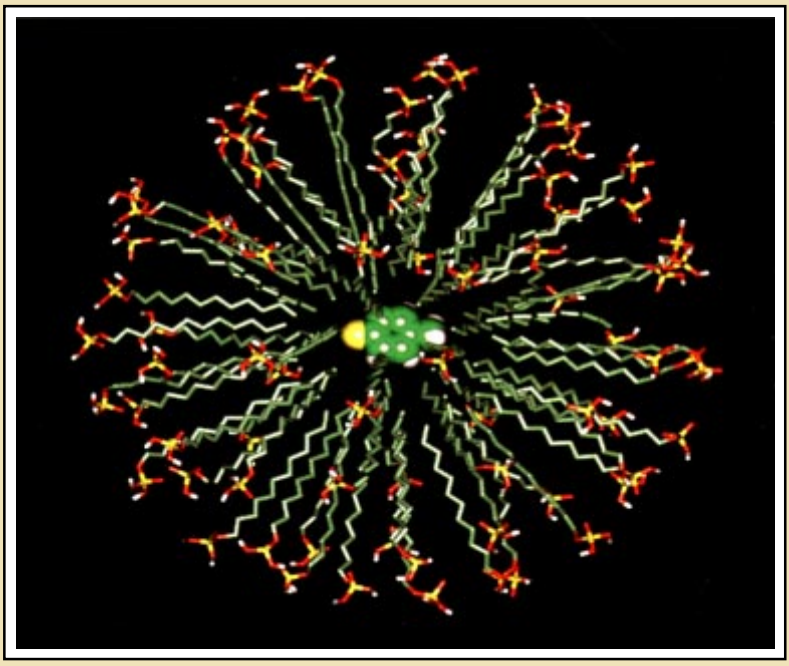
Micellar Electrokinetic Chromatography



BECKMAN

Back to Catalog

Micellar Electrokinetic Chromatography

Shigeru Terabe

Himeji Institute of Technology
Kamigori, Hyogo, Japan

Table of Contents

About the Author

Shigeru Terabe is a professor of analytical chemistry at the Himeji Institute of Technology in Kamigori, Hyogo (Japan). His research interests include the development of high-resolution separation methods such as capillary electrophoresis, electrokinetic chromatography, and open-tubular liquid chromatography. His scientific publications span 65 research papers, 19 review papers, and 2 books. Dr. Terabe is well known for his pioneering work in the field of micellar electrokinetic chromatography. He is a member of the editorial advisory boards of the *Journal of Microcolumn Separations*, *Analytical Chemistry*, the *Journal of Chromatography*, *Chromatographia*, and the *Journal of Biochemical and Biophysical Methods*.

Acknowledgements

We would like to thank Jeff Allen, Ron Biehler, Phyllis Browning, Andy Jacobs, and Kathi Ulfelder at Beckman Instruments for reviewing drafts of the manuscript and for their many suggestions. We are also grateful to Don Gregory at Polygen/Molecular Simulations, Sunnyvale, CA, for the computer drawing of the SDS micelle (front cover) and to Gale Leach at WordsWorth, Pacifica, CA, for the desktop publishing.

Herb Schwartz (technical editor)
Palomar Analytical Services
Redwood City, CA

Acronyms and Symbols Used

The following acronyms and symbols are used in this book.

α	separation factor
CD	cyclodextrin
CDEKC	cyclodextrin electrokinetic chromatography
CDMEKC	cyclodextrin-modified micellar electrokinetic chromatography
CE	capillary electrophoresis
CMC	critical micelle concentration
C_{sf}	surfactant concentration
CTAB	cetyltrimethylammonium bromide
CZE	capillary zone electrophoresis
DTAB	dodecyltrimethylammonium bromide
E	electric field strength
EKC	electrokinetic chromatography
EOF	electroosmotic flow
HPLC	high performance liquid chromatography
IXEKC	ion exchange electrokinetic chromatography
K	distribution coefficient
Kp	Kraft point
k'	capacity factor
LMT	*N*-lauroyl-*N*-methyltaurate
MECC	micellar electrokinetic capillary chromatography
MEEKC	microemulsion EKC
MEKC	micellar electrokinetic chromatography
μ_{eo}	electroosmotic mobility
μ_{ep}	electrophoretic mobility
$\mu_{ep}(mc)$	electrophoretic mobility of the micelle
μ_{ep}^*	effective electrophoretic mobility
N	number of theoretical plates
n	aggregation number
n_{aq}	number of moles of the analyte incorporated into the aqueous phase

n_{mc}	number of moles of the analyte incorporated into the micelle
RPLC	reversed phase liquid chromatography
SDS	sodium dodecyl sulfate
SDVal	sodium *N*-dodecanoyl-*L*-valinate
STS	sodium tetradecyl sulfate
t_0	migration time of the bulk solution
t_{mc}	migration time of the micelle
t_R	migration time of the analyte
UV	ultraviolet
V_{aq}	volume of the nonmicellar phase
V_{mc}	volume of the micellar phase
$\bar{\boldsymbol{v}}$	migration velocity
	partial specific volume of the micelle

I: Introduction

Electrokinetic chromatography (EKC) is a family of electrophoresis techniques named after electrokinetic phenomena, which include electroosmosis, electrophoresis, and chromatography. Micellar electrokinetic chromatography (MEKC) is a mode of EKC in which surfactants (micelles) are added to the buffer solution. Surfactants are molecules which exhibit both hydrophobic and hydrophilic character. They have polar "head" groups that can be cationic, anionic, neutral, or zwitterionic and they have nonpolar, hydrocarbon tails. The formation of micelles or "micellization" is a direct consequence of the "hydrophobic effect." The surfactant molecules can self-aggregate if the surfactant concentration exceeds a certain *critical micelle concentration* (CMC). The hydrocarbon tails will then be oriented toward the center of the aggregated molecules, whereas the polar head groups point outward. Micellar solutions may solubilize hydrophobic compounds which otherwise would be insoluble in water. The front cover picture shows an aggregated SDS molecule. In the center of the aggregate, *p*-fluorotoluene is situated depicting the partitioning of a neutral, hydrophobic solute into the micelle. Every surfactant has a characteristic CMC and aggregation number, *i.e.*, the number of surfactant molecules making up a micelle (typically in the range of 50-100). (See also Table 1 and the discussion on page 10). The size of the micelles is in the range of 3 to 6 nm in diameter; therefore, micellar solutions exhibit properties of homogeneous solutions. Micellar solutions have been employed in a variety of separation and spectroscopic techniques. In 1980, Armstrong and Henry pioneered the use of micellar solutions as mobile phases for reversed-phased liquid chromatography (RPLC).

In the literature, MEKC is also often referred to as MECC (micellar electrokinetic capillary chromatography) since the separations are most often performed in a capillary tube. Other modes of EKC are cyclodextrin EKC (CDEKC), ion-exchange EKC (IXEKC), and microemulsion EKC (MEEKC). Cyclodextrin derivatives, polymer ions, and microemulsions are used in CDEKC, IXEKC, and MEEKC, respectively, instead of the micelles used in MEKC. The references listed on page 3 provide further detail on the differences between the various kinds of EKC techniques. In the following chapters, relevant references are listed in reverse chronological order after each chapter. All EKC techniques are based on the same

separation principle: the differential partitioning of an analyte between a two-phase system (*i.e.*, a mobile/aqueous phase and a stationary phase).

The same instrument that is used for capillary zone electrophoresis (CZE) is also used for MEKC. Both MEKC and CZE are modes of capillary electrophoresis (CE), as are capillary gel electrophoresis, capillary isoelectric focusing, and capillary isotachophoresis (for an introduction to CE, see the Beckman Primer *Introduction to Capillary Electrophoresis*, part number 360643). MEKC is different in that it uses an ionic micellar solution instead of the simple buffer salt solution used in CZE. The micellar solution generally has a higher conductivity and hence causes a higher current than the simple buffer does in CZE. MEKC can separate both ionic and neutral substances while CZE typically separates only ionic substances. Thus MEKC has a great advantage over CZE for the separation of mixtures containing both ionic and neutral compounds. However, in MEKC the size of the sample molecules is limited to molecular weights of less than 5000, whereas CZE has virtually no limitation in molecular size. The separation principle of MEKC is based on the differential partition of the solute between the micelle and water; CZE is based on the differential electrophoretic mobility.

Further Reading
(in reverse chronological order)

Janini, G. M., Isaaq, H. J. Micellar electrokinetic capillary chromatography: basic considerations and current trends. *J. Liq. Chromatogr. 15,* 927-960 (1992)

Kuhr, W. G., Monnig, C. A. Capillary Electrophoresis. *Anal. Chem. 64,* 389R-407R (1992)

Watarai, H. Microemulsion capillary electrophoresis. *Chem. Lett.,* 391-394 (1991)

Nishi, H., Terabe, S. Application of micellar electrokinetic chromatography to pharmaceutical analysis. *Electrophoresis 11,* 691-701 (1990)

Terabe, S., Isemura, T. Ion-exchange electrokinetic chromatography with polymer ions for the separation of isomeric ions having identical electrophoretic mobilities. *Anal. Chem. 62,* 650-652 (1990)

Terabe, S. Electrokinetic chromatography: an interface between electrophoresis and chromatography. *Trends Anal. Chem. 8,* 129-134 (1989)

Khaledi, M. G. Micellar reversed phase liquid chromatography. *Biochromatography 3,* 20-35 (1988)

Burton, D. E., Sepaniak, M. J. Analysis of B_6 vitamers by micellar electrokinetic capillary chromatography with laser-excited fluorescence detection. *J. Chromatogr. Sci. 24,* 347-351 (1986)

Terabe, S., Ozaki, H., Otsuka, K., Ando, T. Electrokinetic chromatography with 2-*O*-carboxymethyl-β-cyclodextrin as a moving "stationary" phase. *J. Chromatogr. 332,* 211-217 (1985)

Terabe, S., Otsuka, K., Ando, T. Electrokinetic chromatography with micellar solution and open-tubular capillary. *Anal. Chem. 57,* 834-841 (1985)

Terabe, S., Otsuka, K., Ichikawa, K., Tsuchiya, A., Ando, T. Electrokinetic separations with micellar solution and open-tubular capillaries. *Anal Chem. 56,* 111-113 (1984)

Armstrong, D. W., Henry, S. J. Use of an aqueous micellar mobile phase for separation of phenols and polynuclear aromatic hydrocarbons via HPLC. *J. Liq. Chromatogr. 3,* 657-662 (1980)

Back to Catalog

II: Separation Principle/Fundamentals

Figure 1 shows a schematic representation of the separation principle of MEKC. When an anionic surfactant such as sodium dodecyl sulfate (SDS) is employed, the micelle migrates toward the positive electrode by electrophoresis. The electroosmotic flow transports the bulk solution toward the negative electrode due to the negative charge on the surface of fused silica. The electroosmotic flow (EOF) is usually stronger than the electrophoretic migration of the micelle under neutral or alkaline conditions and, therefore, the anionic micelle also travels toward the negative electrode at a retarded velocity.

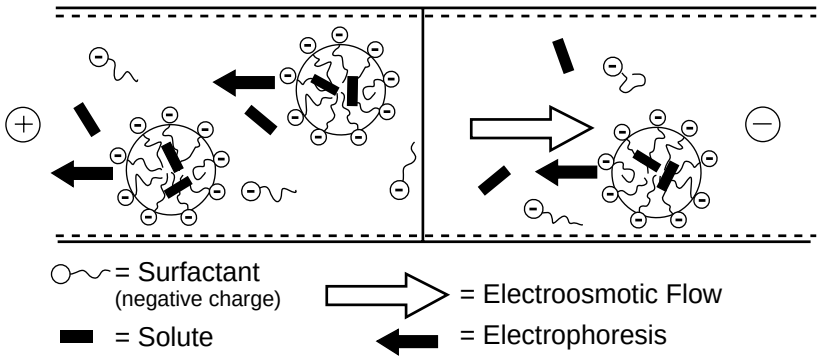


Figure 1. *Schematic of the separation principle of MEKC. The detector window is assumed to be positioned near the negative electrode.*

When a neutral analyte is injected into the micellar solution, a fraction of it is incorporated into the micelle and it migrates at the velocity of the micelle. The remaining fraction of the analyte remains free from the micelle and migrates at the electroosmotic velocity. The migration velocity of the analyte thus depends on the distribution coefficient between the micellar and the non-micellar (aqueous) phase. The greater the percentage of analyte that is distributed into the micelle, the slower it migrates. The analyte must migrate at a velocity between the electroosmotic velocity and the velocity of the micelle (see Figure 2A), provided the analyte is electrically neutral. In other words, the migration time of the analyte, t_R, is limited between the migration time of the bulk solution, t_0, and that of the

micelle, t_{mc} (see Figure 2B). This is often referred to in the literature as the *migration time window* in MEKC.

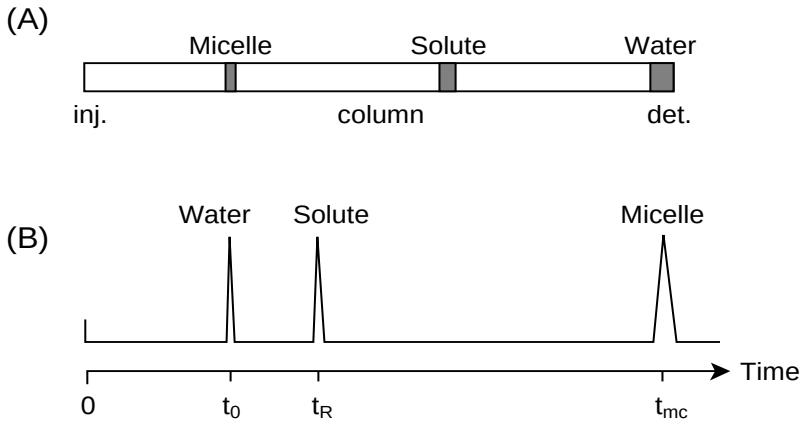


Figure 2. *Schematic of the zone separation in MEKC (A) and chromatogram (B). Reproduced with permission from Terabe,* et al., Anal. Chem. 57, *834 (1985).*

Capacity Factor

We can define the capacity factor, *k*', similarly to that of chromatography as

$$k' = \frac{n_{mc}}{n_{aq}} \tag{1}$$

where n_{mc} and n_{aq} are the amount of the analyte incorporated into the micelle and that in the aqueous phase, respectively. We can obtain the relationship between the capacity factor and the migration times as

$$t_R = \frac{1 + k'}{1 + (t_0 / t_{mc})k'} t_0 \tag{2}$$

The migration time of the analyte is equal to t_0 when $k' = 0$, or when the analyte does not interact with the micelle at all; the migration time becomes t_{mc} when *k*' is infinity or the analyte is totally incorporated into the micelle. Thus, the migration time window is limited between t_0 and t_{mc}.

When t_0 is infinity (electroosmosis is completely suppressed), equation (2) becomes

$$t_R = (1 + 1/k')t_{mc} \quad (3)$$

In this case, the bulk solution remains stationary in the capillary and the micelle migrates only by electrophoresis. If we define the capacity factor as the reciprocal of equation (1), equation (3) becomes identical with the relationship between t_R, t_0, and k' in conventional chromatography.

Figure 3 shows a typical example of MEKC separation. Eight electrically neutral compounds were successfully resolved in 17 min. The capacity factor scale is inserted in the figure to indicate the relationship between the migration time and the capacity factor. The capacity factor of infinity means that analyte has the same migration time as the micelle. Theoretical plate numbers calculated from the peak widths range from 200,000 to 250,000 which is typical for MEKC separations.

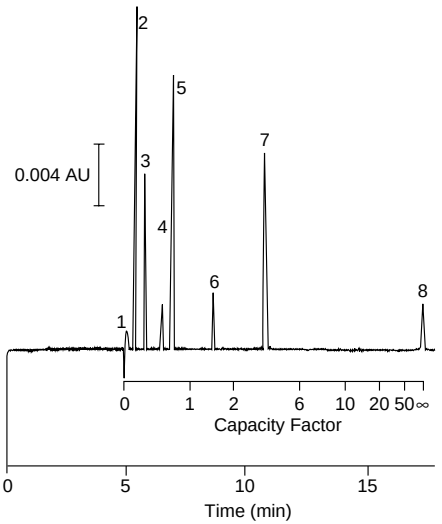


Figure 3*. Micellar electrokinetic chromatogram of a test mixture: 1 = methanol; 2 = resorcinol; 3 = phenol; 4 =* p*-nitroaniline; 5 = nitrobenzene; 6 = toluene; 7 = 2-naphthol; 8 = Sudan III. Conditions: capillary, 50 µm i.d. × 65 cm (effective length 50 cm); run buffer, 30 mM SDS in 50 mM phosphate/100 mM borate (pH 7.0); applied voltage, 15 kV; current, 33 µA; detection, UV absorbance at 210 nm; temperature, 35°C. Reproduced with permission from Terabe,* Trends Anal. Chem. 8, *129 (1989).*

Effective Mobility

The capacity factor is a fundamental term in chromatography and equation (2) is derived from a chromatographic perspective. We can derive a similar equation for electrophoretic processes. In CZE, the migration velocity of the analyte, v_s, is expressed as

$$v_s = \left[\mu_{eo} + \mu_{ep}(s)\right]E \tag{4}$$

where μ_{eo} and $\mu_{ep}(s)$ are the electroosmotic mobility and electrophoretic mobility of the analyte, respectively, and E is the electrical field strength. We can apply this equation to MEKC by defining the effective electrophoretic mobility, $\mu_{ep}^*(s)$, for the neutral analyte as

$$\mu_{ep}^*(s)\frac{k'}{1+k'}\mu_{ep}(mc) \tag{5}$$

where $\mu_{ep}(mc)$ is the electrophoretic mobility of the micelle and $k'/(1+k')$ is the fraction of the analyte incorporated into the micelle, as shown by the following equation:

$$\frac{k'}{1+k'} = \frac{n_{mc}}{n_{aq}+n_{mc}} \tag{6}$$

Thus, the velocity of the neutral analyte in MEKC is given as

$$v_s = \left[\mu_{eo} + \mu_{ep}^*(s)\right]E \tag{7}$$

The effective mobility tells us that even a neutral analyte has an apparent mobility. We can use the effective mobility in a similar way as the electrophoretic mobility is used in CZE to derive a resolution equation which can assist in optimizing separations (see also the discussion regarding equation 8). The effective mobility can simply be calculated according to equation 7, provided v_{eo} (= $\mu_{eo}E$) or μ_{eo} is known. However, in MEKC the calculation of the capacity factor requires both t_0 and t_{mc}. This is the advantage of using the effective mobility as compared to using the capacity factor. It should be emphasized that the capacity factor provides quantitative information about the distribution equilibrium, whereas the effective mobility only gives qualitative information about it. In other words, the capacity factor cannot be obtained according to equation 5 unless $\mu_{ep}(mc)$ is known.

Operating Conditions

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

Since the micellar solution has a relatively high conductivity, a capillary with a small diameter is favored to prevent excessive Joule heating. The length of the capillary usually is not very important, but a longer one can accommodate a larger amount of sample solution at the expense of time. When using relatively large i.d. capillaries for increased sample capacity, efficient capillary cooling (such as the liquid cooling system used in the P/ACE™) becomes important.

The micellar solution is prepared by dissolving a surfactant into a buffer solution at a concentration higher than its CMC (Table 1). The buffer solution is required to keep the pH constant. Concentrations of 30 to 100 mM are typically employed for both the surfactant and buffer components. The separation solution must be filtered through a membrane filter to remove particulates. A disposable, cartridge-type membrane filter can be used with a syringe because the amount of the solution necessary for an MEKC run is usually less than 10 mL. Degassing of the micellar solution is troublesome because of bubbling and is typically unnecessary. When a cationic surfactant (*e.g.*, CTAB) is employed at a high enough concentration, the direction of the electroosmotic flow is reversed. In this case, the polarity of the power supply must be reversed (see page 16).

The applied voltage must be kept to a level that is not too high in order to avoid excessive current. It is also desirable to control the capillary temperature because the migration time in MEKC is even more sensitive to temperature than in CZE (S. Terabe, *J. Microcol. Sep.*, (1992) in preparation).

Table 1. Critical Micelle Concentration, Aggregation Number (n), and Kraft point (*Kp*) of Selected Ionic Surfactants

Surfactant	CMC[a]/10^{-3} M	n	*Kp*
Sodium dodecyl sulfate (SDS)	8.1	62	16
Sodium tetradecyl sulfate (STS)	2.1 (50°C)	138[b]	32
Sodium decanesulfonate	40	40	—
Sodium dodecanesulfate	7.2	54	37.5
Sodium *N*-lauroyl-*N*-methyltaurate (LMT)	8.7	—	<0
Sodium polyoxyethylene dodecyl ether sulfate	2.8	66	—
Sodium *N*-dodecanoyl-*L*-valinate (SDVal)	5.7 (40°C)	—	—
Sodium cholate	13–15	2–4	—
Sodium deoxycholate	4–6	4–10	—
Sodium taurocholate	10–15	5	—
Sodium taurodeoxycholate	2–6	—	—
Potassium perfluoroheptanoate	28	—	25.6
Dodecyltrimethylammonium chloride (DTAC)	16 (30°C)	—	—
Dodecyltrimethylammonium bromide (DTAB)	15	56	—
Tetradecyltrimethylammonium bromide (TTAB)	3.5	75	—
Cetyltrimethylammonium bromide (CTAB)	0.92	61	—

[a] 25°C

[b] In 0.10 M NaCl

Composition of the Micellar Solution

Ionic surfactants are essential for MEKC. Numerous ionic surfactants are commercially available. The surfactants suitable for MEKC should meet the following criteria:

1. The surfactants must have enough solubility in the buffer solution to form micelles.
2. The micellar solution must be homogeneous and UV transparent.
3. The micellar solution must have a low viscosity.

Table 1 lists the CMC, aggregation number, and Kraft point of some selected ionic surfactants available for MEKC. The Kraft point is the temperature above which the solubility of the surfactant increases steeply due to the formation of micelles. In order to obtain a micellar solution, the concentration of the surfactant must be higher than its CMC. The surfactant has enough solubility to form micelles only at temperatures above the Kraft point as
mentioned above. The counter ion of the ionic surfactant *does* affect the Kraft point. For example, the Kraft point of sodium dodecyl sulfate (SDS) is 16°C but potassium dodecyl sulfate has a Kraft point of approximately 35°C. Therefore, if SDS is dissolved in a buffer containing potassium ions, the solubility of SDS will be less than its CMC at ambient temperature because of the exchange reaction of the counter ions. The actual CMC in the buffer solution is usually lower than the value listed in Table 1, as these values were obtained with pure water as solvent.

High concentrations of surfactant (>200 mM) result in relatively high viscosities (and high currents) and should therefore be avoided in MEKC. It is recommended that the concentration of the buffer salt should not be lower than 10 mM. A higher concentration of buffer relative to that of surfactant is preferred to keep the pH constant during the run. It should be remembered that, in electrophoresis, electrolysis occurs at the electrodes, resulting in a reduction of the pH of the anodic solution. This solution may enter the capillary during the run when an anionic micelle (*e.g.*, SDS) is employed. The cathodic solution becomes alkaline during electrophoresis and may enter the capillary when a cationic surfactant (*e.g.*, CTAB) is employed. In this case the EOF is reversed.

Factors Affecting Reproducibility

In our experience, MEKC usually yields better reproducibility in migration times and peak areas than does CZE. Occasionally, however, the reproducibility is poor, probably due to a contaminated capillary surface. In such cases, cleaning of the capillary is necessary. It is recommended to use a strong cleaning solution, *e.g.*, a product used for cleaning lab glassware. Organic solvents such as methanol, acetone, acetonitrile, or tetrahydrofuran are also often effective.

In a clinical application relevant to therapeutic drug monitoring, Nakagawa *et al.* (1989) found that cleaning of the capillary between runs with 0.1 M NaOH was essential to obtain good performance and reproducibility. In this study, an antibiotic, cefpiramide, was determined in plasma by means of a *direct injection method* in an SDS-containing buffer. That is, no sample pretreatment such as deproteination or extraction was necessary. Without SDS (*i.e.*, in the CZE mode), plasma protein peaks interfered with the peaks of interest (see Figure 4A). With SDS, the migration times of the protein peaks had shifted relative to cefpiramide and the internal standard (antipyrine), thus enabling a quantitative plasma assay (see Figure 4B). The plasma proteins solubilized by the SDS have a strong negative charge resulting in a slow net migration whereas the negatively charged cefpiramide and antipyrine will *not* interact with the SDS. Hence, the migration times of the drugs are not affected by the presence of SDS in the buffer as indeed is observed from Figure 4. Recoveries were found to be near 100%, indicating that the method could be used to determine the *total* concentration of the drug in plasma (*i.e.*, the SDS enabled rapid release of protein-bound drug). Furthermore, the assay is fast (≈ 6 min run time) as it is not necessary to wait for the elution of the plasma proteins from the capillary. A rinse with 0.1 M NaOH can be started as soon as the antibiotic peak has been detected. As pointed out above, rinsing of the capillary also aids in generating reproducible surface conditions, which benefits precision and accuracy. A similar *direct sample injection* assay for the penicillin antibiotic aspoxicillin in human plasma was developed by Nishi *et al.* (1990). Both assays showed good linearity and covered the plasma levels typically encountered in clinical therapy.

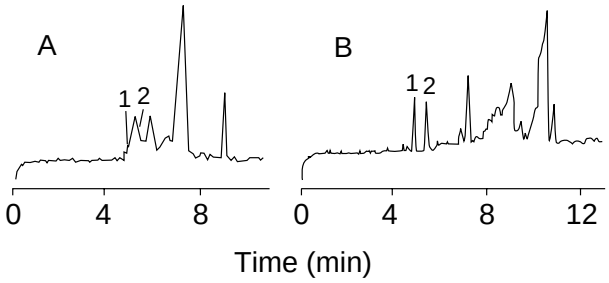


Figure 4. *(A) Separation of cefpiramide (peak 2) and antipyrine (peak 1) in human plasma with a 50 mM phosphate, pH 8.0 run buffer; (B) with the run buffer of A and 10 mM SDS. Detection wavelength, 280 nm. Reproduced with permission from Nakagawa* et al., Chem. Pharm. Bull. 36, *1622 (1988).*

Marker of the Electroosmotic Flow and the Micelle

In order to calculate the capacity factor according to equation (2), it is necessary to know the migration time of the bulk solution, t_0, the migration time of the micelle, t_{mc}, as well as the migration time of the analyte, t_R. Since the whole capillary is filled with micellar solution, the markers of the bulk solution and the micelle are required to obtain t_0 and t_{mc}. Strictly speaking, no ideal marker is available for MEKC.

The marker for the bulk solution must be electrically neutral as well as totally excluded from the micelle. Mesityl oxide, often used in CZE to measure t_0, is *not* an appropriate choice in MEKC, because it is partially incorporated into the micelle. Methanol often serves to measure t_0, because its distribution coefficient is almost negligible. Furthermore, it can be detected by UV absorption due to a change in refractive index as the methanol peak passes through the detection zone.

The marker for the micelle must be totally incorporated into the micelle. Sudan III or IV are often used to measure t_{mc}. Both solutes are not soluble in water and can be dissolved in methanol or in the micellar solution. However, because of the poor solubility in water, it is not always possible to observe the peaks of Sudan III or IV in the electropherograms. As an alternative, compounds that are insoluble in water but soluble in the micellar solution can be employed to measure t_{mc}. Timepidium bromide or quinine hydrochloride are good markers for anionic SDS micellar systems.

Resolution

The resolution equation for MEKC is given as

$$R_s = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k_2'}{1 + k_2'}\right)\left(\frac{1 - t_0 / t_{mc}}{1 + (t_0 / t_{mc})k_1'}\right) \quad (8)$$

where N is the theoretical plate number, α the separation factor equal to k_2'/k_1', and k_1' and k_2' are capacity factors of analytes 1 and 2, respectively. The equation predicts the effect of N, α, k', and t_0/t_{mc} on resolution. Each effect is briefly discussed below.

Plate Number

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

Separation Factor

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

Capacity Factor

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

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

$$k' = K \frac{V_{mc}}{V_{aq}} \tag{9}$$

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. The capacity factor is approximately related to the surfactant concentration, C_{sf}, by

$$k' = K\bar{v}\,(C_{sf} - \text{CMC}) \tag{10}$$

where $\bar{v}$ is the partial specific volume of the micelle. Equation 10 indicates that the capacity factor increases linearly with an increase of the surfactant concentration. It should be noted that the capacity factor is *not* linearly correlated to the migration time (see axes in Figure 3). Equation 10 suggests that we can easily vary the capacity factor by adjusting the surfactant concentration, provided the CMC is known. As mentioned above, the surfactant concentration should preferably be in the range of 20 to 200 mM to avoid excess currents.

Electroosmotic Velocity

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

$$t_0 / t_{mc} = [1 + \mu_{ep}\,(mc) / \mu_{eo}]E \tag{11}$$

where E is the electrical field strength. The mobilities μ_{eo} and $\mu_{ep}(mc)$ usually have different signs and the ratio $\mu_{ep}(mc)/\mu_{eo}$ is smaller than zero and larger than minus one. Therefore, t_0/t_{mc} is less than one. The t_0/t_{mc} is also directly related to the width of the migration time window. The smaller the value of t_0/t_{mc}, the wider the migration time window, hence the

higher resolution. A longer run time is required, however. The value of the migration time ratio t_0/t_{mc} is in the range of 0.2 to 0.3 for most ionic micelles under the conditions of pH above 6.

In order to reduce the value of $t_0/t_{mc,}$ it is necessary to reduce μ_{eo} relative to μ_{ep}(mc) because, in practice, increasing μ_{ep}(mc) is rarely possible. The addition of an organic solvent, *e.g.*, methanol or 2-propanol (<20%) is a possible way to reduce the electroosmotic flow velocity. It is also possible to reduce v_{eo} by changing the pH of the buffer to acidic conditions. Additives such as methylcellulose derivatives or ethylene glycol are often used in CE to increase the viscosity of the run buffer. Although an increase in viscosity of the solution reduces v_{eo}, it also reduces v_{ep}(mc) and, therefore, it does not help to increase resolution as predicted from equation (8).

Use of Coated Capillaries to Control the EOF

Coated capillaries can be used in CZE and MEKC to reduce the zeta potential at the capillary wall and, consequently, the electroosmotic flow. Currently, coated capillaries for CE are commercially available from several manufacturers (*e.g.*, J & W Scientific, Supelco, Scientific Glass Engineering, Chrompack). In MEKC, *untreated* fused silica capillaries have mainly been used. An example of the utility of coated capillaries is presented in Figure 5. A mixture of nucleobases was separated under identical conditions on a nonpolar, polymethylsiloxane (OV-1) capillary (panel A) and on a polar, polyethylene glycol (CW20M) coated capillary (panel C). For comparison, panel B of
Figure 5 shows the separation on an *untreated* fused silica capillary. It can be seen that with the polymethylsiloxane capillary actually an *increase* in the electroosmotic flow is obtained with a corresponding decrease in resolution. Presumably this is because of an increase in negative charge density at the capillary wall as the micellar reagent, SDS, binds to the nonpolar polysiloxane network. This is *not* the case with a relatively polar coating. The polyethylene glycol capillary shows a increase in migration times (*decrease* in the electroosmotic flow) and superior resolution. For example, the resolution for the cytidine/guanosine pair is 3.9 with the polymethylsiloxane capillary, 6.9 with the untreated capillary, and 9.7 with the polyethylene glycol capillary. Another way to manipulate the electroosmotic flow is to use cationic surfactants as will be discussed in the next section.

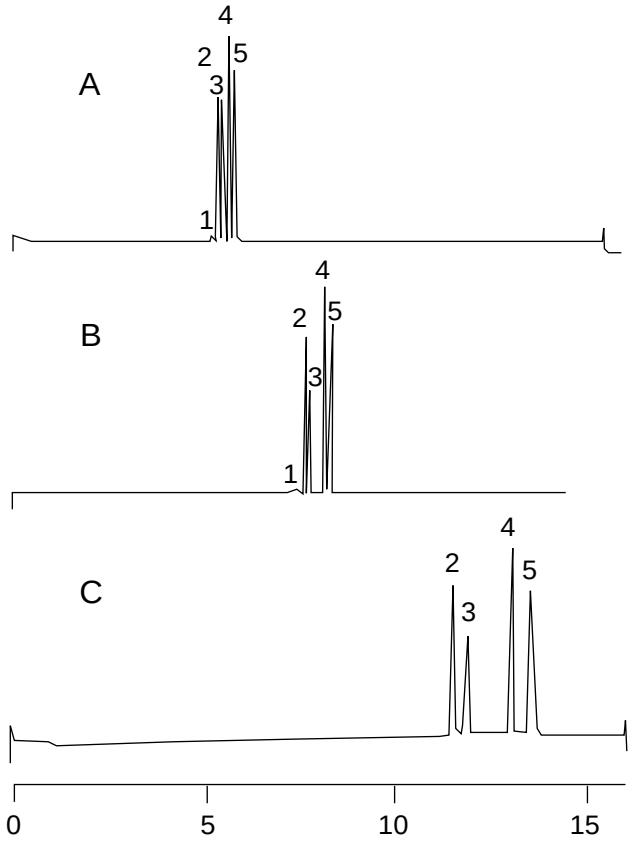


Figure 5. *MEKC separations of nucleobases using capillaries coated with different polymers: 1 = water; 2 = uridine; 3 = cytidine; 4 = guanosine; 5 = adenosine. (A) polymethylsiloxane OV-1 coating; (B) uncoated; (C) polyethylene glycol CW20M coating. Run buffer, 0.02 M phosphate, 0.05 M SDS. Reproduced with permission from Lux* et al., J. High Resolut. Chromatogr. HRC 13, *145 (1990).*

EOF Reversal

Cationic surfactants such as cetyl-, dodecyl-, and hexadecyltrimethylammonium salts can be used in MEKC to reverse the charge on the capillary wall. These surfactants are absorbed on the capillary wall surface by a mechanism involving electrostatic attraction between the positively charged ammonium moieties and the negatively charged Si-O-groups. The

non-polar chains (C10, C14, C16, *etc.*) create a hydrophobic layer and, at a high enough surfactant concentration, the negative surface charge will be completely neutralized. At even higher surfactant concentrations (*e.g.*, 0.35 mM for CTAB), a bilayer is formed through hydrophobic interaction between the nonpolar chains. Schematically, this situation is depicted in Figure 6. In this case, the cationic head groups are facing the buffer solution and the charge at the capillary wall is reversed from negative to positive. Consequently, under the influence of an electric field, a reversal of the EOF takes place.

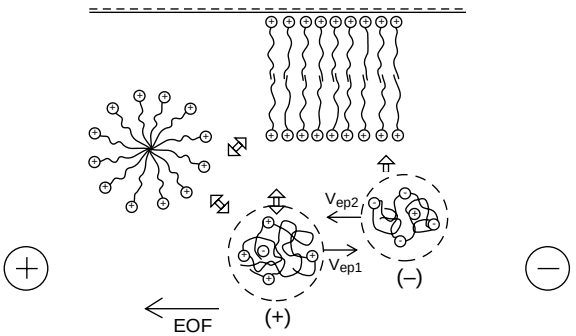


Figure 6. *Separation mechanism with EOF reversal. A bilayer is formed at the capillary wall. The EOF is directed toward the anode. Proteins with a net positive charge will electrophoretically migrate (V_{ep1}) toward the cathode. Net migration is toward the anode if EOF > V_{ep1}. Negatively charged proteins migrate toward the anode by both EOF and electrophoretic flow (V_{ep2}).*
Positively charged micelles are formed and may interact with proteins. Reproduced with permission from Emmer et al., J. High Resolut. Chromatogr. HRC 14, *738 (1991).*

Figure 7 shows examples of the charge reversal mode of MEKC. Opiates and ephedrine are separated with high efficiency and relatively short analysis times, made possible by operating at high pH conditions. It should be noted that with charge reversal MEKC, the polarity of the power supply must be reversed since the bulk flow is now towards the positive electrode, *i.e.*, the bulk flow must always go in the direction of the detector. Whereas the majority of applications of charge reversal MEKC have involved small molecules (e.g., ions, pharmaceuticals, and drugs), applications of biologically important molecules such as peptides and proteins have also been demonstrated (Liu *et al.*, 1990; Emmer *et al.*, 1991). For example, in the

separation of basic proteins, Emmer *et al*. (1991) suggested a fluorocarbon surfactant, FC-134 (commercially available from 3M Company, St. Paul, MI). This surfactant has a non-sticking, Teflon*-like chain, thus minimizing interactions with many proteins. In addition, proteins at a pH below their pI will be repelled from the capillary surface. As shown in Figure 6, the electrophoretic migration of a positively and a negatively charged protein is in opposite direction but in both cases, the net migration is in the anodic direction (assuming that the EOF is greater than electro- phoretic migration). Interactions between the micelles and the protein may enhance the above described repellant effect.

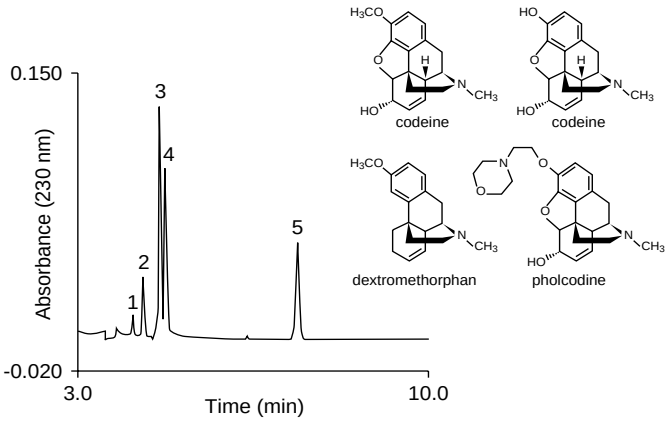


Figure 7. *Separation of ephedrine and opiates with EOF reversal. 1 = ephedrine; 2 = pholcodine; 3 = codeine; 4 = morphine; 5 = dextromethorphan. Run buffer, 50 mM CTAB, 50 mM triethylamine, pH 12.0 in 20% acetonitrile. Capillary, 75 µm i.d. ×57 cm (50 cm to detector). Reproduced from Kerr and Jung,* Beckman Application Note DS-783, *1990.*

* A registered trademark of E. I. du Pont de Nemours & Company.

Further Reading
(in reverse chronological order)

Emmer, A., Jansson, M., Roeraarde, J. Improved CZE separations of basic proteins using a fluorosurfactant buffer additive. *J. Chromatogr. 547,* 544-550 (1991)

Gowsi, K., Foley, J. P., Gale, R. J. Micellar electrokinetic capillary chromatography theory based on electrokinetic parameters: optimization for three modes of operation. *Anal. Chem. 62,* 2714-2721 (1990)

Liu, J., Cobb, K. A., Novotny, M. V. Capillary electrophoretic separations of peptides using micelle-forming compounds and cyclodextrins as additives. *J. Chromatogr. 519,* 189-197 (1990)

Lux, J. A., Yin, H., Schomburg, G. Influence of polymer coating of capillary surfaces on migration behavior in micellar electrokinetic capillary chromatography. *J. High Resolut. Chromatogr. 13,* 145-147 (1990)

Nishi, H., Fukuyama, T., Matsuo, M. Separation and determination of aspoxicillin in human plasma by micellar electrokinetic chromatography with direct sample injection. *J. Chromatogr. 515,* 245-255 (1990)

Nakagawa, T., Oda, Y., Shibukawa, A., Fukuda, H., Tanaka, H. Electrokinetic chromatography for drug analysis. Separation and determination of cefpiramide in human plasma. *Chem. Pharm. Bull. 37,* 707-711 (1989)

Otsuka, K., Terabe, S. Effects of pH on electrokinetic velocities in micellar electrokinetic chromatography. *J. Microcol. Sep. 1,* 150-154 (1989)

Terabe, S., Otsuka, K., Ando, T. Band broadening in electrokinetic chromatography with micellar solutions and open-tubular capillaries. *Anal. Chem. 61,* 251-260 (1989)

Hinze, W. L. Organized surfactant assemblies in separation science, in "Ordered media in chemical separations," Hinze, W. L., Armstrong, D. W., eds. *ACS Symposium Series 342,* American Chemical Society, Washington D.C., pp. 2-82 (1987)

Terabe, S., Utsumi, H., Otsuka, K., Ando, T., Inomata, T., Kuze, S., Hanaoka, Y. Factors controlling electroosmotic flow in open-tubular capillaries in electrokinetic chromatography. *HRC CC, J. High Resolut. Chromatogr. Chromatogr. Commun. 9,* 666-670 (1986)

Armstrong, D. W. Micelles in separations: a practical and theoretical review. *Separation and Purification Methods 14,* 213-304 (1985)

Back to Catalog

III: Selectivity Manipulation

From the viewpoint of selectivity manipulation, the micellar phase in MEKC corresponds to the stationary phase in reversed-phase liquid chromatography (RPLC) as does the surrounding aqueous phase to the mobile phase in RPLC. There are five factors we can control to manipulate selectivity:

1. Temperature
2. Choice of surfactant
3. Modification of the micelle
4. Choice of the aqueous phase
5. Modification of the aqueous phase

Each variable is discussed below.

1. Temperature

The distribution coefficient is significantly dependent on temperature. An increase in temperature causes a reduction in migration time because of the decrease in the distribution coefficient as well as the viscosity. Since the dependence of the distribution coefficient on temperature is different among analytes, temperature will also affect selectivity. The temperature effect on selectivity is not dramatic but temperature does, however, affect the migration time. For reproducible results, it is essential to keep the run temperature constant. It should be noted that even if the temperature of the capillary is carefully controlled, a high current will cause a substantial rise in the temperature of the solution inside the capillary. This is because heat dissipation under these conditions is not complete and instantaneous. Run conditions which result in high currents should therefore be avoided. A CE system which has efficient capillary cooling (such as that used in P/ACE) is mandatory under high current conditions.

2. Choice of Surfactant

A surfactant molecule has a hydrophobic part and hydrophilic part and both groups affect the selectivity in MEKC. Since most analytes interact with the micelles at their surfaces, the hydrophilic group, or ionic group, is generally more important in terms of selectivity. That is, SDS and sodium tetradecyl sulfate (STS) show very similar selectivity, but SDS and sodium *N*-lauroyl-*N*-methyltaurate (LMT) yield considerably different selectivity, provided the analytes are polar (see Figure 8 for example). In practice, it is easy to change the micellar solution. When the resolution is not adequate

with a particular surfactant solution, it is recommended to try another surfactant solution.

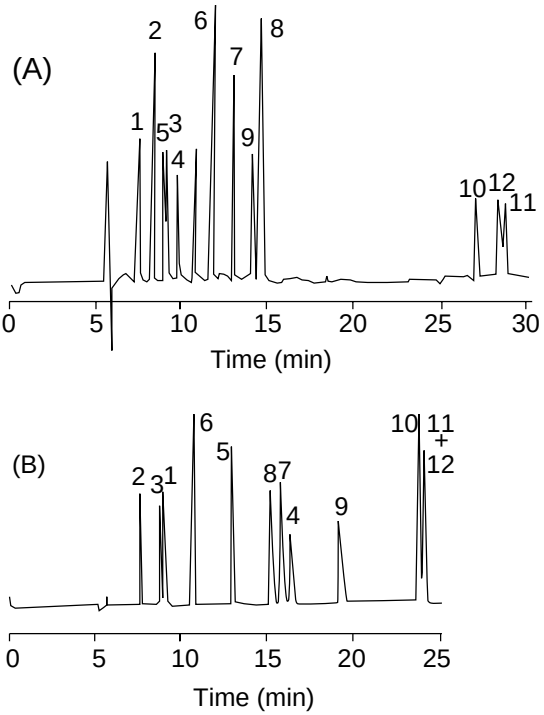


Figure 8. *Comparison of selectivity between surfactants with different polar groups: 1 = caffeine; 2 = acetaminophen; 3 = sulpyrin; 4 = trimetoquinol; 5 = guaifenesin; 6 = naproxen; 7 = ethenzamide; 8 = phenacetin; 9 = isopropylantipyrine; 10 = noscapine; 11 = chlorpheniramine; 12 = tipepidine. Conditions: Run buffer, (A) 0.1 M LMT in 20 mM phosphate-borate (pH 9.0); (B) 0.1 M SDS in the same buffer as in A; applied voltage, 20 kV. Reproduced with permission from Nishi* et al., J. Pharm. Sci. 79, *519 (1990).*

Bile salts such as sodium cholate, sodium deoxycholate, and their taurine conjugates are natural surfactants. Bile salts form helical micelles and yield selectivities significantly different from the long alkyl chain surfactants. In particular, bile salts are useful for the separation of very

hydrophobic compounds. In Figure 9, for example, corticosteroids were *not* successfully separated with SDS but they were completely resolved with sodium cholate as the micelle.

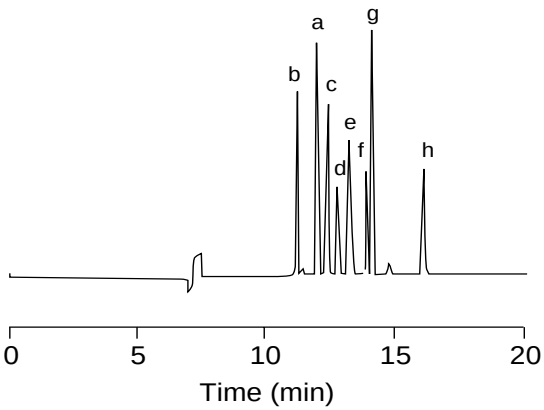


Figure 9. *Separation of eight corticosteroids: a = hydrocortisone; b = triamcinolone; c = betamethazone; d = hydrocortisone acetate; e = dexamethasone acetate; f = fluocinolone; g = fluocinolone acetonide; h = fluocinonide. Conditions: Run buffer, 100 mM sodium cholate in 20 mM phosphate-borate (pH 9.0). Reproduced with permission from Nishi* et al., J. Chromatogr. 513, *279 (1990).*

Although MEKC is used mainly for separating neutral compounds, it sometimes also allows an improved separation of ionic analytes. MEKC may work for the separation of ionic analytes when they are not successfully separated by CZE. Since the micelles used in MEKC are charged on the surface, an analyte with the opposite charge of the micelle will strongly interact with the micelle through electrostatic forces and an analyte with the same charge as the micelle will interact weakly, due to the electrostatic repulsion. In case of ionic analytes, hydrophobicity and charge affect the distribution coefficient. Therefore, the use of a cationic surfactant will result in an entirely different selectivity than when an anionic surfactant is used for the separation of ionic analytes. This is illustrated in Figure 10 for the separation of PTH amino acids.

Some classes of surfactants possess very specific selectivity. For example, sodium *N*-dodecanoyl-*L*-valinate (SDVal) and many bile salts enable enantiomeric separations (see Section IV). Surfactants with perfluorinated alkyl chains may exhibit enhanced selectivity towards flu-

orinated compounds. It is expected that in the near future, surfactants specifically designed for the separation of certain groups of analytes will be commercially available.

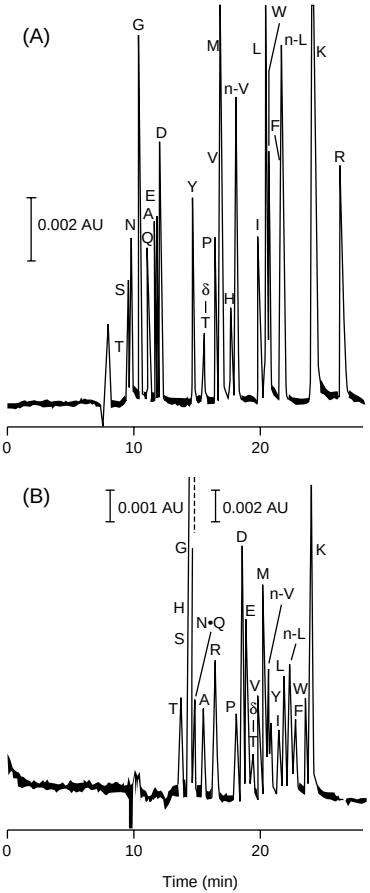


Figure 10. *Comparison of selectivity between anionic and cationic surfactants for the separation of 22 phenylthiohydantoin (PTH)-amino acids by MEKC: The peaks are labeled with one-letter abbreviations for amino acid. Conditions: (A) Run buffer, 50 mM SDS in 50 mM phosphate-borate (pH 7.0);*
(B) 50 mM DTAB in 100 mM Tris-HCI buffer (pH 7.0); applied voltage, (A) 10 kV, (B) 15 kV; detection, UV absorbance at 260 nm; temperature, 35°C. Reproduced with permission from Otsuka et al., J. Chromatogr. 332, *219 (1985).*

3. Modification of the Micelle

The micellar phase can be modified by adding a second surfactant to form a mixed micelle or by selecting a different counter ion. Since a mixed micelle of an ionic and a nonionic surfactant has a lower surface charge and a larger size, its electrophoretic mobility will be lower than a single ionic micelle. The addition of a nonionic surfactant to an ionic micellar solution causes a narrower migration time window. In Figure 11, the separation of several pharmaceuticals is shown. As shown in panel B, the addition of the nonionic surfactant Tween 60 to the buffer resulted in several peak reversals and a shorter analysis time. Typically, the selectivity is dramatically affected by adding a nonionic surfactant because the polar group of nonionic and ionic surfactants are very different. The effect of the counter ion is generally less important, unless the counter ion is replaced by an organic ion.

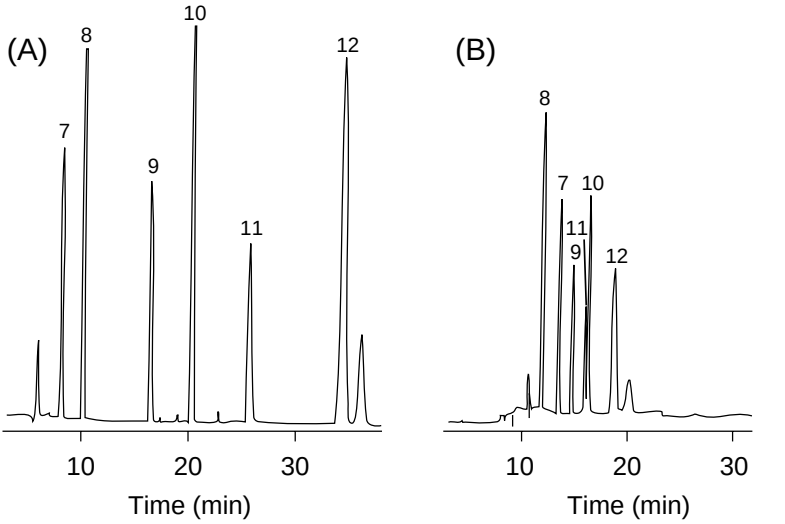


Figure 11. *Effect of nonionic surfactant addition: 7 = acetaminophen; 8 = caffeine; 9 = guaifenesin; 10 = ethenzamide; 11 = isopropylantipyrine; 12 = trimetoquinol. Conditions: (A) Run buffer, 100 mM SDS in 50 mM phosphate/100 mM borate (pH 7.0); capillary, 75 μm i.d. × 57 cm (effective length 50 cm); applied voltage, 18 kV; detection, UV absorbance at 214 nm. (B) as in (A) but with 30 mM Tween 60 added to the run buffer. Unpublished data from Ishihama and Terabe (1991).*

A relevant application of the mixed micellar phase is the separation of enantiomers with a nonionic chiral surfactant. Several nonionic chiral surfactants are effective for the separation of enantiomers. In order to give these surfactants electrophoretic mobilities, an ionic surfactant such as SDS can be employed to form the mixed micelle. For example, digitonin is successfully used with SDS for the enantiomeric separation of PTH amino acids (Figure 12). The method involving the mixed micelle can also be useful when surfactants are used which become nonionic under acidic conditions.

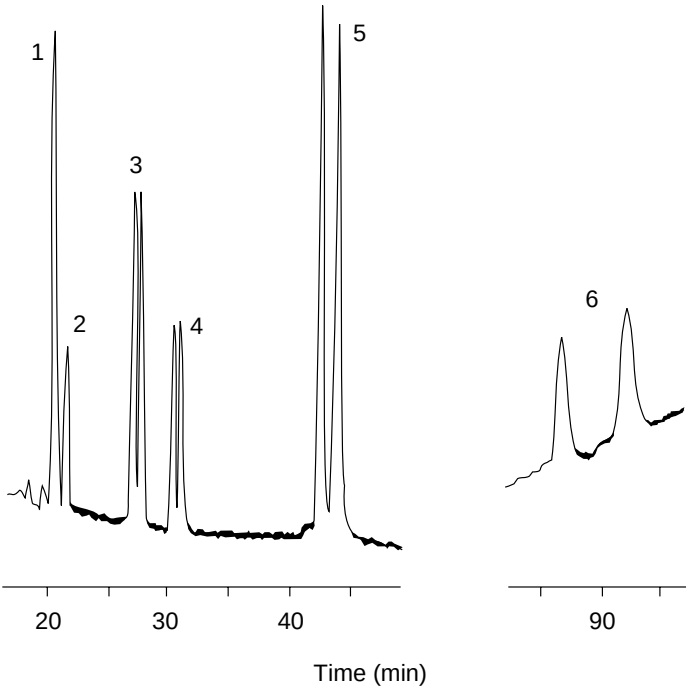


Figure 12. *Enantiomeric separation of six PTH-DL-amino acids by MEKC: 1 = Trp; 2 = Nle; 3 = Nva; 4 = Val; 5 = Aba; 6 = Ala. Conditions: Run buffer, 25 mM digitonin and 50 mM SDS in 50 mM phosphate buffer (pH 3.0); capillary, 50 µm i.d. × 63 cm (effective length 59 cm); applied voltage, 20 kV; detection, absorbance at 260 nm. Reproduced with permission from Otsuka and Terabe,* J. Chromatogr. 515, *221 (1990).*

4. Choice of the Aqueous Phase

The constituents of the buffer solution have very little effect upon selectivity. Organic buffers usually have a relatively low conductivity and, therefore, are recommended if they are stable and UV transparent. Care should be taken not to replace the counter ion of the ionic surfactant with the buffer ion.

The pH of the buffer is a critical parameter for the separation of ionizable analytes. In separations with closely spaced peaks, it is often essential to find the optimum pH. This is demonstrated in Figure 13 for the separation of chlorinated phenols by plotting the apparent capacity factor *vs*. the buffer pH. Here, the apparent capacity factor is calculated according the equation (2), regardless of whether the samples are ionized or not. It should be noted that changing the buffer pH (especially in the acidic region) causes a notable change in the electroosmotic velocity.

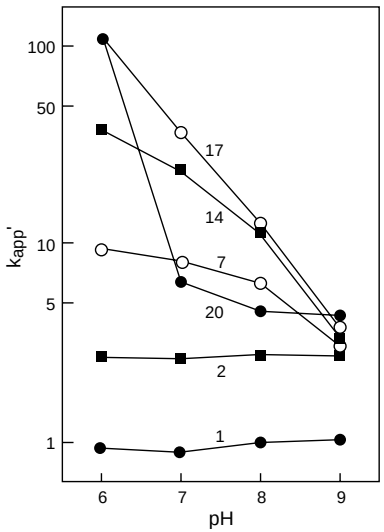


Figure 13. *Dependence of apparent capacity factors of chlorinated phenols on pH: 1 = phenol; 2 = 2-chlorophenol; 7 = 2,5-dichlorophenol; 14 = 2,4,5-trichlorophenol; 17 = 2,3,4,5-tetrachlorophenol; 20 = pentachlorophenol. Conditions: Run buffer, 100 mM SDS in 50 mM phosphate-borate; capillary, 50 µm i.d. × 65 cm (effective length, 50 cm); applied voltage, 15 kV; detection, UV absorbance at 220 nm. Reproduced with permission from Otsuka* et al., J. Chromatogr. 348, *39 (1985).*

5. Modification of the Aqueous Phase

The use of additives to modify the aqueous phase is very effective in manipulating selectivity. Modification of the mobile phase by additives is well documented in HPLC and many of the HPLC additives are also applicable to MEKC. However, we have to consider the difference between the micellar phase in MEKC and the stationary phase in HPLC to take advantage of such additives. Five classes of additives are applicable in MEKC:

1. Cyclodextrins (CDs)
2. Ion-pair reagents
3. Urea
4. Organic solvents
5. Metal salts

Cyclodextrins

CDs are oligosaccharides with truncated cylindrical molecular shapes. Their outside surfaces are hydrophilic, while their cavities are hydrophobic. Traditionally, they have been used to improve the therapeutic usefulness, in animals and humans, of drugs and biologicals that are relatively insoluble in water. CDs tend to include compounds which fit their cavities by hydrophobic interaction. Three CDs, α-, β-, and γ-CD, are widely used and some of their characteristics are listed in Table 2. The size of the cavity differs significantly among the α, β and γ CDs. Only β-CD has a relatively low solubility in water. Many CD derivatives (*e.g.*, O-methylated β-CD derivatives) have been developed for increased solubility in water as well as to modify the cavity shape. CDs can be obtained from a variety of commercial sources. For example, in the U.S., Sigma (St. Louis, MO) and Pharmatec (Alachua, FL) offer a wide choice in chemically modified CDs.

Table 2. Characteristics of Cyclodextrins

Cyclodextrin	**α-CD**	**β-CD**	**γ-CD**
Number of glucose units	6	7	8
Molecular weight	972.9	1135.0	1297.2
Internal diameter of the cavity/nm	0.47–0.52	0.62–0.64	0.75–0.83
Outside diameter/nm	1.46	1.54	1.75
Height of the cavity/nm	0.79–0.80	0.79–0.80	0.79–0.80
Solubility in water at 25°C	14.50	1.85	23.2

In MEKC, CDs are electrically neutral and have no electrophoretic mobility. They are assumed *not* to be incorporated into the micelle, because of the hydrophilic nature of the outside surface of the molecules. A surfactant molecule may, however, be included into the CD cavity. The separation principle of CD-modified MEKC (CDMEKC), in which CD is added to the micellar solution, is shown schematically in Figure 14.

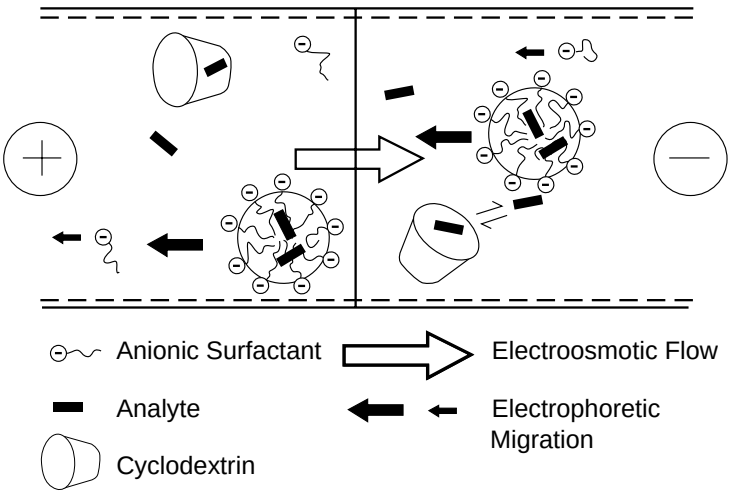


Figure 14. *Schematic of the separation principle of CDMEKC. The detector window is assumed to be positioned near the negative electrode..*

The analyte molecule included by CD migrates at the same velocity as the electroosmotic flow because, electrophoretically, CD behaves as the bulk aqueous phase. Therefore, the addition of CD reduces the apparent distribution coefficient and enables the separation of highly *hydrophobic* analytes, which otherwise would be almost totally incorporated into the micelle in the absence of CD. The higher the concentration of CD, the smaller the capacity factor. In CDMEKC, therefore, the capacity factor is changed by varying the concentrations of both the surfactant and CD. An example of this approach for hydrophobic compounds is shown in Figure 15. All of the isomers of the trichloro- biphenyls migrated with the same velocity as that of the micelle in MEKC. However CDMEKC allowed the baseline separation of the eleven hydrophobic isomers.

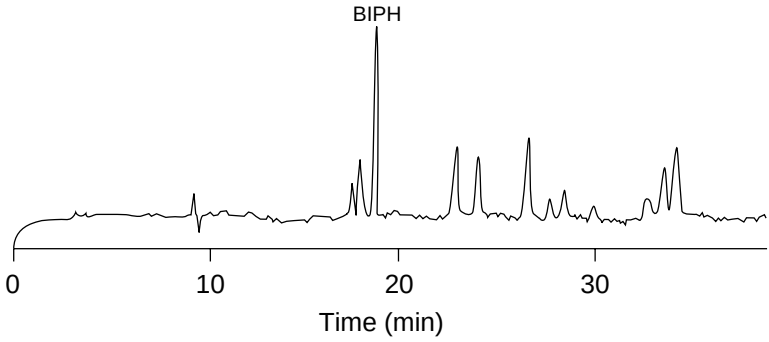


Figure 15. *Separation of eleven trichlorobiphenol isomers by CDMEKC. BIPH = biphenyl. Run buffer, 60 mM γ-CD, 100 mM SDS, and 2 M urea in 100 mM borate-50 mM phosphate (pH 8.0). Reproduced with permission from Terabe* et al., J. Chromatogr. 516, *23 (1990).*

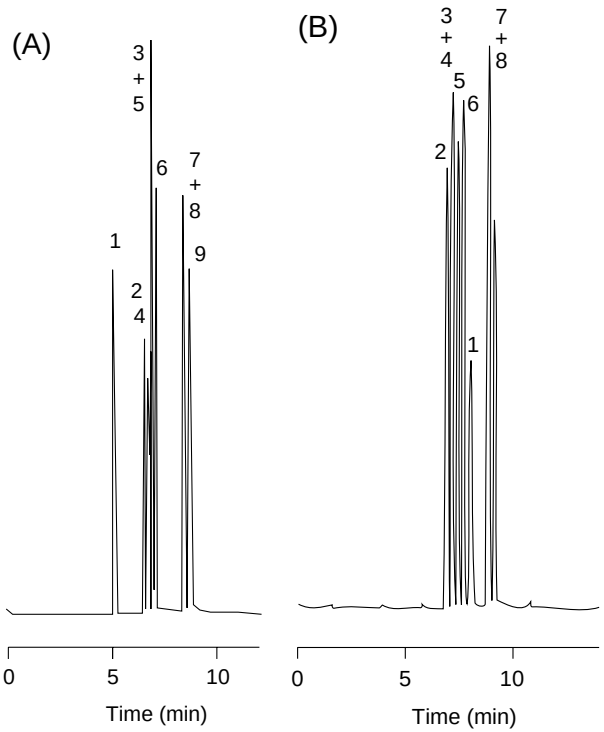
It should be mentioned that CDMEKC is a different technique from CDEKC. In CDMEKC a neutral CD is added to the micellar solution, while in CDEKC an ionic CD derivative without the micelle is employed. CDMEKC is also very effective for enantiomeric separations because of the chirality of CD itself. This will be discussed later in Section IV on Enantiomeric Separations.

The solubility of β-CD is relative low. Addition of a high concentration of urea such as 2 M increases the solubility. It is our experience that, for many compounds, γ-CD is a more effective additive than β-CD in CDMEKC. This is even the case for the compounds successfully separated in HPLC with the β-CD-bonded phase. Conceivably, co-inclusion of the surfactant molecule into the cavity of γ-CD would result in less available space for the analyte molecule. The use of a different CD typically causes changes in selectivity.

Ion-Pair Reagents

There is an essential difference in the separation of *ionic* compounds between MEKC and RPLC, although both have similar characteristics in the separation of *nonionic* compounds. The difference is due to the electric charge on the micelle. When a tetraalkylammonium salt is added to the SDS solution, migration times of *anionic* analytes increase with an in-

crease in the concentration of the ammonium salt, because the ammonium ion interacts with the anionic analyte to form the paired ion. Hence, the electrostatic repulsion between the anionic SDS micelle and the anionic analyte is reduced. In contrast, migration times of *cationic* analytes decrease, because the ammonium ion competes with the cationic analyte in pairing to the anionic micelle. The effect of the ion-pair reagent on selectivity depends significantly on the structure of the reagent, *e.g.*, the length of the alkyl chain. Figure 16 demonstrates the effect of the addition of tetramethylammonium ion to the SDS micellar solution on the selectivity for cephalosporin antibiotics. Also shown in Figure 16 is the CZE separation (*i.e.*, with no SDS). In this case, poor selectivity is obtained. The best separation is obtained when the tetramethylammonium salt is added to the buffer (panel C). It should be mentioned that all cephalosporins are baseline resolved when 100 mM SDS instead of 50 mM is used.



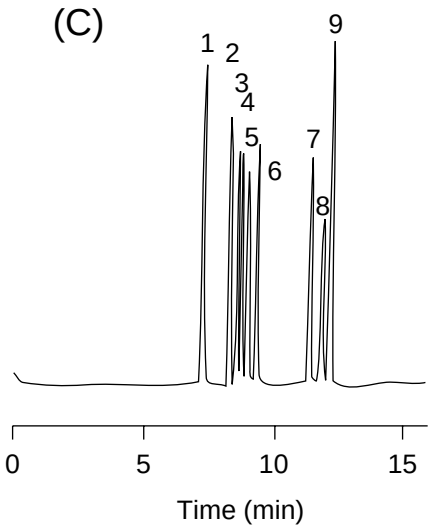


Figure 16. *Separation of cephalosporin antibiotics by CZE (A), MEKC with SDS (B), and MEKC with SDS and tetramethylammonium salt (C): 1 = C-TA; 2 = ceftazidime; 3 = cefotaxime; 4 = cefmenoxime; 5 = cefoperazone; 6 = cefpiramide; 7 = cefpimizole; 8 = cefminox; 9 = ceftriaxone. (A) Run buffer, 20 mM phosphate-borate (pH 9.0); (B) with 50 mM SDS added to run buffer A; (C) with 40 mM tetramethylammonium bromide added to run buffer B. Capillary, 50 μm i.d. × 65 cm (effective length 50 cm); applied voltage 20 kV; detection, UV absorbance at 210 nm. Reproduced with permission from Nishi* et al., Anal. Chem. 61, *2434 (1989).*

Urea

A high concentration of urea is known to increase the solubility of hydrophobic compounds in water. Urea also breaks down hydrogen-bond formation in the aqueous phase. In MEKC, the addition of a high concentration of urea to the SDS solution improves the separation of highly hydrophobic compounds. In Figure 17, the effect of urea is shown for the separation of lipophilic corticosteroids. Urea slightly reduces the electroosmotic velocity and considerably reduces the migration velocity of the micelle, resulting in a reduced capacity factor. Although the addition of urea causes a slight increase in viscosity, the decrease of v_{mc} is more significant than would be expected from the viscosity change alone. Selectivity is not remarkably altered by the addition of urea, but minor changes are noticeable, especially for the separation of closely related compounds.

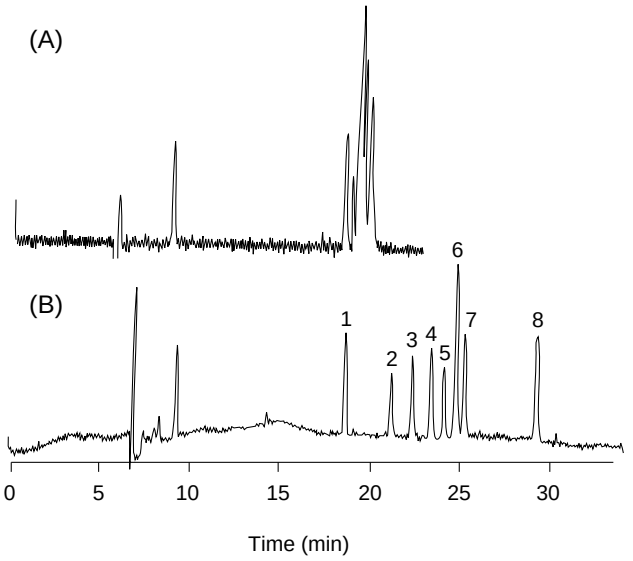


Figure 17. *The effect of urea addition to the SDS solution: 1 = hydrocortisone; 2 = hydrocortisone acetate; 3 = betamethasone; 4 = cortisone acetate; 5 = triamcinolone acetonide; 6 = fluocinolone; 7 = dexamethasone acetate; 8 = fluocinonide. (A) without urea; (B) with the addition of 6 M urea to the run buffer. 50 mM SDS in 20 mM phosphate-borate (pH 9.0); capillary, 50 µm i.d. × 65 cm (effective length 50 cm); applied voltage, 20 kV; detection, UV absorbance at 210 nm. Reproduced with permission from Terabe* et al., J. Chromatogr. 545, *359 (1991).*

Organic Solvents

Organic solvents miscible with water are widely used as mobile phase modifiers in HPLC. The main objective of using organic solvents in RPLC is to adjust the capacity factor close to the optimum value. The result is a reasonable analysis time, even for very hydrophobic compounds. We may expect a similar effect with the use of organic solvents in MEKC. However, it should be noted that a high concentration of the organic solvent may break down the micellar structure. Generally, concentrations of up to 20% organic solvent can be used without difficulty in MEKC. The use of organic solvents contributes to the improvement of resolution or the alteration of selectivity. In general, the addition of methanol, 2-propanol or acetonitrile reduces the electroosmotic velocity and, hence, expands the migration time window. This is illustrated in Figure 18 for the separation of a number of aromatic sulfides with the addition of methanol to the run buffer.

Metal Salts

It has been reported that the addition of certain metal salts to the SDS micellar solution improves selectivity. In particular, the MEKC separation of oligonucleotides is enhanced by the addition of magnesium, zinc, or copper (II) ion. Metals ions are electrostatically attracted to the surface of the micelle where they can be selectively complexed with analytes. (Care should be taken not to cause precipitation of metal salts with SDS.) The analyte/metal ion/micelle complexation results in a widened migration time window and enhanced selectivity. An example of this approach is shown in Figure 19. 3 mM Zn (II) was added to the buffer to significantly enhance the resolution of oligonucleotides. Relatively low concentrations of metal salt (0.3–3.0 mM) give a good compromise between zone broadening and resolution.

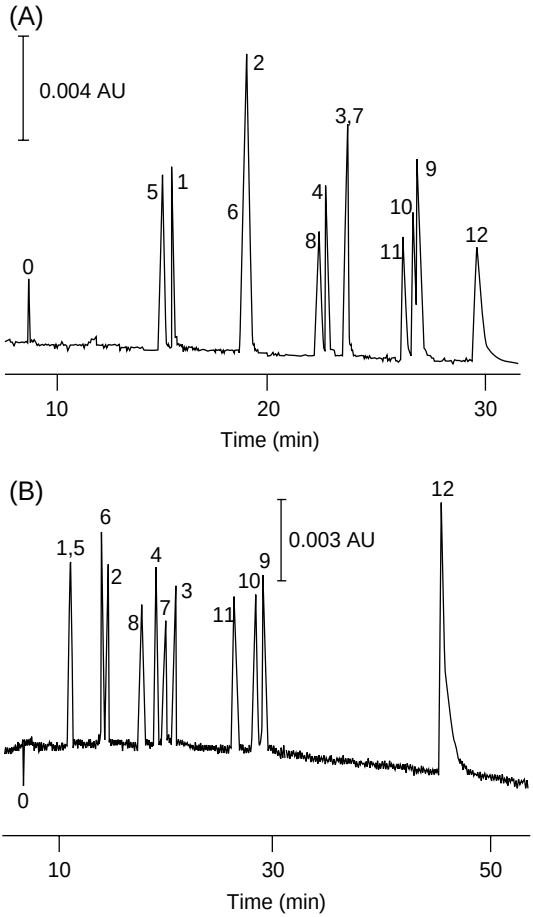


Figure 18. *Effect of methanol addition to the SDS solution: 1 = benzyl methyl sulfide; 2 = benzyl ethyl sulfide; 3 = benzyl propyl sulfide; 4 = benzyl isopropyl sulfide; 5 = methyl phenyl sulfide; 6 = ethyl phenyl sulfide; 7 = phenyl propyl sulfide; 8 = isopropyl phenyl sulfide; 9 = butyl phenyl sulfide; 10 = isobutyl phenyl sulfide; 11 =* s*-butyl phenyl sulfide; 12 = Sudan III. (A) without methanol; (B) with 20% methanol added. Run buffer, 20 mM SDS in 50 mM phosphate-borate (pH 7.0); capillary, 50 μm i.d. × 90 cm (effective length 75 cm); applied voltage 21 kV; detection, UV absorbance at 210 nm; temperature, 35°C. Reproduced with permission from Otsuka* et al., Nippon Kagaku Kaishi, no. 7, *950 (1986).*

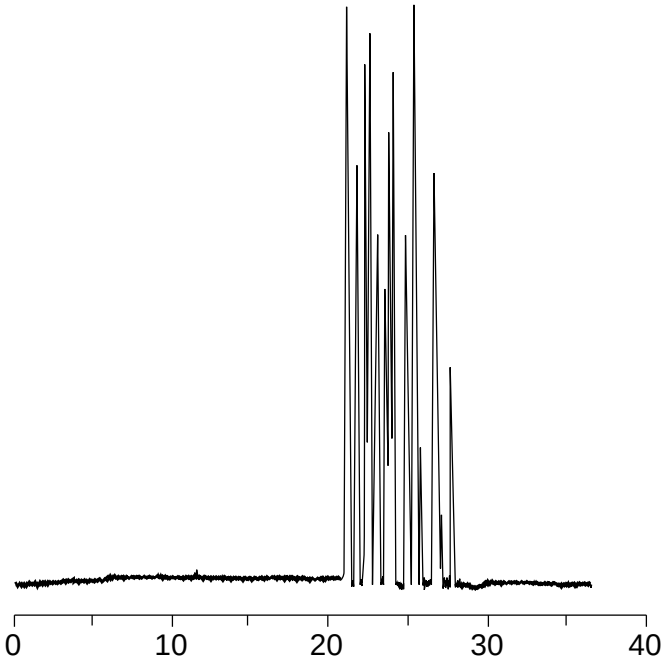


Figure 19. *Separation of a mixture of 18 oligonucleotides, each with 8 bases. Run buffer, 7 M urea, 20 mM Tris, 5 mM sodium phosphate, 50 mM SDS, 3 mM Zn (II). Reproduced with permission from Cohen* et al., Anal. Chem. 59, *1021 (1987).*

Further Reading
(in reverse chronological order)

Ackermans, M. T., Everaerts, F. M., Beckers, J. L. Determination of some drugs by micellar electrokinetic capillary chromatography: the pseudo-effective mobility as parameter for screening. *J. Chromatogr. 585,* 123-131 (1991)

Amankwa, L. N., Kuhr, W. G. Indirect fluorescence detection in micellar electrokinetic chromatography. *Anal. Chem. 63,* 1733-1737 (1991)

Cole, R. O., Sepaniak, M. J., Hinze, W. L., Gorse, J., Oldiges, K. Bile salt surfactants in micellar electrokinetic capillary chromatography: application to hydrophobic molecule separations. *J. Chromatogr. 577,* 113-123 (1991)

Lecoq, A.-F., Leuratti, C., Marafante, E., Di Biase, S. Analysis of nucleic acid derivatives by micellar electrokinetic capillary chromatography. *J. High Resolut. Chromatogr. 14,* 667-671 (1991)

Thormann, W., Meier,P., Marcolli, C., Binder, F. Analysis of barbiturates in human serum and urine by high performance capillary electrophoresis-micellar electrokinetic capillary chromatography with on-column multi-wavelength detection. *J. Chromatogr. 545,* 445-460 (1991)

Terabe, S., Ishihama, Y., Nishi, H., Fukuyama, T., Otsuka, K. Effect of urea addition in micellar electrokinetic chromatography. *J. Chromatogr. 545,* 359-368 (1991)

Weinberger, R., Lurie, I. S. Micellar electrokinetic capillary chromatography of illicit drug substances. *Anal. Chem. 63,* 823-827 (1991)

Nishi, H., Fukuyama, T., Matsuo, M., Terabe, S. Effect of surfactant structure on the separation of cold medicine ingredients by micellar electrokinetic chromatography. *J. Pharm. Sci. 79,* 519-523 (1990)

Nishi, H., Fukuyama, T., Matsuo, M., Terabe, S. Separation and determination of lipophilic corticosteroids and benzothiazepin analogues by micellar electrokinetic chromatography using bile salts. *J. Chromatogr. 513,* 279-295 (1990)

Rasmussen, H. T., Goebel, L. K., McNair, H. M. Micellar electrokinetic chromatography employing sodium alkyl sulfates and Brij 35. *J. Chromatogr. 517,* 549-555 (1990)

Terabe, S., Miyashita, Y., Shibata, O., Barnhart, E. R., Alexander, L. R., Patterson, D. J., Karger, B. L., Hosoya, K., Tanaka, N. Separation of highly hydrophobic compounds by cyclodextrin- modified micellar electrokinetic chromatography. *J. Chromatogr. 516,* 23-31 (1990)

Nishi, H., Tsumagari, N., Kakimoto, T., Terabe, S. Separation of β-lactam antibiotics by micellar electrokinetic chromatography. *J. Chromatogr. 477,* 259-270 (1989)

Nishi, H., Tsumagari, N., Kakimoto, T., Terabe, S. Separation of water-soluble vitamins by micellar electrokinetic chromatography. *J. Chromatogr. 465,* 331-343 (1989)

Nishi, H., Tsumagari, N., Terabe, S. Effect of tetraalkylammonium salts on micellar electrokinetic chromatography. *Anal. Chem. 61,* 2434-2439 (1989)

Back to Catalog

Balchunas, A. T., Sepaniak, M. J. Gradient elution for micellar electrokinetic capillary chromatography. *Anal. Chem. 60,* 617-621 (1988)

Fujiwara, S., Iwase, S., Honda, S. Analysis of water-soluble vitamins by micellar electrokinetic capillary chromatography. *J. Chromatogr. 447,* 133-140 (1988)

Gorse, J., Balchunas, A. T., Swaile, D. F., Sepaniak, M. J. Effects of organic mobile phase modifiers in micellar electrokinetic capillary chromatography. *HRC CC, J. High Resolut. Chromatog. Chromatogr. Commun. 11,* 554-559 (1988)

Snopek, J., Jelinek, I., Smolkova-Keulmansova, E. Micellar, inclusion and metal-complex enantioselective pseudophases in high-performance electromigration methods. *J. Chromatogr 452,* 572-590 (1988)

Balchunas, A. T., Sepaniak, M. J. Extension of elution range in micellar electrokinetic capillary chromatography. *Anal. Chem. 59,* 1466-1470 (1987)

Cohen, A. S., Terabe, S., Smith, J. A., Karger, B. L. High-performance capillary electrophoresis separation of bases, nucleosides, and oligonucleotides: retention manipulation via micellar solution and metal additives. *Anal. Chem. 59,* 1021-1027 (1987)

Szejtli, J., Zsadon, B., Cserhati, T. Cyclodextrin use in separations, in *"Ordered Media in Chemical Separations,"* Hinze, W. L., Armstrong, D. W., eds. *ACS Symposium Series 342,* American Chemical Society, Washington, D.C., pp. 200-217 (1987)

Otsuka, K., Terabe, S., Ando, T. Separation of aromatic sulfides by electrokinetic chromatography with micellar solution. *Nippon Kagaku Kaishi, No. 7,* 950-955 (1986)

Otsuka, K., Terabe, S., Ando, T. Electrokinetic chromatography with micellar solutions: separation of phenylthiohydantoin-amino acids. *J. Chromatogr. 332,* 219-226 (1985)

Otsuka, K., Terabe, S., Ando, T. Electrokinetic chromatography with micellar solutions: retention behavior and separation of chlorinated phenols. *J. Chromatogr. 348,* 39-47 (1985)

IV: Enantiomeric Separations

In the pharmaceutical industry, the determination of the optical purity or separation and determination of enantiomers is becoming increasingly important. The non-active enantiomer in a drug formulation may be considered as an impurity. High resolution separation methods are required to achieve chiral separations. The approaches used in CE, discussed next, are relatively simple compared to HPLC methods in which expensive, chiral stationary phases are frequently used. In CE only minute amounts of chiral selectors are required to determine enantiomeric purity.

Two approaches can be used to perform enantiomeric separations in MEKC:

1. Use of chiral surfactants
2. Use of chiral additives

1. Chiral Surfactants

Bile salts are widely available commercially and have shown to be useful chiral surfactants. Sodium cholate (see the separation in Figure 9) or sodium deoxycholate can be used under neutral or alkaline conditions to ionize the carboxyl group of the surfactant. Taurine conjugates of bile salts can also be used in acidic conditions because taurine has a sulfonic acid group. Amino acid-derived surfactants (*e.g.*, sodium *N*-dodecanoyl-*L*-valinate, SDVal) are another group of chiral surfactants that are commercially available. They also must be used under neutral or alkaline conditions. In order to use these surfactants under acidic conditions, SDS can be added to form mixed micelles with appreciable electrophoretic mobilities. The addition of a small amount of methanol and/or a relatively high concentration of urea often improve resolution while sharpening peak profiles. An example of the enantiomeric separation of PTH amino acids with SDVal is shown in Figure 20. Alternatively, digitonin can be used to form mixed micelles for these types of applications (see Figure 12).

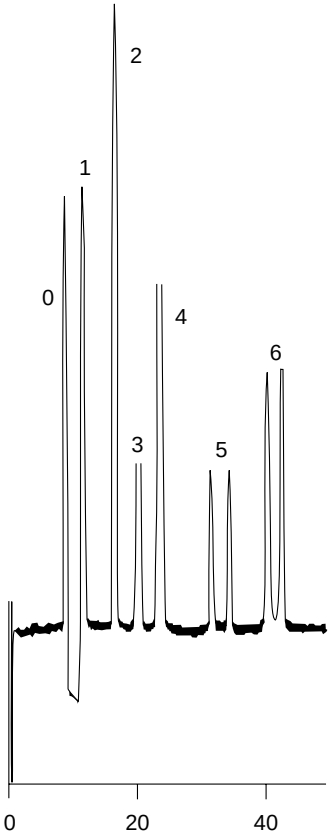


Figure 20. *Enantiomeric separation of six PTH-DL-amino acids by MEKC with SDVal: 0 = acetonitrile; 1 = Ser; 2 = Aba; 3 = Nva; 4 = Val; 5 = Trp; 6 = Nle. Conditions: Run buffer, 50 mM SDVal-50 mM SDS/ 0.5 M urea in 50 mM borate buffer (pH 9.0) containing 10% methanol; detection, UV absorbance at 260 nm. Reproduced with permission from Otsuka* et al., J. Chromatogr. 559, *209 (1991).*

2. Chiral Additives

The second, more popular, method of enantiomeric separation by MEKC is to add CD to the micellar solution (see also discussion on page 27, CDMEKC). The SDS micelle may be conveniently used for this approach. Various CDs or CD derivatives may be tried. The concentrations of SDS and CD should be optimized to yield optimal capacity factors (see Chapter V). An example of this approach is shown in Figure 21. Cicletanine is a member of a new class of antihypertensive drugs, the fusopyridines. Chiral selectivity was obtained by using a buffer consisting of 100 mM borate, 50 mM SDS, pH 8.6 and 50 mM β-CD. The addition of methanol and/or urea often enhances solubility and improves resolution. When γ-CD is employed, a second chiral component, such as *d*-camphor-10-sulfonate or *l*-menthoxyacetic acid, may enhance resolution. This is illustrated in Figure 22 for the separation of several barbiturates.

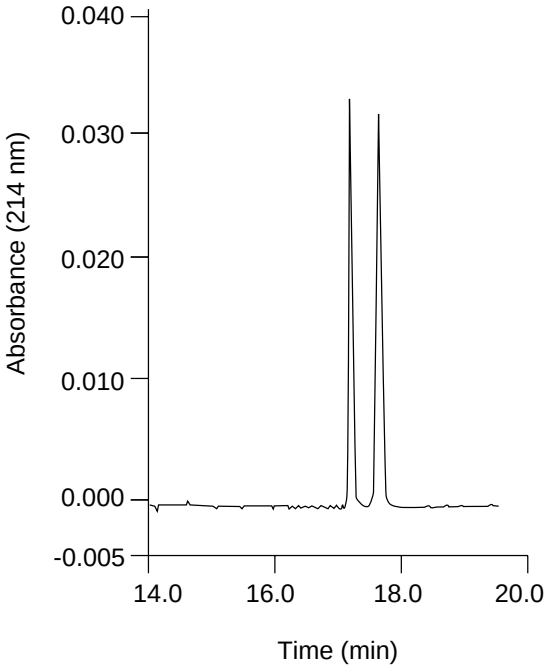


Figure 21. *Enantiomeric separation of R(-) and S(+) cicletanine. Run buffer, 100 mM sodium borate, 50 mM SDS, pH 8.5. Reproduced from Pruñonosa* et al., Beckman Application Note DS-798, *1990.*

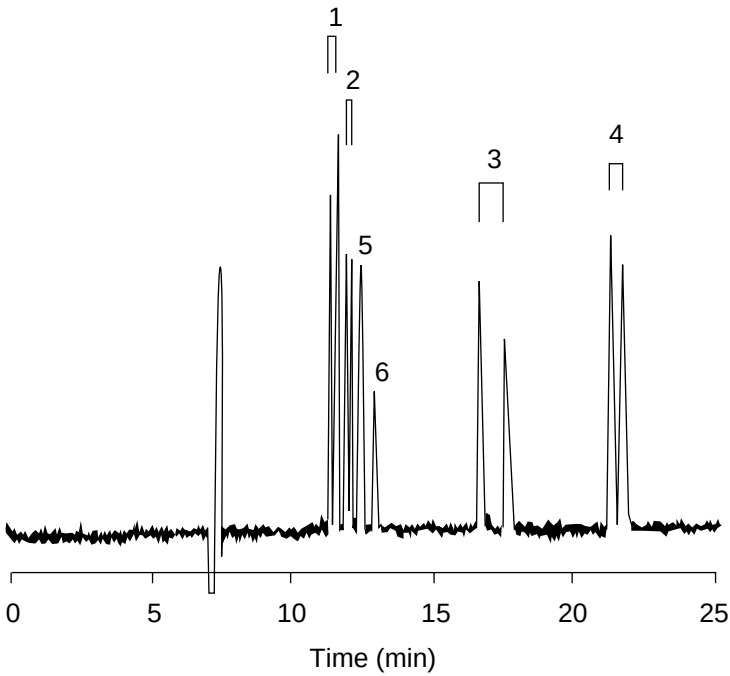


Figure 22. *Enantiomeric separation of several barbiturates by CDMEKC with* d*-camphor-10-sulfonate: 1 = thiopental (sodium salt); 2 = pentobarbital (calcium salt); 3 = 2,2,2-trifluoro-1-(9-anthryl)ethanol); 4 = 2,2'-dihydroxy-1,1'-dinaphthyl; 5 = phenobarbital; 6 = barbital (sodium salt). Run buffer, 50 mM SDS, 30 mM γ-CD, 20 mM sodium* d*-camphor-10-sulfonate in 20 mM phosphate-borate (pH 9.0); capillary, 50 μm i.d. × 65 cm (effective length 50 cm); applied voltage, 20 kV; detection, UV absorbance at 220 nm. Reproduced with permission from Nishi* et al., J. Chromatogr. 553, *503 (1991).*

Since the separation factor, α, of enantiomeric pairs is generally small, the separation conditions must be carefully optimized to maximize resolution. This can be achieved, for example, by using a longer capillary, a higher voltage, adjustment of the capacity factors, expansion of the migration time window, *etc.* Suppression of electroosmosis often enhances resolution (see page 14, equation 11), although analysis times may become long. The use of acidic buffer conditions or the addition of an organic solvent is useful in reducing the electroosmotic flow.

Further Reading
(in reverse chronological order)

Nishi, H., Fukuyama, T., Terabe, S. Chiral separation by cyclodextrin-modified micellar electrokinetic chromatography. *J. Chromatogr. 553,* 503-516 (1991)

Otsuka, K., Kawahara, J., Takekawa, K., Terabe, S. Chiral separation by micellar electrokinetic chromatography with sodium *N*-dodecanoyl-*L*-valinate. *J. Chromatogr. 559,* 209-214 (1991)

Otsuka, K., Terabe, S. Enantiomeric resolution by micellar electrokinetic chromatography with chiral surfactants. *Chromatogr. 515,* 221-226 (1990)

Dobashi, A., Ono, T., Hara, S., Yamaguchi, J. Optical resolution of enantiomers with chiral mixed micelles by electrokinetic chromatography. *Anal. Chem. 61,* 1984-1986 (1989)

Nishi, H., Fukuyama, T., Matsuo, M., Terabe, S. Chiral separation of optical isomeric drugs using micellar electrokinetic chromatography. *J. Microcol. Sep. 1,* 234-241 (1989)

Terabe, S., Shibata, M., Miyashita, Y. Chiral separation by electrokinetic chromatography with bile salt micelles. *J. Chromatogr. 480,* 403-411 (1989)

V: Strategy for Optimizing Resolution

After discussion of the separation principles, selectivity effects, and various applications, this final section of the primer provides a method development guide for MEKC. In many cases, the novice practitioner of MEKC will be surprised how easy it is to get a good separation on the first try. This is often the case with other modes of CE as well. Indeed, many separation scientists feel that, in general, it is easier and quicker to get satisfactory results in CE than in HPLC. Many separation problems can be solved with a "standard" MEKC run buffer and operating conditions. These standard conditions are listed in Table 3. When an electropherogram obtained with the standard operating conditions is not satisfactory, other options must be pursued. These are discussed below and are summarized in the flow chart shown in Figure 23.

Table 3. Suggested Standard Operating Conditions

Running solution:	50 mM SDS in 50 mM borate buffer* (pH 8.5 – 9.0)
Capillary:	50 – 75 μm i.d. × 20 – 50 cm (from the injection end to the detector)
Applied voltage:	10 – 20 kV (keep current below 100 μA)
Temperature:	25°C or ambient
Sample solvent:	water or methanol
Sample concentration:	0.1 – 1 mg/mL
Injection end:	the positive or anodic end
Injection volume:	below 2 nL (or less than 1 mm from the end of the capillary)
Detection:	200 – 210 nm (depends on the sample)

* The buffer solution in which to dissolve SDS may be a 50 mM phosphate (pH 7.0) or 20 mM Good's buffer (pH 7.0), if neutral conditions are desired.

Optimize the Capacity Factor

The optimum value of the capacity factor, k', is around 2 under the above conditions. Useful and practical capacity factors are in the range of 0.5 to 10. When the migration time of the micelle, t_{mc}, is difficult to measure, assume t_{mc} is roughly equal to four times t_0.

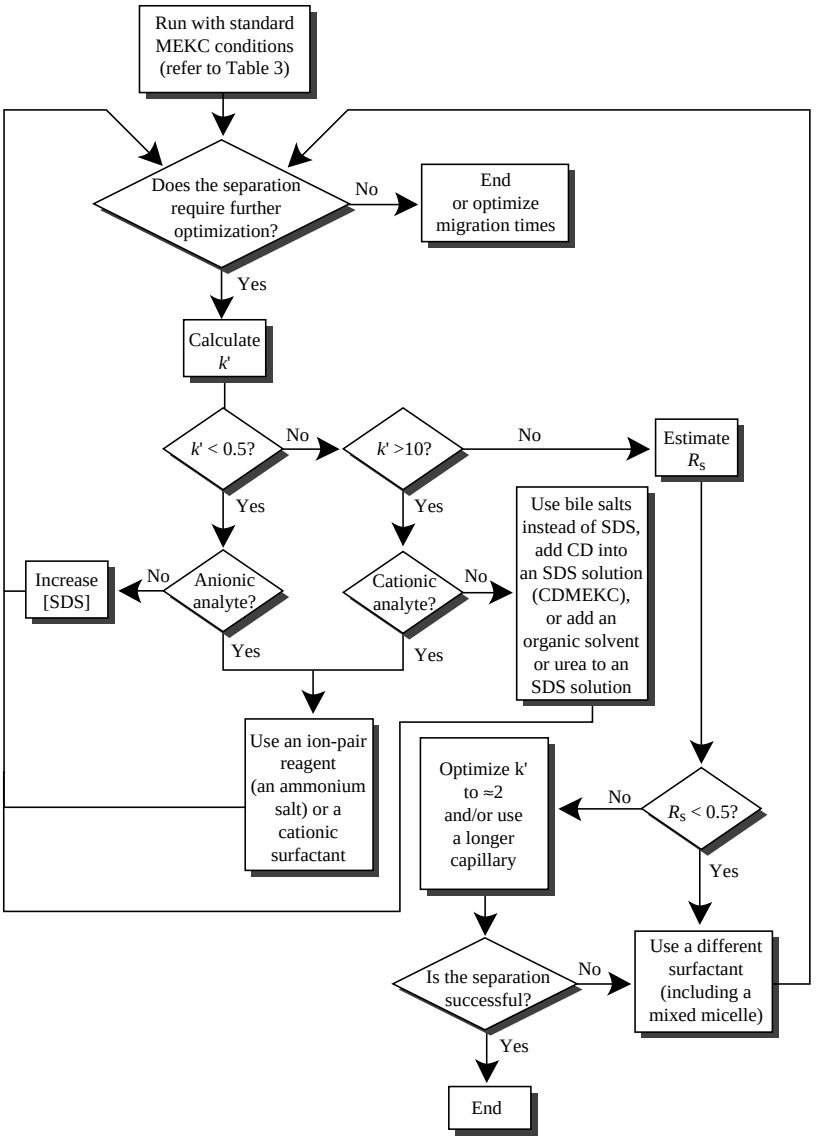


***Figure 23**. Optimization scheme for MEKC separations.*

If k' is less than 0.5, the concentration of SDS may be increased up to 200 mM, (see equation 10). When k' is still too small even when the SDS concentration is relatively high, the surfactant should be altered. In such a case, the analytes are negatively charged, and pH manipulation should be considered as will be described later.

When k' is too large (*i.e.*, $k'>10$), many options are available. For capacity factors less than 10, a reduction of the SDS concentration should be effective. However, the SDS concentration must be kept higher than 10 mM, as low SDS concentrations limit the sample loading capacity. When $k'>10$ or when the migration times are close to t_{mc}, other parameters should be changed.

Change the Surfactant

Even when the capacity factors are in the optimum range, separations may not always be successful. One of the easiest ways to improve resolution is to change the surfactant. At present, it is hard to predict which surfactant will be suitable for the separation of particular analytes. In general, *the polar group of the surfactant molecule affects selectivity more than the nonpolar group*. Therefore we recommend using surfactants with different polar groups to change selectivity. When the capacity factors are too large, the use of a bile salt may give successful results. Cationic surfactants show significantly different selectivities from anionic surfactants. The substitution of a cationic surfactant for an ionic surfactant should dramatically change selectivity for *ionic* analytes. The addition of a nonionic surfactant to the ionic micellar solution also affects selectivity but, as we have seen in Figure 12, this approach results in an increase of the capacity factors and a narrower migration time window.

Optimize the pH of the Buffer

As in CZE, the pH of the micellar solution is an important parameter in optimizing the separation of ionizable analytes. The pH does not significantly affect the selectivity of neutral analytes. In general, when an anionic surfactant is employed, the increase in pH will decrease the capacity factors of acidic compounds. Acids ionize at a high pH and have their own electrophoretic mobilities which are usually less than the mobility of the SDS micelle. That is, the acids will migrate at velocities that are slower than the electroosmotic flow at a high pH, although their interaction with the SDS micelles will be weak. The simplest way to find the optimum pH is to change the pH by one or two pH units and to run the separation in a certain pH range.

Modify the Aqueous Phase

Cyclodextrin is one of the most useful additives, not only for the separation of very hydrophobic or enantiomeric analytes but also for changing the selectivity. Among the various CDs, γ-CD in general will be most effective for most analytes in MEKC. The other CDs also change selectivity and should be used in case γ-CD is *not* very effective. The concentration of CD affects resolution and should be optimized. In CDMEKC, the CD concentration is dependent on the *k*' (in the absence of CD): for the separation of water-soluble compounds or enantiomers, 10 mM of CD is generally sufficient if the *k*'s are in the proper range *without* CD. For the separation of highly hydrophobic compounds (*k*' usually >10), CD concentrations of >40 mM will be necessary.

Methanol or acetonitrile are other useful additives for changing selectivity as well as expanding the migration time window. It is recommended that the concentration of the organic solvent remains below 20% in order to avoid breaking up the micellar structure. Other additives (ion-pair reagents, urea, metal salts) were discussed earlier and may also be useful in certain applications.

Further Reading

(in reverse chronological order)

Khaledi, M. G., Smith, S. C., Strasters, J. K. Micellar electrokinetic capillary chromatography of acidic solutes: migration behavior and optimization strategies. *Anal. Chem. 63,* 1820-1830 (1991)

Strasters, J. K., Khaledi, M. G. Migration behavior of cationic solutes in micellar electrokinetic capillary chromatography. *Anal. Chem. 63,* 2503-2508 (1991)

Vindevogel, J., Sandra, P. Resolution optimization in micellar electrokinetic chromatography: use of Plackett-Burman statistical design for the analysis of testosterone esters. *Anal. Chem. 63,* 1530-1536 (1991)

Foley, J. P. Optimization of micellar electrokinetic chromatography. *Anal Chem. 62,* 1302-1308 (1990)