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Application of electrokinetic chromatography to pharmaceutical analysis*

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Abstract: Electrokinetic chromatography (EKC) is one branch of capillary electrophoresis that permits the separation of electrically neutral solutes by the electrophoretic technique. The separation principle of EKC is based on that of chromatography, and various modes of EKC have been developed along with the partition mechanism. Micellar electrokinetic chromatography (MEKC), in which an ionic surfactant solution is employed instead of a buffer solution in capillary zone electrophoresis (CZE) at a higher concentration than the critical micelle concentration (CMC), has become the most popular technique among various EKC modes. Besides the separation of the electrically neutral or non-ionic solutes, MEKC is also effective for the separation of ionic solutes as well as CZE, hence it suits the analysis of pharmaceuticals, including cationic, anionic and neutral. Separation selectivity in MEKC can be manipulated easily through the modification of the buffer as well as changing the surfactants. Direct enantiomeric separation is successful by using chiral surfactants or chiral additives. Determination of drugs in plasma is also successfully achieved by a direct sample injection method, similar to micellar HPLC. In this paper, we summarize the principle separation characteristics of MEKC and some applications to pharmaceutical analysis, including direct enantiomeric separation and direct assay of drugs in plasma.

Keywords: *Micellar electrokinetic chromatography; selectivity manipulation; direct injection method; chiral separation.*

Introduction

Pharmaceuticals are required to be of high quality to ensure biological activity. Many testing items, especially purity testing and assay of active ingredients, are necessary and important for quality control. The assay of drugs in body fluids is also necessary to clarify absorption, distribution, etc. For these purposes, high-performance liquid chromatography (HPLC) has usually been used.

Capillary zone electrophoresis (CZE), first developed by Jorgenson *et al.* in the early 1980s [1, 2], is one of the rapidly developing separation techniques that has a high resolving power and separation speed. However, only ionic or charged solutes can be separated by this method, because its separation principle is based on the difference in electrophoretic mobilities. By introducing the chromatographic principle to CZE, electrically neutral or uncharged solutes can be separated by the CZE technique. This method is called electrokinetic chromatography (EKC) [3, 4] from its separation principle. EKC is named after electrokinetic phenomena, which include electrophoresis and electroosmosis, and chromatography.

The charged molecules or charged molecular aggregates are employed in EKC as a separation carrier or a pseudo-stationary phase, which corresponds to the stationary phase in conventional chromatography from the viewpoint of separation mechanism. The separation carrier is transported by electrophoresis at a different velocity from the surrounding buffer solution. The solute distributes itself between the carrier and the surrounding medium. The most popular mode of EKC is micellar electrokinetic chromatography (MEKC) [5, 6], in which an ionic surfactant solution is employed instead of a buffer solution in CZE at a concentration higher than the critical micelle concentration (CMC), although various modes of EKC have been developed according to the type of interaction of the separation carrier [7].

Other than changing the surfactant, selectivity is manipulated easily through the modifi-

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cation of the buffer solutions in MEKC, similar to HPLC, without losing CZE's high resolution capability. Attractive characteristics of MEKC, such as high resolving power, ease of selectivity manipulation, relatively rapid analysis, small sample amounts or reagents requirement, etc., make the technique a strong competitor or a useful complement to HPLC. The analysis of pharmaceuticals can be one of the largest areas of the application of MEKC.

In the present paper, the separation principle and selectivity manipulation in MEKC is briefly described. Examples of MEKC applications to the purity testing and assay of pharmaceuticals are shown and some successful enantiomeric separation of drugs and a direct plasma injection method are also reported.

MEKC Separation

Apparatus and separation principle of MEKC

Instrumentation of MEKC is the same as that of CZE. The basic system of MEKC is relatively simple and can be assembled at low cost. However, from moderate to very expensive commercial systems are recently available. Typical separation of MEKC is performed in a 0.05 mm i.d. fused-silica capillary tube, with a high-voltage dc power supply delivering up to about 30 kV. Optical detection methods, especially UV absorbance because of its universal character are used by the on-column method. Migration time, peak area and peak height are measured with a data processor.

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 groups orient toward the centre and the charged head groups toward the outer surface. The formation of the micelle permits the separation of electrically neutral or non-ionic solutes by the CZE technique.

A schematic representation of the separation principle of MEKC is shown in Fig. 1. The system is composed of two phases. One is the micellar phase and the other is the surrounding aqueous phase, which corresponds to the mobile phase in HPLC. When an anionic surfactant is employed, the micelle migrates toward the positive electrode by the electrophoresis. However, the strong electroosmotic flow (EOF), which has a flat velocity profile,

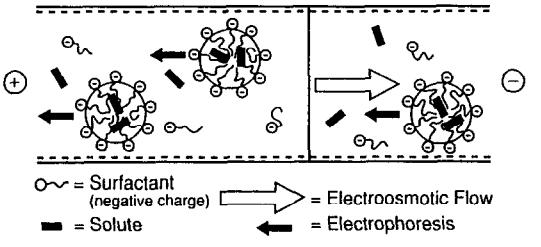

Figure 1
A schematic representation of the separation principle of MEKC. The detector is assumed to be positioned near the negative electrode.

transports the buffer solution toward the negative electrode due to the negative charge on the surface of the fused-silica capillary. The velocity of EOF is usually faster than that of the electrophoretic migration of the micelle under the neutral or alkaline conditions, resulting in a fast-moving aqueous phase and a slow-moving micellar phase.

When a non-ionic solute is injected to the solution, a fraction of it is incorporated into the micelle and it migrates at the same velocity as that of the micelle. The remaining fraction migrates at the velocity of EOF. That is, electrically neutral solute can be separated by the difference in the distribution coefficients between the micellar phase and the surrounding aqueous phase. MEKC can provide enhanced selectivity for the separation of ionic solutes as well as the separation of electrically neutral or non-ionic solutes through the ionic interaction between the solute and the micelle.

The migration velocity in MEKC generally depends on the hydrophobicity of the solute. More hydrophobic solutes interact more strongly with the micelle and migrate more slowly than the hydrophilic solutes. A schematic of the zone separation in MEKC and the corresponding chromatogram are shown in Fig. 2. All solutes injected at the positive end

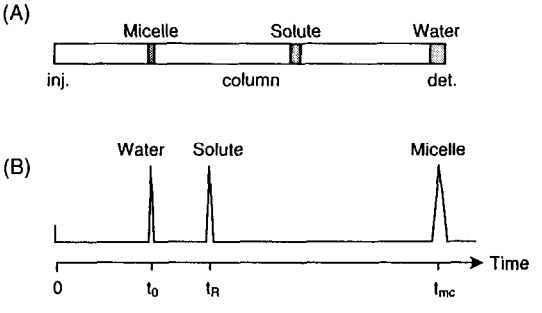

Figure 2
A schematic of separation in MEKC. (A) Zone separation, (B) the corresponding chromatogram. (Reprinted with permission from *Anal. Chem.* [4], copyright 1985 American Chemical Society.)

of the capillary tube move toward the negative end, and must migrate between the migration of the bulk solution and that of the micelle, provided they are electrically neutral. In other words, the migration time of the solute (t_R) is limited between the migration time of EOF (t_0) and that of the micelle (t_{mc}). In MEKC, the capacity factor k' of the solute can be calculated by [4]

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

$$k' = \frac{t_R - t_0}{t_0(1 - t_R/t_{mc})} . \quad (2)$$

As t_{mc} becomes infinite (micellar phase becomes stationary), equations (1) and (2) reduce to the analogous equations for conventional chromatography. To evaluate k' values in MEKC, the parameters 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, Sudan IV, or hydrophobic cations such as timepidium [8], halofantrine [9], which are assumed to be totally solubilized into the micelle.

Resolution and selectivity manipulation in MEKC

Resolution R_s in MECK is given by [4]

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

where N is the theoretical plate number and α is the separation factor given by k'_2/k'_1. The last term of the equation 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 to that of conventional HPLC.

The equation predicts the effect of N, α, k' and t_0/t_{mc} on R_s. Resolution R_s increases in proportion to the square root of N. In MEKC and CZE, the higher the applied voltage, the higher N, unless the temperature increase is too high. Usually a high voltage (10 ~ 30 kV) can be used to perform MEKC separation. About 250,000 theoretical plate numbers can be obtained from a 0.05 mm i.d. and 500 mm length capillary tube within a relatively short time, although those of a conventional HPLC analytical column exhibits *ca* 10000.

The capacity factor k' is more important in MEKC because R_s depends significantly on its value. One can easily realize that the maximum R_s will be obtained when k' approaches the value $-t_{mc}/t_0$ [10], or when $k' = (t_{mc}/t_0)^{1/2}$ [11]. Since the capacity factor k' is proportional to the phase ratio of the volume of the micelle to that of the aqueous phase, 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 the migration time window, hence the higher R_s. The value of t_0/t_{mc} is in the range of 0.2–0.4 for the most ionic micelles under the neutral or alkaline conditions. It is necessary to reduce the velocity of EOF to obtain smaller value of t_0/t_{mc}. One of the possible ways is the addition of an organic solvent such as methanol, acetonitrile or 2-propanol. Changing pH to acidic, is also a possible way. However, in practice, a longer run time is required.

The separation factor, α, is the most important and most effective term to increase R_s, and it is a measure of selectivity. The selectivity manipulation has been described elsewhere [12]. Therefore, a general strategy to improve selectivity is briefly given in this paper. The type of surfactant significantly affects the α value. As an example, the use of cationic surfactants such as dodecyltrimethylammonium bromide results in a different selectivity for the separation of non-ionic solutes as well as ionic solutes [13]. The structure of surfactant also affects the α value. Bile salt micelles, whose structures are quite different from those of the usual long-chain alkyl type surfactants, have unique characteristics and the selectivity is often improved when separation is not successful in MEKC using sodium dodecyl sulphate (SDS).

Selectivity (α) is easily manipulated through the modification of the buffer solution, similarly to HPLC. The addition of tetraalkylammonium salts to the SDS solution was remarkably effective for the separation of ionic solutes [8]. The addition of a high concen-

tration of urea to the SDS solution improved the separation of hydrophobic solutes, which were not resolved with SDS [14]. The addition of cyclodextrins (CDs) to the SDS solution was effective for the separation of highly hydrophobic solutes, which were not separated with an SDS solution alone [15, 16]. Direct enantiomeric separation can be also successful by the addition of CDs to the SDS solution as reported below. This useful separation mode, where CDs are added to conventional MEKC solutions, is called CD–MEKC.

The addition of organic solvents also contributed to the improvement of resolution due to the expansion of the migration time window and alteration of selectivity [17, 18]. A successful application of adding an organic solvent to the micellar solution was reported in the separation of closely related large peptides such as insulin [19]. An example of separation of closely related insulins in MEKC, where 15% acetonitrile and 50 mM SDS is employed, is shown in Fig. 3.

Of course, the migration time can be altered by changing buffer constituents, ionic strength, pH, in addition to applied voltage (current), capillary length, inside diameter or inside surface characteristic, and temperature. These parameters have profound effects on the velocity of EOF as in the usual CZE [1, 2].

In general, we can state the characteristic feature of MEKC separation as follows: (1) high resolution capability and rapid analysis; (2) separation capability of electrically neutral or non-ionic solutes; (3) easy manipulation of separation selectivity; (4) small sample volumes or reagents requirement.

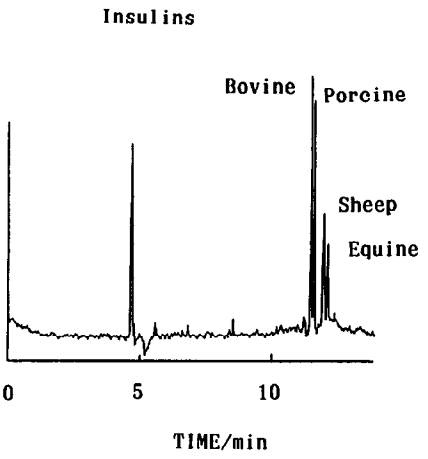

Figure 3
Separation of insulins by MEKC with acetonitrile. Conditions: buffer, 50 mM borate buffer (pH 8.5) with 50 mM SDS and 15% acetonitrile; capillary, 50 μm × 65 cm (effective length, 50 cm); applied voltage, 20 kV; detection, ~210–220 nm; temperature, ambient. (Reprinted with permission from *Anal. Chem.* [19], copyright 1992 American Chemical Society.)

MEKC Application to Pharmaceutical Analysis

Quantitation by MEKC

One of the important factors in the testing method of pharmaceuticals is its quantitative precision. In MEKC (or CZE), precision depends largely on the reproducibility of sample injection. Either the hydrodynamic injection method (siphoning, positive pressure and vacuum) or the electrokinetic injection method is usable, and both are available in commercial instruments. It is not practical to use an in-line injection loop as in HPLC. The reproducibility in both injection systems is satisfactory when injection times are long. However, the electrokinetic method should be avoided for the purity testing of drugs, because there must be a possibility of contamination by electrolytic products and injected amount depends on the electrophoretic mobility (charge). Minor components having slow migration velocities are difficult to detect when the electrokinetic injection is employed. Despite this, in certain cases such as capillary gel electrophoresis, electrokinetic injection must be selected. Preparation of sample solutions of relatively high concentrations, in comparison with HPLC analysis, is needed in MEKC purity testing, to detect less than 0.1% related substances, because of relatively low concentration sensitivity of capillary electrophoresis instruments. Development of high sensitivity detection system is desirable. In assay of drugs by MEKC, it is recommended to use an internal standard (I.S.), that is, I.S. method as in HPLC, to neglect the variance in sample loading.

Besides reproducible sample injection, it is also necessary to control the EOF velocity to obtain reproducible migration times, leading to good analytical precision. However, it is difficult to control the velocity of EOF exactly, because it depends on the condition of the surface of the capillary wall. In practice, capillary washing and capillary temperature control are effective. Some reproducibility data of migration times using a home-made apparatus are summarized in Table 1 [20]. The relative standard deviation was less than 1%

Table 1
Reproducibility (relative standard deviation) of migration times*

Date	Repetition	Acetaminophen (%)	Caffeine (%)	I.S. (%)	Ethenzamide (%)
16 June	6	0.55	0.46	0.64	0.76
17 June	6	0.59	0.69	0.67	0.68
20 June	7	0.78	0.85	0.77	0.88
23 June	5	0.69	0.76	0.89	0.76
Total 4 days		1.77	2.07	2.66	2.72

*Buffer, 20 mM phosphate–borate buffer (pH 8.0) with 100 mM SDS.
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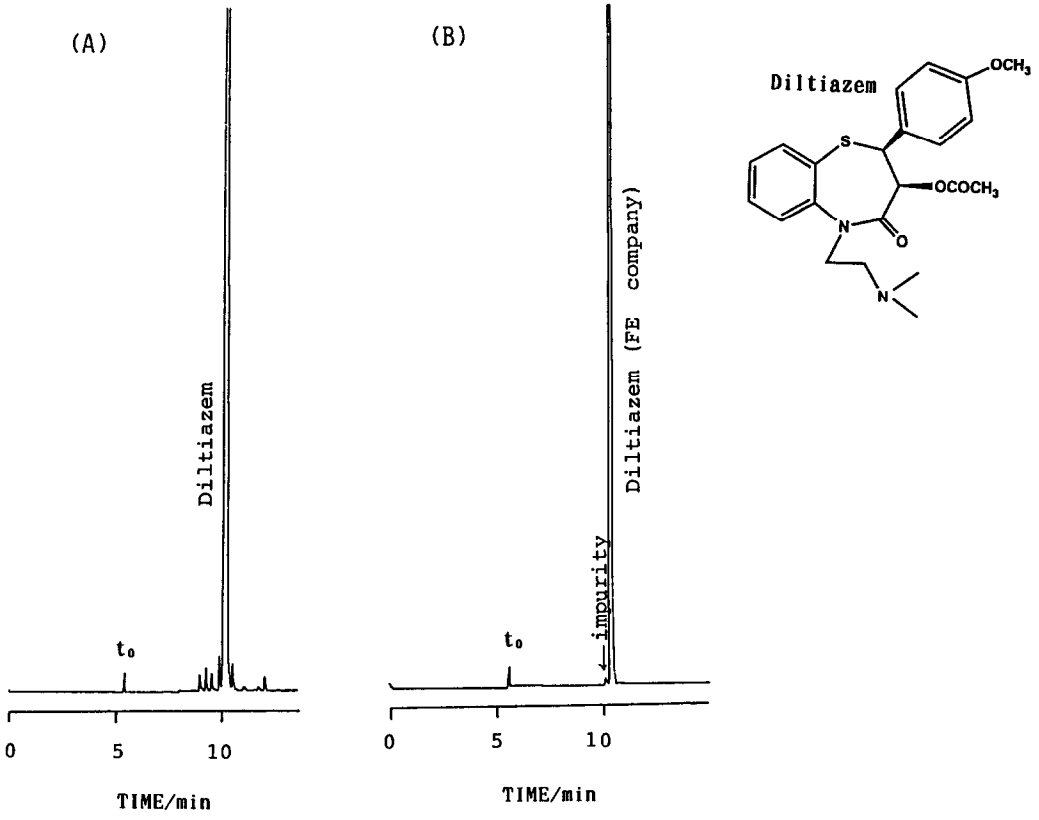

Figure 4
Chromatograms in the purity testing of diltiazem drug substances by MEKC. (A) Authentic diltiazem hydrochloride spiked ~0.3–0.5% of each related substance, (B) diltiazem hydrochloride supplied by FE company. Buffer, 20 mM phosphate–borate buffer (pH 8.0) with 100 mM sodium cholate. Other conditions are the same as in Fig. 3. (Reprinted with permission from *J. Chromatogr.* [21].)

from run-to-run and less than 3% from day-to-day without temperature control. MEKC in constant current mode may improve the reproducibility of the migration times, rather than constant voltage mode because the EOF velocity and electrophoretic mobility is linearly proportional to current irrespective of temperature as long as an identical buffer is used. It is also essential to use an integrator with a fast sampling rate.

Purity testing and assay of pharmaceuticals

The purity determination of drug substances and those in pharmaceutical formulations can be performed by the area per cent method, similar to HPLC. In MEKC (or CZE), it is relatively easy to detect a minor component, which elutes just after the major peak. In HPLC analysis, a minor peak will be sometimes included in the major peak in such cases. Chromatograms of diltiazem standard spiked

ca 0.3–0.5% of each related substance and diltiazem drug substance obtained from FE company are shown in Fig. 4. Impurity (deacetyl diltiazem) of 0.27% was detected from FE company as shown in Fig. 4(B). Detection limit was *ca* 0.1% at signal-to-noise ratio (*S/N*) = 3. The results in the purity determination of diltiazem drug substance and its tablets from several sources are summarized in Table 2 [21]. The values obtained by MEKC agreed well with those by HPLC.

One of the recent demands on HPLC purity testing is the analysis of enantiomeric purity. Chiral separation is successful by MEKC as described below in detail. An example of enantiomeric purity determination of trimetoquinol hydrochloride (*S*-form), which is a broncodilator, is shown in Fig. 5, where sodium taurodeoxycholate solution of pH 7.0 was employed [22]. About 1% minor enantiomer (*R*-form) of the major enantiomer (*S*-form) could be directly detectable at *S/N* = 3.

MEKC assay of active ingredients in pharmaceutical formulations have all been performed using an IS method [20, 21, 23]. Typical chromagrams of the standard and sample solutions in the assay of diltiazem tablets by MEKC using bile salts are shown in Fig. 6 [21]. The assay results were almost 100% with the relative standard deviation of *ca* 1–3% without temperature control. The simultaneous determination of several active ingredients in a cold medicine was also successfully achieved [20, 24]. It is difficult to determine these solutes simultaneously by conventional reversed-phase HPLC in the isocratic mode because the polarity and hydrophobicity of the solutes are widely scattered. However, these solutes, 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 of pharmaceuticals. These results are acceptable for assay and purity testing of pharmaceuticals. Therefore, MEKC can be used as a complementary or alternative method to HPLC.

Table 2
Purity testing of diltiazem hydrochloride drug substances and those in tablets*

Source	Detected impurity† (%)
TA-diltiazem drug substance	Not detected‡
FE-diltiazem drug substance	0.22
FA-diltiazem drug substance	0.27
TA-tablet	Not detected
TO-tablet	1.46

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† Area percentage method.
‡ Detection limit *ca* 0.1%.

Clinical analysis by a direct injection method

In a clinical analysis, solutes at low concen-

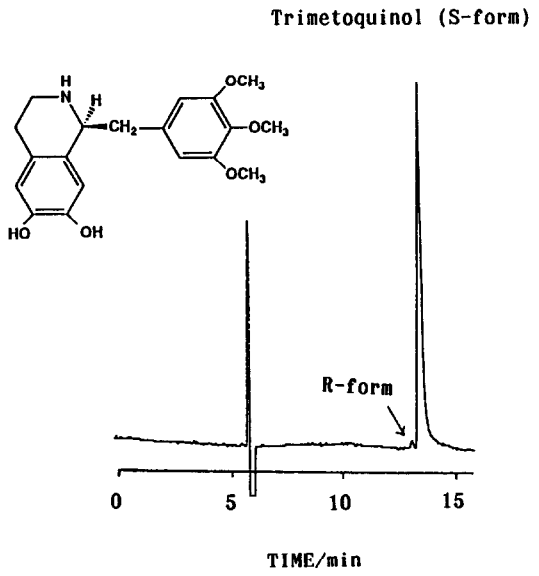

Figure 5
Optical purity testing of trimetoquinol hydrochloride by MEKC. Authentic *S*-trimetoquinol was spiked with *ca* 1% *R*-enantiomer. Buffer, 20 mM phosphate–borate buffer (pH 7.0) with 50 mM sodium taurodeoxycholate. Other conditions are the same as in Fig. 3.

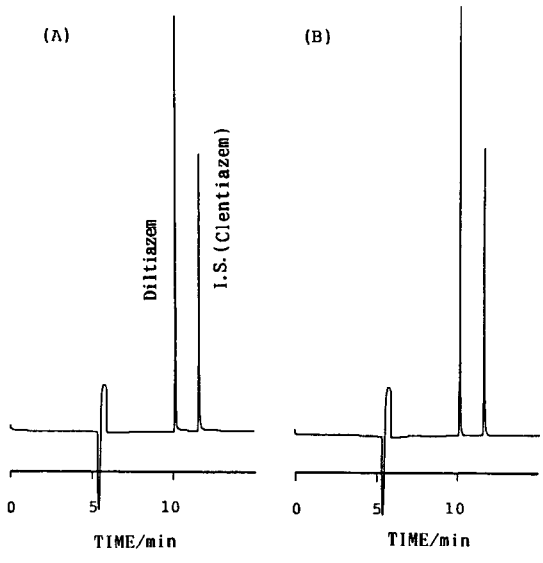

Figure 6
Chromatograms in the assay of diltiazem tablets by MEKC. (A) Standard solution and (B) sample solution. Clentiazem (8-chloro-derivative of diltiazem) was used as I.S. Conditions are the same as in Fig. 4. (Reprinted with permission from *J. Chromatogr.* [21].)

tration in a complex matrix such as plasma and urine must be analysed. Therefore, some sample pre-treatment methods, i.e. deproteination, extraction and concentration are often necessary to obtain good results in CZE or MEKC, as in HPLC analysis. Especially, protein adsorption on the capillary wall should be avoided to keep reproducible migration times of the solute in CZE. However, a direct injection method is successful in MEKC [25–28], similar to micellar HPLC. Without a surfactant, i.e. in CZE mode, plasma protein peaks interfered with the peaks of the solute and protein adsorption occurred, causing a change of the velocity of EOF. Rinsing of the capillary with alkaline solutions is required to recover reproducible migration times after injection of plasma samples in CZE mode. With a surfactant, the migration times of the protein peaks shifted longer, and successful separation between the solute peak and the protein peaks was achieved. The surfactant also breaks the drug–protein complexes, hence, 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.

The assay of the cephalosporin antibiotic cefpiramide in human plasma [25], the penicillin antibiotic aspoxicillin in human plasma [26], creatinine and uric acid in human plasma [27] and barbiturates in human serum and urine [28] was performed successfully by the direct injection method. It should be noted, however, that there is a time window which is free of interferences between the migration time of EOF and that of the solubilized plasma

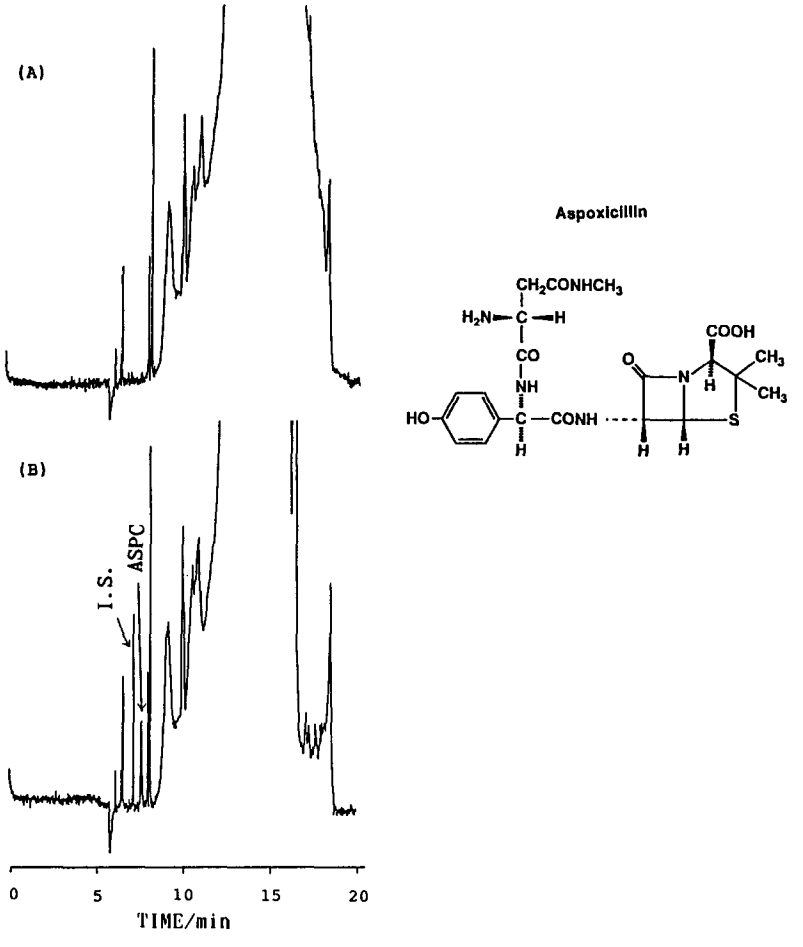

Figure 7
Chromatograms in the assay of penicillin antibiotic aspoxicillin in human plasma by a direct sample injection method. (A) Blank plasma, (B) plasma spiked with aspoxicillin (ASPC) and internal standard (I.S.), acetaminophen. Buffer, 20 mM phosphate–borate buffer (pH 8.5) with 50 mM SDS. Other conditions are the same as in Fig. 3. (Reprinted with permission from *J. Chromatogr.* [26].)

proteins, which migrated slowly. Typical chromatograms in MEKC determination of aspoxicillin in human plasma are shown in Fig. 7, where acetaminophen was used as an internal standard substance [26]. 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^{-1}). The detection limit of aspoxicillin was about 1 μg ml^{-1} at S/N of 3. The recovery was almost 100% as shown in Table 3, indicating that total amount of bound and unbound drugs could be determined.

Chiral separation

Direct chiral separation can be achieved by MEKC using various techniques. One approach is using a chiral micelle, that is, employing a chiral surfactant such as bile salts or sodium *N*-dodecanoyl-L-valinate. Enantiomeric separation of some dansylated DL-amino acids [29], trimetoquinol hydrocholoride [22], tetrahydro-β-carboline derivatives [30], binaphthyl compounds [30, 31] and diltiazem hydrochloride [32] was successful by using bile salts as a chiral selector. Some DL-amino acid derivatives [33] and phenylthiohydantoin DL-amino acids [34] were successfully optically resolved by using sodium *N*-dodecanoyl-L-valinate. A typical chromatogram of enantiomeric separation of diltiazem and its chloro-derivatives is shown in Fig. 8. Among the bile salts employed, sodium taurodeoxycholate was the most effective for the chiral separation of the enantiomeric solutes and has two advantages: one is the increased solubilizing capability by the lack of the hydroxyl group at C-7 position, and the other is a low pK_a value due to the sulphonate group in comparison with other bile salts having a carboxyl group. Nonionic chiral surfactants such as digitonin [34], β-escin or glycyrrhizic acid [35] was also successfully used for enantiomeric separation by MEKC. The nonionic surfactants must be used together with an ionic surfactant such as SDS to form a mixed micelle having an electrophoretic mobility.

Other than employing chiral surfactants, chiral separation was achieved by using some

Table 3
Recovery test of aspoxicillin

ASPC added to plasma (μg ml^{-1})	Repetition	Recovery (%)
50	6	94.3
100	5	103.9
150	6	100.1
200	5	103.4
250	5	97.7

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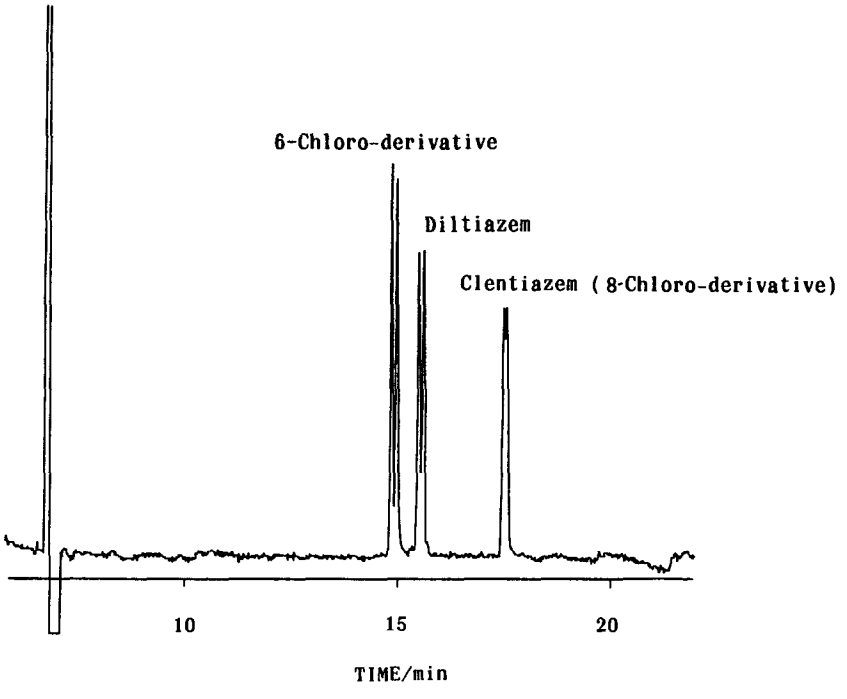

Figure 8
Direct separation of enantiomers of diltiazem and its chloro-derivatives by MEKC. Conditions are the same as in Fig. 5.

chiral additives to the SDS micelle such as *N*,*N*-didecyl-L-alanine in the presence of copper (II) [36]. The addition of CDs to micellar solutions (CD–MEKC), as well as the addition of CDs to the CZE buffer solutions [37–40], is a useful method for the chiral separation. The SDS micelle may be conveniently used for this approach. By adding CDs to the SDS solutions, some dansylated DL-amino acids [41], barbiturates [42], naphthalene-2,3-dicarboxaldehyde-labelled DL-amino acids [43] and cicletanine [44] were optically resolved. A chromatogram of enantiomeric separation of thiopental and pentobarbital is shown in Fig. 9. The separation solution was a 20 mM phosphate–borate buffer (pH 9.0) containing 50 mM SDS, 30 mM γ-CD and 20 mM sodium *d*-camphor-10-sulphonate. In this case, the addition of sodium *d*-camphor-10-sulphonate to the SDS solution containing CD improved the enantioselectivity. Among various CDs or CD derivatives tested in CD–

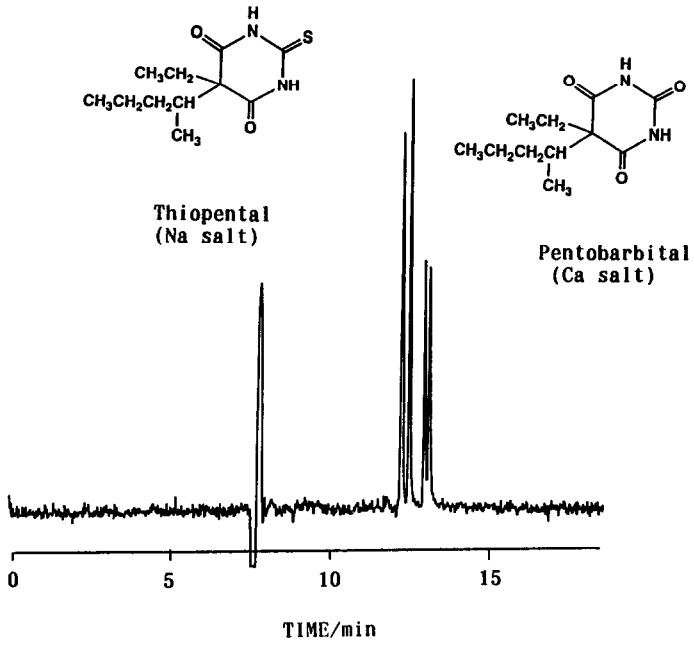

Figure 9
Direct chiral separation of thiopental and pentobarbital by CD–MEKC. Buffer, 20 mM phosphate–borate buffer (pH 9.0) with 50 mM SDS, 30 mM γ-CD and 20 mM *d*-camphore-10-sulphonate. Other conditions are the same as in Fig. 3.

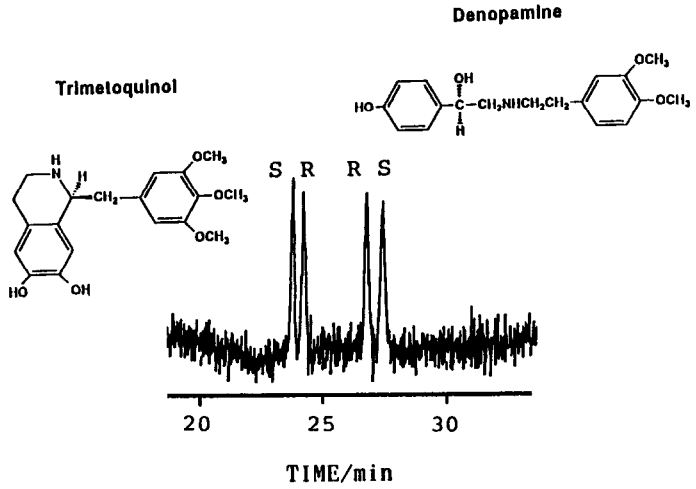

Figure 10
Direct separation of enantiomers of trimetoquinol and denopamine by CD modified CZE. Buffer, 25 mM phosphate buffer (pH 2.2) with 2 M urea and 20 mM 2,6-di-*O*-methyl-β-CD. Capillary length, 55 cm (effective length, 40 cm). Other conditions are the same as in Fig. 3.

MEKC, γ-CD was most successful for chiral recognition [41–44], although β-CD or its analogues such as 2,6-di-*O*-methyl-β-CD were more effective in the chiral recognition by CD modified CZE [38, 45]. An example of enantiomeric separation of trimetoquinol hydrochloride and denopamine by CD modified CZE is shown in Fig. 10 using a 25 mM phosphate buffer (pH 2.2) containing 2 M urea and 20 mM 2,6-di-*O*-methyl-β-CD [45]. The difference in CD which shows the best enantioselectivity between CD–MEKC and CD modified CZE will probably be interpreted in terms of the presence of a surfactant monomer [42].

When a direct chiral separation of a solute by MEKC or CZE mentioned above is not successful and the solute has some reactive groups such as amino or carboxyl groups in a molecule, a diastereomeric derivatization method should be applied to the MEKC separation of the solute, similar to HPLC. Separation of DL-amino acids, which were derivatized with a chiral reagent such as 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate (GITC) [46] or Marfey's reagent [47] as diastereomers, was successfully achieved by MEKC with an SDS solution alone. An example of chromatogram of GITC derivatized DL-amino acids is shown in Fig. 11.

Besides MEKC, chiral separation of dansylated DL-amino acids was achieved by employing cyclodextrin derivatives, which have both chirality and an ionic group in a molecule, as a separation carrier [7]. A chiral copper (II)-L-histidine [48] and a chiral copper (II)-aspartame [49] complex were also successfully used in the chiral separation of dansylated DL-amino acids. Recently, chiral separation was achieved by employing oligosaccharides [50], bovine serum albumin [51] or crown ether [52] to the CZE buffers.

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 without hesitation because of small required volumes in miniaturized techniques like MEKC and CZE. Development of versatile chiral recognition systems in MEKC or CZE will be one of the important areas of application [53].

Conclusions

Besides the separating capability of electri-

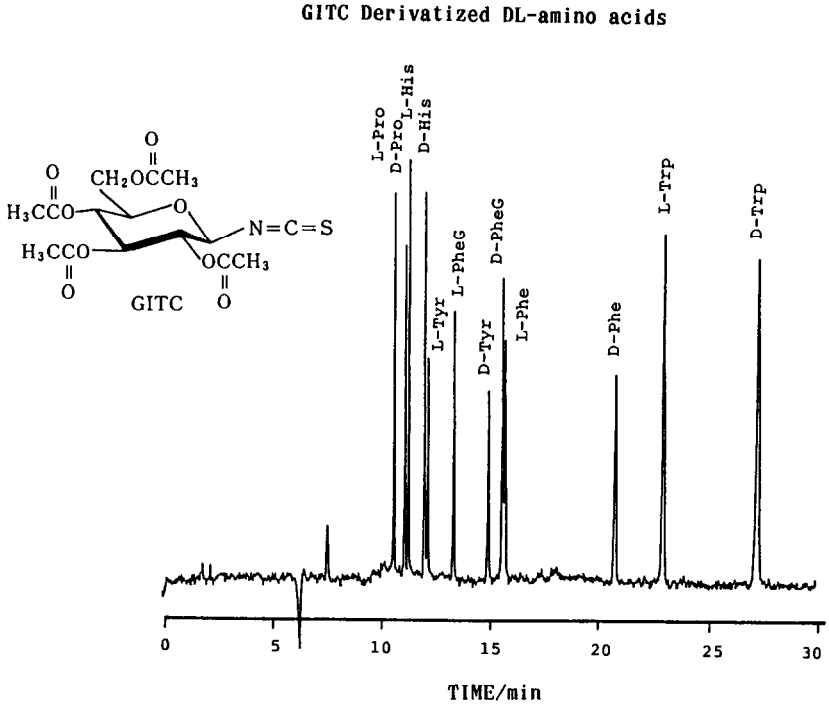

Figure 11
MEKC separation of GITC derivatized DL-amino acids. Buffer, 20 mM phosphate–borate buffer (pH 9.0) with 200 mM SDS. Other conditions are the same as in Fig. 3.

cally neutral or non-ionic solutes, MEKC has many attractive advantages over CZE or HPLC as an analytical method of pharmaceuticals. One-run separation of every kinds of solutes, including cationic, neutral and anionic, is possible in a relatively short time. Direct chiral separation can also be successful by employing chiral selectors such as chiral surfactants or chiral additives to the micellar solutions. The enhancement of selectivity can be easily manipulated through the various techniques. A direct injection method can be applicable in MEKC. However, in the quantitative aspects, there still remains problems to be solved: more precise injection techniques for minute amount volumes and more sensitive detection systems should be developed [54, 55].

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