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Review

Micellar electrokinetic chromatography Perspectives in drug analysis

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Abstract

Micellar electrokinetic chromatography (MEKC) has become a popular mode among several capillary electromigration techniques. Most drug analyses can be performed by using MEKC because of its wide applicability. Enantiomer separation, separation of closely related peptides and isotopic compounds, separation of very complex mixtures, determination of drugs in the biological samples, etc., can be successfully achieved by MEKC. This review surveys typical applications of MEKC analysis. Recent advances in MEKC, especially with pseudo-stationary phases, are described. Modes of electrokinetic chromatography including MEKC, a separation theory of MEKC and selectivity manipulation in MEKC are also briefly mentioned.

Keywords: Micellar electrokinetic chromatography; Reviews; Pseudo-stationary phases; Enantiomer separation; Distribution coefficients; Chinese crude drugs; Steroids; Vitamins; Peptides; Amino acids; Isotopes

Contents

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1. Introduction

Electrokinetic chromatography (EKC) is one branch of capillary electrophoretic (CE) techniques. EKC permits the separation of electrically neutral analytes by the electrophoretic technique [1–3], because the separation principle of EKC is based on that of chromatography. Various EKC modes have been developed along with the partition mechanism, that is, the charged molecule or charged aggregates employed. Among them, micellar electrokinetic chromatography (MEKC), in which an ionic surfactant solution is used as a running buffer solution in capillary zone electrophoresis (CZE) at a higher concentration than the critical micelle concentration (CMC), has become the most popular technique [4,5]. MEKC is effective for the separation of ionic analytes and also electrically neutral or non-ionic analytes, hence it is suitable for drug analyses involving cationic, anionic and neutral drugs.

Drugs are required to be of high quality to ensure biological activity. Many testing methods, e.g., purity testing and assay of active ingredients, are necessary for quality control. Recently, analytical validation has been required for the registration of new drugs [6]. The assay of drugs in body fluids is also necessary to clarify absorption, distribution, etc. For these purposes, HPLC has been widely used. However, a high-resolution separation method or another type of separation method is still desired.

CE shows high resolution and a great capability for the separation of drugs. MEKC is especially powerful for the separation of complex mixtures such as natural products and Chinese crude drugs [7–21]. The direct separation of enantiomers of drugs has been successful by MEKC using chiral surfactants or chiral additives [22–25]. The separation of highly hydrophobic drugs and compounds has been performed by MEKC using bile salts [26–29] or adding organic solvents or cyclodextrins (CDs) [30–34]. Determination of drugs in plasma is also successfully achieved by a direct sample injection method [35–43], similarly to micellar HPLC. Validation exercises have been performed in some CE methods [44,45].

In this review, modes of EKC, the separation principle and selectivity manipulation in MEKC are briefly described. Some recent advances in the type of surfactant used are also presented. Applications of MEKC to purity testing, assay, separation analysis and the determination of some physico-chemical properties of drugs are summarized.

2. Fundamentals

2.1. Modes of EKC

EKC has the capability to separate electrically neutral analytes, because the chromatographic separation principle is combined with capillary

electrophoretic techniques. Technically, charged molecules or charged aggregates, which operate as the pseudo-stationary phase, are added to the running buffer solution in CZE. Along with pseudo-stationary phases, several partition mechanisms have been developed for the separation of a wide variety of compounds. These are summarized in Table 1.

The term "micellar EKC (MEKC)" is used for an EKC mode with a micellar solution. Highly hydrophobic drugs have been separated by MEKC through the addition of CDs [30–32]. This mode is one branch of MEKC and called CD-modified MEKC (CD-MEKC). Enantiomeric separation has been successfully achieved by CD-MEKC [46–49] because CDs are optically active compounds. Microemulsions, which consist of oil, water, a surfactant and a co-surfactant, are effective for the separation of neutral analytes as micelles [50–52].

Proteins and ionic polysaccharides such as mucopolysaccharides also can be used for the separation of both ionic and neutral analytes as pseudo-stationary phases of EKC. This mode is called affinity EKC (AEKC). AEKC has a great capability to separate enantiomers [53–61].

Charged CDs are useful for the separation of neutral analytes [62–65]. This mode is called CD-EKC, although CDs may not be stationary "phases". Polymer ions such as poly(dialkyldimethylammonium chloride) [66,67] and polybrene [66,68] have been used for the separation of ionic analytes such as naphthalenesulfonates. The analytes were separated based on the ion-exchange mechanism. We term this mode ion-exchange EKC [66–68]. EKC utilizing a ligand-exchange mechanism will be successful for the separation of amino acids or carbohydrates. Recently, molecular pseudo-stationary phases such as starburst dendrimers [69–73] and resorcarenes [74] have been successfully employed for the separation of neutral analytes. Chromatographic particles have been also employed as a pseudo-stationary phase in EKC [75]. In addition to the EKC modes listed in Table 1, many other EKC modes can be constructed along with other partition mechanisms.

2.2. Separation principle of MEKC

An ionic surfactant is added to an operating buffer solution in MEKC. Surfactant molecules tend to form micelles or aggregates above the CMC, in which the hydrophobic tail group orients towards the centre and the charged head group towards the outer surface. A schematic representation of the separation principle of MEKC is shown in Fig. 1, where an uncoated capillary and an anionic surfactant are used under neutral or alkaline conditions. When a high voltage is applied, the anionic micelle migrates toward the positive electrode by its electrophoretic mobility. However, the strong electroosmotic flow (EOF) transports the buffer solution towards the negative electrode owing to

Table 1
Modes of EKC

Mode	Abbreviation	Pseudo-stationary phase	Main applicable analyte
Micellar EKC	MEKC	Micelle	Neutral, ionic drugs
Cyclodextrin mediated MEKC	CD-MEKC	Micelle	Hydrophobic, enantiomers
Microemulsion EKC	MEEKC	Microemulsion	Hydrophobic
Affinity EKC	AEKC	Protein, polysaccharide	Enantiomers
Cyclodextrin EKC	CDEKC	Charged cyclodextrin	Enantiomers
Ion-exchange EKC	–	Polymer ion	Ionic drugs
Ligand-exchange EKC	–	Ligand + metal ion	Amino acids
Dendrimer EKC	–	Dendrimer	Hydrophobic
Resorcarene EKC	–	Resorcarene	Hydrophobic
Suspension EKC	–	Chromatographic particles	Hydrophobic

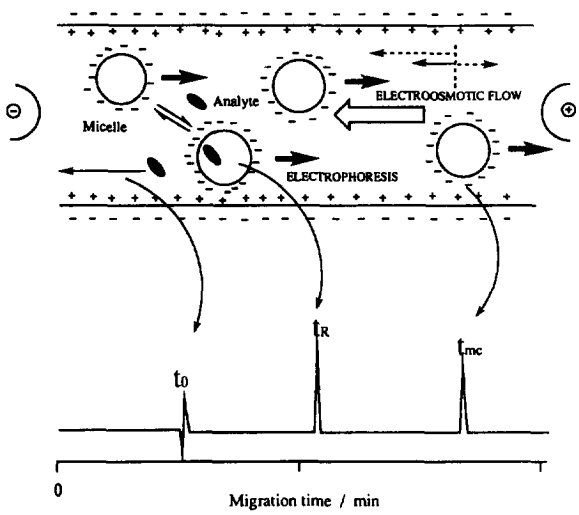


Fig. 1. Schematic illustration of separation principle of MEKC. An electrically neutral solute and an anionic micelle system.

the negative charge on the surface of the fused-silica capillary. The velocity of the EOF is faster than the electrophoretic velocity of the micelle in the opposite direction, resulting in a fast-moving aqueous phase and a slow-moving micellar phase. When neutral analytes are injected from the positive end, a fraction is incorporated into the micelle and migrates at the same velocity as that of the micelle. The remaining fraction migrates at the velocity of EOF. The distribution equilibrium is quickly established and hence neutral analytes are separated by the difference in the distribution coefficients between the micellar phase and the surrounding aqueous phase. The migration order for neutral analytes in MEKC generally relates to the hydrophobicity of the analyte. More hydrophobic analytes interact more strongly with the micelle and migrate more slowly than the hydrophilic analytes. Besides the separation of electrically neutral or non-ionic analytes, MEKC can provide enhanced selectivity for the separation of ionic analytes through the ionic interaction between the analyte and the micelle.

All analytes, including anionic, cationic and neutral molecules, injected at the positive end of the capillary tube move towards the negative end under the conditions shown in Fig. 1. For neutral analytes, the migration time (t_R) is limited between the migration time of the EOF (t_0) and that of the micelle (t_{mc}), and the capacity factor k' of the analyte can be calculated by [1,2]

$$k' = \frac{t_R - t_0}{t_0(1 - t_R/t_{mc})} \quad (1)$$

When t_{mc} becomes infinite (the micellar phase becomes stationary), Eq. 1 reduces to the analogous equations for conventional chromatography. To evaluate k' values in MEKC, t_0 and t_{mc} must be experimentally determined by injecting acetone, formamide or methanol, which are assumed not to interact with the micelle, and lipophilic dyes, Sudan III or Sudan IV, or hydrophobic cations such as timepidium or halofantrine, which are assumed to be totally solubilized into the micelle [4].

On the other hand, the capacity factor k' for an acidic analyte and an anionic micelle, where ion pairing between the two is absent, is given by [76,77]

$$k' = \frac{\mu_{ep,S^*} - \mu_{ep,S}}{\mu_{ep,mc} - \mu_{ep,S^*}} \quad (2)$$

where $\mu_{ep,S}$ is the electrophoretic mobility of the analyte in the absence of the micelle. For basic solutes, there must be ion pairing and the model or equation becomes more complex [78].

2.3. Optimization of separation

The resolution R_s in MEKC is given by [2]

$$R_s = \frac{N^{1/2}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k'_2}{1 + k'_2}\right)\left(\frac{1 - t_0/t_{mc}}{1 + (t_0/t_{mc})k'_1}\right) \quad (3)$$

where N is the theoretical plate number and $\alpha = k'_2/k'_1$ is the separation factor. The last term accounts for the difference between MEKC and conventional HPLC. As t_{mc} becomes infinite, the last term becomes equal to unity and the resultant equation is identical with that of conventional HPLC. The resolution equation for the neutral analytes can be also described as in CZE [79]:

$$R_s = \frac{N^{1/2}}{4}\left(\frac{\mu_{ep,S1^*} - \mu_{ep,S2^*}}{\mu_{ep,S^*} + \mu_{eo}}\right) \quad (4)$$

where $\mu_{ep,S1^*}$, $\mu_{ep,S2^*}$ and μ_{ep,S^*} are effective electrophoretic mobilities of the enantiomeric pair 1 and 2 and the average effective mobility of the two enantiomers, respectively.

Eq. 3 predicts the effects of N, α, k' and t_0/t_{mc} on resolution. Resolution increases in proportion to the square root of N. In MEKC, as in CZE, the higher the applied voltage, the higher is N, unless the temperature increase is too high. Usually a high voltage of 10–30 kV is applied to perform the MEKC separation and 100 000–300 000 theoretical plates are obtained with a 500 mm × 0.05 mm I.D. capillary tube within a relatively short time.

The capacity factor k' is important in increasing the resolution in MEKC. The optimum k' to give the highest resolution is given by $k' = (t_{mc}/t_0)^{1/2}$, provided that N is independent of k' [80]. One can easily realize that the maximum R_s will be obtained when k' approaches the value $-t_{mc}/t_0$ [81] in Eq. 3 when we assume a negative t_{mc} for the migration of the micelle in the opposite direction to the analyte. If we can control the EOF giving close to $-\mu_{ep,S^*}$, an extremely high R_s value will be obtained at the expense of a longer analysis time, as shown in Eq. 4. The k' values can be easily adjusted by changing the concentration of the micelle.

The migration time ratio, t_0/t_{mc}, is directly related to the width of the migration time window. The smaller the value of t_0/t_{mc}, the wider is the migration time window and hence the higher is the R_s value. The value of t_0/t_{mc} is in the range 0.2–0.4 for most ionic micelles under neutral or alkaline conditions. It is necessary to reduce the velocity of the EOF to obtain a smaller value of t_0/t_{mc}. Addition of an organic solvent such as methanol, acetonitrile or 2-propanol is a useful method, and changing the pH to acidic is also a possible approach. However, in practice, a longer run time is required.

The separation factor α is the most important and most effective term to increase resolution. The type of surfactant significantly affects the α values [4,5,82,83]. As an example, bile salt micelles, whose structures are different from those of the usual long-chain alkyl-type surfactants, have unique characteristics and selectivity for hydrophobic drugs, which were not separated by MEKC with sodium dodecyl sulfate (SDS). Detailed recent advances in surfactants employed for MEKC are described below. In addition to changing surfactants, selectivity is easily manipulated through the modification of the buffer solution, similarly to HPLC. The addition of tetraalkylammonium salts [84], CDs [30–32,46–49], urea [85–87], organic solvents [33,34], etc., has been found to be effective for the improvement of selectivity in MEKC. The detailed manipulation of selectivity is described elsewhere [82].

Of course, the migration time can be altered by changing the buffer constituents, ionic strength, pH, applied voltage (current), capillary length, inside diameter or inside surface characteristics and temperature. These parameters have profound effects on the velocity of the EOF as in the usual CZE.

2.4. Surfactants employed for MEKC

2.4.1. Long-alkyl-chain surfactants

Long-alkyl-chain surfactants, typically anionic types such as SDS, have been employed in most MEKC applications. These surfactants are believed to form spherical micelles having ionic groups on the surface and the hydrophobic core. Non-ionic long-alkyl-chain surfactants such as Brij and Tween have also been successfully used for MEKC separations with other ionic surfactants such as SDS. Remarkably different selectivity has been obtained by using a mixed micelle. When ionic interactions are strong in MEKC with SDS alone, i.e., the analytes migrate at around t_{mc}, the addition of non-ionic surfactants will be successful because of the decrease in the surface charge in the mixed micelle. Cationic surfactants are effective for the separation of basic drugs such as catecholamines [88], β-blockers [89] and imipramine analogues [90]. Alkylsaccharide surfactants such as octyl-β-D-glucopyranoside have been utilized in MEKC under alkaline borate conditions by El Rassi and co-

workers [91–94]. In situ charged micelles are formed and the charge density of the micelle can be adjusted by varying the pH and/or the borate concentration. Recently, Tanaka et al. [95] utilized double-chain surfactants, in which two ionic groups and two hydrophobic chains are present, as micelle-forming agents in MEKC. Typical long-alkyl-chain surfactants except for chiral types are summarized in Table 2 [5,87,96,97].

2.4.2. Bile salt surfactants

Bile salts are anionic surfactants found in biological sources. They have steroidal structures as shown in Fig. 2 and form helical micelles

Table 2
Long-alkyl-chain surfactants employed in MEKC

Surfactant	CMC (m*M*)	n^a	Kp^b
Cationic			
Decyltrimethylammonium bromide (DeTAB)	61	–	–
Decyltrimethylammonium chloride (DeTAC)	68	–	–
Dodecyltrimethylammonium bromide (DTAB)	15	56	–
Dodecyltrimethylammonium chloride (DTAC)	20	–	–
Tetradecyltrimethylammonium bromide (TTAB)	3.5	75	–
Tetradecyltrimethylammonium chloride (TTAC)	4.5	75	–
Cetyltrimethylammonium bromide (CTAB)	0.92	61	–
Cetyltrimethylammonium chloride (CTAC)	1.3	–	–
Anionic			
Sodium dodecyl sulfate (SDS)	8.1	62	16
Sodium tetradecyl sulfate (STS)	2.1	138	32
Sodium decanesulfonate	40	40	–
Sodium dodecanesulfonate	7.2	54	37.5
Sodium N-lauroyl-N-methyltaurate (LMT)	8.7	–	<0
Sodium polyoxyethylene(3) dodecyl ether sulfate	2.8	66	–
Sodium tetradecene sulfonate (OS-14)	–	–	–
Sodium polyoxyethylene(3) dodecyl ether acetate	–	–	–
Potassium perfluoroheptanoate	28	–	25.6
Sodium di-2-ethylhexyl sulfosuccinate	2.5	–	–
Disodium 5,12-bis(dodecyloxymethyl)-4,7,10,13-tetraoxa-1,16-hexadecanedisulfonate	ca. 1	–	–
Neutral			
Polyoxyethylene(6) dodecyl ether	0.09	–	400
Polyoxyethylene(23) dodecyl ether (Brij 35)	0.09	–	40
Polyoxyethylene(20) sorbitanmonolaurate (Tween 20)	0.95	–	–
Polyoxyethylene(20) sorbitanmonooctadecanate (Tween 60)	–	–	–
Heptyl-β-D-glucopyranoside	79	–	–
Octyl-β-D-glucopyranoside	25	–	–
Nonyl-β-D-glucopyranoside	6.5	–	–
Decyl-β-D-glucopyranoside	2–3	–	–
Octanoyl-N-methylglucamide	58	–	–
Nonanoyl-N-methylglucamide	19–25	–	–
Decanoyl-N-methylglucamide	6–7	–	–
Octyl-β-maltopyranoside	23	–	–
n-Octanoylsucrose	24.4	–	–

[a] Aggregation number.
[b] Krafft point.

Bile salt	Abbreviation	R_1	R_2	R_3	R_4
Sodium cholate	SC	OH	OH	OH	ONa
Sodium taurocholate	STC	OH	OH	OH	$NHCH_2CH_2SO_3Na$
Sodium deoxycholate	SDC	OH	H	OH	ONa
Sodium taurodeoxycholate	STDC	OH	H	OH	$NHCH_2CH_2SO_3Na$

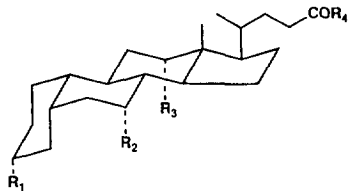


Fig. 2. Structure of bile salt surfactants employed for MEKC.

having a reversed micelle conformation [27]. Non-conjugated types of bile salts must be used at pH > 5, although taurine-conjugated bile salts can be applicable even at pH 3. Compared with long-alkyl-chain surfactants mentioned above, bile salts have a relatively weak solubilization power. Hence corticosteroids and benzothiazepin analogues, which were almost totally solubilized in SDS micelles because of high hydrophobicity and were not separated by MEKC with SDS, have been successfully separated [26,29]. Apart from the separation power for hydrophobic compounds, enantiomer separations are successful with bile salts [98–104] because these are natural chiral surfactants.

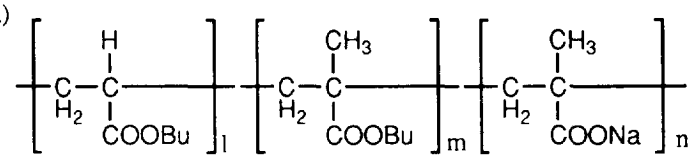
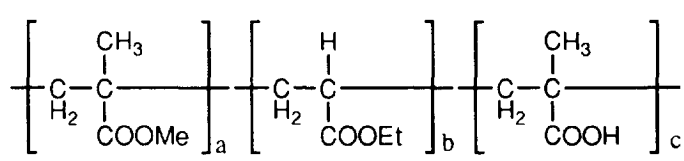
2.4.3. High-molecular-mass surfactants

Most of the surfactants mentioned above form molecular aggregates of surfactants. There must be a CMC and aggregation number for each surfactant. On the other hand, high-molecular-mass surfactants such as block copolymers [105–107] can form a micelle with one molecule. This leads to some advantages over normal low-molecular-mass surfactants, e.g., stability, rigidity and controllable size. The CMC is essentially zero, and therefore the micelle concentration is independent of temperature, buffer concentration and additives such as organic solvents. No monomeric surfactant that may reduce the selectivity in CD-MEKC by interaction with CD is present. The high background in MEKC–MS with normal surfactant micelles may be reduced with high-molecular-mass surfactants [106]. High-molecular-mass surfactants used for MEKC are summarized in Table 3. Terabe and co-workers [105,106] and Yang et al. [107] used copolymers of methacrylate types. These surfactants showed remarkably different selectivity for substituted aromatic compounds from SDS. The copolymer surfactant with β-CD gave a better enantioseparation for dansylated amino acids compared with the β-CD–SDS system [106]. Another type of high-molecular-mass surfactants has been synthesized by polymerization of vinyl group-terminated surfactants such as sodium 10-undecylenate and utilized for MEKC by Palmer and co-workers [108–110] and Wang and Warner [111]. In addition to high-molecular-mass surfactants, macrocyclic molecules built up by four alkylidene-bridged resorcinol units (resorcarenes) were employed as a molecular pseudo-stationary phase in EKC by Bachmann et al. [74] for the separation of polycyclic aromatic hydrocarbons. Tanaka and co-workers [69,72] and others [70,71,73] synthesized several starburst dendrimers and employed them for the separation of neutral analytes. Schematic illustrations of the structures of several micelles and micelle-like molecular pseudo-stationary phases mentioned above are shown in Fig. 3. Different pseudo-stationary phases give different selectivities for the separation of drugs.

2.4.4. Chiral surfactants

Chiral surfactants are used for the separation of enantiomers of drugs. Bile salt surfactants have been found to be effective for the enantioseparation of several drugs [98–104]. Acylamino acid surfactants such as sodium N-dodecanoyl-L-valinate (SDVal) [112–117] and alkoxyacylamino acids [118,119] have also been employed for the separation of enantiomers by MEKC. Some saponins such as digitonin [114], glycyrrhizic acid [120] and β-escin [120] have been used for MEKC enantiomer separations. Chiral surfactants employed for MEKC are summarized in Table 4. Sometimes enantiomer separations by

Table 3
High-molecular-mass surfactants used in MEKC

Surfactant	Ref.
Block copolymer	
Butyl acrylate–butyl methacrylate–methacrylic acid copolymer[a]	[105,106]
Methyl methacrylate–ethyl acrylate–methacrylic acid copolymer[b] (Elvacite2669)	[107]
(a) 	
(b) 	
Polymerized surfactant	
Poly(sodium 10-undecylenate) (polySUA)	[108,109]
Poly(sodium N-undecylenyl-L-valinate) [poly(L-SUVal)]	[111]
Poly(sodium 10-undecenyl sulfate) (polySUS)	[110]

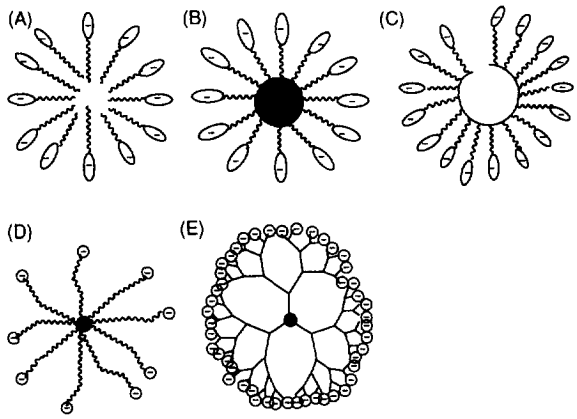


Fig. 3. Schematic illustration of structure of micelle and molecular pseudo-stationary phase (anionic). White circles indicate hydrophilic part of the micelle. (A) Micelle by long-alkyl-chain surfactant; (B) microemulsion (oil–water type; black circle shows oil core); (C) polymerized surfactant (polySUA, etc.); (D) star polymer; (E) dendrimer.

MEKC are performed with a mixed micelle of two chiral surfactants or a combination of a chiral surfactant and a chiral additive such as CDs (CD-MEKC) [104]. Detailed enantiomer separations by MEKC have been described elsewhere [22–25].

3. General considerations in drug analysis by MEKC

3.1. Sample preparation

Drugs which may be soluble in water or an organic solvent can be analysed by CE techniques. Usually only a filtration is performed on the sample solution prior to use. However, drugs having no UV absorbance must be derivatized as in HPLC analysis because most of the commercial instruments are equipped with a UV detector

Table 4
Chiral asurfactants employed in MEKC

Surfactant	CMC	n^a
Bile salts and saponins		
Sodium cholate (SC)	13–15	2–4
Sodium deoxycholate (SDC)	4–6	4–10
Sodium taurocholate (STC)	10–15	5
Sodium taurodeoxycholate (STDC)	2–6	–
Digitonin	–	–
Glycyrrhizic acid	–	–
β-Escin	–	–
Amino acid head type		
Sodium N-dodecanoyl-L-valinate (SDVal)	6.2	–
Sodium N-dodecanoyl-L-alaninate	12.5	–
Sodium N-dodecanoyl-L-glutamate	–	–
N-Dodecanoyl-L-serine	–	–
Sodium N-dodecanoyl-L-threoninate	–	–
Sodium N-undecylenyl-L-valinate	36	–
Poly(sodium N-undecylenyl-L-valinate)		
(*R*)-N-Dodecoxycarbonyvaline	–	–
(*S*)-N-Dodecoxycarbonyvaline	–	–
(*S*)-2-[(Dodecoxycarbonyl)amino]-3(*S*)-methyl-1-sulfoxypentane	–	–
Sugar head type		
n-Heptyl-β-D-thioglucopyranoside	30	–
n-Octyl-β-D-glucopyranoside	24	–
n-Octyl-β-D-thioglucopyranoside	9	–
n-Dodecyl-β-D-glucopyranoside	2	–
n-Dodecyl-β-D-glucopyranoside monophosphate	0.5	–
n-Dodecyl-β-D-glucopyranoside monosulfate	1	–
Others		
L-α-Palmitoyllysophosphatidylcholine	–	–

[a] Aggregation number.

only. Carbohydrates and oligosaccharides are typically analysed as pyridylaminated (PA) [121–125] or 1-phenyl-3-methyl-5-pyrazolone (PMP) derivatives [126,127]. Amino acids are derivatized with dansyl (DNS) chloride, *o*-phthaldialdehyde (OPA), 3,5-dinitrobenzoyl chloride, etc. (see Table 7). Diastereomeric derivatizations have also been employed for MEKC enantiomer separations [128–132]. Chiral derivatization reagents or systems employed for HPLC are utilized in the CE techniques. That is, the concept of sample preparation or treatment in CE is similar to those employed in HPLC.

The preparation of sample solutions with relatively high concentrations, in comparison with HPLC analysis, is needed in MEKC purity testing to detect less than 0.1% of related substances, because of the relatively low concentration sensitivity of CE instruments, or low-concentration samples must be concentrated previously. As for on-column preconcentration techniques, several methods have been developed. Isotachophoretic preconcentration [133–135] has been employed for ionic solutes. The capillary consists of a 1.0-mm packed bed of a polymeric reversed-phase chromatographic packing materi-

als [136] and capillaries having surface-bound octadecyl functions [137] have been employed for on-column concentration of dilute neutral samples. The field-amplified sample stacking technique [138,139], which has been developed for the on-line preconcentration of dilute ionic solutes, has been employed for MEKC [140]. The sample stacking was accomplished by dissolving the neutral solutes in a low-concentration micellar solution.

3.2. Capillary and detection system

Capillaries generally consist of fused silica protected by a polyimide coating. Usually 50–100 μm I.D. cylindrical capillaries are used. For sensitivity enhancement, rectangular capillaries [141], Z-cells [142, 143], bubble cells [144], etc., have been employed to obtain longer optical paths. The most common detector is the variable-wavelength UV detector or photodiode-array detector. Fluorescence [145] and MS detection [107,146,147] are available in some commercial instruments, although they are expensive. Electrochemical detection has also been applied in CE [148]. Sensitivity enhancement for CE has been summarized elsewhere [149].

3.3. Validation

The instrumentation for MEKC is the same as that for CZE. Migration time, peak area and peak height are measured with a data processor. One of the important factors in the testing of drugs is validation of analytical procedures. According to the ICH Harmonized Tripartite Guideline [6], the validation characteristics which should be considered are accuracy, precision (repeatability, intermediate precision), specificity, detection limit, quantification limit, linearity and range. These characteristics are summarized in Table 5 with type of analytical procedure.

For precision of peak areas and migration times, injection methods and control of inner surface of the capillary are important. Either the hydrodynamic injection method (siphoning, positive pressure and vacuum) or the electrokinetic injection method is usable, and both are available in commercial instruments. The repeatability of peak areas in both injection systems is satisfactory when the injection times are long. However, the electrokinetic method should be avoided for the purity testing of drugs, because the amount injected depends on the electrophoretic mobility (charge). Minor components having slow migration velocities are difficult to detect when electrokinetic injection is employed.

To obtain reproducible migration times, control of the EOF, i.e., strict control of the capillary surface, is important. Fortunately, the surface of the capillary in MEKC is more stable than that in CZE because of the presence of a surfactant. Typically the migration time precision (R.S.D.) obtained in MEKC is around 1% when capillary

Table 5
Validation of analytical procedures

Characteristic	Type of analytical procedure[a]			
	Identification	Purity testing		Assay
		Qualitative	Limit	
Accuracy	–	+	–	+
Precision:				
Repeatability	–	+	–	+
Intermediate precision	–	+	–	+
Specificity	+	+	+	+
Detection limit	–	–	+	–
Quantification limit	–	+	–	–
Linearity	–	+	–	+
Range	–	+	–	+

[a] –: Signifies that this characteristic is not normally evaluated. +: signifies that this characteristic is normally evaluated.

conditioning with running buffer solutions and capillary temperature control are employed. Temperature control will be more important in MEKC than that in CZE because the distribution coefficient is sensitive to temperature. Washing with an alkaline solution is sometimes effective for obtaining reproducible migration times. MEKC in the constant-current mode may improve the repeatability of migration times, rather than the constant-voltage mode because the EOF velocity and electrophoretic mobility are strictly proportional to current, irrespective of temperature. Therefore, constant-current operation must be recommended for quantitative analysis.

A validation study was performed for the MEKC determination of *p*-toluenesulfonic acid impurity in a pharmaceutical intermediate [44]. The reported R.S.D. values for migration times and peak areas (eight repeated injections by pressure) were 0.6% and 3%, respectively. The R.S.D. for quantification of 0.11% of minor component was 7%. The recovery, detection limit and quantification limit were 99%, 0.02% and 0.08%, respectively. The results obtained by MEKC was compared with those obtained by a validated HPLC method and a correlation coefficient of 0.995 was obtained. The MEKC method was found to be acceptable for routine drug analysis. Intermediate precision exercises in MEKC have also been performed in seven companies by Altria et al. [150]. Dose uniformity of paracetamol was determined by using the internal standard method. The precisions of the relative migration times and peak areas were good and the results obtained agreed well between companies and with both the HPLC data and the label claim, showing successful transfer of the MEKC method.

4. Applications

4.1. Purity testing of drugs

Ratios of peak areas (area percentage method) are generally used for purity testing of drugs as in HPLC. In CE, including MEKC, normalization of peak areas with migration times is essential because the peak area increases with an increase in migration time [151]. According to the guidelines for registration applications on the content and qualification of impurities in new drugs [152], impurities above apparent levels of 0.1% must be identified. These levels are not so difficult for typical pharmaceuticals in MEKC. Examples of normal purity testing [153] and optical purity testing [154] of diltiazem are shown in Fig. 4, where affinity EKC with chondroitin sulfate C was employed for the enantiomer separation. The purity testing, including optical purity, of drug substances and those in formulations are successful by MEKC [155].

4.2. Assay of drugs

It is recommended to use an internal standard (I.S.) for the assay of drugs to compensate for the variance in sample roading. MEKC assay of active ingredients in pharmaceutical formulations have all been performed using an I.S. method. The results obtained corresponded well with

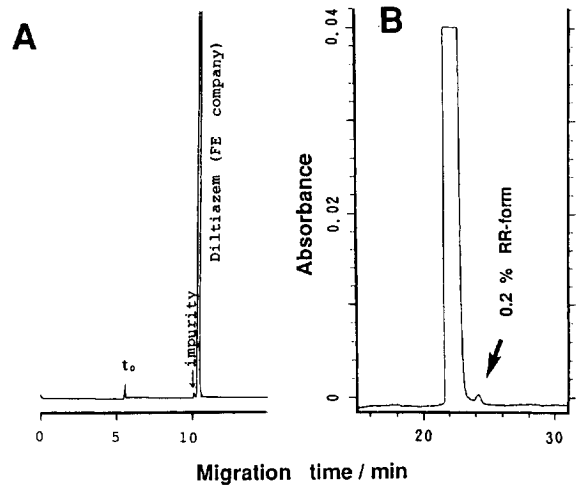


Fig. 4. Purity testing of diltiazem hydrochloride. (A) Normal purity testing by MEKC with bile salt and (B) optical purity testing by affinity EKC with chondroitin sulfate C. Samples: (A) diltiazem extracted from tablets from FE Company; (B) standard diltiazem hydrochloride (2*S*,3*S*-form) spiked with 0.2% of (2*R*,3*R*)-form. Capillary, (A) 50 μm I.D. × 65 cm (effective length, 50 cm) and (B) 75 μm I.D. × 57 cm (effective length, 50 cm); applied voltage, 20 kV; detection wavelength, (A) 220 nm and (B) 235 nm; temperature, 23°C. From Refs. [153,154].

HPLC results with R.S.D. of 1–3%. The simultaneous determination of several drugs in a formulation was successfully achieved by MEKC. A typical example is a cold medicine [83,156–158]. Except for chlorpheniramine, whose label claim is the smallest among five active ingredients, R.S.D. values of 1–3% and assay results of almost 100% were obtained [158]. In general, it is difficult to determine these drugs simultaneously by conventional reversed-phase HPLC in the isocratic mode because the polarity and hydrophobicity of each drug are widely scattered. However, these drugs, including cationic, anionic and neutral, could be separated by MEKC in a relatively short time, indicating a great potential of MEKC as an assay method for pharmaceuticals. The assay results obtained by MEKC and the validation exercises mentioned above are good enough for assay and purity testing methods of pharmaceuticals.

The determination of drugs and their metabolites in biological fluids such as urine and serum has been successfully performed by MEKC [29,35–43,49,89,90,159–178], as summarized in Table 6. Typically drugs at low concentration in complex matrices must be analysed. Therefore, extraction procedures with disposable cartridges are often employed. Proteins in serum samples are precipitated by adding an organic solvent. These pretreatment procedures are similar to those in HPLC except for the direct injection method in MEKC as mentioned below. Desiderio et al. [162] have extensively investigated MEKC determinations of drugs in body fluids employing a photodiode array-detector. One example is shown in Fig. 5. The confirmation of the stereoselectivity of the aromatic hydroxylation of mephenytoin and phenytoin in human urine has been determined by CD-MEKC. In extensive metabolizers, only the (*S*)-mephenytoin [(*S*)-MEPH] enantiomer of the racemic administered drug was metabolized to the (*S*)-

Table 6
Determination of drugs in body fluids by MEKC

Drug/metabolite	Sample composition	Ref.
Abuse drugs	Urine	[171,221,222]
Antidepressant	Plasma	[90]
Antiepileptics	Plasma	[173]
Benzodiazepines	Urine	[43,161]
β-Blockers	Urine/serum	[89,165]
Cicletanine (enantiomer)	Plasma	[49]
Cimetidine	Plasma	[169]
Flavoxate metabolite	Urine	[168]
Hippuric acid	Serum	[163]
Mephenytoin	Urine	[162]
Methadone	Urine	[166]
Thiopental	Serum	[174]
Theophylline	Body fluids	[167,176]
Uric acid, creatinine	Urine	[178]
Vitamin B_6 (vitamer)	Urine	[172]
Xanthine and uric acid derivatives	Urine	[164,175]
(Direct injection of plasma or serum samples)		
Amino acids	Serum	[43]
Antipyrine	Serum	[40]
Antiepileptics	Plasma	[41–43,177]
Aspoxicillin	Plasma	[36]
Barbiturates	Serum/urine	[37]
Cefpiramide	Plasma	[35]
Paracetamol	Plasma	[39,41,170]
Substituted purines	Serum/saliva/urine	[38]

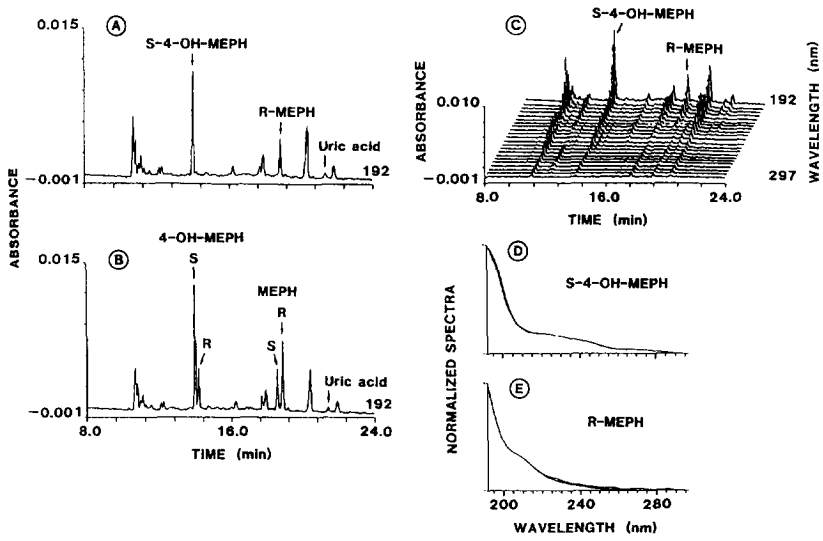


Fig. 5. Determination of mephenytoin and its 4-hydroxy metabolite enantiomers in urine by cyclodextrin-mediated MEKC. (A) Single- and (C) multi-wavelength chromatograms of the sample of an extensive metabolizer. (B) Chromatogram obtained from the sample spiked with two racemic drugs. (D) and (E) UV spectra of peaks of (A) with those of the standard compounds for identification. (From Ref. [162]).

hydroxy derivative (4-OH-MEPH). Peaks were identified in the UV spectra obtained with a photodiode-array detector. These results indicate that MEKC can be used for drug analysis as a complementary or alternative method to HPLC. Application of CE to the determination of pharmaceuticals and drug-related impurities from the viewpoint of quantitative aspects [155] and MEKC monitoring of drugs and their metabolites in body fluids have been reviewed recently [42,159,160].

4.3. Determination of drugs in biological fluids by the direct injection method

Protein adsorption on the capillary wall should be avoided in CZE in order to maintain reproducible migration times of the analytes. However, a direct injection method has been successfully employed in MEKC [35–43], similarly to micellar HPLC. Without a surfactant (CZE mode), plasma protein peaks interfered with the peaks of the solute and protein adsorption occurred, causing a change in the velocity of the EOF. Rinsing of the capillary is required to recover the capillary surface or reproducible migration times when plasma samples are injected in the CZE mode. With a surfactant, the migration times of the protein peaks increase and the useful analytical window, which is defined between the t_0 and the plasma protein peaks, appears. The migration time of the analyte of interest must be manipulated so that it migrates before the protein peaks. The effects of buffer pH and SDS concentration on the analytical window were investigated by Watzig and Lloyd [179]. pH 7 gave an acceptable analytical window with acceptable analysis times over a wide range of SDS concentration; pH ≥ 8 is most useful when [SDS] > 50 m*M*. The surfactant also breaks the drug–protein complexes, and therefore the total amount of bound and unbound drug could be determined by this method. The use of a micellar solution also prevents protein adsorption on the capillary wall, due to the solubilization of the protein hence the electrostatic repulsion between the solubilized proteins and the capillary wall. Rather than washing with NaOH, an SDS solution has been found to be an effective between-run rinse for CE of drugs in body fluids [180]. MEKC drug determinations in body fluids with direct sample injection are included in

Table 6. Typical chromatograms for the MEKC determination of aspoxicillin in human plasma are shown in Fig. 6, where acetaminophen was used as an I.S. [36]. The assay showed good linearity with a correlation coefficient $r = 0.999$ and covered the plasma levels typically encountered in clinical analysis (up to 300 μg/ml). The detection limit of aspoxicillin was about 1 μg/ml at $S/N = 3$. The recovery was almost 100%.

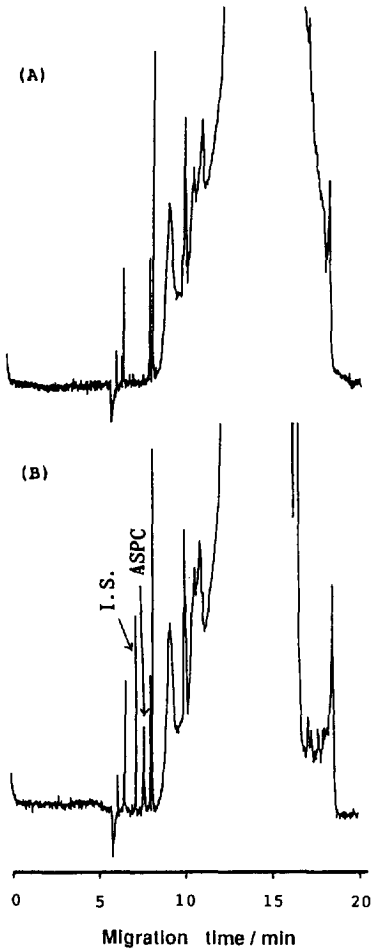


Fig. 6. Determination of aspoxicillin in human plasma by a direct sample injection method. (A) Blank plasma; (B) plasma spiked with aspoxicillin (ASPC) and internal standard (I.S.), acetaminophen. Buffer, 20 m*M* phosphate–borate buffer (pH 8.5) with 50 m*M* SDS. Other conditions as in Fig. 3. (From Ref. [36]).

4.4. Separation analysis

4.4.1. Complex mixtures such as amino acids and vitamins

MEKC is effective for the separation of complex mixtures. Amino acids and vitamin mixtures are two typical examples. These are used as injections or tablets for nutrition supply. Most amino acids are employed as DNS, phenylthiohydantoin (PTH) and OPA derivatives for UV detection, as mentioned previously. Chiral derivatization with 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate (GITC) [128] and L-Marfey's reagent [131] has been used for enantiomer separation of amino acids. Many papers have been published on the separation of amino acids by MEKC [85,181–201], as summarized in Table 7 (except for enantiomer separations of amino acid derivatives). A simultaneous separation of amino acid derivatives (ca. 20) has been achieved by MEKC through the optimization of various parameters. An example is shown in Fig. 7, where 23 DNS-amino acids were separated by MEKC with SDS [196]. On the other hand, water-soluble vitamins can be separated by using the CZE mode because these are mostly ionic compounds. However, a simultaneous separation by MEKC has been successful [192, 202–209]. For fat-soluble vitamins such as retinoic acid, CDs [207] or organic solvents [208] were added to the micelle solution. MEKC separations of vitamins are also summarized in Table 7.

4.4.2. Closely related peptides, drugs and isotopes

Peptides are ionic solutes and can be separated by CZE. The advantage of MEKC over CZE in the separation of peptides is the wide choice of selectivity manipulation. Many peptides are biologically active compounds and pharmaceuticals can be produced by derivatization or imitation of these compounds. For synthetic peptides, related peptides having similar electrophoretic mobilities are sometimes contaminated. MEKC separation will prove to be useful for these samples. A typical example is the separation of [Leu^{13}]motilin and [Met^{13}]motilin [210], in which

Table 7
Separation of amino acid derivatives and vitamins by MEKC

Analyte (Label)	Mixture[a]	Additives[b]	Ref.
Amino acids (AAs)			
o-Phthaldialdehyde (OPA) AAs	17/18	50 m*M* SDS 15% MeOH + 1% THF	[182]
o-Phthaldialdehyde (OPA) AAs	–	100 m*M* SDS	[190]
Dansyl (DNS) AAs	15/15	40 m*M* SDS	[192]
Dansyl (DNS) AAs	20/21	100 m*M* SDS	[183]
Dansyl (DNS) AAs	23/23	103 m*M* SDS	[196]
Dansyl (DNS) AAs	23/24	100 m*M* Tween 20	[199]
Dabsyl (DBS) AAs	16/18	10 m*M* SDS + 50% ACN	[184,185]
Dabth AAs	15/19	10 m*M* SDS + 40% ACN	[188]
Phenylthiohydantoin (PTH) AAs	22/22	50 m*M* SDS	[181]
Phenylthiohydantoin (PTH) AAs	21/22	50 m*M* CTAB	[181]
Phenylthiohydantoin (PTH) AAs	23/23	100 m*M* SDS + 4.3 M urea	[85]
Phenylthiohydantoin (PTH) AAs	18/–	35 m*M* SDS + 9% MeOH	[195]
Phenylthiohydantoin (PTH) AAs	18/–	33 m*M* SDS + 10% ACN	[194]
Phenylthiohydantoin (PTH) AAs	9/9	40 m*M* SDS + 2.5 m*M* CTAB	
Fluorescein isothiocyanate (FITC) AAs	–	100 m*M* SDS	[190]
Fouorescein isothiocyanate (FITC) AAs	17/21	75 m*M* *SDS*	[197]
3-(4-Carboxybenzoyl)-2-quinolinecarboxaldehyde (CBQCA) AAs	17/17	50 m*M* SDS	[189]
Naphthalene-2,3-dicarboxaldehyde (CBI) AAs	14/14	50 m*M* SDS + 10 m*M* β-CD	[193]
Dinitrophenyl fluoride AAs	17/17	48 m*M* SDS + 20 m*M* TBA	[200]
Tetramethylrhodamine thiocarbamyl AAs	13/20	10 m*M* SDS	[186]
9-Fluorenylmethyl chloroformate (FMOC) AAs	–	100 m*M* SDS	[190]
9-Fluorenylmethyl chloroformate (FMOC) AAs	18/18	25 m*M* SDS	[201]
N-2,4-Dinitrophenylhydrazone AAs	–	100 m*M* SDS	[191]
Dichlorotriazinylaminofluorescein AAs	19/21	50 m*M* SDS + 20 m*M* Brij + 4% IPA	[197]
Vitamins			
Water-soluble vitamins		50 m*M* SDS	[202,203,205]
Water-soluble vitamins		60 m*M* SDS + 15% MeOH	[204]
Water-soluble vitamin Bs		50 m*M* SDS	[206]
Water-soluble vitamin Bs		100 m*M* SDS + 13% ACN	[209]
B_6, B_6 vitamers		50 m*M* SDS	[172]
B_1 and B_{12}		50 m*M* SDS + 20 m*M* TAA	[84]
Water- and fat-soluble vitamins		30 m*M* SDS +1% IPA or γ-CD	[207]
Vitamins		40 m*M* SDS	[192]
Retinoic acids		25 m*M* SDS + 20% ACN	[208]

[a] Number of species separated/number of species in mixture.
[b] THF = tetrahydrofuran; ACN = acetonitrile; MeOH = methanol; TBA = tetrabutylammonium salt; IPA = 2-propanol; TAA = tetraalkylammonium salt. For others, see Tables 2 and 4.

only one neutral amino acid residue is different among 22 amino acid residues. The successful separation was achieved by MEKC through the addition of organic solvents [210] or MEKC with the non-ionic surfactant Tween 20 [206]. Enkephalins (five amino acid residues) and an-

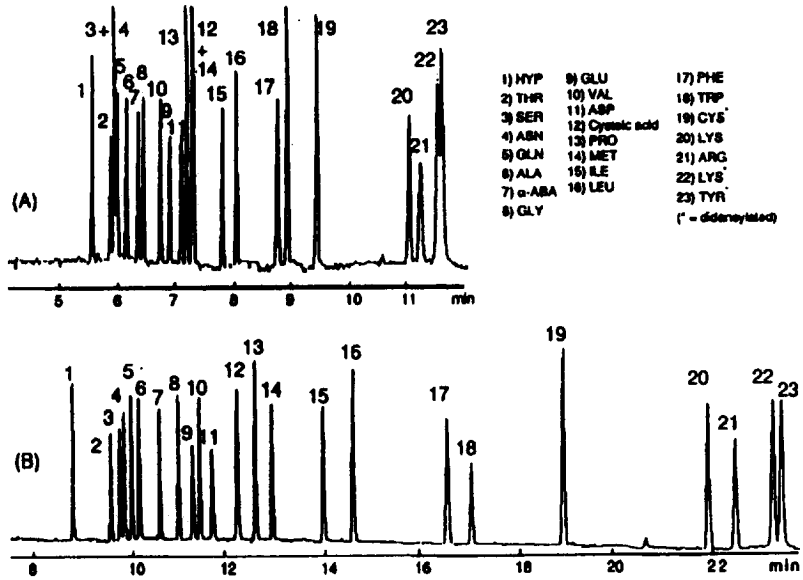


Fig. 7. Separation of 23 Dns-amino acids by MEKC with SDS. Buffer, 20 m*M* borax (pH 9.2) with (A) 100 m*M* SDS, 20°C and (B) 102.5 m*M* SDS, 10°C. Capillary, 50 μm I.D. × 57.5 cm (effective length, 50 cm); applied voltage, 25 kV; detection wavelength, 214 nm. (From Ref. [196]).

giotensins (seven to ten amino acid residues) were successfully separated by MEKC with SDS [212] or Tween 20 [211]. Insulins of different origins were successfully separated by MEKC with organic solvents, as shown in Fig. 8 [210]. It has been reported that MEKC gave the best selectivity among CZE, MEKC and reversed-phase HPLC for the separation of neurohypophyseal peptides and analogues [213].

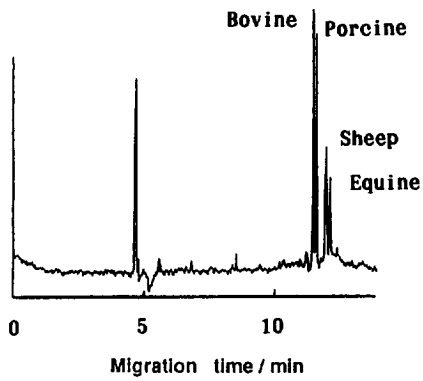


Fig. 8. Separation of insulins by MEKC and acetonitrile. Buffer, 50 m*M* borate buffer (pH 8.5) with 50 m*M* SDS and 15% acetonitrile. Capillary, 50 μm I.D. × 65 cm (effective length, 50 cm); applied voltage, 15–20 kV; detection wavelength, 215 nm; temperature, ambient. (From Ref. [210]).

Another example to demonstrate the high resolution capability of MEKC is the separation of isotopes. The separation of deuterated and non-deuterated benzene, benzoic acid, pyridine, benzyl alcohol, etc. was much improved by MEKC with SDS or Nonidet P-40 neutral micelles [214]. A coated capillary and a buffer close to each p*K* value or pH 7.2 were used. The separation of a mixture of closely related drugs, which are not used in practice for medical treatment, as model samples was investigated. Antibiotics [205,215], corticosteroids [153], barbiturates [212,216], xanthine derivatives [217] and benzodiazepines [218], most of which were not separated by CZE because of their similar electrophoretic mobilities or electrically neutral drugs, have been successfully separated by MEKC.

4.4.3. Natural products and Chinese crude drugs

Chinese crude drugs and Chinese drug preparations contain many biologically active components which are ionic and hydrophobic. In

order to estimate the quality of these samples, a simple and high-resolution method must be developed. For the separation of complex mixtures MEKC is effective as mentioned above. Applications of MEKC to natural products and Chinese crude drugs are summarized in Table 8. Flavonoids [7–13,219], cardiac glycosides [14], lignans [15] and lappaconitine [16] in plant extracts and illicit drugs [220–227] such as codeine and cocaine have been separated by MEKC with SDS or cetyltrimethylammonium bromide (CTAB). Determinations of glycyrrhizin in Chinese drug preparations [17,18], ginsenosides in Ginseng Radix [19] and sennoside A and B [20,21] in Chinese drug preparations were successful by MEKC with SDS or bile acids. Organic solvents such as acetonitrile or CDs were found to be effective for the separation of relatively hydrophobic or basic Chinese drugs. The separation of sennoside A and B, which have cathartic activity, and four other components by MEKC with sodium cholate and acetonitrile is shown in Fig. 9 [21].

4.4.4. Hydrophobic drugs such as steroids

Steroids and aromatic compounds are electrically neutral. Therefore, the MEKC mode must be applied for these analytes. However, most of

Table 8
Application of MEKC to Chinese crude drugs and illicit drugs

Analyte	Additives[a]	Ref.
Flavonoids (rutin, isoquercitrin, hyperosid, quercitrin, avicularin, etc.)	50 m*M* SDS	[7]
Flavonoids (kaempferol, quercetin, etc.)	35 m*M* SC + 4–10% PA	[12]
	20–60 m*M* CTAB	[12]
Flavonoids (baicalin, baicalein, wogonin, oroxylin A, glucoronides)	20 m*M* SDS	[13]
Flavonol-2-O-glycoside	60 m*M* SDS	[8]
Flavonol glycones	30 m*M* SDS	[9]
Flavanones, flavonols, flavones	50 m*M* SDS	[10]
Flavonol glycosides	50 m*M* SDS	[11]
Flavonoids (quercetin, morin, chrysin, rutin, hesperetin, naringenin, narignin)	42 m*M* SDS	[219]
Cardiac glycosides (lanatoside A, C,	25–50 m*M* SDS + γ-CD, urea	[14]
purpureaglycoside A	25 m*M* SC	[14]
glucodigifucoside, evatromonoside, digitoxin, etc.)		
Podophyllotoxin, kaempferol, quercetin	50 m*M* SDS + MeOH, DMF	[15]
Lappaconitine	50 m*M* SDS + 2–4% PEG	[16]
Glycyrrhizin, peoniflorin, ephedrine	100 m*M* SDS	[17]
Glycyrrhizin, peoniflorin, geniposide	25 m*M* SDS + 100 m*M* SC	[18]
Ginsenoside Rb_1, Rb_2, Rc, Rd, Re, Rf, Rg_1	75 m*M* SC + 25% ACN	[19]
Sennoside A	75 m*M* STC	[20]
Sennoside A, B, naringin, emodin, honokiol, magnolol	50 m*M* SC + 40% ACN	[21]
Illicit drugs (various)	75 m*M* SDS	[220]
Illicit drugs (various)	75 m*M* SDS	[221]
Illicit drugs (various)	85–100 m*M* SDS	[222]
Opium alkaloids (6 mixture)	12 m*M* SDS + 25 m*M* Tween 20	[223]
Amphetamines and related substances	25 m*M* CTAB + 11% DMSO + 1% EA	[224]
Cocaine and related substances	50 m*M* CTAB + 7.5% ACN	[225]
Heroin and its impurities	45 m*M* SDS + β-CD SBE + 10% ACN	[226]
Codeine and its by products	40 m*M* SDS	[227]

[a] PA = propanol; DMF = dimethylformamide; PEG = polyethylene glycol; MeOH = methanol; ACN = acetonitrile; EA = ethanolamine; β-CD SBE = sulfobutyl ether β-CD. For others, see Tables 2 and 4.

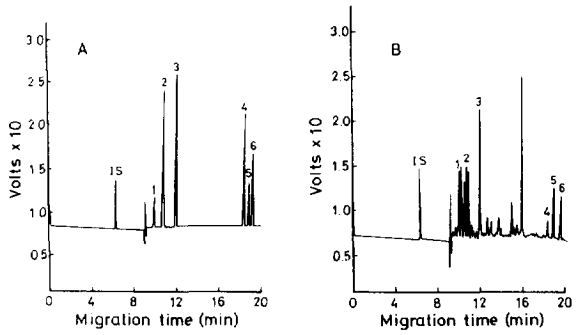


Fig. 9. Separation of (A) a mixture of six authentic standards and (B) extract of Hsiao-cheng-chi-tang. Peaks: IS = internal standard; 1 = naringin; 2 = honokiol; 3 = magnolol; 4 = sennoside B, 5 = emodin; 6 = sennoside A. Buffer, phosphate–borate buffer with 50 m*M* SC and 40% acetonitrile. Capillary, 75 μm I.D. × 80 cm (effective length, 72.5 cm); applied voltage, 25 kV; detection wavelength, 254 nm; temperature, 22.5–23.5°C. (From Ref. [21]).

these analytes are too hydrophobic to be separated by conventional MEKC with SDS. The CD-MEKC mode has been found to be effective for the separation of polycyclic aromatic hydrocarbons [30–32,46]. MEKC with bile salts or MEKC with SDS and organic solvents or urea were successful for the separation of corticosteroids [153,228] and steroid hormones [29, 229, 230]. Hydrophobic basic drugs have been separated by MEKC with cationic surfactants [88–90,96,163,165,223–225]. Separation of explosives was successful by MEKC with SDS [231,232]. Environmental application of CE have been summarized elsewhere [233].

4.4.5. Enantiomers

Direct enantiomer separations can be achieved by MEKC using various techniques. One approach is to use chiral micelles such as bile salts or SDVal as mentioned in Section 2.4.4. Some amino acid derivatives [112,113] and PTH-amino acids [114,115] were successfully enantioseparated by using SDVal. Enantiomeric separation of some DNS-amino acid derivatives [99], trimetoquinol hydrochloride [101,102], β-carboline derivatives [98], binaphthyl compounds [98,100], diltiazem hydrochloride [102], etc., was successful by using bile salts as chiral selectors. Among the bile salts employed, sodium taurodeoxycholate was the most effective for the enantiorecognition of the solutes. This may ascribe to the increased solubilizing capability due to the lack of a hydroxyl group at C-7 and the low pK_a value due to the sulfonate group in comparison with other bile salts having a carboxyl group. Non-ionic chiral surfactants such as digitonin [114], β-escin or glycyrrhizic acid [120] have also been successfully used for enantiomeric separations. In this case, these non-ionic surfactants were used together with an ionic surfactant such as SDS to form a mixed micelle having an electrophoretic mobility.

Besides employing chiral surfactants, enantiomer separations have been achieved by incorporating some chiral additives such as CDs (CD-MEKC) [46–49] and N,N-didecyl-L-alanine in the presence of copper(II) [234] in the SDS micelles. By CD-MEKC with SDS or bile salts, some DNS-amino acids [46], barbiturates [47], naphthalene-2,3-dicarboxaldehyde-labelled (CB-I) amino acids [48], cicletanine [49], etc., were enantioseparated.

When the direct enantiomer separation of an analyte by MEKC is not successful and the analyte has some reactive groups such as amino or carboxyl groups in a molecule, diastereomeric derivatization methods should be applied to the MEKC separation of the analyte [128–132], similarly to HPLC. The separation of DL-amino acids, which were derivatized with a chiral reagent such as GITC or Marfey's reagent as diastereomers, was successfully achieved by MEKC with an SDS solution alone.

The above-mentioned approaches are relatively simple compared with HPLC in which expensive chiral stationary phases are frequently used. Further, relatively expensive reagents can be used in MEKC because of its small volume requirement. More detailed reviews concerning enantiomer separations by CE including MEKC have been published elsewhere [22–25]. The development of versatile enantiomer recognition systems in MEKC or CZE will be one of the important areas of application.

4.5. Determination of some physico-chemical properties of drugs

4.5.1. Distribution coefficient and thermodynamic quantities in micellar solubilization

The capacity factor k' can be related to the distribution coefficient K of an analyte between the micellar and aqueous phases by

$$k' = K \cdot \frac{V_{mc}}{V_{aq}} \tag{5}$$

where V_{mc} and V_{aq} are the volumes of the micellar and aqueous phases. V_{mc} can be determined from the micellar concentration, c_{mc}, which is equal to the concentration of a surfactant, c_{sf}, minus the CMC, and partial specific volume of micelle, v; v can easily be determined (e.g., 0.87 ml/g for SDS) [235]. V_{aq} is equal to $1 - V_{mc}$. Thus, V_{mc}/V_{aq} is given

$$\frac{V_{mc}}{V_{aq}} = \frac{v(c_{sf} - \mathrm{CMC})}{1 - v(c_{sf} - \mathrm{CMC})} \tag{6}$$

When micellar concentrations are low, the denominator on the right-hand side of Eq. 6 may be approximated as equal to unity, i.e.,

$$k' = Kv(c_{sf} - \mathrm{CMC}) \tag{7}$$

The plots of the capacity factor vs. surfactant concentration have shown linear relationships for both neutral [2] and charged analytes [77,78] and the distribution coefficient has been obtained from the slopes of these plots. Terabe and coworkers [2,236] determined the distribution coefficients of analytes in SDS, sodium tetradecyl sulfate and sodium dodecyl sulfonate. The data indicated that the distribution coefficients of analytes partially soluble in water are more sensitive to changes in the polar moiety of a surfactant molecule than to that of the non-polar moiety and that the distribution coefficients of solutes that are almost insoluble in water are sensitive to the length of the alkyl chain.

Distribution coefficients depend on temperature according to the van't Hoff relationship:

$$\ln K = \frac{-\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} \tag{8}$$

where ΔH° is the standard enthalpy change associated with micellar solubilization, ΔS° is the corresponding entropy change, R is the gas constant and T is absolute temperature. ΔH° and ΔS° are determined from the plots of $\ln K$ vs. $1/T$. The dependence of selectivity on temperature can be evaluated with the ΔH° value. The enthalpy change decreased with increase in the alkyl chain length of phenols [237] and phthalate esters [238]. Analytes having longer alkyl chains are more advantageous with regard to enthalpy in the distribution of the micelle. These data will be helpful in understanding this phenomenon and MEKC may provide a useful technique to determine these thermodynamic qualities.

4.5.2. n-Octanol–water partition coefficient

Hydrophobicity of drugs is one of the important physico-chemical properties for evaluating the biological effects because a drug has to pass across various biomembranes, which have a lipoid nature. In the development of a new drug, logarithmic partition coefficients between *n*-octanol and water (P_{oct}) have usually been determined. Traditionally, $\log P_{oct}$ values are measured using the "shake-flask" method combined with UV or HPLC assay. Recently, MEKC was used to determine the $\log P_{oct}$ values as in HPLC [239–241]. Linear log–log relationships were found between both the micelle–water partition coefficient ($\log K$) in MEKC and *n*-octanol–water partition coefficient ($\log P_{oct}$) [242]. The capacity factor k' and the micelle–water partition coefficient K are proportional, as shown in Eq. 7. Therefore, these linear relationships can be expressed as

$$\log k' = a \log P_{oct} + b \tag{9}$$

where a and b are constants that can be determined from the observed data. Thus, the technique is performed by analysing a set of standards of known $\log P_{oct}$ under the given MEKC conditions. Log k' of each standard obtained is plotted against its $\log P_{oct}$ to form a

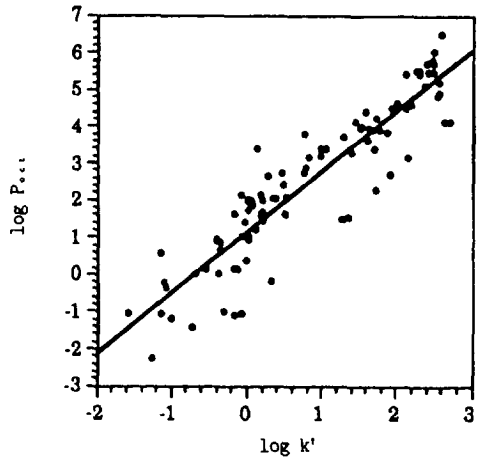


Fig. 10. Plot of log k' vs. log P_{oct}. The correlation is described by the line log $P_{oct} = 1.63 \pm 0.07$ log $k' \pm 1.14$, $r^2 = 0.853$, $n = 101$. (From Ref. [240]).

linear calibration graph. Then log P_{oct} of an unknown analyte is calculated from the obtained capacity factor and the calibration graph. An example is shown in Fig. 10, where over 100 analytes with widely varying functionality were investigated. Recently, Ishihama et al. [243] employed microemulsion EKC for the determination of the hydrophobicity of analytes and compared the results with those obtained by MEKC with SDS.

5. Conclusions

In addition to the separation capability of electrically neutral or non-ionic analytes, MEKC has many attractive advantages over CZE or HPLC as an analytical method for pharmaceuticals. One-run separation of every kind of drug, including cationic, neutral and anionic, is possible within a relatively short time. MEKC is especially powerful for the separation of complex mixtures such as natural products and crude drugs because of its high resolution. Direct enantiomer separation also can be successful using chiral selectors such as chiral surfactants or chiral additives. The enhancement of selectivity can be easily manipulated through the various techniques. The direct injection method for biological samples such as serum can be usable in MEKC. Physico-chemical properties can also be determined by MEKC. MEKC seems to be a very promising method for drug analysis. However, for much wider use it is still desirable for the precision in quantitative analysis and sensitivity in qualitative analysis to be improved to be comparable to those in HPLC.

6. Abbreviations

AEKC	Affinity electrokinetic chromatography
CD	Cyclodextrin
CE	Capillary electrophoresis
C_{me}	Micellar concentration
CMC	Critical micellar concentration
CZE	Capillary zone electrophoresis
DNS	Dansylated
EKC	Electrokinetic chromatography
EOF	Electroosmotic flow
GITC	2,3,4,6-Tetra-O-acetyl-β-D-glycopyranosyl isothiocyanate
MEKC	Micellar electrokinetic chromatography
N	Theoretical plate number
OPA	o-Phthaldialdehyde
PA	Pyridilamine derivatives
PMP	1-Phenyl-3-methyl-5-pyrazolone derivatives
PTH	Phenylthiohydantoin
P_{oct}	Partition coefficient between n-octanol and water
t_{mc}	Migration time of micelle
t_0	Migration time of the electroosmotic flow
t_R	Migration time of solute
v	Specific volume of micelle
V_{aq}, V_{mc}	Volumes of micellar and aqueous phases
α	Separation factor
μ	Electrophoretic mobility of the analyte
μ_{ep}	Effective electrophoretic mobility

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