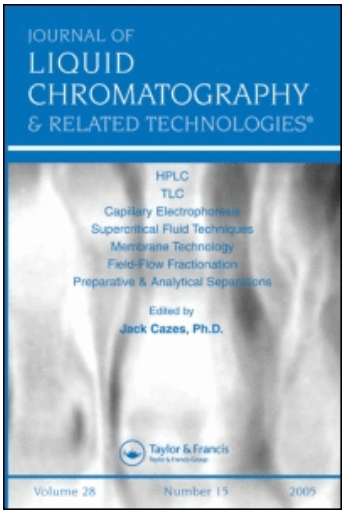

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Micellar Electrokinetic Capillary Chromatography: Basic Considerations and Current Trends

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MICELLAR ELECTROKINETIC CAPILLARY CHROMATOGRAPHY: BASIC CONSIDERATIONS AND CURRENT TRENDS

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ABSTRACT

A brief review of micellar electrokinetic capillary chromatography is presented. Basic theory of MECC, a discussion of types of micelles in MECC and its application to different classes of compounds is presented. Selected examples, which illustrate the advantages of MECC over capillary zone electrophoresis are also given.

INTRODUCTION

Andrews, in the introduction to his book on electrophoresis, wrote "Electrophoresis has evolved within the last thirty years from a general low resolution method of relatively limited application into a wide variety of analytical and small scale preparative techniques of unrivalled resolving power and exceptional versatility. These qualities have resulted in a virtual explosion in their use especially in the field of biochemical research. Methods are being constantly improved and modified, new variations introduced and new equipment built and yet new areas of exploitation opened up" (1).

In particular, the recent development of capillary zone electrphoresis (CZE) (2-5) have not only presented the researchers with the potential for

achieving rapid high-resolution of macromolecules, but it also opened the way for the application of electrophoresis to the separation of small molecules such as inorganic and organic ions, amino acids, peptides and oligonucleotides (3,6-8). Furthermore, the development of micellar electrokinetic capillary chromatography (MECC) has allowed the extention of this method for the separation of neutral compounds (4,9).

Although CZE is compared to chromatography, yet as a separation technique it has its specific features. The separation is achieved by differential migration of charged solute molecules in a semi-conducting buffer under the influence of an electric field gradient. MECC is more like a combination of CZE and micellar liquid chromatography. Neutral solutes are separated by differential partitioning while ionic solutes are influenced by differential partitioning and differential migration mechanisms.

Micellar liquid chromatography was first proposed in 1977 by Armstrong and co-workers (10). This technique involves the addition of surfactant ions above their critical micelle concentration (CMC) to the mobile phase in order to control or adjust solute retention. Compounds are separated based on their differential partitioning between the aqueous mobile phase and the hydrophobic interior of the micelles. The multiplicity of interactions which micellar systems provide (hydrophobic, electrostatic and hydrogen bonding) cannot be duplicated by conventional normal or reversed mobile phase systems.

The first use of micelles (surfactant aggregates as buffer modifiers) in CZE was reported by Terabe et al. (4). By adding sodium dodecyl sulphate (SDS) to the buffer at concentrations above its CMC they were able to separate fourteen phenol derivatives within 19 minutes, figure 1. Theoretical plate numbers were not as high as could be achieved by CZE because of the resistance to mass transfer that is introduced by solute partitioning between the bulk buffer and the micelles. However, column efficiency was much higher than could be achieved by high-pressure liquid chromatography (HPLC) because the buffer is electroosmotically driven through the capillary resulting in a flat-flow profile.

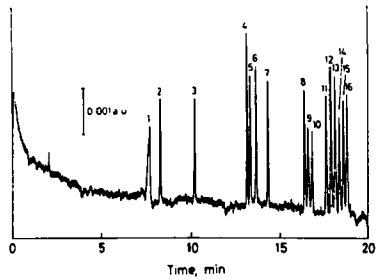

FIGURE 1. Electrokinetic separation of phenols with an SDS solution: (1) water, (2) acetylacetone, (3) phenol, (4) o-cresol, (5) m-cresol, (6) p-cresol, (7) o-chlorophenol, (8) m-chlorophenol, (9) p-chlorophenol, (10) 2,6-xylenol, (11) 2,3-xylenol, (12) 2,5-xylenol, (13) 3,4-xylenol, (14) 3,5-xylenol, (15) 2,4-xylenol, (16) p-ethylphenol; micellar solution, 1 mmol of SDS in 20 mL of borate-phosphate buffer, pH 7.0; current, 28 μA; detection wavelength, 270 nm; temperature, ca. 25°C. (Reprinted with permission).

Since this initial report (4) the general method termed MECC by Burton et al. (11) has developed into a practical separation technique (5,8,9,11-95). Barring unintentional omissions this list of references covers the MECC literature rather comprehensively. Various aspects of MECC were summarily covered in reviews on capillary electrophoresis (96-98). To date, the most comprehensive reviews of MECC are those published by Sepaniak (99) and Kuhr (100).

Specifically, MECC was used for the separation of nucleic acid constituents (8,20,39,67,78), amino acids and peptides (23,45,64,70,85,111) vitamins (11,28,-29,31,47,70,88), drugs and pharmaceuticals (15,27,28,37,43,47,48,50,56,57,71,-72,74,75,86,89), chiral compounds (22,23,44-46,50,66,94), acidic solutes (37,55,77,82), phenols (4,60), phthalates (69), isotopically substituted compounds (34,42), hydrophobic and aromatic compounds (11,65,66,91,92), metal chelates (38), catechols and catechol amines (68), amines (17,26,48), cationic compounds (90), and other miscellaneous classes of compounds (32,33,37,38,58,-76,77,93).

The purpose of this paper is to preview MECC basic separation principles and optimization procedures; to present selected examples in order to illustrate typical application and to identify potential future directions.

MICELLES

Micelles are aggregates of surfactant molecules which are a class of compounds that exhibit amphiphilic properties. Amphiphilic molecules possess both hydrophilic and hydrophobic regions that are spatially separated. The hydrophobic region of the molecule is either a straight or a branched chain hydrocarbon or a steroidal skeleton. The hydrophilic head, on the other hand, is structurally more diverse with cationic, anionic, zwitterionic and nonionic possibilities. Surfactants can be classified as anionic ($R\text{-}X^-M^+$), cationic ($R\text{-}N^+(CH_3)_3X^-$), zwitterionic ($R\text{-}(CH_3)_2N^+CH_2X^-$) or nonionic ($R(OCH_2CH_2))_mOH$ where R is a long aliphatic chain, M^+ is a metal ion, X^- is a halogen, CO_2 or SO_4^{-2} and n is an integer. Sodium dodecyl sulfate (SDS) is an example of an anionic surfactant, hexadecyltrimethylammonium bromide is an example of a cationic surfactant, N-decylsultaine [SB-10] is an example of a zwitterionic surfactant and polyoxyethylene(23)dodecanol (Brij-35) is an example of a neutral surfactant. Bile salts (steroidal skeleton) can associate in water to form micelles which passes a hydrophilic and a hydrophobic face. Each bile salt aggregate is formed of 2-8 monomers held together by hydrophobic interactions. Regardless of the structure of the hydrophilic moiety, it is generally accepted that hydrophobic interactions are the main driving force for micelle formation in aqueous media. The structure of the micelle is either spherical or cylindrical depending, to a large extent, on the structure of the surfactant molecules and their concentration. Micellar structure is also affected by experimental parameters such as temperature, pressure, pH, ionic strength and presence of impurities. At the onset of formation of micelles from the monomers, the structure is spherical for a large number of amphiphiles. At higher surfactant concentrations the physico-chemical properties including micelle structure change differently for different systems.

For most surfactants the physico-chemical properties remain approximately the same or vary to a constant rate up to very high surfactant concentration. For some systems, a number of physico-chemical properties, including micelle shape, size and hydrocarbon chain conformation, undergo rather dramatic changes as the surfactant concentration is increased. At high surfactant concentrations amphiphile association is not limited to the formation of relatively small spherical micelles but that association may progress to the formation of very large aggregates and lyotropic liquid-crystalline phases.

One of the most striking properties of micellar systems, that is relevant to their use in chemical separation, is their ability to solubilize compounds that are normally insoluble or only slightly soluble in water. Solubilization starts at the critical micelle concentration (CMC), and increases in proportion to micelle concentration. The extent of solubilization depends on the properties of the solubilizate and surfactant and is also affected by experimental parameters.

The most important parameter, with respect to micelle formation, is the surfactant concentration. At low concentration and at temperatures above the critical micelle (Krafft) temperature the surfactant is dispersed in the aqueous media at the molecular level. As the surfactant concentration exceeds a minimum value, the molecules associate to form micellar assemblies. The average number of molecules per micelle is termed the aggregation number. Typically, micelles are composed of 40-140 molecules each. The surfactant concentration at the onset of micelle aggregation is termed the critical micelle concentration (CMC). At 25°C and 1 atm. the CMC is typically less than 20 mM.

The molecular organization of micelles in aqueous solutions is described as follows. The hydrophobic moieties are oriented inward forming a non-polar core and the hydrophilic head groups are in contact with the bulk aqueous phase. From a physico-chemical point of view this could be looked at as an example of molecular complex formation or a pseudo phase separation; the two pseudo phases formed being the aqueous phase and the micellar phase. Both models have been

used to describe the physico-chemical properties of micelles, however, the phase separation model is particularly useful for describing solubilization properties of micellar systems and how solute solubility varies with surfactant concentration.

Because of the great diversity of organized surfactant aggregates and their unique solubilization properties, they have found numerous practical applications in many areas of separation science. Micelles can differentially solubilize and bind a variety of solute molecules via hydrophobic, electrostatic and hydrogen-bonding interactions. The solubilization properties for a specfic solute can be controlled by: variation of surfactant head-group type or hydrocarbon region; variation of surfactant concentration; addition of appropriate additives such as organic solvents, ionic salts, cyclodextrins, ion-pairing and complexing agents; or by manipulation of the experimental parameters such as pH (for ionizable solutes) and temperature. When used as additives to the mobile phase in HPLC or the buffer in CZE the resulting enhanced selectivity is unmatched by any other single separation technique. Micelles differentially solubilize structurally similar solutes. Both hydrophilic (ionic and polar) and hydrophobic solutes can be simultaneously separated, because solutes can interact with the micelles via a combination of electrostatic, hydrophobic and hydrogen-bonding forces. Micelles allow for direct injection of untreated biological fluids because solubilization of the fluids by the surfactant prevent protein precipitation. Furthermore, micellar systems allow, in some specific applications for new or enhanced modes of detection. On the negative side, some micellar systems are optically opaque which limits the wavelength range available for solute spectroscopic detection. Micellar systems are viscous which may cause complications in HPLC but not in CZE. Finally, one of the most serious disadvantages of using micellar additives is the resulting diminished column efficiency in comparison with HPLC with hydro-organic mobile phases and CZE with unmodified buffers.

For information on the formation and properties of micellar systems the reader is referred to the excellent books by Mukerjee and Mysels (101), Tanford (102) and the excellent reviews by Wennerstrom and Lindman (103) and by Hinze (104).

THEORY

Electrophoresis involves the migration of charged particles in a semi-conducting fluid under the influence of an electric field. Ionic and ionizable solutes are separated based on differences in charge, size and shape. When a charged particle is placed in an electric field (E) it experiences a force which is proportional to its effective charge (q) and the electric field strength. The translational movement of the particle is opposed by a viscous drag force which is proportional to the particle velocity (V), hydrodynamic radius (r) and medium viscocity (η). When the two forces are counterbalanced the particle moves with a steady state velocity (3):

$$V_{ef}=\mu_{ef} E \qquad (1)$$

where μ_{ef} is the electrophoretic mobility and E is the applied voltage per unit column length (L). Electroosmosis in capillary tubes, on the other hand, refers to the propulsion of the bulk solvent in the tube under the influence of an applied electric potential. The surface of silica consists of Si-OH groups which are ionized to SiO^- in alkaline and slightly acidic media (PH>2). The negatively charged surface is counterbalanced by positive ions from the buffer and a double layer is formed. Under the influence of an applied potential the positive ions in the diffuse region migrate towards the cathode and in so doing they entrain the water of hydration resulting in electroosmotic flow. The equations of flow are identical to those developed for electrophoretic migration since both phenomena are complementary. The electroosmotic velocity (V_{eo}P is given by:

$$V_{eo}=\mu_{eo} E \quad (2)$$

where μ_{eo} is the electoosmotic mobility. μ_{eo}, depends, to a large extent, on the magnitude of the Zeta potential at the capillary wall bulk buffer interface. The Zeta potential is largely dependent on the electrostatic nature of the wall surface and, to a smaller extent, on the ionic nature of the buffer.

Electroosmotic flow is directly proportional to the Zeta potential and for untreated capillary walls it, generally, decreases with decreasing pH, because the hydrogen ions deactivate the column surface causing a decrease in the Zeta potential. At moderate pH values (>3) electroosmotic flow with untreated capillary columns is generally higher than electrophoretic flow causing all solutes (cationic, neutral, anionic) to migrate towards the detection end of the column. Cationic and anionic solutes are separated based on differential electrophilic migration while neutral solutes co-migrate with the electroosmotic flow velocity and are not separated (97,105).

When an ionic surfactant is added to the buffer at concentrations above its CMC, surfactant monomers tend to aggregate forming micelles. The surface of the micelles acquire a charge which gives them an electrophoretic mobility. Negatively-charged micelles (such as SDS) migrate electrophoretically towards the anode and positively-charged micelles (such as CTAB) migrate electrophoretically towards the cathode. The electrophoretic motion is opposed by the electroosmotic motion (4,9,97). Since the electroosmotic velocity for most systems (see ref. 51 for exceptions) is higher than the electrophoretic velocity, the net migration of negatively-charged micelles will be towards the cathode and the net migration of positively-charged micelles will be towards the anode. In both cases, neutral solutes, which are not separated by conventional CZE, partition between the slow moving micellar phase and the faster moving aqueous buffer phase resulting in retention and separation based on differential solubilization in the micellar phase. The mechanism of retention in MECC is, therefore, similar to that of

liquid-liquid chromatography with a micellar stationary phase and an aqueous buffer stationary phase. The major difference is that solute retention in MECC falls in a time interval between the retention time of a solute that has no interaction with the micelles, i.e. moves with the buffer's osmotic velocity and the retention time of a solute that is totally solubilized in the micelles (4,9).

The fundamental equations of retention in MECC were first derived by Terabe and co-workers (4). For neutral solutes the capacity factor ($\tilde{k}'$) is given by:

$$\tilde{k}' = \frac{t_{Rm} - t_o}{t_o[1 - t_{Rm}/t_{mc}]} \qquad (3)$$

where t_{Rm} is solute retention time, t_o is the retention time of a solute with no interaction with the micelles (for example, mesityl oxide) and t_{mc} is the retention time of a solute that is completely solubilized by the micelles (for example, sudan III). Terabe et al. (9) used $\tilde{k}'$ as a symbol for the capacity factor in MECC to emphasize the difference from conventional chromatography. Algebraic manipulation of eq. 3 readily yield the following expression for t_{Rm} (9):

$$t_{Rm} = \frac{1 + \tilde{k}'}{1 + [t_o/t_{mc}]\tilde{k}'} t_o \qquad (4)$$

As $t_{mc} \to \infty$ which is the condition for a stationary micellar phase, eq. 3 reduces to the fundamental equation of chromatography:

$$\tilde{k}' = \frac{t_R - t_o}{t_o} \qquad (5)$$

Rather than deriving eq. 3 from basic principles as was done by Terabe and co-workers (4), we arrived at eq. 3 from eq. 5 as follows: Solute corrected retention time in chromatography format (t_R-t_o) is longer than solute corrected retention time with MECC format (t_{Rm}-t_o) by a factor equal to the ratio of solute velocity in MECC format (V_s) to solute velocity assuming the micelles are stationary (V_s-V_{mc}), where V_{mc} is the velocity of the micelles. Substituting for t_R-t_o in eq. 5 we get:

$$\tilde{k}' = [\frac{t_{Rm}-t_o}{t_o}] \; [\frac{V_s}{V_s-V_{mc}}] \qquad (6)$$

Eq. 3 is obtained from eq. 6 by simple algebraic rearrangement after substituting for the velocities according to the relationships $V_s = L/t_{rm}$ and $V_{mc} = L/t_{mc}$.

The resolution equation was also given by Terabe et al. (9):

$$R_s = \frac{N^1/2}{4} \; (\frac{\alpha-1}{\alpha}) \; (\frac{\tilde{k}_2'}{1+\tilde{k}_2'}) \; (\frac{1-t_o/t_{mc}}{1+(t_o/t_{mc})\tilde{k}_1'}) \qquad (7)$$

where N is the plate number, and α is the separation factor which is equal to $\tilde{k}'_2/\tilde{k}'_1$. Here again the analogy to conventional chromatography is obvious. As $t_{mc} \to \infty$ eq. 7 reduces to the equation of resolution in chromatography. Unlike chromatography, large $\tilde{k}'$ values do not lead to better resolution because the last term in eq. 7 decreases as $\tilde{k}'$ increases. From their discussion of the significance of the last two terms of eq. 7, Terabe and co-workers (9) conclude that for a given value of t_o/t_{mc}, there is an optimum value of $\tilde{k}'$ that maximizes the resolution at a given value of N and α.

Foley (106) presented an interesting discussion of optimization in MECC which included a comprehensive review of the relevant literature, and concluded that all the variables, except surfactant identity, have very little effect on selectivity for neutral solutes. In his view, MECC is much restricted with respect to control of selectivity in comparison to HPLC. A variety of factors have been studied for their effect on selectivity, including applied voltage (21), pH (15,29,80,105,107), organic modifier (30,80,105), surfactant type (11,21,35), and surfactant concentration (15,20,29). Factors affecting column efficiency were also examined by several workers including applied voltage (16,35), electroosmotic velocity (35), column dimensions (16,107), buffer concentration (16), temperature (26,35), surfactant concentration (16,26,35), sample injection techniques (20,35), and the elution range (9,17,21).

More recently, Ghowsi et al. (108) derived MECC retention and resolution equations in a manner analogous to CZE. Column efficiency was treated with the appropriate parameters of the Van Deemter equation and resolution was optimized for the different possibilities of micelle migration. For positive and zero migration mobility the range of capacity factor for optimum resolution (2-5) is comparable to that for HPLC.

The theory of retention in MECC was further extended by Khaledi and co-workers to cover anionic (82) and cationic (90) solutes. A mathematical model was advanced that would allow the prediction of migration behavior of solutes based on a limited number of experiments. The pH and micelle concentration were found to be interactive and it was concluded that the most effective strategy to enhance the separation of ionic solutes is to optimize these two parameters simultaneously.

TYPES OF MICELLS USED IN MECC

Terabe et al. (4), developed MECC for the separation of neutral solutes. However, today MECC is used to enhance the selectivity for the separation of neutral as well as ionic solutes. Wellingford and Ewing (25) were able to use

MECC to resolve a mixture of neutral, ionic and zwitterionic compound. In this section we will present examples that will illustrate the use of MECC in it's various modes including buffer modification in order to give the reader a general idea of the utility and application of MECC.

Anionic, Cationic, Zwitterionic and Neutral Surfactants:

The first MECC work was published in 1984 by Terabe et al. (4) who were able to resolve a mixture of 14 phenol derivatives using 1mM SDS in a buffer made by titrating 0.025 M tetraborate solution with 0.05 M sodium dihydrogen phosphate to pH 7.0. The mixture was resolved in less than 20 minutes (figure 1).

Sodium tetradecyl sulphate (STS) was used with a borate-phosphate buffer (pH 7.0) to resolve a mixture of aromatic compounds (9). Burton et al. (21) evaluated the use of four common surfactants as "pseudo stationary" phases in MECC. Two anionic surfactants, SDS and STS and two cationic surfactants, dodecyltrimethylammonium chloride (DTAC) and cetyltrimethylammonium chloride (CTAC) were studied, using a mixture of acidic, basic, electron-withdrawing and neutral compounds. They concluded that the SDS micellar system is: (a) similar to reversed phase in HPLC for moderately water soluble compounds; and (b) stable and suitable for a wide range of compounds. Using STS to extend the elution range was unsatisfactory, due to poor retention reproducibility (21). The cationic surfactant CTAC was found to be useful for large molecular weight solutes. The acidic compounds were more retained by CTAC and DTAC (21). In a previous study Otsuka et al. (11,19) compared the separation of 22 PTH-amino acids using SDS and dodecyltetraammonium bromide (DTAB). McNair and co-workers (84) studied the use of sodium alkyl sulphates, namely SDS and STS, as pseudo phases to resolve the ASTM test mixture LC-79-2. They were able to achieve good resolution of benzyl alcohol, acetophenone, methyl benzoate and dimethyl-terepthalate from benzene and benzaldehyde which coeluted using SDS. In the range of 0.025-0.075 M SDS it was not possible to resolve benzene from benzaldehyde. Attempts to improve resolution by using STS of different concentrations

were not feasible. Minor differences in the selectivity of both SDS and STS were observed.

Non ionic and zwitterionic surfactants were used as micelles to enhance the selectivity of the separation by CE (56). Octyl β-D-glucoside and CHAPS above their CMC were used in a phosphate buffer to resolve desipramine from nortriptyline, which differed only by one proton. Also, they were used to resolve two heptapeptides which differed by the substitution of isoleucine for valine at the fourth residue from the N-terminus.

Bile Salts:

Bile salts are biological surfactants which are synthesized in the liver. They form small micelles, up to ten monomers, by the hydrophobic interaction between the nonpolar sides of the monomers. Terabe et al. (45) used bile salts which extends the utility of MECC to hydrophobic species to resolve a mixture of racemic DNS-ammino acids. Later, Nishi et al. (50) studied 4 bile salts, namely, sodium cholate, sodium deoxycholate, sodium taurocholate and sodium taurodeoxycholate, as chiral micelles for the separation of optical isomeric drugs. A solution of 0.05 M bile salt was prepared in a 0.02M phosphate-borate buffer at two different pH values 7.0 and 9.0. Differences in the separation were observed when the different bile acids were used at pH 7.0 and pH 9.0. The reduction in buffer solution pH, using these anionic bile salts, greatly increases chiral recognition for compounds that can be either positively charged or basic (45). Sepaniak et al. (109) also used bile salts to resolve a mixture of polycyclic aromatic hydrocarbons.

ADDITIVES TO MICELLAR SYSTEMS

Organic and Inorganic Modifiers:

The use of anionic, cationic and neutral surfactants as pseudo phases in MECC may not always lead to the desired separation. In this section modification of the pseudo phase with organic and inorganic compounds will be discussed and

representive examples presented. Sepaniak and his co-workers (17,26,30,80,92), Fujiwara and Honda (15), and Terabe et al. (105) studied the effect of the addition of an organic solvent to the micellar phase. It was found that the addition of organic solvents to the pseudo phase serves few purposes: (a) interact with the capillary wall and as a result slows down electroosmotic flow; (b) decreases the polarity of the mobile phase; (c) alters the partition coefficient; (d) may improve the selectivity; (e) extends the utility of the technique to more hydrophobic compounds; and (f) allows the development of gradient elution in MECC. Selectively in MECC is altered by the addition of organic solvents (15,17,26,30,92). The organic additive modifies the retention mechanism by shifting the equilibrium of the solute towards the bulk aqueous buffer phase. Selectivity is enhanced by the addition of organic modifiers because the shift in equilibrium is greater for hydrophobic solutes compared to hydrophilic solutes (110). However, the addition of an organic solvent at high percentages, above 15% (v/v) drops column efficiency and migration times become impractical. For example, the addition of 22.5% isopropanol extended the elution window from 30 min. to over 80 min., figure 2. It was found that analysis time in MECC is dependent on the amount and type of organic modifier; methanol and isopropanol extended the elution range, while acetonitrile and dioxane did not affect the flow appreciably (26). The addition of 20% (v/v) methanol to a phosphate-borate buffer, pH 8, containing 25 mM SDS allowed the resolution of dansylated methylamine from dansylated methyl-d_3-amine in 83 minutes (34,42), figure 3.

Balchunas and Sepaniak (26) were the first to report the use of gradient elution in MECC to resolve a mixture of ten primary and secondary amines by stepwise gradient elution, figure 4. Note that these amines were not resolved using isocratic elution. The gradient forming solvent was composed of Triton X-100 and isopropanol. This was added stepwise to the starting mobile phase which was made of 1.5ml 0.05M SDS, 0.01M Na_2HPO_4, 0.005M $Na_2B_4O_7$ and 10% (v/v) 2-propanol. A gradient solution, consisting of identical SDS and buffer salt

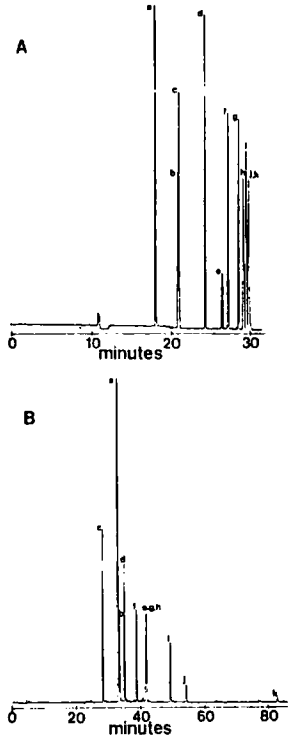

FIGURE 2. (A) Separation of a test mixture by using a 0.05 mm i.d. x 850 mm long column with 0.05 M SDS, 0.01 M Na_2HPO_4/0.005 M $Na_2B_4O_7$ (pH 7) mobile phase. (B) Separation of test mixture with 22.5% 2-propanol in the mobile phase. (Reprinted with permission).

concentrations, and 50% (v/v) 2-propanol and 2.5% (v/v) Triton X-100 was added in four 0.5ml increments every 5 minutes. The addition of the nonionic surfactant poly(ethyleneglycol)p-isooctyl-phenylether (Triton X-100) to the micellar phase resulted in a reduction of the electrophoretic velocity of the micelles. This was done in order to compensate for solvent-related reductions in electroosmotic velocity. Sepaniak et al. (40) reported in 1989 the building of an apparatus for continuous gradient elution. This apparatus was later used

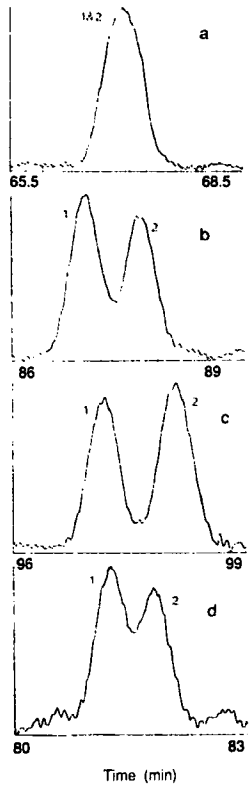

FIGURE 3. Electropherogram of DNS-$NHCD_3$ and DNS-$NHCH_3$. Run conditions are as follows: 25 mM SDS, 20% MeOH, 25 mM Na_2HPO_4, 0.625 mM $Na_2B_4O_7 \cdot 10H_2O$; capillary, 50 μm i.d., 150 μm o.d., 120 cm (105 cm to detector); applied potential, 30kV, 23 μA current; 365 nm excitation wavelength, 270 nm cut on emission filter wavelength. Figure 3a is 0% MeOH, 33 μA current. Figure 3b is 10% MeOH, 25 μA current. Figure 3c is 20% MeOH, 23 μA current. Figure 3d is 30% MeOH, 20 μA current. Peak 1 in each case is DNS-$NHCD_3$, and peak 2 in each case is DNS-$NHCH_3$, 10%, 20%, and 30% data have all undergone a 9-point Savitzky-Golay smooth. (Reprinted with permission).

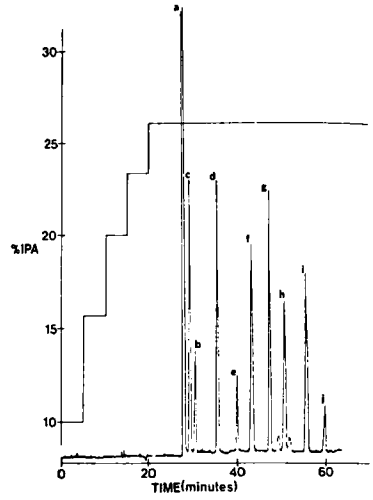

FIGURE 4. Separation of a test mixture using a stepwise solvent program. Initial mobile phase, 1.5 mL of 0.05 M SDS, 0.01 M Na_2HPO_4, 0.005 M $Na_2B_4O_7$ and 10% (v/v) isopropanol. Other conditions, same as figure 2. (Reprinted with permission).

for the resolution of a mixture of alkyl amines, figure 5, using a SDS-borate/phosphate buffer and linear and concave acetonitrile gradients (80).

Brij 35 (polyoxyethylene (23) dodecanol) is a nonionic surfactant which has little use in MECC because it cannot migrate electrophoretically. However, when added to an ionic surfactant it can effect the separation. Also, Brij 35 can be added to the micellar phase without increasing the Joule heating. McNair et al. (84) were able to resolve benzene from benzaldehyde by adding 0.025 M Brij 35 to 0.025 M SDS in 0.01 M Na_2HPO_4.

Towns and Regnier (111) examined the effect of polyoxyethylene surfactant size and structure on protein exclusion and small molecular separation. Brij 35 and other polyoxyethylene surfactants (Tween 20, Tween 40, Tween 80 and Brij 78) were used to coat the polysiloxane inner surface and to create a hydrophilic network which allows the separation of acidic and basic proteins (110). It was

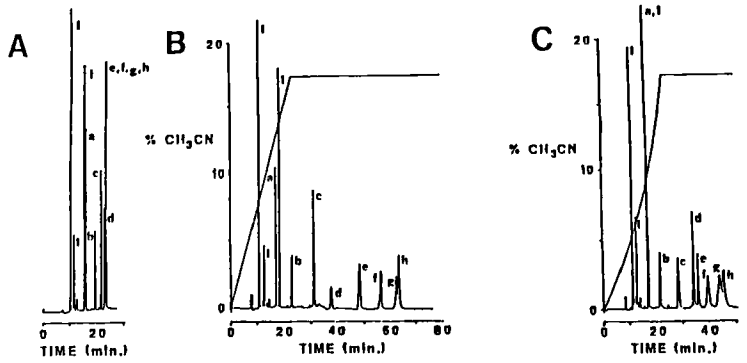

FIGURE 5. MECC chromatograms for separations of a mixture of (a) NBD-n-propylamine, (b) NBD-n-butylamine, (c) NBD-n-pentylamine, (d) NBD-n-hexylamine, (e) NBD-n-heptylamine, (f) NBD-n-octylamine, (g) NBD-n-decylamine, (h) NBD-n-dodecylamine, and (i) impurities using a mobile phase consisting of 0.01 M Na_2HPO_4, 0.006 M $Na_2B_4O_7$, 0.05 M SDS with (A) no solvent gradient, (B) a linear acetonitrile gradient, and (C) a concave acetonitrile gradient. (Reprinted with permission).

found that when the head-group size was held constant (TWEEN series), the hydrocarbon chain length had no influence on the capillary performance. However, there was a large change in capillary performance when head-group size and structure were varied.

Nishi et al. (37) studied the effect of the addition of tetraalkylammonium salts (TAAS) to an SDS micellar phase on the separation of ionic substances. The results show that the addition of TAAS to the SDS solution resulted in better separation of the solutes in the mixture. For example, the separation of nine closely related antibiotics having both anionic and cationic groups in a molecule were best resolved when TAAS (40 mM) was added to the SDS (50 mM) micellar solution, figure 6. The addition of these salts to the micellar system decreases the migration times of cationic solutes and increases the migration times of anionic solutes. It was suggested that an ion-pair type mechanism is involved whereby the TAAS ion are likely to combine with the anionic SDS micelle as a counterion instead of the sodium ion and alter the character of the micelles.

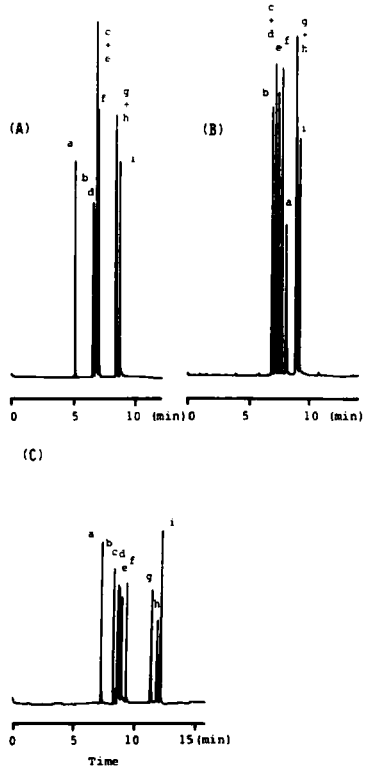

FIGURE 6. Effect of TAA salts on MECC of nine cephalosporin antibiotics: (a) C-TA, (b) ceftazidime, (c) cefotaxime, (d) cefmenoxime, (e) cefoperazone, (f) cefpiramide, (g) cefpimizole, (h) cefminox, (i) ceftriaxone; (A) CZE with 0.02 M phosphate-borate buffer 9pH 9.0), (B) MECC with 0.02 M phosphate-borate buffer (pH 9.0) containing 0.05 M SDS, (C) 0.04 M TMAB added to the same SDS solution as in (B). (Reprinted with permission).

Yu and Dovichi (112) were able to resolve a mixture of DABSYL amino acids using a 1:1 acetonitrile:20 mM phosphate buffer (pH 7.0) which contained 5 mM SDS. Of the 18 amino acids injected 2 pairs coeluted, isolucine and valine, and cysteine and tyrosine. Cohen et al. (22) resolved a chiral mixture of dansylated amino acids in MECC by complexation with Cu(II) and N,N-didecyl-L-alanine. Dobashi et al. (46) reported the MECC separation of racemic mixtures of N-

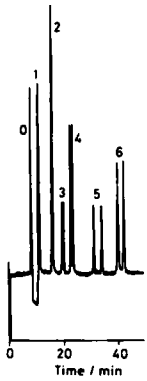

FIGURE 7. Chiral separation of six PTH-DL-AA by MECC. Corresponding amino acids: 1 = Ser; 2 = Ala; 3 = Nva; 4 = Val; 5 = Trp; 6 = Nle. 0 = acetonitrile. Micellar solution, 50 mM SDVal-30 mM SDS-0.5 M urea (pH 9.0) containing 10% (v/v) methanol; separation column, 650 mm x 0.05 mm i.d.; length of the tube used for separation, 500 mm; total applied voltage, 20 kV; current, 17 μA; detection wavelength, 260 nm; temperature, ambient. (Reprinted with permission).

acetylated amino acids by employing a chiral micelle made of surfactants derivatized with L-amino acids. The best results were obtained when N-dodecanoyl-L-valinate was used. Modification of the micellar solution with methanol affected the selectivity of the solutes. Otsuka et al. (94) investigated the use of SDVal for chiral separations in MECC, figure 7, and reported that the addition of SDS, methanol and urea improved the peak shapes and resolution, and affected the selectivity.

Gozel etal (23) were able to use a micellar solution composed of 20 mM sodium tetradecylsulfate (pH 7.81), 2.5 mM copper sulfate, 5.0 mM aspartame and 10 mM ammonium acetate to resolve a chiral mixture of dansylated D-amino acids from L-amino acids, figure 8. The addition of STS resulted in the resolution of the enantiomer pairs which were not completely separated in its absence.

Terabe and Isemura (52) resolved a mixture of naphthalene disulfonates by modifying the buffer with a soluble cationic polymer. The polymer acts as a pseudostationary phase mimicking an ion exchanger.

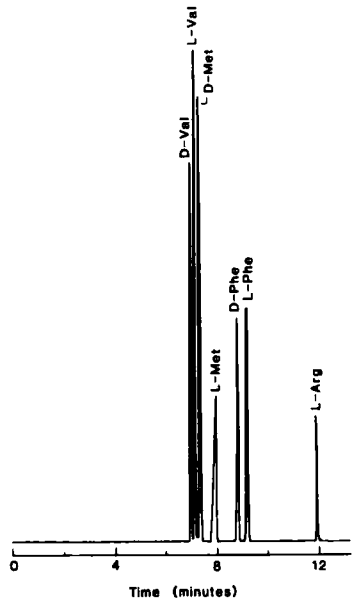

FIGURE 8. Electropherogram of a mixture of three DNS-DL-AAs. DNS-L-Arg is used as an internal standard. Electrolyte composition is as follows: 2.5 mM $CuSO_4 \cdot 5H_2O$, 5.0 mM aspartame, 10 mM NH_4OAc, and 20 mM STS, pH 7.81, applied voltage, -30 kV; current, -38 μA. (Reprinted with permission).

Nishi et al. (37) showed that the addition of tetraalkylammonium salts to the micellar solution improved the separation of some ionic substances. Terabe et al. (45) found that the addition of 10 mM SDS to the micellar bile salt solution cut the retention time in half without affecting the resolution considerably. Karger and his co-workers (8) were able to resolve 14 out of a mixture of 18 oligonucleotides of 8 bases, each with a different sequence, in less than 30 minutes by adding zinc and SDS to the buffer system. Their explanation is that the addition of low concentrations of divalent metals and SDS to the buffer system leads to a significant enhancement of the time window and good separation of the oligonucleotides. Metal ions are electrostatically attracted

to the surface of the micelle where they can be selectively complexed with appropriate solutes, which can manipulate selectivity. Zn(II) was used because it is intermediate in oligo-nucleotide binding strength between Mg(II) and Cu(II); also Zn(II) is transparent in the range of oligonucleotide detection (48). Cu(II)-L-histidine (23) and Cu(II)-aspartame (23) were used for chiral separations in MECC.

The Cyclodextrins:

The alpha, beta and gamma cyclodextrins are cyclic oligosaccharides which contain 6-, 7-, and 8- glucose units, respectively. These compounds which have the shape of a truncated cone have a hydrophobic inner cavity and hydrophilic outer surface. In addition, they are chiral molecules where each glucose unit contains five chiral atoms. This means that the β-CyD contains thirty-five chiral atoms. These properties, hydrophobic, hydrophilic and chiral can be used to effect different separation problems (113-116). In addition, CyD form inclusion complexes with compounds in solution, which means that compounds are separated by their geometrical fit into the CyD cavity (114). Depending on the size of the molecules to be separated, the analyst can select the right cavity size. For example, α-CyD is most useful for small molecules such as amino acids, inorganic ions...etc. The β-CyD has an inner diameter of 7.8A and is used for molecules larger than a benzene ring but smaller than benzo(a)pyrene. The γ-CyD is used for large molecules. The most widely used one in HPLC is the β-CyD (113,116). The primary mode of separation by CyD is the formation of inclusion complexes (114) between the guest molecules to be separated and the host CyD hydrophobic cavity. These separations are effected in general by methanol/water or acetonitrile/water and in certain cases by the addition of a buffer. Other separations (normal phase type) are carried out in hydrocarbon or hydrocarbon/alcohol mixtures. The mechanism of separation by normal phase mode is not well understood. However, it is not through an inclusion complex formation

(115). It may be the result of the interaction between the guest compounds and the outside hydrophilic surface of the CyD. Other factors such as VanDer Waals, dipole-dipole interaction and hydrogen bonding may play a role in the separation process. The mechanism of separation of a wide variety of isomers (optical, geometrical and structural) is carried out by inclusion complex formation. The isomer separations are readily achieved due to different strengths of the inclusion complexes formed in the hydrophobic cavity, the better the fit the stronger the complex. The CyD have been used as multimodal phases for the separation of small molecules (114) and chiral compounds in HPLC (113,116), GC (117), isotachophoresis (118), and gel filled HPCE (119). Terabe et al. (120) were the first to use an ionic CyD derivative as a substitute for the micelle in MECC, which can form inclusion complexes. They modified a 0.1 M phosphate buffer (pH 7.0) with 0.025M 2-O-carboxymethyl-β-cyclodextrin and were able to resolve a series of substituted benzene isomers. Fanali investigated the effect of β-CyD and derivatized β-CyD in the buffer solution on the resolution of optical isomers (48). The results show poor resolution of the enantiomers of ephedrine and isoproterenol, although high concentrations of β-CyD were added to the buffer solution. However, the addition of 18 mM Heptakis (2,6-di-O-methyl-β-CyD) to the buffer (10 mM Tris-H_3PO_4, pH 2.4) resulted in the separation of the optical isomers studied. Moreover, Terabe et al. (65) explored the applicability of CyD-MECC to the separation of highly hydrophobic and closely related compounds, such as polychlorinated biphenyls (PCBs) tetrachlorodibenzodioxin (TCDD) isomers and polycyclic aromatic hydrocarbons (PAHs). The results show that the addition of 40 mM γ-CyD to the separation solution (100 mM SDS in 100 mM borate buffer, pH 8.0, containing 2 M urea) enabled the separation of all chlorinated benzene congeners, which were not resolved in the absence of γ-CyD, figure 9. Eleven trichloro-biphenyl isomers which comigrated with the micelle were completely resolved when 60 mM γ-CyD was added. The separation using β-CyD instead of γ-CyD was not very successful. The TCDD isomers were also resolved by γ-CyD MECC. Nishi and Matsuo (73) reported that the addition of CyD to the SDS solution

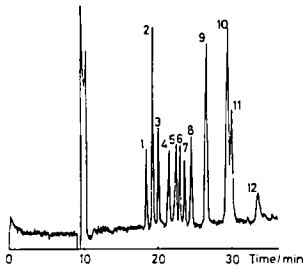

FIGURE 9. γ-CyD-MECC separation of chlorinated benzene congeners: 1 = 1,2,3,5-tetra-, 2 = 1,2,3-tri-, 3 = 1,3,5-tri-, 4 = 1,2-di-, 5 = 1,2,4,5-tetra-, 6 = mono-, 7 = 1,3-di-, 8 = 1,2,4-tri-, 9 = 1,2,3,4-tetra-, 10 = penta-, 11 = 1,4-di- and 12 = hexachlorobenzene. Capillary, 700 mm (Polymicro Technologies); separation solution, 100 mM SDS in 100 mM borate buffer (pH 8.0) containing 2 M urea and an additional 40 mM γ-CyD; applied voltage, 15 kV; current, 23 μA. (Reprinted with permission).

improved the resolution of lipophilic compounds corticosteroids and aromatic hydrocarbons.

FACTORS THAT INFLUENCE RETENTION TIMES, RESOLUTION AND SELECTIVITY IN MECC

The surfactants are molecules which contain hydrophobic and hydrophilic parts. They might be anionic, cationic, zwitterionic or neutral depending on the charge of the polar head group. The surfactants at a certain concentration above their CMC form aggregates or micelles. The aggregation number and CMC values depend on various physiochemical parameters including the addition of organic solvents, ionic strength, pH, temperature, and the presence of electrolytes. Separation in MECC is a function of the partitioning of the solutes between the micelle pseudo phase and the bulk solvent, and the difference in mobility of the bulk solvent and the micelle. Therefore, selectivity can be manipulated by changing the type and composition of the surfactant and by variations in experimental parameters.

The discussion in the previous section clearly shows the influence of different micelles and additives to the micellar solution on separation, peak shape, retention times and selectivity. In this section, selected specific examples are presented that highlight the effect of experimental conditions on separation parameters. Terabe et al. (9,105) found that the solute velocity is linearly dependent on the current. Also, at constant applied voltage the v_{eo} remained virtually constant irrespective of SDS concentration (0.025 M to 0.15 M), while the reciprocal of v_{mc} and capacity factor increased with an increase in SDS concentration (9). The current was observed to increase linearly with SDS concentration. This increase in current with SDS concentration is probably due to the increase in the sodium ion concentration of the SDS (121). It was observed by McNair et al. (84) that the current did not increase with an increase in the concentration of Brij 35 which does not contain a metal ion. The effect of pH on v_{eo} at 0.1 M and 0.2 M SDS in a phosphate-borate buffer was negligible in the pH range of 6-9. This may be due to the adsorption of the SDS on the inner wall of the capillary. No substantial effect on the electroosmotic velocity was observed when 0.1% of Brij 35, Tween 20, hydroxypropylcellulose or hydroxypropyl-methylcellulose were added to SDS solution (110).

The effect of surfactant type (11, 21,35), concentration (15,20,29) and pH (9,15,29,41,68,69,80,91,105,107) on selectivity and on column efficiency (16,26,35) were studied by several workers. Sepaniak et al. (21) found that the type of surfactant used; SDS, STS or DTAC affects the elution order (selectivity) of the solutes. Ong et al. (68,69) in their study of parameters (pH, SDS concentration and applied voltage) which influenced the separation of six phthalate esters and catecholamines found that changing the SDS concentration changed solute retention times, figure 10, due to the solubilization of the phthalates by the micelle. Sharper peaks were observed at higher voltages, with an increase in efficiency and resolution. Although they stated (69) that the elution order does not change with pH, it is clear that above pH 7.0 two of the solutes reversed their elution order (figure 2, in ref. 69). Rasmussen and

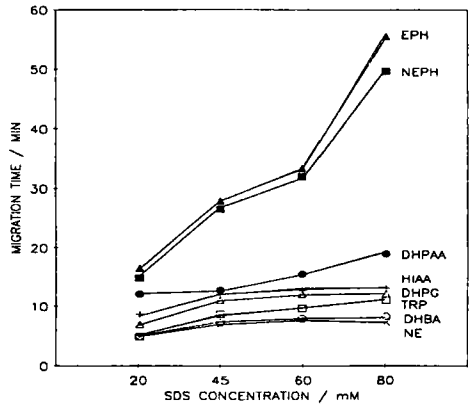

FIGURE 10. Plot of migration times of the catechols and catecholamines with variation in SDS concentration. Experiments were carried out at pH 7.00. (Reprinted with permission).

McNair (41) found that the elution order of alkylparabanes in MECC using 0.01M Na_2HPO_4/0.05M SDS, pH 6.75 was reversed when the pH was adjusted to 3.37. It was also observed that there were changes in the capacity factor with changes in the pH of the micellar solution (68-69,91). Ong et al. (68) and Vinevogel and Sandra (91) observed that the migration times decreased with increasing pH. This finding contradicts what was reported earlier for both CZE (3) and MECC (9). Ong et al. (68) reported that migration times for ephedrine and norephedrine dropped from 60 min. and 54 min. to about 25 min. when the pH was increased from 6 to 7, see figure 11. Other examples are given in reference 91. Otsuka and Terabe (51) studied the effect of pH on solute migration in MECC, and concluded that the electro-osmotic velocity decreased with increasing pH below 5.5, while the electrophoretic velocity of the SDS micelles was almost constant in the pH range 3.0-7.0. Figure 12 shows how changes in pH affect the direction and magnitude of the migration veolcity in SDS, MECC.

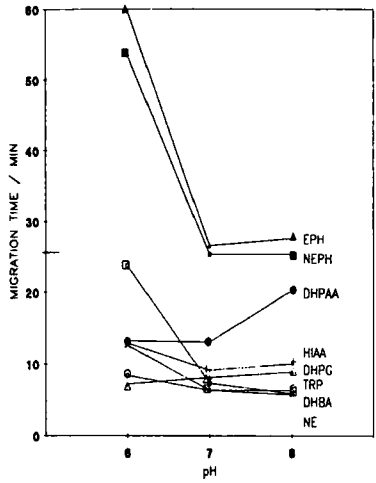

FIGURE 11. Plot of migration times of the catechols and catecholamines with variation of pH. Experiments were carried out at 80 mM SDS. (Reprinted with permission).

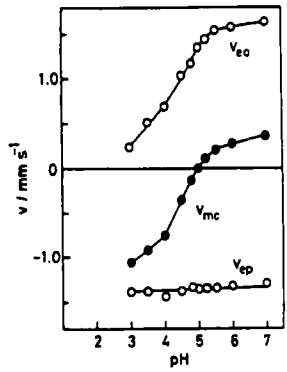

FIGURE 12. Dependence of electrokinetic velocities on pH. Micellar solution, 0.10 M SDS (pH 6.0); column, 0.05 mm i.d. x 650 mm; length of the column used for separation: 500 mm; current, 50 μA; applied voltage, about 14.7 kV; detection wavelength: 220 nm. (Reprinted with permission).

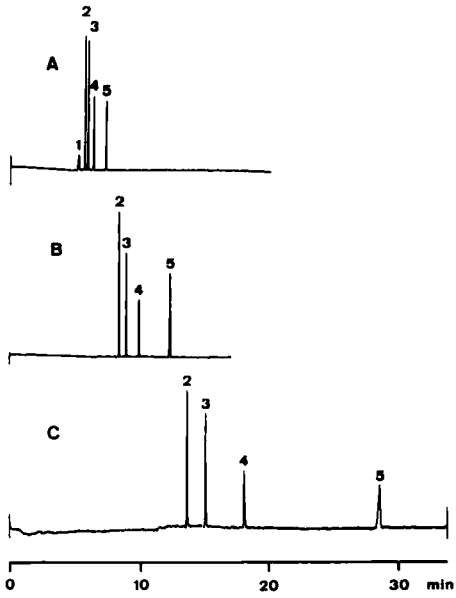

FIGURE 13. MECC separations of purine derivatives using capillaries coated with polymers of different chemical structure: Sample: 0.2 mg/ml each; 1 water; 2 theobromine; 3 theophylline; 4 caffeine; 5 uric acid; Column: 80 cm effective, 120 cm total length (A) polymethylsiloxane OV-1 on FS; 50 μm i.d., film thickness 0.09 μm; (B) uncoated FS; 50 μm i.d.; (C) polyethylene glycol CW20M on FS; 50 μm i.d., film thickness 0.04 μm; Temperature: 298 K; Buffer: 0.02 M phosphate buffer/0.05 M SDS; Injection: electrokinetic; 7500 V; (A) 2 s; (B) 5 s, (C) 8 s; Separation voltage: 35 kV; Detection: UV/254 nm. (Reprinted with permission).

Column Coating Effects in MECC:

The influence of two polymeric coatings (DB-1 and DB-WAX) of the inner surface of the capillary on electroosmotic flow was first studied by Terabe et al. (105), however, the geometry of capillaries was different and direct comparison of retention data is inappropriate (53). A systematic investigation was undertaken by Schomburg and his co-workers (53) who used fused silica capillaries of identical geometry coated with different thicknesses of polymethylsiloxane (PMS) or polyethyleneglycol (PEG), using two test mixtures. The

results show that in MECC, the electroosmotic flow and the resolution can be effectively varied by polymer coating of the capillary surface. With the non-polar PMS coating the electroosmotic flow is increased giving shorter analysis time, but μ_{eo} decreased with the polar PEG coating, resulting in improved resolution, figure 13. They (53) also reported that only thin layers of polymer are necessary to modify the inner surface of the capillary. Towns and Regnier (110) were able to decrease the adsorption of proteins to the capillary walls by coating the inner surface of the capillary with an alkylsilane followed by water soluble non-ionic surfactants, such as Brij 35 and Tween. Balchunans and Sepaniak (17) used capillaries deactivated with trimethylsilane for the separation of small amines by MECC. The deactivation resulted in reduced electroosmotic flow, allowing the separation of small molecular weight compounds.

El Rassi (122) had introduced new micellar systems with surfactants of adjustable surface charge density that allow the tailoring of the separation window to any desired level for a particular separation.

Knox (123) suggested the use of colloidal sols as pseudostationary phases whereby solutes can absorb and desorb from the surface of the charged colloidal particles (pH 7.0), which results in the separation of a mixture.

Weinberger and Alban (95) investigated the parameters that affect the linear dynamic range in quantitative MECC. Their results show good linearity at low solute concentration (less than 100 μg/ml); and by employing high ionic strength buffers and small diameter capillaries.

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