REVIEW ARTICLE

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Micellar Electrokinetic Capillary Chromatography [MECC]

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Micellar Electrokinetic Capillary Chromatography (MECC) is one of the recently developed separation techniques. It is a fast method with high resolution, high efficiency and precision and useful for separation of charged molecules as well as neutral solutes. It can be coupled on-line with detectors such as U.V., laser-induced fluorescence and mass spectrometry, thus increasing its selectivity. The present review article briefly outlines the principle, instrumentation, theory and applications of MECC.

Electrophoresis involves the movement of a charged particle through a liquid under the influence of an applied potential difference. The charged components to be separated migrate in an electric field at different rates which are governed primarily by the charges and by the strength of the electric field. The development of electrophoresis in capillary tubes offers several exciting methods for fast highly efficient separation of ionic species and is known as Capillary Electrophoresis (CE). It offers an advantage of enhanced heat dissipation that permits the use of high potentials for separation. The ultra small volume flow rates obtainable in capillary electrophoresis permit sampling from picoliter environments. There are four modes of CE[1].

a) Capillary Zone Electrophoresis (CZE)
b) Capillary Gel Electrophoresis
c) Isoelectric Focussing
d) Micellar Electrokinetic Capillary Chromatography (MECC)[1]

In CZE, a buffer-filled capillary is placed between two buffer reservoirs and a potential field is applied across this capillary (Fig. 1). In this systems, electroosmotic flow is towards the cathode hence a detector is placed at this end. Injection of solutes is performed at the anode end. It offers the ability to analyze a nanoliter or less of sample with over 1 million theoretical plates and a detection sensitivity of injected components at the attomole (10^{-18} mole) level or less. The separation medium is in a fused silica capillary tube (e.g. 10-100 μm i.d.) 1m. long containing an appropriate electrolyte. High voltage source, capable of delivering current up to 250 micro amperes at voltage ranging from 1000 to 30000 V.

In capillary gel electrophoresis, gel filled capillary columns are used. Gels are potentially useful for electrophoretic separations because they minimize solute diffusion, which contributes to zone broadening, they are an anticonvective media. Proteins can be separated based on their size using the sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) system.

The technique of Isoelectric focussing involves separation of amphoteric sample components in a pH gradient, which is running from low pH at the anode and to high pH at the cathode end. When a sample is placed in this pH gradient and an electric field is applied. The amphoteric sample components begin to migrate. Eventually it arrives at a pH where its net charge is zero i.e. its isoelectric point and it ceases to migrate. Each sample component migrates to its own isoelectric point and then stops (Fig. 2). A mixture of synthetic ampholyte mixture allow generation of pH gradients from 1 to 11. Isoelectric focussing is a powerful technique that permits resolution of substances differing in isoelectric points by only 0.001 pH unit.

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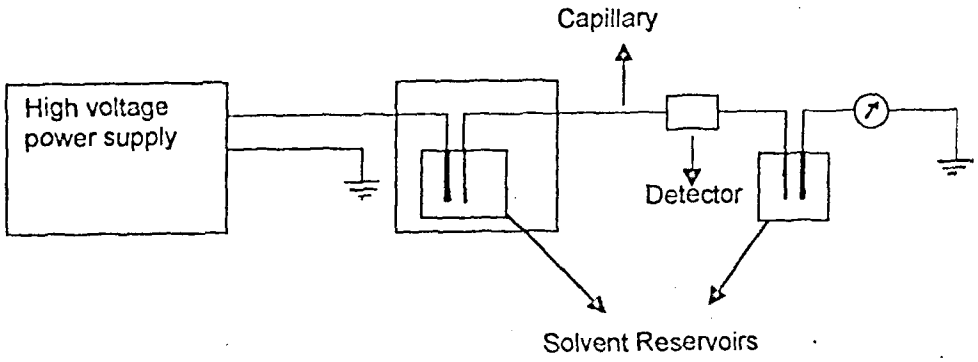

Fig. 1: Schematic diagram of Capillary Electrophoresis

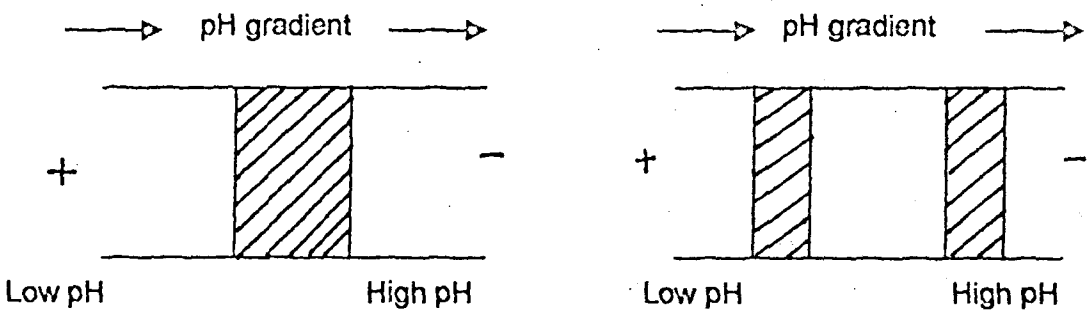

Fig. 2: Isoelectric focussing

In the micellar electrokinetic capillary chromatography (MECC), micelles of an ionic surfactant can migrate in an aqueous solution by electrophoresis. When a solubilizate is added into a micellar solution, some portion of the solubilizate may be solubilized into the micelles. Thus the solubilization by micelles can constitute a mechanism of retention in chromatography. In 1984, Terabe *et al.*[11] introduced the use of electroosmotically pumped micelles in a capillary electrophoresis system to affect chromatographic separations of neutral compounds. Above critical micelle concentration surfactant monomers tend to form roughly spherical aggregates or micelles, with the hydrophobic tail groups oriented toward the center and charged head groups along the outer surface. MECC provides formation of an additional phase and is composed of two phases aqueous and micellar. In MECC surfactant forms charged or neutral micelles constitute an intracapillary matrix which interacts with migrating molecules by means of electrostatic hydrogen bonding and hydrophobic forces.

A combination of such forces on the one hand creates unique opportunities for new separation mechanisms, making on the other hand interpretation of the results more complicated. Because the micellar phase is similar to a chromatographic stationary phase, the micelles have been termed as pseudostationary phase. The MECC system was originally developed for analysis of nonionic solutes and retention in this system is generally based on hydrophobicity. Solutes can partition between the two phases resulting in retention based on differential solubilisation by the micelles[1].

Advantages of MECC

A fundamental advantage with respect to MECC is the formation of on additional phase which gives additional selectivity and specificity[2].

Mixing of different surfactants can be used to achieve separation of highly hydrophobic compounds[3].

Micelles provide both ionic and hydrophobic sites of interaction simultaneously making MECC preferable to CZE for the separation of mixtures of charged and uncharged solutes[4].

The major advantage of MECC is the high efficiency such as plate numbers of ~200000[5].

MECC technique is the facile manner in which reten-

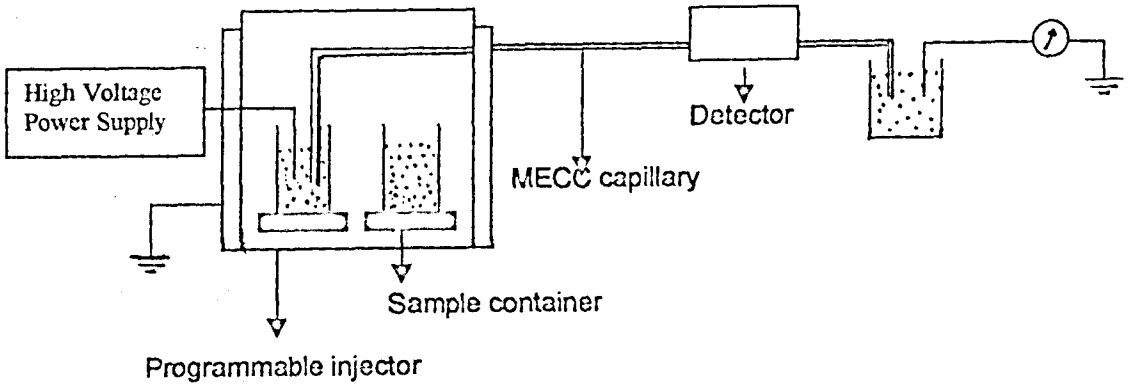

Fig. 3: Schematic diagram of MECC

tion can be manipulated through simple adjustment of the composition of the mobile phase.

Both the primary (mobile) and secondary (micellar) phases can be rapidly changed to get changes in retention[5].

MECC separates in a single run, both neutral and charged molecules in an electroosmotically driven system, an application that was not possible before its inception[6].

Disadvantages of MECC

The micelles may interact with the migrating molecules by means of electrostatic, hydrogen bonding and hydrophobic forces. A combination of such forces make the interpretation of the results more complicated[2].

The major disadvantage of MECC is concentration sensitivity where HPLC has an 80 fold advantage[6].

The complexity of the system and the large number of parameters would require a systematic approach to optimization of MECC separation in order to achieve the best results with a minimum amount of experimental effort[7].

Instrumentation of MECC

The basic instrumentation of MECC involves same set up of instruments as used for capillary electrophoresis with the only difference that the separation buffer contains surfactant/s above it's critical micelle concentration. Figure 3 gives simple set up of MECC.

High voltage power supply is used to apply potential difference. Programmable injector for capillary electrophoresis was used for pressurized injection at the inlet of the capillary in which MECC was performed. Normally the solution of the sample is prepared in the running buffer used. The concentration of the sample changes in each experiment. Mobile phase contains an appropriate concentration of the surfactant. The mobile phase is prepared in the buffer solution. The choice of buffer system normally depends on the chemical nature of analyte. Normally phosphate and borate buffers are used. Low ionic strength and high pH produce the fastest velocities in glass and fused silica capillaries. The solvation parameter model is used to prove the efficiency of surfactants used. The choice of surfactant depends on individual intermole interaction to retention properties of the surfactants and the effect of experimental parameters of surfactant selectivity.

The difference in selectivity in MECC can easily be achieved by changing the micellar system. Sodium dodecyl sulfate (SDS) is the most commonly used surfactant. The micellar systems used in MECC are classified according to solvatochromic parameters. Linear solvation energy relationships and retention indexes are used to characterise the micellar system in MECC. Thus use of proper micellar system plays important role in separation of various compounds in MECC. Capillary set up in MECC consisted of two fused-silica capillaries that were coupled via an open liquid junction. Normally the capillary used in different studies in MECC differs only in size. Fused silica coated capillaries are more common[8].

In MECC the modes of detection used are spectrophotometric, absorption, fluorescence, indirect fluorescence, thermal lens, Raman, mass spectrophotometric, electrochemical, conductometric, potentiometric, amperometric[9]. U.V. detectors are most commonly used

detectors for MECC. U.V. detection must be accomplished on columns such that the path length is defined by the diameter of the capillary. Fluorescence detectors are most easily adapted for use in capillary electrophoresis, since its sensitivity is not dependent on the dimensions of the capillary. The system produces low stray light and allows scanning excitation between 200 and 800 nm.

Laser light sources are most amenable to focussing in small capillary diameters, thus loading to higher mass sensitivity helium, cadmium and argon ion lasers are used extensively since they have much higher power at much longer wavelength. The laser light, which is monochromatic and very well collimated is easily focussed into small capillaries. Use of laser based detectors is not that common incase of MECC[9].

On-line coupling of MECC with various other systems can be done to get proper selectivity or high resolution. The selectivity is enhanced by on-line coupling of MECC to electrospray masspectrometry fast scanning multiwavelength detection.

Theory of MECC

In a recent work, Terabe *et al.*[10] examined several possible sources of nonequilibrium dispersion in MECC, including sorption-desorption kinetics, intermicelle diffusion, the effect of temperature gradients and electrophoretic dispersion. The magnitudes of the dispersions will be estimated from the theory of a random walk and these estimates will then the compound to experiment. A significant distinction between MECC and the parent techniques on which it is based, capillary zone electrophoresis (CZE), is that the plate heights of certain analytes resolved in MECC, under typical experimental conditions, appear to be formed by nonequilibrium effects when the electric field is high exceeds roughly 7 -20 kV/m.

To explain the micellar mass transfer a new equation is derived for this plate heights contribution by extending the nonequilibrium theory of chromatography to MECC. In this case mass transfer originates from the kinetics of partitioning i.e. the rate of adsorption of analyte molecules to and their desorption from the micellar phase. The origin of nonequilibrium effect in MECC in the result of adsorption of analyte molecules to the capillary wall. So dispersion of this type has its origin in transchannel mobile-phase mass transfer. Desorption kinetics exist and complicate the phenomenon of transchannel mass transfer. In MECC, analyte molecules can diffuse by two means. first as in CZE and other forms of chromatography they can diffuse through the mobile phase with diffusion coefficient D_m Secondly, in contrast to CZE and most other forms for chromatography they are also transported by the diffusion of analyte micelle adduct which has diffusion coefficient D_{mc}. In MECC both mobile and micellar phases more through the channel and an analyte molecule can consequently assume two extreme values of velocities[10].

Migration Behavior of Various compounds in MECC:

The charged and neutral molecules exhibit different interactions with micellar phase. The two migration parameter in MECC (retention factor and mobility) and the two Important experimental parameters (pH and micelle concentration) have great influence on the migration behavior and selectivity.

1) Neutral solutes:

The electrophoretic mobility of a neutral solute in MECCμ_n is proportional to the mobility of the micellar mobile phase μ_{mc}. (Fig. 4) presents the typical migration behavior of a neutral solute in MECC. n=Neutral solute mc=micelle

$$\mu_n = \frac{K'}{K'+1}\,\mu_{mc}$$

The retention factor K' is defined as the ratio of the velocities of solute associated with micelles to that in the mobile phase the term K'/(K'+1) represents the mole traction of the solute in micellar pseudophase[4].

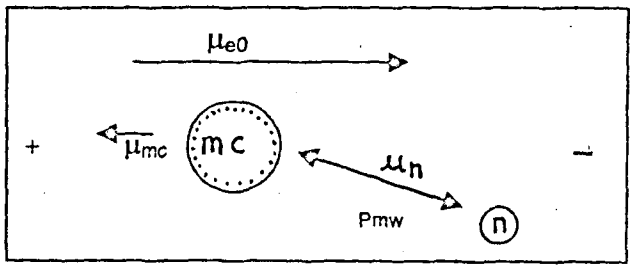

Fig. 4: Typical migration behavior of a neutral solute in MECC

n is a Natural solute. mc is a micelle. μ_{eo} is electroosmotic mobility

The elcectrophoretic mobility of a neutral solute in MECC is μ_n. The mobility of the micellar mobile phase is μ_{mc}. P_{mw} is solute's partition coefficient into micelles

2) Anionic solutes:

Anionic solutes is a special case of an acid that dissociates. (Fig. 5) represents the typical migration behavior of anionic solutes.

In contrast to uncharged solutes, not all retention of anions is explained by interactions with the help of pseudophase. Since these compounds have a negative electrophoretic mobility in the aqueous phase. Therefore in contrast the mobility of an anion would be the weighted average of the mobility of the micellar phase and its own mobility in aqueous phase. This can be accounted for by including overall mobility in the absence of micelles μ_o[4].K'

$$\mu = \frac{K'}{K'+1}\ \mu_{mc} + \frac{1}{K'+1}\ \mu o$$

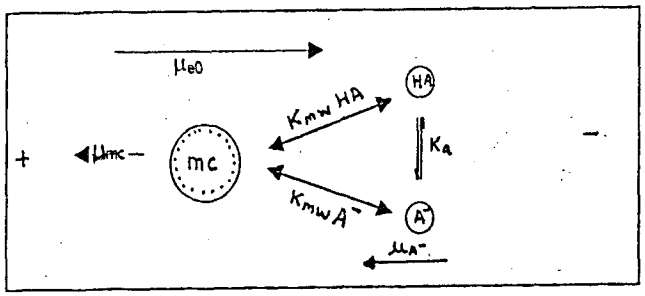

Fig. 5: The typical migration behaviour of Anionic solutes

A is Acid and Ha is base. Kmw HA is equlibrium constant for HA
Kmw A⁻ is equlibrium constant for A
μ_A⁻ is the electrophoretic mobility of A

3) Cationic Solutes:

It can be expected that the observed migration will be more difficult to describe. This is due to the electrostatic interaction between the free surfactant ions in the mobile phase and the cations, which introduces an additional equilibrium (ion-pairing formation) into the equations. Fig. 6 depicts a chematic representation of the migration mechanism in the MECC of a cationic solute.

This ion-pairing equilibrium is defined by the equilibrium constant K_{IP}. (Fig.6). The mobility of cations can be obtained by directly using the various fractions μ and the electrophoretic mobility of the micellar phase μ_{mc} and the electrophoretic mobility of various-paired cationic species μ_c.

$$\mu = \frac{K_b/(1+K_b+K_bK_i)}{1+K'}\ \mu_c + \frac{K'}{1+K'}\ \mu_{mc}$$

μ_c can be related to the observed electrophoretic mobility in the absence of the micellar Pseudophase μ_o[7].

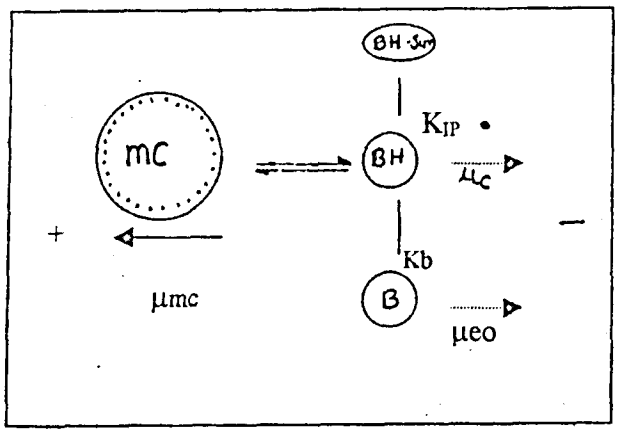

Fig. 6: Schematic representation of the migration mechanism in the MECC of a cationic solute

BH-surf is a cation-surfactant complex. BH is a conjugate acid and B is a base. Kb is equlibrium constant for B. This ion-pairing equilibrium is defined by the equilibrium constant K_{IP}. The electrosphoretic mobility of the micellar phase μ_{mc} and the electrophoretic mobility of various - paired cationic species is μ_c.μ_c can be related to the observed electrophoretic mobility in the absence of the micellar Pseudophase μ_o.

Modifications in MECC

Modifications in various components of MECC can be for good selectivity, good sensitivity, good specificity. Allowing the use of MECC for different solutes like highly hydrophobic solutes and chiral solutes etc. These modifications are done mainly in micellar phase.

1) Mixed micelles of sodium dodecyl sulfate and sodium cholate in MECC:

Physicochemical properties of mixed micellar system of sodium dodecyl sulphate (SDS) and sodium cholate (SC) were investigated. The micelle-micelle and micelle-buffer interactions in an SDS/SC[C(1,1-dimethyl-2-hydroxyethyl) amino]-2-hydroxypropane-sulfonic acid buffer system for separation by MECC were studied. The corticosteriods separated are cortisol, dexamenthasone, corticosterone, 11-deoxycortisol, 4-androstene-3,17-dione and fludrocortisone acetate[3].

2) On-line Concentration of Neutral Analytes by sample stacking in reversed Migration MECC:

Micellar solutions of sodium dodecyl sulfate are prepared with acidic buffers to reverse the direction of the migration velocity of neural analytes owing to reduced electroosmotic flow. Samples are prepared in nonmicellar matrix of lower conductivity to achieve field enhancement in the sample zone. This is commonly known as sample stacking, which result from the movement of sample ions across a distinct concentration boundary found between the high resistivity sample zone and low resistivity separation zone. The electrophoretic velocity of ions found in such a zone is much greater than that found in the low resistivity separation zone[11].

3) Use of polymerized surfactant:

Enantio selectivity is achieved by differential interaction of each enantiomer with the chiral micelle. The utility of the chiral MECC approach has been demonstrated using sodium N-dodecanoyl-L-valinate (SDval) bile salts, digitonin saponins and glucopyranoside based phosphate and sulfate surfactants.

The chiral separation was done for compounds such as BINOL, DABN, Troger's base, laudunosolien, norlaudanosoline laudanosine, warfarin, Coumachoir. All of the enantiomers separated also had polar groups that seemed to be necessary for hydrogen bonding with L-valinate on the micelle. Negative electrophoretic mobilities confirms enantiomers associated with the anionic micelle. In all cases hydrophobic and electrostatic interaction as well as hydrogen bonding appears to be important for chiral recognition[12].

Application of MECC

Micellar electrokinetic capillary chromatography is a subclass of capillarelectrophoresis. MECC provides the opportunity to separate a single run, both neutral and charged molecules in an electroosmotically driven system. A great variety of molecular types are amerable to MECC including phenol, chlorinated penols, phenylthiohydantoin, aminoacids, nucleosides and oligonucleotides, nucleic acids, catechols, vitamins, antibiotics, chiral substances, isotopic substituents, peptides, barbiturates and prophyrins. MECC gave significantly greater efficiency, selectivity, peak capacity and speed compared to high-performance liquid chromatography for the determination of illicit drug substance. Some of the applications are listed below.

1. Determination of heroin and its impurities like acidic basic, neutral impurities and various adulterants. MECC was used to analyse cocaine and its basic impurities. The separation was about 3 times faster than liquid chromatography[6].

2. Analysis of mycotoxins: The acidic mycotoxins citrinin, penicillic acid and ochratoxin and the neutral mycotoxins zearalenone, aflatoxins G_1, G_2, B_1, B_2 roridin A and sterigmatocystin were analysed[5].

3. A MECC method has been developed for the qualitative assay of amoxycillin and its degradation products and clavulanic acid. Together with amoxycillin the latter acid is an important constituent in the antibiotic augmentin[13].

4. Separation of water soluble vitamins by MECC. The retention behavior of eleven water soluble vitamins in MECC was investigated in comparison with CZE. SDS and sodium lauroylmethyl taurate were used as anionic surfactant[14].

5. Purity indication assays of commercially available sennoside A standard. In this separation sodium taurocholate (TCA) was used as a micelle forming agent[15].

6. pH Dependent isoform transitions of Monoclonal antibody monitored by MECC. In pH range 2-12 The monoclonal antibody BR96 occurs in 1-5 isoforms which are detected by MECC[2].

7. Analysis of illicit drugs in Human Urine by MECC with on-column fast scanning polychrome Absorption Detection.

Screening and confirmation of drugs of abuse in body fluids, including urine, are important for the investigation of intoxications, detection of potential users of drugs and control of drug addicts following withdrawal therapy. The effectiveness of this approach was demonstrated with the analysis of barbiturates in human serum and urine analysis of illicit drug in human urine, including opioids cocaine metabolites, amphetamines and hypnotic[16].

8. Evaluation of a separation based fiber optic sensor in a MECC with laser induced flurescence detection. The fiber optic sensor that integrate the separation based selectivity afforded by laser induced flurescence detection[17].

9. Separation of the diastereoisomers and enantiomers of the fugicide triadimenol by MECC[18].

10. Determination of creatinin and other uremic toxins in human blood sera with MECC[19].

11. MECC for analysis of platelet activating factors in human blood by indireat U.V. detection[20].

12. Separation of adducted and modified nucleic acid constituent by MECC[21].

13. Separation and detection of peptides like Bradikinin neurotensis derivatives and angiotensin by MECC coupled with electrospray ionization mass spectrometry with sucrose monododecanoate as micelle forming agent[22].

14. MECC of algal toxins for the detection of toxins like nudularin and microcrystin at nanogram levels[23].

In conclusion, MECC has proven to be an important tool to the pharmaceutical industry for chiral separation of drugs and for separation of biological compounds. It is a fast method giving high resolution and efficiency. Changing micellar system can change the selectivity in the MECC. The sensitivity can be achieved by coupling MECC with various other sensitive detection methods such as MS fiber optics etc. MECC is applicable to a large variety of forensic studies especially those which are difficult to analyse via GC. Excellent resolution is obtained due to a combination of good selectivity and very high separation efficiency.

Thus MECC has proven to be an excellent technique for analysis and separating various compounds along with its own limitation of interaction of micelles and solutes making it more complex. It has been proved to be superior with regard to simplicity, rapidity, precision and sensitivity over CZE.

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