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Review

Separation of enantiomers by capillary electrophoretic techniques

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ABSTRACT

This review surveys the separation of enantiomers by capillary electrophoretic (CE) techniques for those who are not highly acquainted with CE techniques. The CE techniques described are capillary zone electrophoresis, electrokinetic chromatography, isotachophoresis, capillary gel electrophoresis and electrochromatography. The fundamental separation theory of each CE technique is discussed from the viewpoint of the optimization of selectivity and resolution. Enantiomeric separations by the CE techniques are mostly based on complex formation between the analytes and the chiral selectors added to the separation system, and therefore the chiral additives and their effects are discussed, including interaction models. The indirect separation of enantiomers by the prederivatization to diastereomers is also briefly discussed.

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1. INTRODUCTION

Electrophoretic techniques are rapidly becoming popular among separation analytical chemists since the advent of capillary electrophoresis (CE) [1–3] as an instrumental analytical technique. Conventional electrophoresis has almost exclusively been employed for the separation of biomolecules and has hardly been utilized for the separation of small molecules. Although the greatest interest of CE still centres around the separation of biopolymers, CE is also an attractive and powerful analytical separation technique even for small molecules because of (1) high separation efficiency; (2) extremely small injection volumes of the sample; (3) separation selectivity complementary to high-performance liquid chromatography (HPLC); (4) fully automated instrumentation; and (5) simple separation mechanism. However, the detector sensitivity is relatively low in terms of concentration, except for laser-induced fluorescence or electrochemical detectors. Some monographs [4–8] and reviews [9–11] are available that provide overviews of CE techniques, and therefore only a brief introduction to CE is given in this review from the viewpoint of the optimization of separation.

Syntheses of chiral compounds and recognition of molecular chirality are major issues in various fields, especially in the pharmaceutical industry. The contribution of analytical chemistry to this field is to develop various methods for the recognition of chiral compounds or the separation of enantiomeric compounds, which may be components of raw materials, pharmaceutical products or medicines, or be included in biological matrices such as urine, serum and saliva. Chromatography, particularly HPLC, is the most successful technique for the analysis of enantiomers, because many useful chiral stationary phases have been developed. HPLC is also suitable for the analysis of biomedical samples. Separation of enantiomers by CE techniques was first reported in 1985 [12], but nevertheless enantiomeric separation by CE is still in its infancy. Some review papers have been published on this subject [13,14], outlining trends in CE methods for enantiomeric separation.

Concerning the separation of enantiomers, CE techniques can take advantage of the high separation efficiency and easy changes of separation media compared with HPLC. CE is advantageous for the separation of solutes having low separation factors, which is often the case with enantiomers, because a high plate number can easily be obtained. CE separation is performed in a homogeneous solution, and the separation solution can be easily altered to find the optimum separation medium, except for the use of a gel-filled capillary. As CE is an ultramicroscale technique, it is generally not suitable for preparative work.

Several CE techniques have been developed: capillary zone electrophoresis (CZE), isotachophoresis (ITP), isoelectric focusing, capillary gel electrophoresis (CGE), electrokinetic chromatography (EKC) and electrochromatography. The boundaries among these techniques are not always distinct. For example, electorphoretic separation of charged molecules using a coated capillary is usually grouped in CZE, but if the partitioning of the analytes to the coated layer

causes separation, it may be electrochromatographic separation. We shall consider this case as electrochromatography irrespective of the presence of the electroosmotic flow. Although CZE and EKC are major techniques employed for enantiomeric separation, some examples of enantiomeric separation by ITP, CGE and electrochromatography have also been published, as discussed later. Most papers on the separation of enantiomers by CE techniques have described direct separation, which can resolve racemic compounds without any prederivatization. A few papers have been published on derivatization to diastereomers before the CE separation. Therefore, most of this review concerns the direct separation method.

The separation principle of CZE is the difference in electrophoretic mobilities. As enantiomers have identical electrophoretic mobilities, some chiral complexing reagents must be added to the separation buffer to form diastereomeric complexes in dynamic equilibra. In CZE, chiral metal complexes [12,15–17], cyclodextrins (CDs) [18–43], crown ethers [23,44,45] and oligosaccharides [46] have been successfully employed. In EKC, where the separation is based on a differential distribution between the pseudostationary phase and the surrounding aqueous phase, the use of charged CDs [47], chiral surfactants [48–58], proteins [59–62], a microemulsion containing a chiral surfactant [63] and addition of CDs to achiral [64–68] or chiral [68–70] surfactant solutions have been reported to be useful even for the separation of neutral compounds. Although the gel matrix used in CGE causes a sieving separation of polymers, the gel is also useful for the immobilization of chiral selectors such as CDs [71,72] and proteins [73], permitting enantiomeric separation based on differential partitioning. CDs have also been very effective additives for enantiomeric separation by ITP [74–79]. In electrochromatography, CD derivatives bonded on the capillary wall work as a chiral stationary phase [80,81], as in capillary gas and liquid chromatography.

The separation of diastereomers by CE has rarely been reported, probably because CZE is not useful for the separation of diastereomers. Diastereomers have identical molecular masses but different molecular structures, and the differences in charge will be minimal. Therefore, the electrophoretic mobilities of diastereomers should be very close each other. This is the reason why the CZE separation of diastereomers has not been reported. On the other hand, EKC is capable of separating many closely related compounds on the basis of differential partitioning, as is HPLC. The separation of enantiomers after derivatization to diastereomers by micellar electrokinetic chromatography (MEKC) has been published [82–87].

In this review, simple theories of enantiomeric separation by CE techniques are described mainly on the basis of the resolution equation, then various CE techniques for enantiomeric separation are overviewed with some examples.

2. BASIC THEORY OF CAPILLARY ELECTROPHORETIC (CE) SEPARATION

2.1. Capillary zone electrophoresis (CZE)

A schematic diagram of the separation principle of CZE is shown in Fig. 1, where a negative surface of the capillary is assumed. Positively charged solutes migrate electrophoretically towards the negative electrode and negatively charged solutes in the opposite direction under the influence of the electric field. The migration direction of the electroosmotic flow depends on the sign of the surface charge or the zeta potential. When the zeta potential is negative, the electroosmotic flow migrates towards the negative electrode. Under neutral or alkaline conditions with an untreated fused-silica capillary, the electroosmotic flow is strong enough to carry

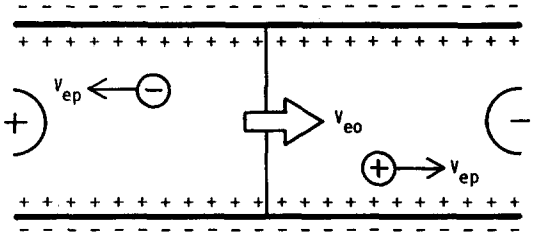


Fig. 1. Schematic diagram of the separation principle of capillary zone electrophoresis: v_{eo} = electroosmotic velocity; v_{ep} = electrophoretic velocity of the solutes.

even the negatively charged solutes towards the negative electrode. The difference in electrophoretic mobility causes separation and the electroosmotic flow itself does not affect the separation selectivity, because it transports all the solutes at equal velocities.

Resolution, R_s, in electrophoresis [88] is given by

$$R_s = \frac{N^{1/2}}{4} \cdot \frac{\Delta v_s}{\bar{v}_s} \quad (1)$$

where N is the average theoretical plate number, $\bar{v}_s$ is the average migration velocity of two solutes and Δv_s in the difference in migration velocities. This equation is equivalent to the equation used in conventional chromatography:

$$R_s = \frac{2\Delta t_R}{W_1 + W_2} \quad (2)$$

where W_1 and W_2 are peak widths on the baseline and Δt_R is the difference in retention times in chromatography or migration times in CE, provided the peak shapes are Gaussian. The average velocity in CZE is given by

$$\bar{v}_s = v_{eo} + \bar{v}_{ep} \quad (3)$$

where v_{eo} is the electroosmotic velocity and $\bar{v}_{ep}$ is the average electrophoretic velocity of the solutes. Eqn. 3 is rewritten with mobilities as

$$\bar{v}_s = (\mu_{eo} + \bar{\mu}_{ep})V/L \quad (4)$$

where μ_{eo} is the electroosmotic mobility or the coefficient of the electroosmotic flow, $\bar{\mu}_{ep}$ is the average electrophoretic mobility, V is the applied voltage and L is the total length of the capillary. If we assume that band broadening in CZE is caused only by the thermal diffusion of the solute along the capillary axis [2], N is given by

$$N = l^2/2Dt_R \quad (5)$$

where l is the effective length of the capillary from the injection end to the detector and D is the diffusion coefficient of the solute. Combining the relationship $t_R = l/v_s$, and eqns. 3–5 with eqn. 1, we obtain resolution equation in CZE [89]:

$$R_s = \frac{1}{4}\left(\frac{V}{2D}\right)^{1/2} \cdot \left(\frac{l}{L}\right)^{1/2} \cdot \frac{\Delta\mu_{ep}}{(\mu_{eo} + \bar{\mu}_{ep})^{1/2}} \quad (6)$$

or

$$R_s = \left(\frac{V}{32}\right)^{1/2}\left(\frac{l}{L}\right)^{1/2} \cdot \frac{\Delta\mu_{ep}}{(D\bar{\mu}_{ep})^{1/2}} \cdot \frac{1}{(1+x)^{1/2}} \quad (7)$$

where $\Delta\mu_{ep}$ is the difference in electrophoretic mobilities, $x = \mu_{eo}/\bar{\mu}_{ep}$ and D is assumed to be identical between the two solutes.

Eqn. 6 or 7 shows that the resolution is a function of four separate factors, of which the last two are important for manipulating resolution, *i.e.*, relative difference in electrophoretic mobilities and electroosmotic flow. The first two factors, the applied voltage and the ratio of the effective length to the total length of the capillary, are not greatly changeable in an advantageous direction. The applied voltage will be limited to 40 kV and the ratio of the capillary lengths can be close to but less than unity for on-column detection. The most important variable for enhancing resolution is the difference in the electrophoretic mobilities. If we define the separation factor, α, in CZE as the ratio of electrophoretic mobilities of solutes 1 and 2:

$$\alpha = \mu_{ep,2}/\mu_{ep,1} \geq 1 \quad (8)$$

then

$$\frac{\Delta\mu_{ep}}{(D\bar{\mu}_{ep})^{1/2}} = 2\left(\frac{\bar{\mu}_{ep}}{D}\right)^{1/2} \cdot \left(\frac{\alpha - 1}{\alpha + 1}\right)$$

$$\approx \left(\frac{\bar{\mu}_{ep}}{D}\right)^{1/2} \cdot \left(\frac{\alpha - 1}{\alpha}\right) \quad (9)$$

where the last equation is derived assuming $\bar{\mu}_{ep} = \mu_{ep,2}$. Finally, eqn. 7 is rewritten as

$$R_s = \left(\frac{Vl}{32L}\right)^{1/2} \cdot \left(\frac{\bar{\mu}_{ep}}{D}\right)^{1/2} \cdot \left(\frac{\alpha - 1}{\alpha}\right) \cdot \left[\frac{1}{(1+x)^{1/2}}\right] \quad (10)$$

The relative difference in electrophoretic mobilities in eqn. 7 can be expressed as a product of the square root of the average ratio of the mobility to the diffusion coefficient and the separation factor function, as shown in eqn. 9. The mobility divided by the diffusion coefficient

may be called a reduced electrophoretic mobility. The equation clearly shows that resolution is affected by the ratio of the average electrophoretic mobility to the diffusion coefficient. We have to consider the relative values of electrophoretic mobility compared with the diffusion coefficient to evaluate resolution. The important contribution of the separation factor to resolution is the same as is in HPLC. For example, if we assume $V = 30$ kV, $l/L = 400$ mm/500 mm = 0.8, $\bar{\mu}_{ep} = 1.0 \cdot 10^{-2}$ mm^2 V^{-1} s^{-1} and $D = 1.0 \cdot 10^{-3}$ mm^2 s^{-1}, the separation factor required to obtain $R_s = 1.5$ is 1.02, when $\mu_{eo} = 0$, giving $t_R = 11.1$ min, $N = 120\,000$. These values are taken as examples but are close to the conditions employed in oxygen isotropic separation of benzoic acids reported previously [89]. The calculation suggests that we need a separation factor more than 1.02 or more than a 2% difference in electrophoretic mobilities in CZE even under favourable conditions.

The electroosmosis factor, $1/(1+x)$, can be also useful for obtaining a high resolution. The value of x can be negative when the electroosmosis is in the opposite direction to the electrophoretic migration. The problem to be solved is the control of the electroosmotic flow to take advantage of this factor. Although various possibilities [90–92] have been proposed, the applications of these techniques are still considerably limited. The control of the electroosmotic flow is a major issue in CE but its discussion it is beyond the scope of this review.

2.2. Electrokinetic chromatography (EKC)

Fig. 2 illustrates schematically the separation principle of EKC, where a pseudo-stationary phase or a separation carrier having electric charge is added to the running buffer of CZE. The pseudo-stationary phase migrates electrophoretically as a solute in CZE, at a velocity different from that of the surrounding aqueous phase. An electrically neutral solute migrates at the velocity of the electroosmotic flow when it is free from the pseudo-stationary phase, while it migrates at the velocity of the pseudo-stationary phase when it is incorporated into the pseudo-

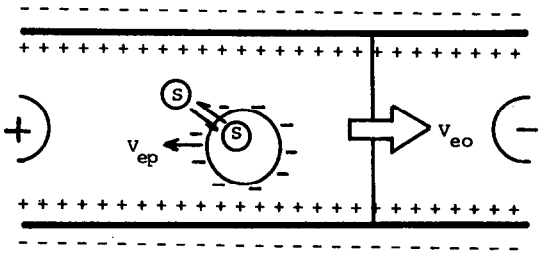


Fig. 2. Schematic diagram of the separation principle of electrokinetic chromatography: S = solute; v_{eo} = electroosmotic velocity; v_{ep} = electrophoretic velocity of pseudo-stationary phase.

stationary phase. Therefore, the distribution coefficient of the solute between the pseudo-stationary phase and the surrounding aqueous phase determines the relative migration order. Several charged substances have been reported as pseudo-stationary phases: ionic micelles [93], CD derivatives having ionic groups [94], microemulsions [95] and proteins [59].

As EKC is a chromatographic method, we can follow the equations used in conventional chromatography with minor modifications. Among EKC techniques, micellar EKC (MEKC) is the most widely accepted, and hence the following discussion refers to MEKC, where ionic micelles are employed as pseudo-stationary places. We can define the capacity factor, k', as

$$k' = n_{mc}/n_{aq} \tag{11}$$

where n_{mc} and n_{aq} are the number of solute molecules incorporated into the micelle and the number dissolved in the surrounding aqueous phase, respectively. The migration time of the electrically neutral solute, t_R, is related to the capacity factor by [93]

$$t_R = \frac{(1+k')t_0}{1+(t_0/t_{mc})k'} \tag{12}$$

where t_0 and t_{mc} are the migration times of the bulk solution and of the micelle, respectively. The migration times t_0 and t_{mc} can be measured by using appropriate tracers of the aqueous phase and the micelle, respectively; methanol or formamide is a typical marker of t_0 and Sudan III or IV that of t_{mc}. Eqn. 12 is different from

the corresponding equation for conventional chromatography:

$$t_R = (1 + k')t_0 \quad (13)$$

The difference comes from the limited migration time window in MEKC between t_0 and t_{mc} because of the migration of the pseudo-stationary phase in the capillary.

The resolution equation of MEKC is given as [96]

$$R_s = \frac{N^{1/2}}{4} \cdot \left(\frac{\alpha - 1}{\alpha}\right) \cdot \left(\frac{k_2'}{1 + k_2'}\right) \cdot \left[\frac{1 - t_0/t_{mc}}{1 + (t_0/t_{mc})k_1'}\right] \quad (14)$$

where N is the theoretical plate number, α the separation factor defined as k_2'/k_1' (≥ 1) and k_1' and k_2' are the capacity factors of solutes 1 and 2, respectively. The equation is similar to that in conventional chromatography, but the limited migration time window in MEKC requires a modification of the conventional equation also as shown above.

The optimum capacity factor, k'_{opt}, which yields the maximum resolution, is a function of the ratio t_0/t_{mc} and is given mathematically as [97]

$$k'_{opt} = (t_{mc}/t_0)^{1/2} \quad (15)$$

where we assume that N is independent of k'. The capacity factor in MEKC is easily adjusted to a desired value without affecting the selectivity by changing the phase ratio, the volume ratio of the micelle to the aqueous phase or the surfactant concentration [96], and this is an advantage of MECK over reversed-phase HPLC. In most instances, a range of k' between 1 and 5 is recommended for optimum performance.

An extra factor that affects the resolution other than those known in conventional chromatography is the effect of the electroosmotic flow as it is in CZE. In most instances, the electrophoretic migration of the micelle is in the opposite direction to the electroosmosis, but the micelle usually migrates in the same direction as the electroosmotic flow but at a slower velocity, because the electroosmotic flow is much stronger than the electrophoretic migration of the micelle. However, under acidic conditions or in a coated capillary, the electroosmosis is often suppressed considerably and the micelle may migrate in a different direction to the electroosmotic flow, under which conditions we may assume that the ratio t_0/t_{mc} is negative. Hence it is possible to obtain an extremely high resolution, when we can control the electroosmotic flow giving close to $t_0/t_{mc} = -(1/k')$ at the expense of a longer separation time. The ratio t_0/t_{mc} is expressed as [98]

$$\frac{t_0}{t_{mc}} = \frac{v_{mc}}{v_{eo}} = 1 + \frac{\mu_{ep}(mc)}{\mu_{eo}} \quad (16)$$

where v_{mc} is the migration velocity of the micelle, which is equal to $v_{eo} + v_{ep}(mc)$, where $v_{ep}(mc)$ is the electorphoretic velocity of the micelle, and $\mu_{ep}(mc)$ is the electorphoretic mobility of the micelle. The mobility $\mu_{ep}(mc)$ is almost constant for a given micelle, whereas μ_{eo} is dependent on the conditions, which may be a change in pH, addition of an organic solvent or the use of a coated capillary. A decrease in v_{eo} enhances the resolution, but requires a longer analysis time. Although viscosity affects μ_{eo}, the effect is also equivalent to $\mu_{ep}(mc)$, and t_0/t_{mc} will not be varied by the viscosity change.

The separation factor is the most important factor, which we can manipulate by consideration of the chemistry. The separation factor is equal to the ratio of the distribution coefficients, which depends on the combination of the micelle and the surrounding aqueous phase. To manipulate α, we may try various techniques as described elsewhere [99]. The minimum α value that can give $R_s = 1.5$ under optimum conditions is roughly 1.02 in MEKC [96].

2.3. Isotachophoresis (ITP)

In CZE, the contribution of the migration of sample solutes to the electric current is not taken into consideration, because we can assume that current is transported only by buffer components. In ITP, however, current is exclusively carried by the sample solutes alone in the capil-

lary. Electroosmosic usually must be suppressed. We shall assume that a sample mixture contains only three anions, A, B and C, and all the anions have the same counter cation, Q, for simplicity. The sample mixture is introduced between the leading (L^-Q^+) and terminating (T^-Q^+) electrolytes. After a transient state for a short time on application of the electric field, the solutes are separated as shown in Fig. 3 and migrate at the identical velocity in order of decreasing electrophoretic mobilities. The isotachophoretic conditions [100] are given by

$$v_L = v_A = v_B = v_C = v_T \quad (17)$$

$$\mu_L E_L = \mu_A E_A = \mu_B E_B = \mu_C E_C = \mu_T E_T \quad (18)$$

where v is the migration velocity, μ the electrophoretic mobility and E the electric field strength, and the subscripts indicate solutes or zones. The zones migrate in contact with each other without any separating buffer zones, as shown in Fig. 3. Eqns. 17 and 18 suggest two important characteristics of ITP: one is that the zone boundary is self-constructed and the other is that E is high when μ is low. The former feature, which comes from the constancy of current in any zone, is explained as follows: if solute A happens to enter zone L, the velocity of solute A will become lower than that in zone A, because E_L is lower than E_A. Therefore, solute A will come back to zone A. The reverse situation will be brought about when solute A enters zone B, where E_B is higher than E_A. The latter feature suggests that joule heating is different among the zones, lower in the foregoing zone than in the following one. Therefore, a thermal detector can be used in ITP as well as a conductivity detector, and ITP usually gives a stepwise electorpherogram, which differs from the electropherograms in CZE or chromatograms.

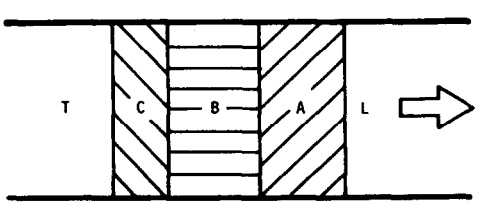


Fig. 3. Schematic diagram of the isotachophoretic separation: L = leading electrolyte zone, A, B and C = sample zones of A, B and C, respectively; T = terminating electrolyte zone.

From the principle of electroneutrality, isotachophoretic conditions and Ohm's law, we can derive the following equation for the boundary between zone 1 and zone 2 under the condition $\mu_A > \mu_B$ [100]:

$$c_{B,2} = c_{A,1} \cdot \frac{\mu_B(\mu_A + \mu_Q)}{\mu_A(\mu_B + \mu_Q)} \quad (19)$$

where c is the concentration and subscripts 1 and 2 indicate zones. Eqn. 19 means that the concentration of B in zone 2 is determined by the concentration of A in zone 1 and the mobilities of A, B and Q, which leads to the important conclusion that the concentrations of all zones are determined by the concentration of L and the mobilities. The equation also suggests the concentration effect of a dilute sample solution by ITP.

A general resolution equation in ITP has not been given. To obtain the resolution by calculation is very complex. However, to obtain separate zones by ITP, it is generally considered that the difference in electrophoretic mobilities of the two contact zones must be more than 2% or the separation factor must be 1.02, obtained by experience [101]. The resolution in ITP is mainly limited by the detector. Most ITP instruments are equipped with a conductivity detector and a UV absorbance detector.

2.4. Capillary gel electrophoresis (CGE)

CGE is usually used to separate polymers by molecular size. The separation of enantiomers by CGE is a minor application. Therefore, only gel-filled capillaries incorporating chiral selectors are mentioned in this review. The gel matrix suppresses electroosmosis and serves as a support of the chiral selector or generally the stationary phase, but will not affect significantly the electrophoretic migration of small ions. The different point of this technique from HPLC is the different migration velocities of the solutes even when they are free from the stationary phase. The separation principle is the same as

that in chromatography. Theoretical treatment of this technique has been discussed by Guttman *et al.* [71].

Starting from eqn. 1 or 2, the resolution equation is given as

$$R_s = \frac{N^{1/2}}{2} \cdot \left(\frac{\alpha - 1}{\alpha + 1}\right) \approx \frac{N^{1/2}}{4} \cdot \left(\frac{\alpha - 1}{\alpha}\right) \quad (20)$$

where the separation factor α is assumed to be the ratio of apparent or net mobilities, which is equal to the ratio of migration velocities or the reciprocal ratio of migration times:

$$\alpha = \frac{\mu_1}{\mu_2} = \frac{v_1}{v_2} = \frac{t_{R,2}}{t_{R,1}} \quad (21)$$

The last part of eqn. 20 is derived assuming that the average velocity is equal to μ_2. In CGE, eqn. 5 for the plate number is probably inapplicable to eqn. 20 owing to the presence of the gel matrix. As electroosmosis is suppressed in CGE, the effect of the electroosmotic velocity on resolution disappears in eqn. 20. The effect of α on resolution is dramatic also in this technique. Eqn. 20 predicts that $\alpha = 1.02$ is required to obtain $R_s = 1.5$ for $N = 100\,000$.

The apparent mobility is expressed as

$$\mu = R\mu_{ep} \quad (22)$$

where R is the fraction of the solute free from the stationary phase and μ_{ep} the electrophoretic mobility of the free solute in the gel matrix. R can be related to the binding constant K of the solute with the stationary phase by an equilibrium reaction:

$$A + C \rightleftharpoons AC \quad (23)$$

$$K = \frac{[AC]}{[A][C]} \quad (24)$$

and

$$R = \frac{[A]}{[A] + [AC]} = \frac{1}{1 + K[C]} \quad (25)$$

Combination of eqns. 22 and 25 with eqn. 21 yields [71]

$$\alpha = \frac{\mu_{ep,1}}{\mu_{ep,2}} \cdot \left(\frac{1 + K_2[C]}{1 + K_1[C]}\right) \quad (26)$$

We can assume that $\mu_{ep,1} = \mu_{ep,2}$ for enantiomers, and when $K \gg 1$ eqn. 24 is simplified to

$$\alpha = K_2/K_1 \quad (27)$$

Hence, the separation factor becomes nearly equal to the ratio of the binding constants.

2.5. Electrochromatography

Electromatography, which is shown schematically in Fig. 4, uses the electroosmotic flow for mobile phase delivery instead of a high-pressure pump as in liquid chromatography. Electrochromatography is usually limited to microscale separations because of serious joule heating. The flow profile of the electroosmotic flow is substantially flat and this characteristic feature causes less band broadening. In addition to the flow profile, electroosmosis provides other merits: no pressure drop is generated and hence small-diameter packing materials can be used, although an open-tubular system is shown in Fig. 4; the flow velocity is independent of the cross-section of flow paths and multi-path diffusion is minimal; and the sample injection is easier than the use of the pressure-driven system, because no high-pressure injection system is necessary. The separation principle is identical with that of conventional liquid chromatography and is not described in the review. It should be noted that the flat flow profile does not dramatically improve the efficiency unless the capacity factor is close to zero [102].

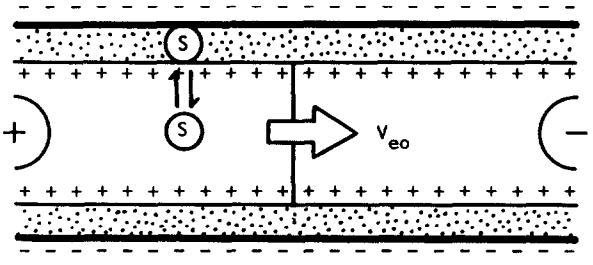


Fig. 4. Schematic diagram of the separation principle of electrochromatography. S = Solute; v_{eo} = electroosmotic velocity.

3. SEPARATION OF ENANTIOMERS BY CZE

3.1. Ligand-exchange complexation

Gassmann *et al.* [12] reported first the separation of enantiomers by CE. They used a Cu(II)–histidine complex as an additive to the electrophoretic buffer and successfully separated dansylated (Dns) amino acids. The copper complex has a positive charge under the experimental conditions (pH 7–8), while the electroosmotic flow migrates towards the negative electrode. Dns-DL-amino acids injected into the capillary will form complexes with the Cu(II)–L-histidine complex by a ligand-exchange mechanism. The ligand-exchange products, ternary complexes of Cu(II) with L-histidine and Dns-L-amino acid or Dns-D-amino acid, are diastereomeric, but they probably have close electrophoretic mobilities. The principle of this enantiomeric separation is the difference in the formation (stability) constants of the complexes. As the Cu(II) complexes have a positive charge, an enantiomer having a larger formation constant has a higher migration velocity towards the negative electrode.

The difference in the formation constants will be observed not only between enantiomers but also among different Dns-amino acids, resulting in the separation of both enantiomers and different Dns-amino acids. However, the formation constants are not always different enough for separation. In particular, this technique was not very successful for the separation of different Dns-amino acids. Gozel *et al.* [15] improved the enantioselectivity by using $Cu^{II}(aspartame)_2$, instead of the Cu(II)–histidine complex. $Cu^{II}(aspartame)_2$ has two six-membered chelate rings formed by the α-amino and β-carboxyl groups of the aspartyl residue of the aspartame molecule. The six-membered ring is less stable than the five-membered ring involving the α-amino and α-carboxyl groups of amino acids. Thus, when an amino acid is added to the solution containing $Cu^{II}(aspartame)_2$ complex, it can replace one aspartame ligand, giving a ternary complexes. Gozel *et al.* [15] successfully separated the enantiomers of fourteen of eighteen Dns-amino acids. Although the migration order between D- and L-enantiomers was different among different Dns-amino acids when the Cu(II)–histidine complex was used, all the D-isomers had shorter migration times or higher electprohoretic mobilities when $Cu^{II}(aspartame)_2$ was employed.

Most monobasic Dns-amino acid derivatives had similar migration times, and the MEKC technique was applied to separate different Dns-amino acids based on the difference in distribution to the sodium tetradecyl sulphate micelle, giving better resolutions of many monobasic Dns-amino acid derivatives [15]. A problem with this technique is detection. In the above example, a laser-excited fluorescence detector was employed, but absorbance of the complex in the UV region will give an adverse effect on photometric detector sensitivity.

Cohen *et al.* [16] reported similar work, in which they use a mixed micelle consisting of sodium dodecyl sulphate (SDS) and N,N-didecyl-L-alanine in the presence of copper(II). The L-alanine derivative itself does not form a micelle. Amino acids will form ternary complexes at the surface of the mixed micelle. Fanali *et al.* [17] successfully separated enantiomers of racemic (+)-$[\Lambda Co(en)_3]^{3+}$ and (−)-$[\Delta Co(en)_3]^{3+}$ and of (+)-$[Co(en)_2$L-Met$]^{2+}$ and (−)-$[Co(en)_2$L-Met$]^{2+}$ by using an L-(+)-tartrate buffer. The system also could separate diastereomeric complexes of $[Co(en)_2$L-Phe$]^{2+}$ and $[Co(en)_2$D-Phe$]^{2+}$.

3.2. Host–guest complexation with CDs (CD-CZE)

CDs are cyclic oligosaccharides with truncated cylindrical molecular shapes. They have particular names, α-, β- and γ-CD for those having six, seven and eight glucopyranose units, respectively. Their outside surfaces are hydrophilic whereas their cavities are hydrophobic. CDs tend to include compounds that fit their cavities by hydrophobic interaction. The size of the cavity differs significantly among α- β- and γ-CDs. Many CD derivatives have been developed for increased solubility in water and to modify the cavity shape. Separation of enantiomers by CZE with CD as the chiral selector was first intro-

duced by Fanali [18] and is the most successful among techniques for enantiomeric separation by CE; the number of papers describing this technique is increasing dramatically. In this review, only some typical examples are discussed.

CD is an electrically neutral compound and hence it migrates at the electroosmotic velocity in CZE. When a charged solute is included into the cavity of CD, the inclusion complex thus formed has a charge identical with that of the free solute but an increased molecular mass, and hence a lower electrophoretic mobility than the free solute. In an enantiomeric separation, free enantiomers have identical electrophoretic mobilities and the included enantiomers also probably have the same mobilities. Therefore, the separation principle of CD-modified CZE (CD-CZE) for enantiomeric separation is the difference in inclusion-complex formation constants between a pair of enantiomers and CD. The more strongly included enantiomer has a lower effective mobility. The effective mobility means an averaged or apparent mobility of the solute under experimental conditions, but in most instances we shall simply use mobility.

Wren and Rowe [24,28,40] described a separation model and derived some equations describing mobility and differential mobility. The proposed model is

$$\underset{\mu_f}{A} + C \rightleftharpoons \underset{\mu_c}{AC} \quad K_1 = \frac{[AC]}{[A][C]} \tag{28}$$

$$\underset{\mu_f}{B} + C \rightleftharpoons \underset{\mu_c}{BC} \quad K_2 = \frac{[BC]}{[B][C]} \tag{29}$$

where A and B are a pair of enantiomers having identical electrophoretic mobilities, μ_f, C is a neutral chiral selector or CD, AC and BC are diastereomeric inclusion complexes having identical electrophoretic mobilities μ_c and K_1 and K_2 are formation constants for inclusion complexes. For the separation of the enantiomers, it is required that both pairs of K_1 and K_2 and of μ_f and μ_c have different values. The effective mobilities of A is written as

$$\mu_A = \frac{[A]}{[A]+[AC]} \cdot \mu_f + \frac{[AC]}{[A]+[AC]} \cdot \mu_c \tag{30}$$

Then

$$\mu_A = \frac{\mu_f + \mu_c K_1[C]}{1 + K_1[C]} \tag{31}$$

The difference in electrophoretic mobilities between A and B is given as

$$\Delta\mu = \frac{\mu_f + \mu_c K_1[C]}{1 + K_1[C]} - \frac{\mu_f + \mu_c K_2[C]}{1 + K_2[C]} \tag{32}$$

Rearrangement yields

$$\Delta\mu = \frac{[C](\mu_f - \mu_c)(K_2 - K_1)}{1 + [C](K_1 + K_2) + K_1 K_2[C]^2} \tag{33}$$

Eqn. 33 shows the dependence of $\Delta\mu$ on the difference in the mobilities between the free and complexed enantiomer, $(\mu_f - \mu_c)$, formation constants, K_1 and K_2, and the concentration of CD. It is obvious that $\mu_f = \mu_c$ or $K_1 = K_2$ gives no separation.

Wren and Rowe [24] calculated the dependence of the difference in mobilities between enantiomers on the concentration of the chiral selector as shown in Fig. 5 assuming $\mu_f = 2$ and $\mu_c = 1$. As seen in Fig. 5, the optimum concentration of CD that gives the maximum selectivity depends on the formation constants. The results suggest that the solutes must keep themselves free from the chiral selector to considerable extent in order to be resolved. The optimum concentration is given mathematically as [24]

$$[C]_{opt} = 1/(K_1 K_2)^{1/2} \tag{34}$$

The model has been verified by experiments using racemic propranolol as enantiomer and β-CD and methylated β-CD (Me-β-CD) as chiral selectors in 40 m*M* phosphate buffer (pH 3.0). (*R*)-(+)-Propranolol had a greater affinity than (*S*)-(−)-propranolol for Me-β-CD and the optimum concentration of the CD was *ca.* 5.5 m*M*; the average formation constants of K_1 and K_2 were ca. 180 $mmol^{-1}$, differing by about 12%. When β-CD was employed, the optimum concentration was about 14 m*M* and selectivity was worse than with Me-β-CD because of the lower formation constants.

Wren and Rowe [28] successfully applied the above-mentioned model to a CD-CZE system using methanol or acetonitrile as a modifier of

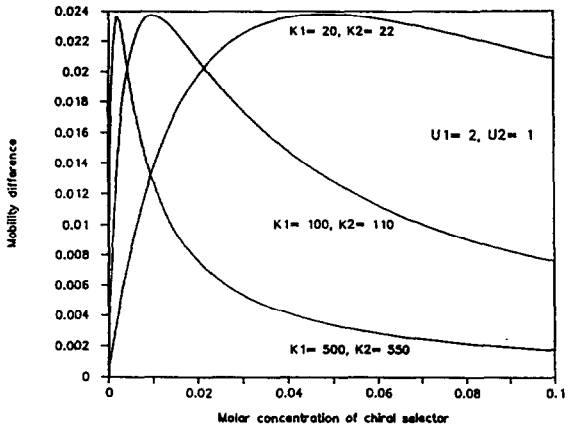


Fig. 5. Theoretical curves generated from eqn. 33 using $\mu_1 = 2$ and $\mu_2 = 1$ with three sets of equilibrium constants as shown. From ref. 24.

the separation solution. They explained the effect of the organic solvent addition in terms of the change in the inclusion-complex formation constants. Acetonitrile or methanol addition decreased the formation constants, affecting the optimum concentration of CD, either enhancing or degrading the separation. The model was also supported by the results obtained with ephedrine, atenolol and practolol [40].

The above model assumes that the mobility of the free solute is constant or one species for one enantiomer when the pH is changed. Rawjee *et al.* [39] developed a more complex model, where the solutes are weak acids, and therefore three pairs of equilibrium constants, acid dissociation constants of the enantiomers and inclusion-complex formation constants for both non-ionized and ionized enantiomers, must be introduced. They derived equations describing selectivity, but they were complex. Then they discussed three simplified models: (1) only a non-ionic form of the enantiomers; (2) only the ionic forms of the enantiomers; and (3) both forms of the enantiomers interact differently with CD. The first model is the simplest, concluding that the pH of the separation solution must be decreased until the desired selectivity is realized. The migration order of the enantiomers cannot be reversed by varying the pH or the CD concentration. The lower pH causes a longer analysis time. The second model also leads to the same conclusion, but selectivity was generally lower than in the first model. The third model is difficult to predict. They performed three sets of specifically designed separation experiments: one at varying pH values without CD in the running buffer, one at high pH with varying concentrations of CD and one at low pH with varying concentration of CD to obtain the parameters of the model. The experimental results with fenoprofen and ibuprofen as test probes were compared with the calculated values, verifying the validity of the model. Both probes were classified as the first model. Non-ionic forms had formation constants about double those of ionized forms, and the mobilities of the free solutes were about three times higher than those of the complexes for both probes.

Table I summarizes reported separations of enantiomers by CD-CZE. For some enantiomers simple α-, β- or γ-CD was useful, but for many enantiomers derivatized CDs such as heptakis(2,6-di-O-methyl)-β-CD (DM-β-CD) and heptakis(2,3,6-tri-O-methyl)-β-CD (TM-β-CD) were required. The pH of the separation solution was preferably selected in acidic regions, probably because suppressed electroosmosis yielded a high resolution. The electroosmotic flow is in the same direction for cationic solutes and in the opposite directions for anionic solutes, and therefore the sign of x in eqn. 10 is positive for cationic and negative for anionic solutes. However, it should be noted that under neutral or alkaline conditions the electroosmotic flow is usually much stronger than the electrophoretic migration of the solute, $|\mu_{eo}/\mu_{ep}| > 2$, hence $1/(1+x)^{1/2}$ becomes less than unity.

In some separations, capillaries coated with polyacrylamide were employed to suppress the electroosmotic flow and adsorption of the solute on the wall. Viscosity modifiers such as hydroxyethylcellulose (HEC) were also used for the same purpose for uncoated capillaries in some instances.

Nielen [36] examined the difference between the different sources of DM-β-CD and found significant differences in resolution. This is probably due to the slight differences in the compositions or purities of the CD derivatives. An example of the separation of some enantiomers

TABLE I

SEPARATION OF ENANTIOMERS BY CD-CZE

Compound	CD[a]	pH	Additive[b]	Ref.
Alanine β-naphthylamide	DA-β-CD	2.0		31
	β-CD, γ-CD			
Atenolol	Me-β-CD	2.5		40
1,2′-Binaphthyl-2,2′-diyl hydrogenphosphate	α-CD,	9		34
	G_1-α-CD			
1,1′-Binaphthyl-2,2′-dicarboxylic acid	β-CD	9		34
	G_1-α-CD			
Bupivacaine	DM-β-CD	2.7, 2.9	MHEC 4000, CTAB	27, 35
Carvedilol	DM-β-CD	2.7, 2.9	MHEC 4000, CTAB	27, 35
Chloramphenicol	DM-β-CD	3.50	MHEC	21
Chlorpheniramine	β-CD	2.5	Urea	37
Clenbuterol	β-CD	4.0		29
	HP-β-CD	2.2		43
Dns-leucine	β-CD, γ-CD,	9.0		30
	TM-α-CD,			
	TM-β-CD			
Dns-phenylalanine	HP-β-CD	9		34
3,4-Dihydroxymandelic acid	DMeNH-β-CD	5		42
ENX 792	β-CD	2.5		23
Ephedrine	DM-β-CD	2.4, 2.5		18, 22, 36
	Me-β-CD	2.5		40
Epinephrine	DM-β-CD	2.4, 25		18, 19, 26, 33
Fenoprofen	β-CD	4.50	HEC	39
Fluoxetine	TM-β-CD	2.7, 2.8	MHEC 1000, CTAB	27, 35
Ibuprofen	β-CD	4.50	HEC	39
Isoproterenol	DM-β-CD	2.4, 2.5		18, 33
m-Hydroxymandelic acid	MeNH-β-CD	5		42
	DMeNH-β-CD			
p-Hydroxymandelic acid	MeNH-β-CD	5		42
	DMeNH-β-CD			
Ketamine	DM-β-CD,	2.5		33
	β-CD			
Ketotifen[c]	β-CD	3.5	MHEC	21
(6*R*,*S*)-Leucovorin	γ-CD	7.1	Urea	38
Mandelic acid	MeNH-β-CD	5		42
	DMeNH-β-CD			
Mepivacaine	DM-β-CD	2.9	MHEC 4000, CTAB	27
	DM-β-CD	2.7		35
N-Methylephedrine	DM-β-CD	2.5		36
(6*R*,*S*)-5-Methyltetrahydrofolate	γ-CD	7.1	Urea	38
Norephedrine	DM-β-CD	2.4, 2.5		18, 36
Norepinephrine	DM-β-CD	2.4, 2.5		18, 33
Octopamine	DM-β-CD	2.5		33
2-Phenyllactic acid	MeNH-β-CD	5		42
	DMeNH-β-CD			
	HP-β-CD	6		
3-Phenyllactic acid	MeNH-β-CD	5		42
	DMeNH-β-CD			
Picumeterol (GGR 1)	β-CD	4.0		29
Pindolol	DM-β-CD	2.9	MHEC 4000, CTAB	27
Practolol	Me-β-CD	2.5		40

TABLE I *(Continued)*

Compound	CD[a]	pH	Additive[b]	Ref.
Propranolol	Me-β-CD	3.0	Methanol, acetonitrile	24, 28
	β-CD	2.5	Urea, methanol	20
Quinagolide	β-CD	2.5		23
Terbutaline	DM-β-CD	2.5		20
	β-CD			
Thioridazine	γ-CD	2.5		21
Tioconazole	HP-β-CD	4.3		41
Tocainides	γ-CD	3.0	HEC	32
Tryptophan	α-CD	2.5		19
Tryptophan derivatives[d]	DM-β-CD,	2.5	Methanol	25
	α-CD			
Verapamil	TM-β-CD	2.7, 2.8	MHEC 1000, CTAB	27, 25

[a] DA-β-CD = Heptakis(2,3-di-O-acetyl)-β-CD; DM-β-CD = heptakis(2,6-di-O-methyl)-β-CD; DMeNH-β-CD = 6^{A},6^{D}-dimethylamino-β-CD; G_1-α-CD = glycosylated α-CD derivative; HP-β-CD = 2-hydroxypropyl-β-CD; Me-β-CD = methyl-β-CD; MeNH-β-CD = 6^{A}-methylamino-β-CD; TM-β(α)-CD = heptakis(hexakis)(2,3,6-tri-O-methyl)-β(α)-CD.

[b] CTAB = cetyltrimethylammonium bromide (hexadecyltrimethylammonium bromide); HEC = hydroxyethylcellulose; MHEC = methylhydroxyethylcellulose.

[c] Three related compounds are also resolved with β-CD or γ-CD [21].

[d] Eight tryptophan derivatives were separated [25].

by CD-CZE is shown in Fig. 6 [36]. Nielen mentioned the importance of the selection of applied voltages. The optimum electric field strength for resolution was 200 V cm^{-1}, or an applied voltage of 15 kV for a 75-cm capillary. The loss of resolution at high electric fields was ascribed to a slow kinetics in inclusion complex formation equilibria.

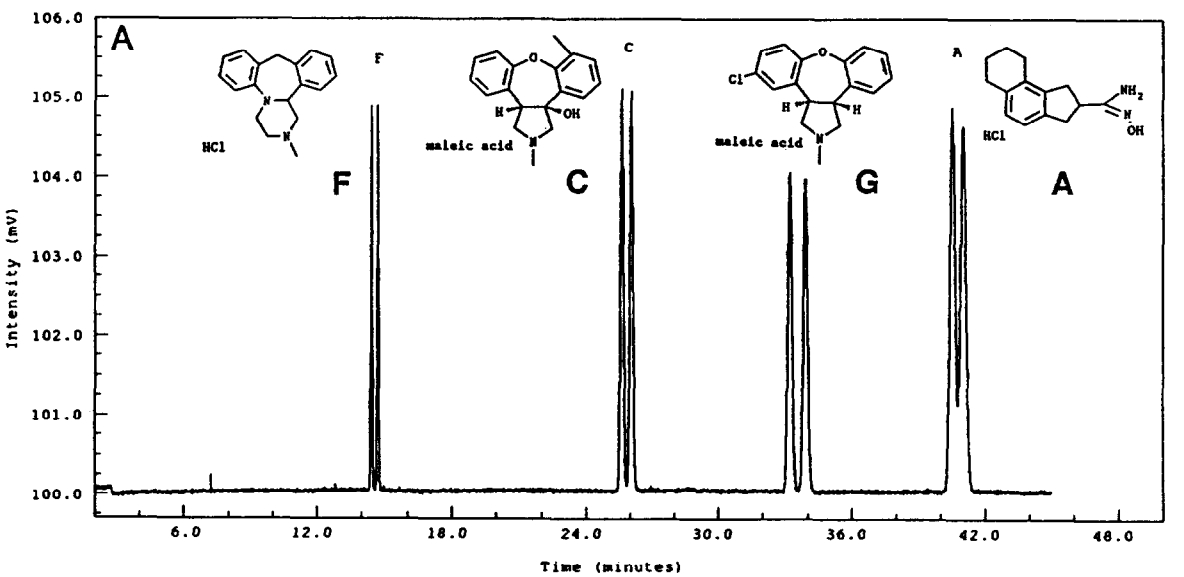


Fig. 6. Separation of some enantiomers by CZE with β-CD. Conditions: capillary, 70 cm (50 cm to the detector) × 50 μm I.D. uncoated fused silica; separation solution, 60 m*M* Tris–phosphate (pH 2.5) containing 20 m*M* β-CD; applied voltage, 15 kV; temperature, 26°C; detection, UV absorbance at 200 nm; samples, 0.1 mg ml^{-1} in the separation solution. From ref. 36; © (1993) American Chemical Society.

Soini *et al.* [27] used cationic surfactants such as cetyltrimethylammonium bromide (CTAB) and cetylpyridinium chloride at a concentration around their critical micelle concentration to improve the reproducibility of migration times and peak shapes of various basic enantiomers with uncoated capillaries. The surfactant also modified the selectivity. They determined some basic drugs extracted from a spiked serum sample at a therapeutic level, and reported that, in general, the CD-CZE approach appeared much easier to develop and optimize than the methodologies based on gas chromatography and HPLC.

Shibukawa *et al.* [38] cleaned up a plasma sample that had been spiked with (6*R*,*S*)-leucovorin and its active metabolite (6*R*,*S*)-5-methyltetrahydrofolate by a pretreatment consisting of denaturation of the plasma proteins by the addition of a high concentration of urea and subsequent ultrafiltration, and separated each pair of the disastereomers with γ-CD at pH 9. They successfully determined each pair of diastereomers, but concluded that the sensitivity was too low for use in clinical applications.

3.3. Host–guest complexation with crown ethers

Crown ethers are known to form stable complexes with alkali metal, alkaline earth metal and primary ammonium cations. Only one crown ether, 18-crown-6-tetracarboxylic acid (18-C-$6H_4$) (Fig. 7), is known to have been successfully used for the separation of primary amines. The amino groups must be protonated in order to be resolved, and therefore acidic conditions are essential. Under acidic conditions at pH 2.2, the ionization of carboxyl groups of 18-C-$6H_4$ are suppressed, and hence the solute migrates towards the negative electrode owing to the positive charge on the ammonium group. As 18-C-$6H_4$ forms stable complexes with alkali metals, the running buffer must be free from alkali metals. Kuhn and co-workers [23,45] reported the separation of 22 amines including many amino acids, phenylalaninol, naphthylethylamine, norepinephrine, normethanephrine and norephedrine with 18-C-$6H_4$ at pH 2.2. They found that the best resolutions are achieved if the chiral centre is adjacent to the amino group. Kuhn and Hoffstetter-Kuhn [7] recognized the synergistic effect of α-CD and 18-C-$6H_4$ on the separation of DL-tryptophan. The resolution increased from 1.29 (α-CD only) and 5.67 (18-C-$6H_4$ only) to 7.37 with a mixture of both chiral selectors. Höhne *et al.* [44] reported the enantiomeric separation of phenylalaninol, phenylglycinol, methoxamine, octopamine and norepinephrine with the same crown ether at pH 2.07.

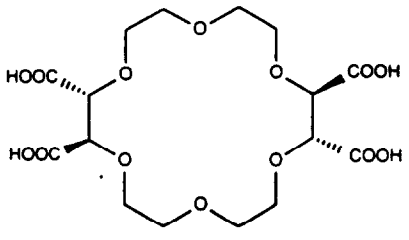


Fig. 7. Structure of 18-crown-6-tetracarboxylic acid.

3.4. Complexation with oligosaccharides

D'Hulst and Verbeke [46] used maltodextrins, mixtures of linear α-(1–4)-linked D-glucose polymers, as a chiral additives in CZE to separate enantiomers of the non-steroidal anti-inflammatory drugs flurbiprofen and ibuprofen and the coumarin anticoagulants warfarin, 3-(α-acetonyl-*p*-chlorobenzyl)-4-hydroxycoumarin, phenprocoumon and chlorophenprocoumon. Diastereomers of cephalosporin antibiotics, cephalexin and cephadroxyl, were also resolved. The above compounds were successfully optically resolved with Glucidex-20 also at pH ≈ 7. The separation mechanism is not well defined.

4. SEPARATION OF ENANTIOMERS BY EKC

There are two techniques for the separation of enantiomers by EKC: one is the use of chiral pseudo-stationary phases and the other is the use of chiral additives in the aqueous phase. Micelles formed from chiral surfactants alone [49–56], or mixtures of chiral and achiral surfactants [48,57,58], CDs having ionic groups [47,103], microemulsions containing chiral surfactants [63] and proteins [59–62] have been reported as chiral pseudo-stationary phases. As modifiers of

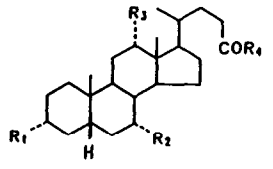
the aqueous phase, CDs [64–70] and maltoheptaose [68] have been reported. Combinations of chiral surfactants and CDs have also been reported [68–70]. Although the use of CDs with achiral surfactants is an MEKC version of CD-CZE, CD modified MEKC (CD-MEKC) can separate neutral compounds with neutral CDs. Each EKC technique for enantiomeric separation is briefly discussed below.

4.1. Micellar EKC (MEKC) with chiral micelles

Many surfactants are commercially available and some of them are chiral. Amino acid-derived synthetic surfactants, bile salts and some other natural surfactants have been successfully used for the separation of enantiomers, including electrically neutral compounds. The enantioselectivity or separation factor function, $(\alpha - 1)/\alpha$, in eqn. 14 depends on the combination of the micelle and the solute. Most polar solutes are supposed to interact with the micelle on the surface through polar–polar interactions in addition to hydrophobic interactions with the core of the micelle [99,104]. Therefore, a surfactant having a chiral centre around the polar group will be suitable for the separation of enantiomers, because most chiral compounds have polar groups near chiral centres. However, as the mechanism of interaction of the solute with the micelle is not well understood we need a more detailed model for the interaction mechanism in order to predict enantioselectivity.

Dobashi *et al.* [48,50] used sodium N-dodecanoyl-L-valinate (SDVal) and sodium N-dodecanoyl-L-alaninate (SDAla) and successfully separated N-(3,5-dinitrobenzoyl), N-(4-nitrobenzoyl) and N-(benzoyl)-O-isopropyl ester derivatives of DL-amino acids. They examined the effect of the fraction of SDVal in the mixed micelle with SDS and the addition of methanol. Methanol addition did not change separation factor, although the capacity factor decreased considerably. The decrease of the SDVal fraction in the mixed micelle reduced the enatioselectivity. Otsuka and Terabe [52] also used SDVal for the separation of phenylthiohydantoin (PTH)-DL-amino acids. When SDVal was used alone to form the micelle, the efficiency was poor owing to seriously tailed peaks, although the separation factors were large. Addition of 10% methanol and 5 *M* urea improved the peak shapes but the efficiency was still not very high [56]. The use of a mixed micelle of SDVal and SDS with 10% methanol and 5 *M* urea enhanced the efficiency and resolution at pH 9.0, resulting in the separation of six PTH-DL-amino acids. Enantiomers of racemic benzoin and wafarin were also resolved under the same conditions [57].

Bile salts are an abundant source of chiral surfactants. The molecular structures of some bile salts are shown in Fig. 8. Sodium cholate (SC) and sodium deoxycholate (SDC) are soluble in neutral or alkaline buffers only, but taurine conjugates are soluble in water even under acidic conditions. Bile salt surfactants are assumed to form helical-structured micelles with a reversed micelle conformation [105,106]. The enantioselectivity with bile salt micelles seems to



Bile salts	Symbol	R_1	R_2	R_3	R_4
Sodium cholate	SC	OH	OH	OH	ONa
Sodium taurocholate	STC	OH	OH	OH	$NHC_2H_4SO_3Na$
Sodium deoxycholate	SDC	OH	H	OH	ONa
Sodium taurodeoxycholate	STDC	OH	H	OH	$NHC_2H_4SO_3Na$

Fig. 8. Structures of some bile salts.

be greater for relatively flat and rigid compounds, which at least have a fused-ring system.

Terabe *et al.* [49] tried to separate enantiomers of Dns-amino acids using sodium taurocholate (STC) and sodium taurodeoxycholate (STDC). Under neutral conditions with an uncoated capillary, the electroosmotic flow was strong and separation was not successful. However, six Dns-DL-amino acids were resolved with 50 m*M* STDC under acidic conditions (pH 3.0). The separation factors of six pairs of Dns-DL-amino acids were 1.01–1.03. Dns-DL-amino acids with a separation factor of 1.01 (Dns-DL-Try, Dns-DL-Leu and Dns-DL-Nva) were partially resolved. Under acidic conditions, the electroosmotic flow was considerably suppressed and the micelle migrated toward the positive electrode, giving shorter migration times for more hydrophobic compounds such as tryptophan. STC gave a poor resolution for these Dns-DL-amino acids.

Nishi *et al.* [51,53,54] successfully separated enantiomers of diltiazem, trimetoquinol and related compounds, carboline derivatives A and B, naphthylethylamine and dinaphthyl derivatives with bile salt micelles under neutral conditions. Enantiomers of carboline derivatives A and B and 2,2′-dihydroxy-1,1′-dinaphthyl were well separated with SC, SDC, STC, STDC with separation factors of 1.06–1.45 [51]. Enantiomers of diltiazem, trimetoquinol and related compounds were separated with only STDC at pH 7.0. An example of the enantiomeric separation of diltiazem and its derivatives with STDC is shown in Fig. 9. Trimetoquinol hydrochloride and related compounds were optically resolved also using only STDC at pH 7.0. An example of optical purity testing of trimetoquinol hydrochloride is given in Fig. 10. Cole *et al.* [55] separated enantiomers of three 1,1′-dinaphthyl derivatives with the SDC or STDC micelle with or without methanol addition.

Otsuka and Terabe [52] used a non-ionic natural surfactant, digitonin, which is a glycoside of digitogenin, for the optical separation of PTH-DL-amino acids. As digitonin is non-ionic, SDS was employed to form a charged mixed micelle, and six PTH-DL-amino acids were resolved under acidic conditions (pH 3.0), which were chosen to enhance resolution, giving a longer analysis time

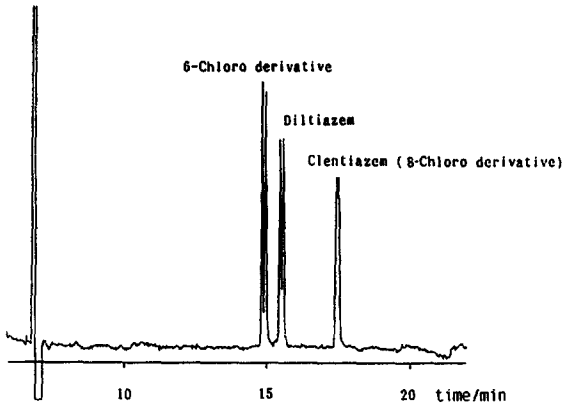


Fig. 9. Separation of enantiomers of diltiazem and its derivatives by MEKC with sodium taurodeoxycholate. Conditions: capillary, 65 cm (50 cm to the detector) × 50 μm I.D.; separation solution, 50 m*M* sodium taurodeoxycholate in 20 m*M* phosphate–borate buffer (pH 7.0); applied voltage, 20 kV; temperature, ambient; detection, UV absorbance at 210 nm.

for less hydrophobic amino acid derivatives such as PTH-Ala. Two other natural surfactants, glycyrrhizic acid and β-escin, were used for the separation of enantiomers of Dns-amino acids and PTH-amino acids by Ishihama and Terabe [58]. Both surfactants are triterpene glucosides and soluble in alkaline solution because they have one or two carboxyl groups. Single micelles of these surfactants were not effective for enantiomeric separation. Glycyrrhizic acid was

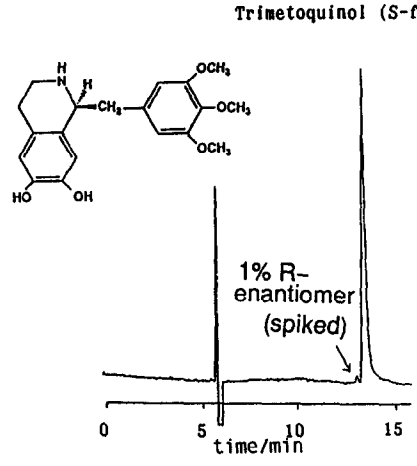


Fig. 10. Optical purity testing of trimetoquinol hydrochloride by MEKC with taurodeoxy cholate: authentic (*S*)-trimetoquinol was spiked with *ca.* 1% of the *R*-enantiomer. Conditions as in Fig. 9.

used with octyl-β-glucoside and SDS at pH 7.0, separating six pairs of Dns-DL-amino acids. Glycyrrhizic acid has a carbonyl group conjugated to a carbon–carbon double bond, and hence a fluorescence detector had to be used. β-Escin showed better enantioselectivity for nine PTH-amino acids than digitonin. This surfactant was also used with SDS to form a mixed micelle at pH 3.0.

So far, separation of enantiomers by MEKC with chiral surfactants has not been applied to many kinds of compounds. However, it should be mentioned that MEKC can be applied to the separation of neutral analytes. Another advantage of MEKC is that the separation of mixtures, which may contain other racemic or achiral compounds in addition to the enantiomers of analytes, can be optimized easily. We hope that new synthetic chiral surfactants will become available for enantiomeric separations by MEKC in near future.

4.2. CD-modified MEKC (CD-MEKC)

CD-MEKC, which employs CD together with an ionic micelle, was originally developed for the separation of highly hydrophobic compounds, which are almost totally incorporated into the micelle and cannot be separated [107]. The addition of CD to the MEKC solution changes the apparent distribution coefficients between the micelle and non-micellar phase, because CD can include hydrophobic compounds in its cavity and increase the apparent solubility to the aqueous phase. As CD has enantioselectivity for various chiral compounds, CD-MEKC can be applied to the separation of enantiomeric compounds.

In CD-MEKC, we can assume that CD is part of the aqueous phase and can affect the distribution of the solute to the micelle. We can define the apparent capacity factor in CD-MEKC as

$$k'_{app} = \frac{n_{mc}}{n_{non\text{-}mc}} = \frac{n_{mc}}{n_{aq} + n_{CD}} \tag{35}$$

where $n_{non\text{-}mc}$ is the numbers of moles of the solute in the non-micellar phase, which is equal to $n_{aq} + n_{CD}$, where n_{CD} is the number of moles of the solute included by CD. It should be noted that k'_{app} in eqn. 35 can be equally used as k' in eqn. 11 for the calculation of various chromatographic parameters. Eqn. 35 can be rewritten as

$$k'_{app} = K_{app} \cdot \frac{V_{mc}}{V_{aq} + V_{CD}} \approx K_{app} \cdot \frac{V_{mc}}{V_{aq}} \tag{36}$$

where K_{app} is the apparent distribution coefficient of the solute between the micelle and non-micellar phase and V_{aq}, V_{mc} and V_{CD} are volumes of the aqueous phase, micelle and CD, respectively. We can assume that the volume of CD in the denominator is negligibly small compared with the volume of the aqueous phase. Taking into consideration the following relationships:

$$\frac{n_{mc}}{n_{aq}} = K_{mc} \cdot \frac{V_{mc}}{V_{aq}} \tag{37}$$

and

$$\frac{n_{CD}}{n_{aq}} = K_{CD} \cdot \frac{V_{CD}}{V_{aq}} \tag{38}$$

where K_{mc} and K_{CD} are distribution coefficients between the micelle and the aqueous phase and between the CD and the aqueous phase, respectively, we can obtain

$$k'_{app} = \frac{V_{mc} K_{mc}}{V_{aq} + V_{CD} K_{CD}} \tag{39}$$

Then, the separation factor α is given as

$$\alpha = \frac{k'_{app,2}}{k'_{app,1}} = \frac{1 + (V_{CD}/V_{aq}) K_{CD,1}}{1 + (V_{CD}/V_{aq}) K_{CD,2}} \cdot \frac{K_{mc,2}}{K_{mc,1}} = \frac{1 + \phi_{CD} K_{CD,1}}{1 + \phi_{CD} K_{CD,2}} \tag{40}$$

where subscripts 1 and 2 indicate enatiomers, ϕ_{CD} is the phase ratio of the volume of CD to that of the aqueous phase and $K_{mc,1} = K_{mc,2}$ is assumed for enantiomers. Eqn. 40 suggests that the separation factor in CD-MEKC is not affected by changes in the concentration of the micelle. However, we can change the concentration of the micelle to optimize the capacity factor as given in eqn. 15 to enhance the resolution. Actually, eqn. 39 indicates that it is more effective to change the concentration of the micelle than to change that of CD, to adjust the appar-

ent capacity factor to the optimum value. The volume of the micelle is related to the concentration of the surfactant, c_{srf}, by [96]

$$V_{mc} = \bar{v}(c_{srf} - CMC) \quad (41)$$

where $\bar{v}$ is the partial specific volume of the surfactant forming the micelle. It is also indicated by eqn. 40 that the separation factor in CD-MEKC is always less than the ratio of the distribution coefficients of the enantiomers to CD, whereas in CGE α is substantially equal to the ratio of the binding constants as given in eqn. 27, although the definitions of α are slightly different. Only when $\phi_{CD}K_{CD} \gg 1$ does α become equal to $K_{CD,1}/K_{CD,2}$ as predicted by eqn. 40.

Miyashita and Terabe [64] reported the enantiomeric separation of Dns-DL-amino acids using γ-CD and SDS at pH 8.3. Nine Dns-DL-amino acids were successfully resolved, but Dns-DL-Trp, -Ser and -Asp were not resolved. The optical resolution of Dns-amino acids was improved by using a mixture of β-CD and γ-CD with SDS [68]. Only Dns-DL-Ser was not resolved among twelve Dns-DL-amino acids. The addition of methanol did not increase the resolution significantly, but the emigration times increased slightly. Ueda et al. [66] also applied CD-MEKC to the separation of naphthalene-2,3-dicarboxaldehyde (CBI)-labelled DL-amino acids using β- or γ-CD with SDS. Generally, γ-CD showed higher enantioselectivity than β-CD for CBI-DL-amino acids in CD-MEKC.

Nishi *et al.* [65] examined the separation of various enantiomers by CD-MEKC. Enantiomers of 2,2,2-trifluoro-1-(9-anthryl)ethanol, 2,2′-dihydroxy-1,1′-dinaphthyl and 1,1′-dinaphthyl-2,2′-diyl hydrogenphosphate were successfully resolved with γ-CD and SDS. Other CDs, β-CD, DM-β-CD and TM-β-CD, were less effective for the separation of these enantiomers. Enantiomers of thiopental and pentobarbital were also resolved with γ-CD and SDS. The resolution was not improved by the addition of methanol but the addition of chiral compounds, *d*-camphor-10-sulphonate or *l*-menthoxyacetic acid, enhanced the selectivity significantly. The effect of these chiral additives in the CD-MEKC system on resolution is probably explained in terms of increased enantioselectivity of the CD by the co-inclusion of the chiral additive and the enantiomeric analyte. An example of CD-MEKC separation of enantiomers of barbiturates is shown in Fig. 11. Enantiomers of an antihypertensive drug, cicletanine, were resolved by CD-MEKC with β-CD [67].

Okafo and co-workers [69,70] used a mixture of STDC and β-CD for the separation of enantiomers of a variety of compounds and the resolution was much superior to that obtained by using either STDC or β-CD. They elucidated a loose association between β-CD and the hydrophilic face of taurodeoxycholate molecules in the micelle by hydrogen bonding, from the observations that the fluorescence intensity measurement of N-phenylaminonaphthalene depended on the β-CD concentration in the presence of STDC and that the solubility of β-CD increased far above the saturation solubility in the presence of STDC. This β-CD–STDC complex enhanced the resolution of enantiomers of Dns-amino acids, mephenytoin and its hydroxy metabolite, fenoldopam, and a closely related derivative with a mixture of 20 m*M* β-CD and 50 m*M* STDC at pH 7.2 [69]. A mixture of β-CD and STDC was used also to resolve the enantio-

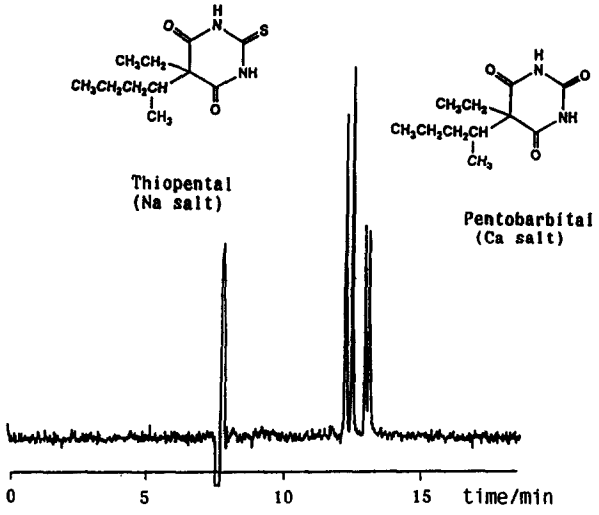


Fig. 11. Separation of enantiomers of thiopental by CD-MEKC. Conditions: separation solution, 30 m*M* sodium γ-CD, 30 m*M* sodium camphor-10-sulphonate and 50 m*M* SDS in 20 m*M* phosphate–borate buffer (pH 9.0). Other conditions as in Fig. 9.

mers of CBI-DL-amino acids and Dns-DL-amino acids [70]. The mixture gave an improved resolution of CBI-DL-amino acids in comparison with the use of a mixture of γ-CD and SDS mentioned above [66]. CBI derivatives of the enantiomers of baclofen and its aminophosphoric acid analogues were also successfully resolved with the same β-CD–STDC system [70]. Okafo and Camilleri [69] reported the *in situ* derivatization of amino acids to dansyl derivatives and the successful separation of enantiomers of Dns-Pro, -Arg and -Ala, which are not commercially available. They also found that the technique separated these dansyl derivatives from the by-product dansylic acid. The technique was successfully applied to the analysis of the hydrolysis products of D-Phe[7]-bradkinin and the expected ratio of Dns-D-Phe and -L-Phe or CBI-D-Phe and -L-Phe was observed. The migration order between Dns-D-Phe and -L-Phe was reversed from that of CBI derivatives. Terabe *et al.* [68] tried to improve the resolution of enantiomers of Dns-DL-amino acids using a mixture of STDC and γ-CD at pH 3.0. The resolution was improved compared with the use of STDC alone at pH 3.0, but it was poorer than the results obtained with γ-CD and SDS at pH 8.6.

4.3. Microemulsion EKC

Only one paper has been published on the use of a microemulsion containing a lipophilic chiral selector, (2*R*,3*R*)-dibutyl tartrate (0.5%, w/w) as a pseudo-stationary phase for the separation of enantiomers of ephedrine [63]. As the separation mechanism of microemulsion EKC has not been examined in detail, further data are needed in order to be able to evaluate this technique.

4.4. Affinity EKC

The idea of using proteins as the pseudo-stationary phases in EKC is natural from the viewpoint of the separation principle of EKC. Problems with using proteins as pseudo-stationary phases are that (1) adsorption of proteins on the capillary wall causes band broadening due to the interaction of the solute with the adsorbed proteins; (2) absorption of UV light especially at short wavelength regions by proteins limits the range of wavelength available for detection; and (3) most proteins are not stable under EKC conditions and their purity is not always high. The presence of different forms of proteins causes band broadening. However, affinity EKC is a challenging topic, particularly for investigating the interaction of drugs with specific proteins. Most proteins are considered to recognize chirality and several papers on enantiomeric separations by affinity EKC with various proteins have been published recently.

Barker *et al.* [59] used bovine serum albumin (BSA) to separate a diastereomeric pair, (6*R*)- and (6*S*)-leucovorin, with an uncoated capillary at pH 7.0 or with a polyethylene glycol-coated capillary at pH 7.2. The migration orders are reversed between the above conditions because of the difference in electroosmotic flow velocities. They developed an equation to calculate the difference in the free energy change between the enantiomers and the protein from the migration times of the the protein and both enantiomers in the absence and presence of the protein.

Busch *et al.* [60] employed four proteins that are used as protein-based stationary phases in HPLC columns for enantiomeric separations, orosomucoid (γ_1-acid glycoprotein), ovomucoid, fungal cellulase and BSA, for the separation of enantiomers of tryptophan, benzoin, pindolol, promethazine and warfarin. BSA, which was the most versatile among the four proteins, could resolve enantiomers of tryptophan, benzoin and warfarin at pH 7.4 or 6.8 with addition of 1-propanol. Ovomucoid was not effective for separating the above test compounds. However, Ishihama *et al.* [108] recently used ovomucoid successfully as a chiral pseudo-stationary phase for the separation of enantiomers of benzoin, tolperisone, eperisone and chlorpheniramine. Orsomucoid recognized (*R*,*S*)-promethazine and in this instance the addition of N,N-dimethyloctylamine improved the peak shapes significantly. Fungal cellulase was effective for the separation of enantiomers of promethazine and pindolol.

Valtcheva *et al.* [61] used a cellulase, cellobiohydrolase I (CBH I), for the separation of enantiomers of the β-blockers propranolol, al-

prenolol, metoprolol, pindolol and labetolol. They performed the experiments under various conditions and pH 5.1 was selected in order for the protein to be negatively charged and to migrate towards the positive electrode (the injection end), whereas the analytes migrated towards the negative electrode (the detector end), avoiding disturbance of detection by UV absorption by CBH I. The injection end had a short agarose plug to prevent any hydrodynamic flow through the capillary. For each pair of enantiomers, the second-eluting peaks were extremely broad and asymmetric. These poor peak shapes were significantly improved by the addition of 25–30% of 2-propanol.

Vespalec *et al.* [62] used human albumin (HA) for the separation of enantiomers of kynurenine, tryptophan, 3-indolelactic acid, 2,3-dibenzoyl-D,L-tartaric acid and N-2,4-dinitrophenyl-DL-glutamin acid under alkaline conditions. Alkaline HA solutions were mildly heated for a short time, *e.g.*, at 60°C for 30 min at pH 9, to stabilize the enantioselectivity, then the pH of the running solution was changed to 8 to protect the coated capillaries. HA was also employed by Lloyd *et al.* [109] as a pseudo-stationary phase in EKC for the separation of enantiomers of promethazine, priomazine, thiridizine and benzoin under slightly acidic conditions with addition of 1-propanol.

5. SEPARATION OF ENANTIOMERS BY ITP

Snopek and co-workers [74–78] extensively studied the separation of enantiomers by ITP, in a series of studies on the use of CD in ITP. Enantiomers of pseudoephedrine alkaloids were successfully separated by using β-CD and DM-β-CD as additives to the leading electrolyte. The enantiomers of *p*-hydroxynorpseudoephedrine was also separated under the same conditions [13]: 5 m*M* sodium acetate (adjusted to pH 5.48 with acetic acid) containing 0.2% HEC and 10 m*M* DM-β-CD was used as the leading electrolyte and 10 m*M* β-Ala as the terminating electrolyte. Enantiomers of ketotifen and its polar intermediate were also successfully resolved by ITP employing β-CD or DM-β-CD under similar conditions [76]. The dependence of resolution and the maximum load of the racemic compound were studied. An increase in the concentration of β-CD from 0 to 5 m*M* increased the resolution and loadability. The ITP technique was also successful for the separation of three pairs of enantiomers of thioridazine, promethazine and cyamepromazine with β-CD or γ-CD [76].

Snopek *et al.* [78] developed a two-dimensional system using commercially available column-coupling equipment to separate two paris of enantiomers in one run, where one pair of enatiomers were separated with a CD derivative, *e.g.*, γ-CD, and the other pair with the other CD derivative, *e.g.*, DM-β-CD. Each separation compartment of the system was filled with the leading electrolyte containing one type of CD. They separated two pairs of enantiomers of pseudoephedrine and thioridazine with γ-CD and DM-β-CD, and norpseudoephedrine and hydrothiadene with the same combination of the CD derivatives. Two-dimensional ITP proved useful for the investigation of complex mixtures of racemates and was advantageous compared with two runs with a one-column system with respect to a shorter analysis time and a low consumption of the samples and chiral selectors. Snopek *et al.* [21] compared CD-CZE and ITP with CD in the separation of some enantiomers listed in Table 1, and concluded that ITP appears more suitable than CZE for the determination, or at least preconcentration, of minute components in a large excess of other components. It is also feasible to work with larger sampling volumes in ITP. However, CZE will be preferable for the separation of multi-component mixtures.

Jelínek *et al.* [77] proposed a simple mathematical model of 1:1 CD–solute complex formation, which includes effective and non-effective complex formation as a possible limiting CD–solute interaction. The effective complex formation means that the formation constants are different between the enantiomers, while non-effective complex formation gives the same values of the formation constants for the enantiomers. Two different complexes are assumed to be formed by the interaction of CD with the different sites of the solute. The dependence of

resolution on the above two types of complex formation was discussed with a computer simulation in addition to the effects of solute molecular mass, its effective charge and the molecular mass of the CD used. A mathematical model describing the electrophoretic migration of strong electrolytes in the presence of a neutral complexing agent was proposed also by Dubrovcáková *et al.* [79]. The model allows the determination of formation constants and mobilities of complex compounds from experimental isotachophoretic data.

6. SEPARATION OF ENANTIOMERS BY CGE

Guttman *et al.* [71] prepared a capillary filled with cross-linked polyacrylamide gel incorporating a CD without bonding by simply adding the CD to the solution prior to polymerization, and used a gel filled capillary for the separation of enantiomers of twelve Dns-amino acids β-CD was most effective and the addition of 10% of methanol enhanced the enantioselectivity slightly. The binding constants of some Dns-amino acids and β-CD in a polyacrylamide gel matrix were calculated from the dependence of migration times on CD concentration. The gel-filled capillary was very stable and even 1000 V cm^{-1} could be applied.

Cruzado and Vigh [72] copolymerized alkyl carbamoylated β-CD (ac-β-CD) with acrylamide to form gels for use in CGE to separate enantiomers. They prepared both cross-linked (solid) and linear (liquid) gels. The solid gel was not stable enough to operate for a long time, although Dns-DL-Phe were well resolved in this gel matrix at pH 8.3. The liquid gel was obtained from 1–2% acrylamide and 0.30 *M* ac-β-CD by copolymerization. The latter was added to the running buffer as a chiral selector. Nine Dns-DL-amino acids were resolved with a liquid gel made of 2% ac-β-CD and 20 m*M* acrylamide at pH 8.3. Enantiomers of homatropine were resolved with the liquid gel but those of atropine were not.

Birnbaum and Nilsson [73] prepared a gel capillary containing BSA cross-linked with glutaraldehyde for the separation of enantiomers of tryptophan. The gel was not filled in the cell part for UV detection. The concentration of BSA within the capillary was *ca.* 0.25 m*M*. They named this technique affinity gel electrophoresis.

7. SEPARATION OF ENANTIOMERS BY ELECTROCHROMATOGRAPHY

Two papers on the separation of enantiomers by electrochromatography have been published, both of which described CD derivative-coated capillaries. Mayer and Schurig [80] prepared immobilized Chiral-Dex as a liquid stationary phase for gas chromatography and supercritical fluid chromatography (SFC), and this capillary was also used for electrochromatography to separate enantiomers of 1,1′-dinaphthyl-2,2′-diyl hydrogenphosphate and 1-phenylethanol, giving almost complete resolution.

Armstrong *et al.* [81] used a capillary coated with a derivatized β-CD and organohydrosilane copolymer in electrochromatography. Racemic mephobarbitol was optically resolved at pH 7.8. They claimed that this technique is CZE, because electroosmosis is completely suppressed. However, in this review, this technique is classified as electrochromatography as mentioned above. The same capillary could resolve enantiomers of 1-aminoindane and perhydroindole by SFC.

8. SEPARATION OF DIASTEREOMERS BY CE TECHNIQUES

As mentioned above, the separation of diastereomers by a simple CZE system is usually difficult, and therefore MEKC is almost exclusively employed for this purpose. The derivatization techniques used were those developed for HPLC. Leopold [82] used Marfey's reagent for the separation of DL-amino acids, isomeric dipeptides, Ala–Ala, and tripeptides, Ala–Ala–Ala, and good resolution was obtained by MEKC using 200 m*M* SDS at pH 8.5. Tran *et al.* [83] also used L- and D-Marfey's reagent for the separation of enantiomers of amino acids. The derivatized enantiomers of Ala, Val, Leu, Phe and Trp co-migrated in CZE under alkaline conditions (pH 8.5). However, under acidic conditions (pH 3.3), the derivatized enantiomers

of Asp, Glu, Ala and Leu were well resolved by CZE, although the peaks were slightly broad. When the D-form of the reagent was used, the migration order between D- and L-amino acids was reversed. It is surprising that these diastereomers have been well resolved by CZE. The resolution is probably due to the difference in the dissociation constants of the carboxyl groups of the diastereomers. MEKC with the SDS micelle at pH 8.5 was much superior to CZE for the separation of the diastereomers mentioned above because of the high efficiency. MEKC separation of the derivatized amino acids using Marfey's reagent was applied to the analysis of D-amino acids in an unknown sample.

Nishi *et al.* [84] reported the separation of 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate (GITC)-derivatized DL-amino acids by MEKC using SDS at pH 7 or 9 with high efficiency. An example of the MEKC separation of GITC-amino acids is shown in Fig. 12. Lurie [85] also separated GITC-derivatized phenylamines, amphetamine, methanphetamine, ephedrine, pseudoephedrine, norephedrine and norpseudoephedrine, by MEKC with SDS and methanol at pH 9.0. He concluded that the MEKC technique is far superior to HPLC with respect to both resolution and speed of analysis.

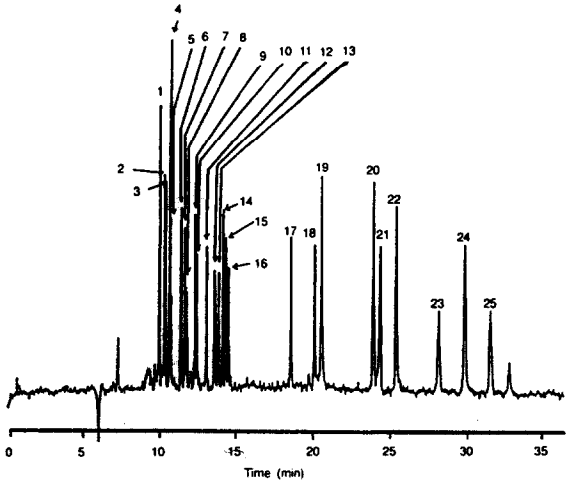


Fig. 12. MEKC separation of fourteen GITC-amino acids: 1 = L-Pro + L-Thr; 2 = L-Ala; 3 = D-Pro; 4 = D-Ala + L-Val; 5 = D-Thr; 6 = L-Thy + L-Met; 7 = D-Val; 8 = L-Leu; 9 = L-phenylglycine (PheG); 10 = D-Met; 11 = L-Leu; 12 = D-Leu; 13 = D-Tyr; 14 = D-PheG; 15 = L-Phe; 16 = D-Leu; 17 = D-Phe; 18 = L-His; 19 = L- Trp; 20 = D-Trp; 21 = D-His; 22 = L-Lys; 23 = L-Arg; 24 = D-Lys; 25 = D-Arg. Conditions: capillary, 65 cm (50 cm to the detector) × 50 μm I.D.; separation solution, 200 m*M* SDS in 20 m*M* phosphate–borate buffer (pH 7.0); applied voltage, 20 kV; detection, UV absorbance at 210 nm. From ref. 84; © (1990) MicroSeparations.

Kang and Buck [86] derivatized amino acids with *o*-phthalaldehyde and either N-acetyl-L-cysteine or N-*tert.*-butoxycarbonyl-L-cysteine to diastereomeric isoindole derivatives, and successfully resolved them by MEKC with SDS and methanol at pH 9.5. The diastereomers were separated by simple CZE at pH 9.5 but the addition of tetrabutylammonium chloride improved the CZE separation of diastereomers. Schützner *et al.* [87] derivatized DL-tryptophan with (+)-diacetyl-L-tartaric anhydride to form the corresponding tartaric acid (mono)amide and separated the derivatized diastereomers by CZE using a polyacrylamide-coated capillary and polyvinylpyrrolidone 15 as an additive. An increase in the concentration of the polymer additive up to 6% increased the resolution. Some interactions between the analytes and the polymer probably caused separation.

So far, derivatization of enantiomers with a chiral reagent, such as Marfey's reagent or GITC-reagent, followed by the separation by MEKC with SDS under neutral or alkaline conditions seems one of the most successful applications of the MEKC technique, taking advantage of the high efficiency and easy optimization of separation.

9. CONCLUSIONS

Most principles of the separation of enantiomers by CE techniques are the same as those in HPLC techniques. The additives or chiral selectors used in CE are simply borrowed from those used in HPLC, except for the chiral surfactants used in MEKC. However, the high efficiency of the CE techniques often makes them more advantageous than HPLC techniques. This is especially true when prederivatized diastereomers are to be separated. The interaction mechanism between the analyte and the chiral selector is not discussed in detail in this review, because the mechanism will be similar to that in other techniques if the chiral selector is the same. The

difference in the circumstance of the chiral selector between CE and HPLC may cause some significant differences in enantioselectivity: the chiral selector is usually immobilized on a solid support in HPLC, whereas it is usually free in the running buffer in CE. Some specially designed chiral selectors will soon become commercially available for CE separations of enantiomers. Recently, some ionic derivatives of CD became commercially available from different sources, and these compounds suggest the potential use of CE techniques taking advantage of the electrophoretic technique. Finally, we have to look forward to a significant increase in the detection sensitivity of the CE instruments in order to use the CE technique for practical analyses.

10. ABBREVIATIONS

ac-β-CD	Alkylcarbamoylated β-cyclodextrin
BSA	Bovine serum albumin
CBH I	Cellobiohydrolase I
CBI	Naphthalene-2,3-dicarboxaldehyde
CD	Cyclodextrin
CD-CZE	Cyclodextrin-modified capillary zone electorphoresis
CD-MEKC	Cyclodextrin-modified micellar electrokinetic chromatography
CE	Capillary electrophoresis
CGE	Capillary gel electrophoresis
18-C-6H_4	18-Crown-5-tetracarboxylic acid
CTAB	Cetyltrimethylammonium bromide
CZE	Capillary zone electrophoresis
DM-β-CD	Heptakis(2,6-di-O-methyl)-β-cyclodextrin
Dns	Dansyl (5-dimethylaminonaphthalene-1-sulphonyl)
EKC	Electrokinetic chromatography
GITC	2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate
HA	Human albumin
HEC	Hydroxyethylcellulose
HPLC	High-performance liquid chromatography
ITP	Isotachophoresis
Me-β-CD	Methylated β-cyclodextrin
MEKC	Micellar electrokinetic chromatography
PTH	Phenylthiohydantoin
SC	Sodium cholate
SDAla	Sodium N-dodecanoyl-L-alaninate
SDC	Sodium deoxycholate
SDS	Sodium dodecyl sulphate
SDVal	Sodium N-dodecanoyl-L-valinate
SFC	Supercritical fluid chromatography
STC	Sodium taurocholate
STDC	Sodium taurodeoxycholate
TM-β-CD	Heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin

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