phosphate buffer	7.4	+25	25	Diode array detector (DAD)	200	21

Voltage influence

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

Influence of surfactant concentration

Sodium dodecyl sulfate has been used as surfactant in order to facilitate the separation of drug through MEKC separation mechanism. The SDS influence was studied for the concentration interval ranging from 10 to 50 mM SDS an increases of 5 mM units. All the other parameters were kept constant while varying the concentration of SDS. As the SDS concentration increases the drug peak becomes very broad due to its non-ionic oligomers starting to be separated. An increase of the SDS concentrations problems related to capillary blockage was found. A concentration of 10 mM SDS was found to be enough in order to have optimum separation of drug.

Ionic strength influence

The effect of ionic strength was study on electrolyte solutions with phosphate concentrations differing in between 10 and 50 mM. Currently ranging from 24 μ A up to 102 μ A was obtained. By increments the ionic strength the buffering capacity increases with help for optimum separation. Above 35 mM the Joule effect becomes noticeable through consequently peak broadening, heating and especially towards the end of the separation.⁷

Advantages

- Can separate molecules too small for gel electrophoresis.
- Can separate both ionic and neutral compounds with short retention time high efficiency and unlike in CE.
- High separation efficiency.
- Minimal consumption of sample as compared to HPLC since it detects concentration on ng/L scale.
- Ability to separate chiral compounds efficiently.
- Equipment is cost less than that of HPLC.
- High sensitivity in small amounts
- Quicker than HPLC for separating complex samples.

Applications

- Determination of paracetamol and its impurity in analgesic preparation
- Determination of sulfacetamide, prednisolone acetate, in local pharmaceutical preparations
- Determination of sunscreen agents
- Verification of level of cefuroxiumin human serum.

- Verification of water soluble vitamin in energy and sport drink
- It has been use for therapeutic and diagnostic and therapeutic drug monitoring.

It is for analyzing of amino acid in neutraceutical substances and uncharged pesticides.⁸

CONCLUSION

MEKC is a new analytical techniques and one of the mode for chromatographic separation in capillary electrophoresis. It is use for separation of several combinational chemistry compound and chiral compound. The formation of micelle by surfactant is further advance techniques in capillary electrophoresis. The compound which cannot be separated by HPLC can be separated by MEKC by using suitable background electrolyte. It is a simple, rapid, high resolution method and required small sample size for separation of compound in pharmaceutical analysis.

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