ELSEVIER

Journal of Chromatography A, 735 (1996) 57–76

JOURNAL OF
CHROMATOGRAPHY A

Review

Enantiomer separation of drugs by electrokinetic chromatography

Hiroyuki Nishi

Analytical Research Laboratory, Tanabe Seiyaku Co., Ltd., 16-89, Kashima 3-chome, Yodogawa-ku, Osaka 532, Japan

Abstract

This review surveys the enantiomer separation of drugs by electrokinetic chromatography (EKC). EKC is one option of capillary electrophoretic (CE) techniques, which permits the separation of electrically neutral drugs. In enantiomer separation by EKC, ionic pseudo-stationary phases such as chiral micelles and proteins, which can migrate with its electrophoretic mobility and can interact with the solutes, are employed as chiral selectors. Addition of electrically neutral chiral selectors such as cyclodextrins and precolumn conversion of solutes into diastereomers are also successful in EKC. A brief theory of separation and modes of EKC enantiomer separation are described with some typical applications.

Keywords: Enantiomer separation; Reviews; Buffer composition; Drugs

Contents

1. Introduction 58
2. Enantiomer separation by electrokinetic chromatography 58
3. Micellar electrokinetic chromatography 60
   3.1. Bile salts and saponins 60
   3.2. Long-alkyl-chain surfactants 63
   3.3. High-molecular-mass surfactants 63
   3.4. Cyclodextrin-mediated micellar electrokinetic chromatography 66
4. Affinity electrokinetic chromatography 67
   4.1. Proteins 67
   4.2. Polysaccharides 68
   4.3. Antibiotics 70
5. Cyclodextrin electrokinetic chromatography 71
6. Enantiomer separation by derivatization to diastereomer 72
7. Conclusions 72
Abbreviations 73
References 74

0021-9673/96/$32.00 © 1996 Elsevier Science B.V. All rights reserved
SSDI 0021-9673(95)01262-1

1. Introduction

Enantiomer separations by capillary electrophoresis (CE) has rapidly attracted attention as a promising technique in the field of pharmaceutical sciences. Enantiomer separation can be achieved with one capillary tube (without a special chiral column and an organic solvent), high resolution and relatively fast separation. Furthermore, the amounts of sample and media required in CE techniques are extremely small. Among various different CE modes, capillary zone electrophoresis (CZE) and electrokinetic chromatography (EKC), in which only a chiral selector is added to the usual running buffer solution, are most widely used for enantiomer separations [1–5].

One of the most attractive advantages of CZE and EKC for the separation of enantiomers is easy changes of separation media in the method development, that is, one can easily alter the separation solution to find the optimum separation media and one can also use an expensive chiral selector because of the small amounts of media required. Electrically neutral chiral selectors are employed for the enantiomer separation of ionic solutes in CZE. Cyclodextrins (CDs) have been found to be most effective in CZE for a wide range of ionic drugs. On the other hand, charged pseudo-stationary phases are added to the running buffer solution in EKC. Therefore, EKC can be applied to both ionic and non-ionic solutes. CDs are also as effective in micellar EKC (CD-MEKC) as in CZE.

We have previously reported enantiomer separations by CE techniques, including EKC [4,5]. In this paper, we focus on the enantiomer separation of drugs by EKC modes, mainly micellar EKC (MEKC) and affinity EKC (AEKC). In the latter, biological components such as proteins or mucopolysaccharides are employed as novel chiral selectors. CD-EKC, in which charged CDs are employed as chiral pseudo-stationary phases, and enantiomer separation through the derivatization of the solutes to diastereomers are also mentioned. A brief separation theory of EKC and typical applications are described. Developments in the technology of novel chiral selectors have been reported in detail.

2. Enantiomer separation by electrokinetic chromatography

The EKC mode was first developed for the separation of electrically neutral solutes by the CE technique [6,7]. Originally micelles such as sodium dodecyl sulfate (SDS) were used for the separation of aromatic compounds [6–8]. This mode was termed micellar EKC or MEKC [9,10]. More recently, EKC, especially MEKC, has been used widely for the separation of both ionic and non-ionic drugs because of its different separation principle to HPLC [3]. In EKC, a buffer solution containing an ionic pseudo-stationary phase, which can migrate itself with a different electrophoretic mobility to the surrounding medium, is employed. According to the type of the pseudo-stationary phase, several EKC modes have been developed. These are summarized in Table 1 with the typical applicable solutes. Among them, micellar EKC has become the

Table 1
Modes of electrokinetic chromatography (EKC)

Mode	Abbreviation	Pseudo-stationary phase	Main applicable analyte	Refs.
Micellar EKC	MEKC	Micelle	Neutral and ionic compounds	[11–38]
CD-mediated MEKC	CD-MEKC	Micelle	Hydrophobic compounds, enantiomers	[39–54]
Microemulsion EKC	MEEKC	Microemulsion	Neutral and ionic compounds	[55–58]
Affinity EKC	AEKC	Protein, polysaccharide	Enantiomers	[76–93]
Cyclodextrin EKC	CD-EKC	Charged cyclodextrin	Enantiomers	[59–75]
Ion-exchange EKC	IEEKC	Ionic polymer	Ionic compounds	[9,94,95]
Ligand exchange EKC	LEEKC	Metal complex	Amino acids, carbohydrates	

most popular mode. Chiral surfactants and/or chiral additives such as CDs are usually employed in enantiomer separations by micellar EKC [11–38]. CDs have also been found to be effective for MEKC enantiomer separation using achiral surfactants such as SDS [39–54]. Microemulsion EKC (MEEKC) uses a microemulsion, which is prepared by mixing oil, water, a surfactant and a co-surfactant such as a medium-alkyl-chain alcohol, as pseudo-stationary phase [55–58]. (2*R*,3*R*)-Di-*n*-butyl tartrate was employed as a lipophilic chiral selector in an SDS–butanol–buffer system [57]. Charged CDs can also be regarded as ionic pseudo-stationary phases and will be classified as a branch of EKC (CD-EKC) from the viewpoint of EKC definition. Electrically neutral enantiomers in addition to ionic ones can be separated by CD-EKC [59–75]. Manipulation of the migration order of enantiomers can also be achieved easily in CD-EKC by changing the buffer pH or the type of capillary (coated or uncoated). Other than micelles, microemulsions and charged CDs, ionic biopolymers such as proteins [76–88] and mucopolysaccharides [89–93] have been successfully employed for EKC enantiomer separations. We call this mode affinity EKC (AEKC), because enantiomer separation is not dependent on simple ion exchange but multiple interaction (affinity). For the EKC mode, in which a simple ion-exchange interaction is essential, the term ion-exchange EKC (IEEKC) is used [9,94,95]. Any kind of ionic compound can be applicable in EKC modes as pseudo-stationary phases. Recently, antibiotics such as vancomycin [96,97], rifamycin B [98] and ristocetin A [99] have been successfully employed as chiral selectors in EKC enantiomer separations. This mode can also be regarded as a mode of EKC.

In EKC, the capacity factor k' for neutral solutes is given from chromatographic theory as [6]

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

where t_R, t_0 and t_{mc} are the migration times of the solute, the bulk solution and the pseudo-stationary phase, respectively. The migration behavior of neutral solutes can be also described from the viewpoint of electrophoretic theory as [100]

$$k' = \frac{\mu_{ep,S*}}{\mu_{ep,mc} - \mu_{ep,S*}} \tag{2}$$

where $\mu_{ep,S*}$ is the effective electrophoretic mobility of the solute in the presence of the pseudo-stationary phase [100] and $\mu_{ep,mc}$ is the electrophoretic mobility of the pseudo-stationary phase. The actual migration mobility of the neutral solute, μ_{S*}, is given as $\mu_{ep,S*} + \mu_{eo}$, where μ_{eo} is the electroosmotic mobility. The effective electrophoretic mobility of a neutral solute can be obtained experimentally from the determination of μ_{S*} and μ_{eo} without knowing the value of the electrophoretic mobility of the pseudo-stationary phase.

For charged solutes, the capacity factor, assuming ion-pair formation between the solute and the pseudo-stationary phase is absent, is given as [101,102]

$$k' = \frac{\mu_{ep,S*} - \mu_{ep,S}}{\mu_{ep,mc} - \mu_{ep,S*}} \tag{3}$$

where $\mu_{ep,S}$ is the electrophoretic mobility of the solute in the absence of the pseudo-stationary phase. If ion pairing is present, the equation of the capacity factor becomes more complex [103].

The resolution equation for the neutral enantiomeric pair 1 and 2 in EKC can be given as [6,7]

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

where N is the number of theoretical plates, α the separation factor, given by k'_2/k'_1, and k'_1 and k'_2 are the capacity factors of the enantiomeric pair. The resolution equation for neutral solutes can be also described with the effective mobility as in the resolution equation in CZE [104]:

$$R_s = \frac{N^{1/4}}{4}\left(\frac{\mu_{ep,S_1^*} - \mu_{ep,S_2^*}}{\bar{\mu}_{ep,S*} + \mu_{eo}}\right) \tag{5}$$

where μ_{ep,S_1^*}, μ_{ep,S_2^*} and $\bar{\mu}_{ep,S^*}$ are the effective electrophoretic mobilities of enantiomers 1 and 2 and the average effective electrophoretic mobility of the two solutes, respectively. For the separation of charged solutes, the resolution equation has not yet been derived, because the parameters are very complex.

For the maximum resolution, N, α and k' are the important factors, as shown in Eq. 4. The plate number depends on the capillary I.D., etc. However, it is impractical to manipulate the plate number, because its value is almost constant (around 100 000–500 000) for the investigated solute under the conditions employed, although the plate number depends on the capacity factor. The separation factor will be manipulated through the choice of pseudo-stationary phases, buffer solution, temperature, additives, etc. [105,106]. Usually in the process of the development of a CE enantiomer separation, one will try to use several running buffers containing different chiral selectors to find the optimum conditions for the enantiomer investigated. Once a pseudo-stationary phase, which is successful for the enantiorecognition of the solute, has been found, the capacity factor must be optimized. The effect of the capacity factor on resolution is shown in the last two functions of the right-hand side of Eq. 4. The optimum value of the capacity factor, k'_{opt}, can be obtained by differentiation as [107,108]

$$k'_{opt} = (t_{mc}/t_0)^{1/2} \quad (6)$$

In micellar EKC with a bile salt in neutral or alkaline solution, $t_{mc}/t_0 \approx 3$; hence $k'_{opt} \approx 1.7$. Generally, capacity factors between 1 and 5 are recommended for the most optimum resolution through changes in the concentration of the pseudo-stationary phase. An extra factor that affects the resolution other than those mentioned above is the effect of the electroosmotic flow, as in CZE. Under acidic conditions or in a coated capillary, electroosmosis is often suppressed considerably and the anionic pseudo-stationary phase may migrate in a different direction to the electroosmotic flow. From the last function in Eq. 4 or 5, when we can control the electroosmotic flow (μ_{eo}) giving close to $t_0/t_{mc} = -(1/k')$ or $-\mu_{ep,S^*}$, we can obtain an extremely high resolution at the expense of a longer analysis time. For the maximum resolution of charged solutes, we can reasonably assume that the resolution is a function of the same parameters as mentioned above. In a simple model proposed by Wren and Rowe [109,110] for the CZE separation of enantiomers with a neutral CD as a chiral additive, the optimum concentration of CD given the maximum mobility difference between the enantiomers, $\Delta\mu$, is given mathematically as

$$[C]_{opt} = 1/(K_1 K_2)^{1/2} \quad (7)$$

where K_1 and K_2 are formation constants for inclusion complexes. This model can be applied to the EKC of charged solutes [84,89], that is, there is an optimum selector concentration in EKC enantiomer separation, although $[C]_{opt}$ does not mean the concentration which gives the maximum resolution. Optimization of the EKC enantiomer separation can be achieved mainly through changes in buffer pH and the selector concentration.

3. Micellar electrokinetic chromatography

3.1. *Bile salts and saponins*

Bile salts are natural anionic surfactants found in biological components. Various bile salts are known such as non-conjugated and taurine- or glycine-conjugated types. They have steroidal structures and form helical micelles having reversed micelle conformations [111,112]. Since bile salts are optically active surfactants, enantiomer separation has been examined [11–20]. The bile acids used in MEKC enantiomer separation are summarized in Table 2. It should be noted that non-conjugated forms must be used at pH >5, although taurine-conjugated forms can be used even at pH 3. Fig. 1 shows the separation of enantiomers of diltiazem and its main decomposition product (desacetyl form) by MEKC with sodium taurodeoxycholate (STDC) [11]. Other

Table 2
Bile salts and saponins employed for MEKC enantiomer separation

Surfactant	CMC/10^{-3} M (25°C)	n	Separated enantiomer	Ref.
Bile salts				[11–20]
Sodium cholate (SC)	13–15	2–4	DNS-AAs, CBI-AAs, diltizem and its analogues, trimetoquinol and its analogues, binaphthyl compounds, carboline derivatives, fenoldopam, its analogs, baclofen (CBI derivatives), mephenitoin and its metabolite, aminophosphates, oxazepam, temazepam, lorazepam, lormetazepam, methylamphetamine, diclofensine, carbuterol, timolol, pindolol, metoprolol, clenbuterol, Cimaterol, nadolol, oxprenolol	
Sodium deoxycholate (SDC)	4–6	4–10		
Sodium taurocholate (STC)	10–15	5		
Sodium taurodeoxycholate (STDC)	2–6	–		
Saponins				[21,22]
Digitonin	Glycyrrhizic acid	β-Escin	PTH-AAs, DNS-AAs	

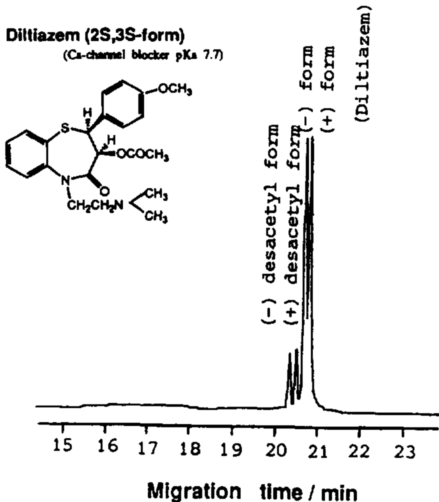

Fig. 1. Separation of enantiomers of diltiazem and its main decomposition product (desacetyl form) by MEKC with bile salts. Conditions: buffer, 50 m*M* STDC in 20 m*M* phosphate–borate buffer (pH 7.0); uncoated capillary, 50 cm effective length × 50 μm I.D.; detection wavelength, 210 nm; temperature, room temperature; applied voltage, +15 kV.

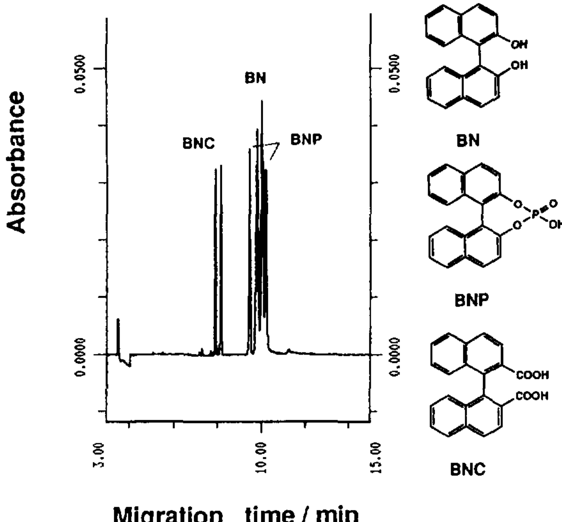

Fig. 2. Separation of enantiomers of three binaphthyl compounds by MEKC with bile salt and cyclodextrins. Conditions: buffer, 50 m*M* SDC and 10 m*M* α-CD in 20 m*M* phosphate–borate buffer (pH 8.0); uncoated capillary, 33 cm effective length × 75 μm I.D.; detection wavelength, 214 nm; temperature, 23°C; applied voltage, +15 kV. (From Ref. [20].)

diltiazem-related compounds [11], trimetoquinol and related compounds [11,12], carboline derivatives [13], binaphthyl compounds [11,13,14], dansylated amino acids (DNS-AAs) [15], etc., have also been successfully enantioseparated by bile salt micelles alone. Among four bile salts used in MEKC, STDC seems to be most effective for enantiomer separation. The mechanism of chiral discrimination by bile salts is not known. However, judged from the fused-ring structure of the successfully resolved compounds, a relatively rigid planar structure may be related to chiral discrimination [11–13].

The addition of CDs to bile salt micelle solutions (CD-MEKC) has also been successful. Baclofen and its analogs, naphthalene-2,3-dicarboxaldehyde-derivatized amino acids (CBI-AAs) [16], DNS-AAs [17,18], mephenytoin, fenoldopam [17], diclofensine, β-blockers such as atenolol, nadolol, oxprenolol, etc. [19], were enantioseparated by the CD-STDC system. A combination of CD with STDC appeared to produce a synergistic effect on enantiomer separation [16,19]. A typical example is shown in Fig. 2. 1,1′-Binaphthyl-2,2′-dicarboxlic acid (BNC), which has two carboxyl groups in the molecule and could not be separated by bile salts alone owing to weak solubilization to bile salt micelles, was successfully enantioseparated through the addition of α-CD to sodium deoxycholate (SDC) [20]. Other than enantiomer separation, MEKC with bile salts has permitted the separation of hydrophobic drugs such as corticosteroids, which could not be separated by MEKC with long-alkyl-chain surfactants [113]. Aromatic choline esters [114], bilirubin [115], polycyclic aromatic hydrocarbons [112], phthalate esters [116] and 1,4-benozodaizepines [117] were also successful separated by MEKC with bile salts. This can probably be ascribed to the relatively weak solubilization power. In the separation of 1,4-benzodiazepine by MEKC with bile salts, peak splitting due to enantiomer separation was observed and this chiral recognition was suppressed by increasing the column temperature [117].

Other than bile salts, some saponins such as digitonin [21], glycyrrhizic acid and β-escin [22], which are all natural chiral surfactants, have been employed in MEKC enantiomer separation

of DNS-AAs and phenylthiohydantoin amino acids (PTH-AAs). In all cases, a mixed micelle of saponin and SDS was essential for enantiomer separation. Many other chiral surfactants from natural sources can be applicable, even though they are non-ionic, by using a mixed micelle system mentioned above.

3.2. Long-alkyl-chain surfactants

Optically active amino acid-derived synthetic surfactants have been employed for enantiomer separation by MEKC [21,23–33]. Typically, acylamino acids such as sodium N-dodecanoyl-L-valinate (SDVal) [21,23–27], sodium N-dodecanoyl-L-alaninate (SDAla) [24], sodium N-dodecanoyl-L-glutamate (SDGlu) [27], N-dodecanoyl-L-serine (DSer) [28] and sodium N-dodecanoyl-L-threoninate (SDThr) [29], which are now mostly commercially available [30], have been used for the enantiomer separation of amino acid derivatives, warfarin and benzoin. An organic solvent such as methanol and/or urea is sometimes added to the micellar solution to obtain peak symmetry. Long-alkyl-chain type chiral surfactants used for MEKC enantiomer separation are summarized in Table 3. Recently, (*R*)- and (*S*)-N-dodecoxycarbonylvaline were employed for the separation of some β-blockers, adrenergic drugs [30,32] and 1-phenyl-3-methyl-5-pyrazolone (PMP)-derivatized DL-glucose (epimer) [33] (see Fig. 3). The separation of benzoin by MEKC with (*S*)-2-[(dodecoxycarbony)amino]-3(*S*)-methyl-1-sulfooxypentane has also been reported [32]. More effective active amino acid-derived surfactants will be designed and synthesized from the investigation of enantiorecognition by MEKC with the above-mentioned surfactants in the near future.

Second, surfactants having a sugar head group such as octyl-β-D-glucopyranoside have been employed in MEKC with SDS [34] or borate buffer [118–120]. Some DNS-AAs were enantioseparated by the former alkylglucoside–SDS system. In the latter system, in situ charged micelles are formed in the presence of alkaline borate and it is not necessary to add an ionic surfactant to form a mixed micelle. The surface charge density of the micelle can be adjustable by utilizing the complexation between alkylglucoside and alkaline borate. Several DNS-AAs were enantioseparated by adding γ-CD to decanoyl-N-methylglucamide and borate at pH 10 [120]. Recently, surfactants having ionic sugar head were employed for MEKC enantiomer separation, viz., dodecyl-β-D-glucopyranoside monophosphate and dodecyl-β-D-glucopyranoside monosulfate surfactants [35]. Several DNS-AAs, cromakalin, binaphthyl compounds, mephenytoin, etc., were successfully enantioseparated by MEKC with the glucopyranoside-based surfactants.

Phospholipids, which are also biological chiral components along with amino acids and sugars, have been applied to MEKC as chiral selectors [36]. Several DNS-AAs were successfully enantioseparated by MEKC with L-α-palmitoyllysophosphatidylcholine, which is an optically active zwitterionic biosurfactant. In this case, a coated capillary was employed to avoid adsorption of the surfactant on the wall.

3.3. High-molecular-mass surfactants

Recently, high-molecular-mass surfactants have been extensively investigated [121–124]. Such surfactants can form a micelle with one molecule, which leads to some advantages over normal low-molecular-mass surfactants, e.g., enhanced stabilities and rigidities and controllable size. The critical micelle concentration (CMC) is essentially zero, and therefore the micelle concentration is constant irrespective of temperature, buffer concentration and additives, etc. A high content of organic solvent will not break the micelle. Furthermore, no monomeric surfactant that may deteriorate the enantioselectivity in CD-MEKC is present. The absence of a monomeric surfactant will be also advantageous for CE–MS analysis.

In MEKC enantiomer separation, poly(sodium N-undecylenyl-L-valinate) (polySUVal) was employed for the separation of binaphthol, laudanosine [37] and 3,5-dinitrobenzoyl-derivatized AAs [29]. Polymerization of the vinyl

Table 3
Long alkyl-chain type chiral surfactants used for MEKC

Surfactant	CMC/10^{-3} *M* (water)	*n*	Separated enantiomer	Ref.
Acylamino acid surfactants				[21,23–29]
Sodium N-dodecanoyl-L-valinate (SDVal)[a]	6.2	498	PTH-AAs,	
Sodium N-dodecanoyl-L-alaninate (SDAla)	12.5	–	benzoin,	
Sodium N-dodecanoyl-L-glutamate (SDGlu)	–	–	warfarin,	
N-Dodecanoyl-L-serine (DSer)	–	–	amino acid derivatives,	
Sodium N-dodecanoyl-L-threoninate (SDThr)	–	–	(N-benzoyl-O-isopropyl, etc.),	
Sodium N-undecylenyl-L-valinate (SUVal)	36	–	laudanosine,	
			binaphthyl compounds	
Other amino acid type surfactants				[31–33]
(*R*)-N-dodecoxycarbonylvaline	–	–	Atenolol, ketamine, benzoin,	
(*S*)-N-dodecoxycarbonylvaline[b]	–	–	bupivacaine, metoprolol,	
(*S*)-2-[(dodecoxycarbony)amino]-3(*S*)-methyl-1-sulfooxypentane	–	–	homatropin, terbutaline,	
			ephedrine, pindolol, octopamine,	
			norephedrine, pseudoephedrine,	
			N-methylpseudoephedrine,	
			norphenylephrine,	
			PMP-derivatized glucose	
Glucopyranoside-based surfactants				[34,35,120]
n-Heptyl-β-D-thioglucopyranoside	30	–	DNS-AAs, metoprolol,	
n-Octyl-β-D-glucopyranoside	24	–	cromakilin, Troger's base,	
n-Octyl-β-D-thioglucopyranoside	9	–	ephedrine, hexobarbital,	
n-Dodecyl-β-D-glucopyranoside	2	–	mephenytoin, fenoldopam,	
n-Dodecyl-β-D-glucopyranoside monophosphate	0.5	–	hydroxymephenytoin,	
n-Dodecyl-β-D-glucopyranoside monosulfate[c]	1	–	binaphthyl compounds	
Phospholipids				[36]
L-α-Palmitoyllysophosphatidylcholine	–	–	DNS-AAs	

[a] R_1 = $-CH(CH_3)_2$ (SDVal)

[b] R_1 = $-CH(CH_3)_2$

[c] (structure with OSO_3Na)

$-OCO(CH_2)_{14}CH_3$; $-OCO(CH_2)_{14}CH_3$; $-O-P(=O)(O^-)-O-CH_2CH_2N^+(CH_3)_3$

group-terminated chiral surfactant monomer was achieved by ^{60}Co γ-irradiation [37] or UV irradiation [29]. The average molecular mass of polySUVal by gel permeation chromatographic (GPC) analysis was 13 600 [29]. Schematic illustrations of normal and polymer-type micelles are shown in Fig. 4. A polymer-type chiral surfactant gave a better resolution than the corresponding monomer-type surfactant at the same concentration. An example is shown in Fig. 5 [37]. However, this type of polymer has been found to undergo a conformational transition at around pH 8.5 [37]. At lower pH, the negatively charged polySUVal micelle has a compact formation,

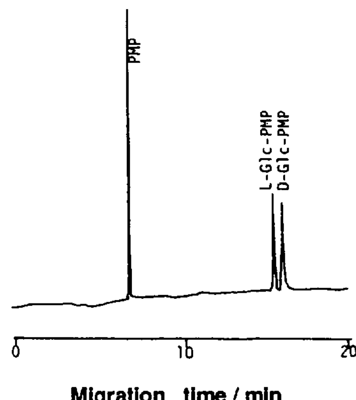

Fig. 3. Separation of epimers of PMP derivatives of DL-glucose by MEKC with N-dodecoxycarbonyl-L-valine. Conditions: buffer, 50 mM N-dodecoxycarbonyl-L-valine in 50 mM phosphate buffer (pH 7.0); uncoated capillary, 75 cm effective length × 50 μm I.D.; detection wavelenth, 245 nm; temperature, room temperature; applied voltage, +20 kV. (From Ref. [33].)

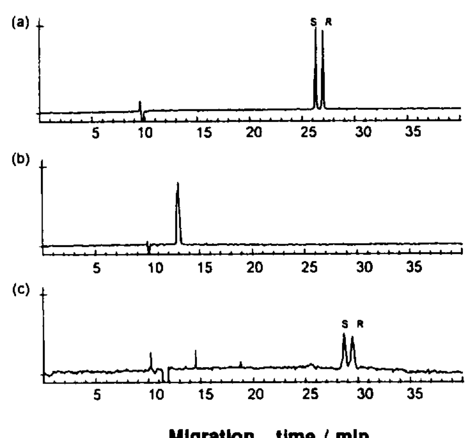

Fig. 5. Separation of enantiomers of binapthyl compounds by MEKC with normal or polymerized chiral micelles. (a) 0.5% poly(SUVal); (b) 0.5% SUVal; (c) 1% SUVal. Conditions: buffer, 25 mM borate buffer (pH 9.0); uncoated capillary, 60 cm effective length × 75 μm I.D.; detection wavelength, 290 nm; applied voltage, +12 kV. (From Ref. [37].)

whereas at high pH it may have a looser conformation because of the electrostatic repulsion. The looser conformation of the micelle leads to better enantioseparation.

A copolymer of butyl acrylate, butyl methacrylate and methacrylic acid (BBMA), with a molecular mass of about 40 000, was also em-

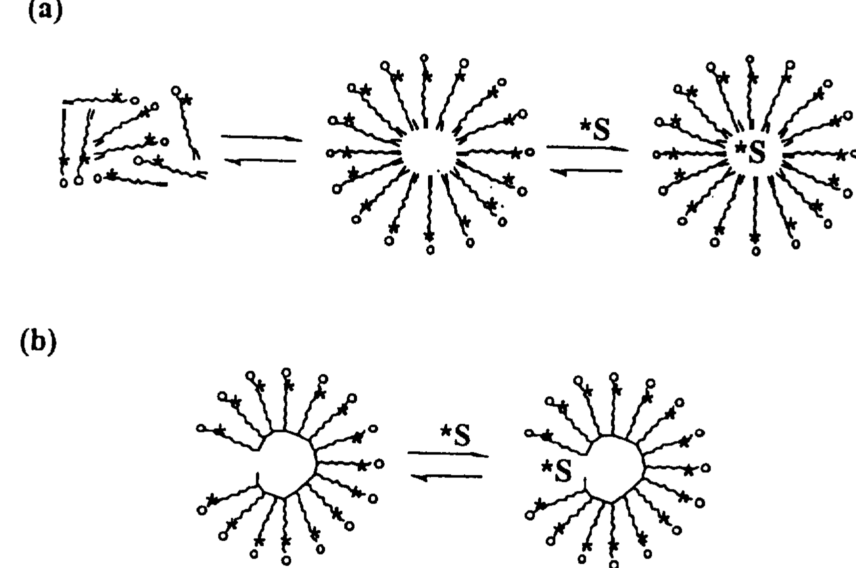

Fig. 4. Schematic illustrations of (a) normal non-polymerized micelles and (b) polymer-type micelles of sodium N-undecylenyl-L-valinate. S, solute; asterisk denotes chiral center. (From Ref. [37]).

ployed in MEKC enantiomer separation by adding CDs (CD-MEKC). Enantiomer separation of DNS-AAa by MEKC with β-CD–BBMA was much better than that in the β-CD–SDS system [38].

3.4. Cyclodextrin-mediated micellar electrokinetic chromatography

CDs are effective additives in MEKC enantiomer separations as in CZE enantiomer separations. MEKC with CDs and a chiral surfactant was described above. In this section, enantiomer separations by MEKC with CDs and an achiral surfactant such as SDS are described. Amino acid derivatives with DNS [39–41], CBI [42,43] or 9-fluorenylmethyl chloroformate (FMOC) [44], cicletanine [45,46], pentobarbital, thiopental [47], diniconazole, uniconazole [48,49], β-blockers [50], etc., were enantioseparated by CD-MEKC with SDS. The reported enantiomer separations by CD-MEKC are summarized in Table 4 [39–54]. Mostly γ or β-type CDs were employed in CD-MEKC, probably because the cavity size is close to the size of a benzene or naphthalene ring. In general, γ-CD seems to be most effective for a wide range of enantiomeric solutes in CD-MEKC. This can probably be ascribed to the presence of the monomeric surfactant, which will be included in CD cavity and will disturb the solute inclusion or interaction. In contrast, addition of some other chiral additives such as l-menthoxyacetic acid and *d*-camphor-10-sulphonate to an SDS and CD solution improved the enatioseparation [47]. They may be included or interact with CD, resulting in more effective conditions for the enantiorecognition of the solute.

Table 4
Enantiomer separations by CD-MEKC with achiral surfactants

Cyclodextrin	Surfactant	pH	Additives	Separated enantiomer	Refs.
β-CD, γ-CD	SDS	8.6	Methanol	DNS-AAs	[39–41]
β-CD, γ-CD	SDS	9.0		CBI-AAs	[42,43]
β-CD, γ-CD	SDS	8.6	Acetonitrile	Cicletanine	[45,46]
β-CD, DM-β-CD	SDS	7.0	Urea, methanol	Hexobarbital, fadrozole	[51]
DM-β-CD	SDS	7.0	Urea, methanol	Aminoglutethimide analogues	[51]
β-CD	SDS	7.0	Urea, methanol	Mephobarbital, glutethimide	[51]
γ-CD	SDS	7.0	Urea, methanol	Secobarbital	[51]
γ-CD	SDS	9.0	*l*-Menthoxyacetic acid *d*-Camphorsulfonic acid	Pentobarbital, thiopental, binaphthyl compounds, trifluoroanthrylethanol	[47]
γ-CD	SDS	9.0	Urea, 2-methyl-2-propanol	Uniconazole	[49]
γ-CD, DM-β-CD	SDS	9.0	Urea, acetonitrile	Diniconazole	[48]
α-CD + β-CD	SDS	9.3		Alprenolol, atenolol, propranolol	[50]
HP-β-CD	SDS	9.3		BMS-180431-09 (cholesterol-lowering drug)	[52]
β-CD	SDS	9.0	Urea	SM-8849, analogs (immunomodulating antirheumatic drug)	[53]
β-CD	SDS	9.0		Aminoglutethimide	[54]
β-CD	SDS	7.0	2-Propanol	FMOC-AAs	[44]

4. Affinity electrokinetic chromatography

4.1. Proteins

Proteins have been found to be effective chiral selectors in both HPLC and CE. Most proteins used for chiral columns in HPLC have already been employed in CE. A protein has charge and consequently has electrophoretic mobility. Therefore, proteins can be used for the separation of neutral solutes and this mode can be classified as a branch of EKC. We term this mode affinity EKC (AEKC) because an affinity interaction may operate on the separation. Proteins used in CE enantiomer separations are summarized in Table 5 [76–88]. Other proteins, which are valuable and/or expensive, can also be used in CE because only small amounts are required. A typical example of enantiomer separation by AEKC with avidin is shown in Fig. 6 [84]. In AEKC with proteins, the most important factors for enantioseparation are the buffer pH and the protein concentration. Depending on the solute pK_a and protein pI, the buffer pH must be selected and typically 100 μM of protein are added. Organic modifiers such as methanol, ethanol and 2-propanol, which are miscible with buffer and used for reversed-phase HPLC, have been also found to be important factors for improving the enantioseparation [84].

From the viewpoint of practical use, AEKC with proteins has been used for enantiomer separation principally because of some problems: (1) adsorption of the protein on the capillary

Table 5
Proteins employed for AEKC enantiomer separation

Protein	Molecular mass	pI	Carbohydrate (%)	Separated enantiomer	Refs.
BSA	67 000	4.7	–	Benzoin, DNS-AAs, ibuprofen, leucovorin, warfarin, Trp, promethazine, mandelic acid, homochlorcyclizine, propranolol, trimebutine, epinastine, DNP-AAs, ofloxacin, DR-3862, oxyphencyclimine	[76–81]
HSA	68 000	4.7	–	2,3-Dibenozoyl tartaric acid, Trp, N-2,4-dinitrophenyl glutamic acid, 3-iodolelactic acid, kynurenine, benzoin, promethazine thioridazine	[82,87,88]
Fungal cellulase	60 000–70 000	3.9	6	Pindolol	[78]
Cellobiohydrolase I	60 000–70 000	3.9	6	Alprenolol, labetolol, pindolol, metoprolol, propranolol	[83]
α_1-AGP	44 000	2.7	45	Promethazine, clorprenaline, DNP-AAs	[78–81]
Avidin	70 000	10	20.5	Vanillylmandelic acid, warfarin, atrolactic acid, flurbiprofen, ketoprofen, ibuprofen, iopanoic acid, leucovorin	[84]
OVM	28 000	4.5	30	Benozoin, chlorpheniramine, eperisone, tolperisone, atorinolol, epinastine, bunitrolol, pindolol, oxyphencyclimine, tolperisone, DNP-AAs, verapamil, primaquine, trimebutine	[79,81,85]
Conalbumin	–	–	–	Trimetoquinol	[79]
Casein (α-casein)	26 200	4.7	–	DNP-AAs	[81]
Ovoglycoprotein	25 000	3.9	20	Chlorpheniramine, tolperisone	[86]

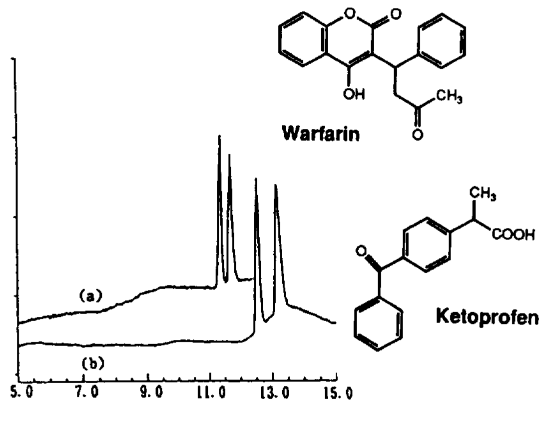

Fig. 6. Separation of enantiomers of (a) warfarin and (b) ketoprofen by AEKC with avidin. Conditions: buffer, 25 μ*M* avidin in 50 m*M* phosphate buffer (pH 6.0) containing 10% 2-propanol; coated capillary, 31.5 cm effective length × 50 μm I.D.; detection wavelength, (a) 300 and (b) 260 nm; temperature, 25°C; applied voltage, −12 kV. (From Ref. [84].)

wall; (2) absorption of UV light in the shorter wavelength region; and (3) low performance (low peak theoretical plate number). Concerning point (1), coated capillaries are used and some of them are commercially available [125]. A capillary coating is also achieved with an ease according to the literature [126,127]. Some approaches in which proteins do not pass the detection cell and do not interfere with the solute detection have been reported. One technique employed a gel plug to prevent hydrodynamic flow [83] and another is named the "partial separation zone technique" [79]. In the latter method, the capillary was partially filled with a solution containing a protein and the protein was not in the detector cell when solutes reached that cell. This technique can be performed automatically by using a commercial CE instrument. For broad peak shapes, addition of an organic modifier is a remedy. However, an asymmetric peak shape like a triangle, especially in the secondly migrated enantiomer, seems to be due to slow kinetics of the protein–solute interaction as in protein chiral columns.

Other than separation, AEKC with proteins is an attractive mode for investigating the interaction of drugs with the protein. As in chromatography, there are a variety of ways in which binding studies may be performed. One is achieved by injecting the protein as a solute into the running buffer solution containing various concentrations of ligand (drug), and then performing a Scatchard analysis of the data [128–130]. The other is AEKC using proteins as chiral additives. The advantage of the latter method is that the racemic drugs may be separated if there is stereoselectivity in the binding. A simple model as in CD-CZE can be used in AEKC, making an assumption of one-to-one binding. The apparent mobility of the solute, μ_S^*, is given as

$$\mu_S^* = \mu_{eo} + \frac{\mu_S + \mu_{PS}[P]}{1 + K[P]} \quad (7)$$

where μ_S and μ_{PS} are electrophoretic mobilities of the free solute and the protein–solute complex, respectively, K is the binding constant and [P] is the free protein concentration. Typically the protein concentration is varied over a wide range, and a non-linear least-squares curve fitting procedure is used to model the data to Eq. 7 [79,87,88].

4.2. Polysaccharides

Polysaccharides such as amylose and cellulose have been found to be effective for enantiomer separations in HPLC [131,132]. For CE enantiomer separation, electrically neutral maltodextrins were first employed in CE enantiomer separation (CZE) [133–135]. Recently, ionic polysaccharides were successfully employed as chiral selectors in CE. These are dextran sulfate [89,90] and mucopolysaccharides such as heparin [89–93] and chondroitin sulfate C [90,91]. These ionic polysaccharides are biopolymers and can be applicable to neutral solutes. Thus, ionic polysaccharides such as mucopolysaccharides can be included as chiral selectors of AEKC. The unit structures of these ionic polysaccharides are shown in Fig. 7 [136] and some characteristics are summarized in Table 6. Enantiomers of trimetoquinol, diltiazem, propranolol [91], several antimalarial drugs, antihistamines, etc. [92], were

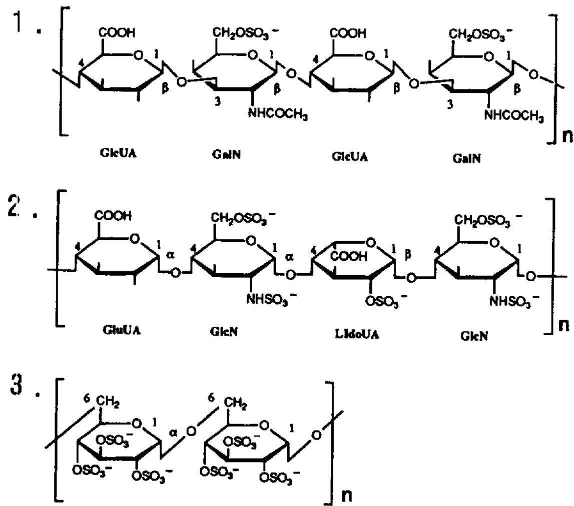

Fig. 7. Unit structures of ionic polysaccharides employed for AEKC enantiomer separation. (1) Chondroitin sulfate C; (2) heparin; (3) dextran sulfate. (From Ref. [136].)

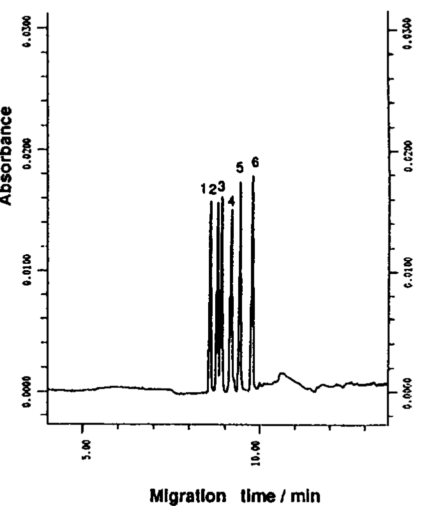

Fig. 8. Separation of diltiazem and clentiazem by AKEC with chondroitin sulfate C. Conditions: buffer, 3% chondroitin sulfate C in 20 m*M* phosphate–borate buffer (pH 4.0); uncoated capillary, 50 cm effective length × 75 μm I.D.; detection wavelength, 235 nm; temperature, 23°C; applied voltage, +20 kV. Solutes: 1 and 2 = (2*S*,3*R*)- and (2*R*,3*S*)-diltiazem; 3 = (2*S*,3*S*)-diltiazem; 4 = (2*R*,3*R*)-diltiazem; 5 = (2*S*,3*S*)-clentiazem; 6 = (2*R*,3*R*)-clentiazem. (From Ref. [90].)

successfully separated by AEKC with ionic polysaccharides. A typical example by AEKC with chondroitin sulfate C is shown in Fig. 8 [90]. The

Table 6
Ionic polysaccharides employed for AEKC enantiomer separation

Polysaccharide	Molecular mass	S content (%)	Separated enantiomer	Refs.
Chondroitin sulfate C	30 000–50 000	7	Diltiazem, chlorodiltiazem, trimetoquinol, trimetoquinol isomer, laudanisine, norlaudanosoline, laudanosine, sulconazole, primaquine, propranolol, verapamil	[90,91,138]
Chondroitin sulfate A	30 000–50 000	7	Diltiazem, clentiazem, trimetoquinol, verapamil, primaquine, sulconozole	[138]
Heparin	7000–20 000	11	Diltiazem, chlorodiltiazem, chlorpheniramine, trimetoquinol, oxamniquine, chloroquine, primaquine, mefloquine, enpiroline, pheniramine, carbinoxamine, doxylamine, dimethindene, tetramisole, tryptophan methyl ester, anabasine, nornicotine	[89–93]
Dextran sulfate	~7300	18	Trimetoquinol, trimetoquinol isomer, diltiazem, chlorodiltiazem, laudanosine	[89,90]

separation of possible four enantiomers of diltiazem was first achieved by this mode.

Among three type ionic polysaccharides, dextran sulfate and heparin must be employed under neutral conditions because of their strong ionic character. Basic solutes were not detected at the negative end below pH 5.0, indicating that the solute binding to ionic polysaccharides migrated very slowly or toward the positive end. On the other hand, chondroitin sulfates could be usable under both acidic and neutral conditions [90,91]. This may be ascribed to the weak ionic interaction due to the smaller ionic residues per unit structure (see Fig. 7). The wider applicability of chondroitin sulfate C permitted the enantiomer separation of a wide variety of compounds.

The separation mechanism of AEKC with ionic polysaccharides is not clear, as in AEKC with proteins. Multiple interactions such as ionic interactions by sulfate or carboxyl groups, hydrophobic or hydrogen bonding through glucose units (i.e., affinity) must contribute the enantiorecognition. Recently, dextran and dextrin, which are both neutral polysaccharides and have high molecular masses, have been found to be effective in CE enantiomer separations [137]. However, AEKC with mucopolysaccharides gave better enantiorecognition than CZE with dextran or dextrin [138]. This may indicate that ionic interactions together with hydrophobic interactions are important for successful enantiorecognition.

4.3. Antibiotics

Recently, some macrocyclic antibiotics such as rifamycin B [98] and vancomycin [96] have been employed for CE enantiomer separations. These are natural compounds having molecular masses of 700–2100 and can be utilized in either charged or uncharged states. Therefore, macrocyclic mediated CE can be regarded as an AEKC mode. Some characteristics of macrocyclic antibiotics employed in EKC enantiomer separations are summarized in Table 7 [96–99]. They have multiple stereogenic centers in addition to a variety of functional groups that are known to be advantageous for enantiorecognition: a rigid "pocket" that can provide a site for hydrophobic interactions and polar, flexible arms (i.e., pendent sugar moieties) that can rotate and form hydrogen bonds, etc. As an example, the structure of vancomycin is shown in Fig. 9. Over 100 racemates, including a variety of N-blocked amino acids and non-steroidal anti-inflammatory drugs, have been successfully separated by AEKC with antibiotics. However, in this mode, the UV detection wavelength must be set at over ca. 260 nm because of its strong absorption below 250

Fig. 9. Structure of vancomycin.

Table 7
Macrocyclic antibiotics used for AEKC enantiomer separation

Macrocyclic antibiotic	Molecular mass	pK	pI	Sugar units	Refs.
Vancomycin ($C_{66}Cl_2N_9O_{24}$)	1449	2.9, 7.2, 8.6, 9.6, 10.5, 11.7	7.2	2	[96,97]
Ristocetin A ($C_{95}H_{110}N_8O_{44}$)	2066	–	7.5	6	[99]
Rifamycin B ($C_{39}H_{49}NO_{14}$)	756	2.8, 6.7	–	0	[98]

nm. The stability of these selectors is also an important consideration. There must be a possibility for a better chiral selector usable at low UV regions without loss of the great enantioselectivity of macrocyclic antibiotics. The separation efficiency was much increased by adding SDS to the buffer solution containing vancomycin, as in CD-MEKC [97]. Analysis times were decreased and a reversed migration order of the enantiomers was observed.

5. Cyclodextrin electrokinetic chromatography

Charged CDs have been successfully employed for enantiomer separations of both ionic and non-ionic solutes [9,59–75]. Reported charged CDs used for enantiomer separations are summarized in Table 8. Most of them are commercially available [139]. Compared with native or electrically neutral CDs (i.e., CD-CZE mode), enantiorecognition of charged CDs seems to be better judged from the number of enantioseparated solutes [73,75]. Especially phosphated and sulfated β-CD and γ-CD have been found to be successful for a wide range of enantiomeric solutes. This may be ascribed to the ionic effect in the process of inclusion, leading to the enhancement of the difference in the stability of the complexed compound. Typical separations of some drugs by CD-EKC with CD phosphates are shown in Fig. 10 [75]. α-CD phosphate was effective for the enantioseparation of chlorpheniramine, which was not separated using neutral α-type CDs in HPLC and CZE. CZE enantiomer separation by using neutral α-type CDs has been found to be effective for only very few compounds, such as BNC [75,140]. Generally, the cavity size of α-type CDs does not fit the size of the drugs, which are typically small aromatic compounds. Denopamine, for which β-type CDs have been found to be essential for CZE enantioseparation using neutral CDs [141,142], was separated by CD-EKC with γ-CD phosphate. Furthermore, the concentrations of phosphated CDs used were much lower than those usually required for electrically neutral CDs. These

Table 8
Charged CDs employed for CD-EKC

Charged cyclodextrin	Degree of substitution (per CD ring)	Average molecular mass	Refs.
Cationic			
Mono(6-β-aminoethylamino-6-deoxy)-β-CD	1	ca. 1200	[9]
6^A-Methylamino-β-CD	1	ca. 1170	[59]
6^A,6^D-Dimethylamino-β-CD	2	ca. 1200	[59]
Anionic			
α-CD phosphate sodium salt	6	1651	[75]
2-O-Carboxymethyl-β-CD	1	ca. 1200	[60]
Carboxymethyethyated β-CD	–	–	[61]
Carboxymethylated β-CD	3.5	ca. 1330	[62–65, 72, 73]
Carboxymethylated β-CD polymer	–	ca. 10000	[64]
Carboxyethylated β-CD	6	ca. 1570	[63–65]
Succinylated β-CD	3.5	1493	[63,64]
Sulfobutyl ether(IV) β-CD sodium salt	3.5	1683	[66–72,73]
Sulfoethyl ether β-CD sodium salt	0.4	ca. 1200	[72]
β-CD phosphate sodium salt	6	1879	[20,73]
Carboxymethylated γ-CD	3.2	1472	[73]
γ-CD phosphate sodium salt	6	1997	[73,75]
Sulfated β-CD sodium salt	7–11	ca. 1900	[74,75]

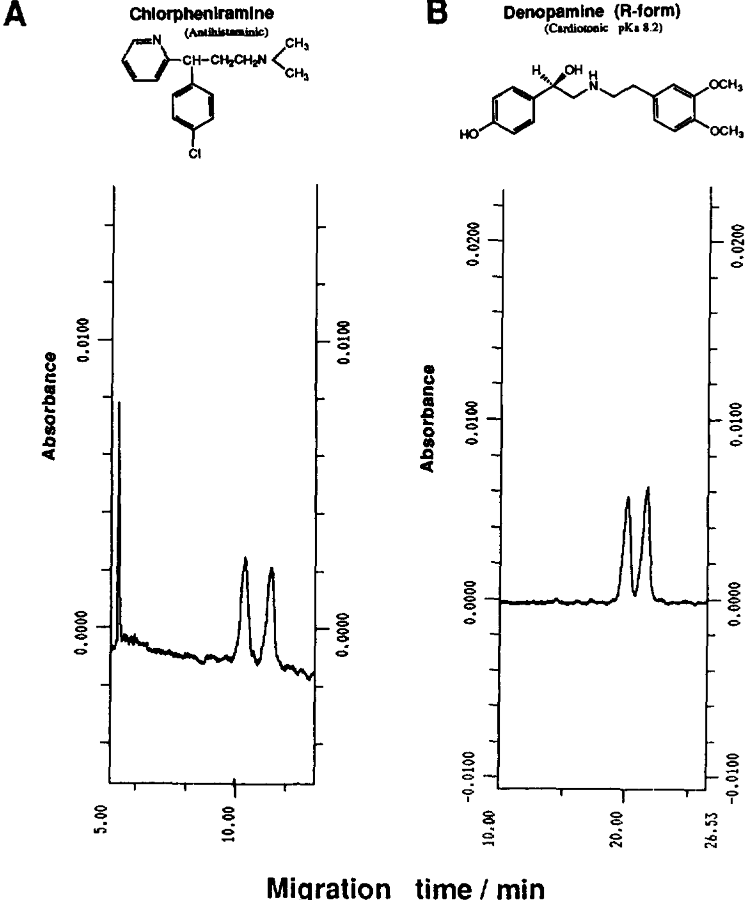

Fig. 10. Enantiomer separation of (A) chlorpheniramine and (B) denopamine by MEKC with CD phosphates. Conditions: buffer, (A) 5 mM α-CD phosphate and (B) 5 mM γ-CD phosphate in 20 mM phosphate–borate buffer (pH 6.5); coated capillary, 50 cm effective length × 75 μm I.D.; detection wavelength, 220 nm; temperature, 20°C; applied voltage, −20 kV.

results may be interpreted by the effective ionic interaction mentioned above.

6. Enantiomer separation by derivatization to diastereomer

Diastereomeric analytes have been successfully separated by EKC as in reversed-phase HPLC analysis. The same chiral derivatization reagent as used for HPLC analysis and typically MEKC with SDS have been employed, as summarized in Table 9 [108,143–148]. Although CZE can be applicable and some papers have reported diastereomer separations by CZE utilizing polyvinylpyrrolidone [149,150], poly(ethylene glycol) [151] or CD [148], MEKC must be employed because of its wider selectivity and easy manipulation of selectivity. For example, for the separation of a large number of derivatized amino acids in a single run, MEKC with SDS and/or CD will be successful.

7. Conclusions

CE enantiomer separations, including EKC and CZE, have many advantages over HPLC enantiomer separations. It is usually easy to find successful conditions for the enantiomer sepa-

Table 9
Separation of diastereomers by MEKC

Chiral derivatization	Analyte	Mode	Ref.
Marfey's reagent	AAs, dipeptides	MEKC with SDS	[143]
GITC reagent	AAs	MEKC with SDS	[108]
GITC reagent	Amphetamine, methamphetamine, ephedrine, pseudoephedrine, norephedrine, pseudonorephedrine,	MEKC with SDS	[147]
GITC reagent	3-Aminoalkyl-6-carboxamido--1,2,3,4-tetrahydrocarbazole (serotonin agonist)	MEKC with STDC	[146]
OPA and TATG reagent	AAs	MEKC with SDS	[145]
OPA and N-acetyl-L-cysteine or Boc-L-cysteine	AAs	MEKC with SDS	[144]
OPA or CBI and N-acetyl-L-cysteine or N-acetyl-D-penicillamine	Amphetamine, heptaminol, norephedrine	MEKC with SDS	[148]
OPA or CBI and norephedrine or L-Phe or L-Tyr or DOPA	*rac*-N-Acetyl-D-penicillamine, *rac*-N-(2-mercaptopropionic)acid, *rac*-N-(2-mercaptopropionic)glycine	MEKC with SDS	[148]

ration of an investigating analyte. Most enantiomers have been separated by applying one of several CE modes. Chiral method development kits are now commercially available from several sources. Although the reproducibilities of the migration times and peak areas and the sensitivity of UV detectors are inferior to those given by fully automated modern HPLC instruments, the performance of CE enantiomer separation is satisfactory for optical purity testing of drugs. The separation principle of EKC is essentially the same as that applied in HPLC. Most chiral selectors employed for EKC have already been employed for HPLC, except for the chiral surfactants. However, in CE, a novel chiral selector will be more easily applicable. There are great possibilities for a specially designed selector having wide enantioselectivity.

Abbreviations

α_1-AGP	α_1-Acid glycoprotein
AEKC	Affinity electrokinetic chromatography
BBMA	Butyl acrylate–butyl methacrylate–methacrylic acid copolymer
BN	1,1′-Bi-2-naphthol
BNC	1,1′-Binaphthyl-2,2′-dicarboxylic acid
BNP	1,1′-Binaphthyl-2,2′-diyl hydrogenphosphate
BSA	Bovine serum albumin
CBI-AAs	Naphthalene-2,3-dicarboxaldehyde
CD(s)	Cyclodextrin(s)
CD-EKC	Cyclodextrin electrokinetic chromatography
CD-MEKC	Cyclodextrin-mediated micellar electrokinetic chromatography
CE	Capillary electrophoresis
CE–MS	Capillary electrophoresis–mass spectrometry
CMC	Critical micelle concentration
CZE	Capillary zone electrophoresis
DNS-AAs	Dansylated amino acids
DSer	N-Dodecanoyl-L-serine
EKC	Electrokinetic chromatography
FMOC	9-Fluorenylmethyl chloroformate
GITC	2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate
GPC	Gel permeation chromatography
HPLC	High-performance liquid chromatography
HSA	Human serum albumin

IEEKC	Ion-exchange electrokinetic chromatography
LEEKC	Ligand-exchange electrokinetic chromatography
MEEKC	Microemulsion electrokinetic chromatography
MEKC	Micellar electrokinetic chromatography
OPA	*o*-Phthalaldehyde
OVM	Ovomucoid
PMP	1-Phenyl-3-methyl-5-pyrazolone
poly(SUVal)	Poly(sodium N-undecylenyl-L-valinate)
PTH-AAs	Phenylthiohydantoin-derivatized amino acids
SC	Sodium cholate
SDAla	Sodium N-dodecanoyl-L-alaninate
SDC	Sodium deoxycholate
SDGlu	Sodium N-dodecanoyl-L-glutamate
SDS	Sodium dodecyl sulfate
SDThr	Sodium N-dodecanoyl-L-threoninate
SDVal	Sodium N-dodecanoyl-L-valinate
STC	Sodium taurocholate
STDC	Sodium taurodeoxycholate
SUVal	Sodium N-undecylenyl-L-valinate
TATG	2,3,4,6-Tetra-O-acetyl-1-thio-β-D-glucopyranose

References

[1] T.J. Ward, Anal. Chem., 66 (1994) 633A–640A.
[2] M. Novotny, H. Soini and M. Stefansson, Anal. Chem., 66 (1994) 646A–655A.
[3] J. Vindevogel and P. Sandra, Introduction to Micellar Electrokinetic Chromatography, Hüthing, Heidelberg, 1992.
[4] S. Terabe, K. Otsuka and H. Nishi, J. Chromatogr. A, 666 (1994) 295–319.
[5] H. Nishi and S. Terabe, J. Chromatogr. A, 694 (1995) 245–276.
[6] S. Terabe, K. Otsuka and K. Ichikawa, A. Tsuchiya and T. Ando, Anal. Chem., 56 (1984) 111–113.
[7] S. Terabe, K. Otsuka and T. Ando, Anal. Chem., 57 (1985) 834–841.
[8] K. Otsuka, S. Terabe and T. Ando, Nihon Kagaku Kaishi, (1986) 950–955.
[9] S. Terabe, Trends Anal. Chem., 8 (1989) 129–134.
[10] H. Nishi and N. Tsumagari, S. Terabe, Anal. Chem. 61 (1989) 2434–2439.
[11] H. Nishi, T. Fukuyama, M. Matsuo and S. Terabe, J. Chromatogr., 515 (1990) 233–243.
[12] H. Nishi, T. Fukuyama, M. Matsuo and S. Terabe, Anal. Chim. Acta., 236 (1990) 281–286.
[13] H. Nishi, T. Fukuyama, M. Matsuo and S. Terabe, J. Microcol. Sep., 1 (1989) 234–241.
[14] R.O. Cole, M.J. Sepaniak and W.L. Hinze, J. High Resolut. Chromatogr., 13 (1990) 579–582.
[15] S. Terabe, M. Shibata and Y. Miyashita, J. Chromatogr., 480 (1989) 403–411.
[16] G.N. Okafo and P. Camilleri, J. Microcol. Sep., 5 (1993) 149–153.
[17] G.N. Okafo, C. Bintz, S.E. Clarke and P. Camilleri, J. Chem. Soc., Chem. Commun., (1992) 1189–1192.
[18] M. Lin, N. Wu, G.E. Barker, P. Sun, C.W. Huie and R.A. Hartwick, J. Liq. Chromatogr., 16 (1993) 3667–3674.
[19] A. Aumatell and R.J. Wells, J. Chromatogr. A, 688 (1994) 329–337.
[20] H. Nishi, J. High Resolut. Chromatogr., 18 (1995) 659–664.
[21] K. Otsuka and S. Terabe, J. Chromatogr., 515 (1990) 221–226.
[22] Y. Ishihama and S. Terabe, J. Liq. Chromatogr., 16 (1993) 933–944.
[23] A. Dobashi, T. Ono, S. Hara and J. Yamaguchi, Anal. Chem., 61 (1989) 1984–1986.
[24] A. Dobashi, T. Ono, S. Hara and J. Yamaguchi, J. Chromatogr., 480 (1989) 413–420.
[25] K. Otsuka and S. Terabe, J. Chromatogr., 559 (1991) 209–214.
[26] K. Otsuka and S. Terabe, Electrophoresis, 11 (1990) 982–984.
[27] K. Otsuka, M. Kashihara, Y. Kawaguchi, R. Koike, T. Hisamitsu and S. Terabe, J. Chromatogr. A, 652 (1993) 253–257.
[28] K. Otsuka, K. Karuhara, M. Higashimori and S. Terabe, J. Chromatogr. A, 680 (1994) 317–320.
[29] A. Dobashi, M. Masaki and Y. Dobashi, Anal. Chem., 67 (1995) 3011–3017.
[30] S. Sakamoto, Oil Chem., 44 (1995) 256–265.
[31] J.R. Mazzeo, E.R. Grover, M.E. Swartz and J. S. Petersen, J Chromatogr. A, 680 (1994) 125–135.
[32] J.R. Mazzeo, M.E. Swartz and E.R. Grover, Anal. Chem., 67 (1995) 2966–2973.
[33] S. Honda, M. Kotani, A. Miyoshi, A. Taga and E.R. Grover, Chromatography, 16 (1995) 88–89.
[34] N. Nimura, C. Mitsuno, H. Itoh, T. Kinoshita and T. Hanai, in Proceedings of 12th Symposium on Capillary Electrophoresis, Himeji, 1992, pp. 45–46.
[35] D.C. Tickle, G.N. Okafo, P. Camilleri, R.F.D. Jones and A.J. Kirby, Anal. Chem., 66 (1994) 4121–4126.
[36] N. Nimura, H. Itoh, C Mitsuno and T. Knoshita, in Proceedings of Separation Sciences '94, Tokyo, 1994, pp. 283–285.

[37] J. Wang and I.M. Warner, Anal. Chem., 66 (1994) 3773–3776.
[38] H. Ozaki, Y. Tanaka, A. Ichihara and S. Terabe, J. Chromatogr. A, 709 (1995) 3-10.
[39] Y. Miyashita and S. Terabe, Chromatogram (Beckman, Fullerton, CA), 11 No. 2 (1990), 6–7.
[40] Y. Miyashita and S. Terabe, Application Data DS-767, Beckman, Fullerton, CA, 1990.
[41] S. Terabe, Y. Miyashita, Y. Ishihama and O. Shibata, J. Chromatogr., 636 (1993) 47–55.
[42] T. Ueda, F. Kitamura, R. Mitchell, T. Metcalf, T. Kuwana and A. Nakamoto, Anal. Chem., 63 (1991) 2979–2981.
[43] T. Ueda, R. Mitchell, F. Kitamura, T. Metcalf, T. Kuwana and A. Nakamoto, J. Chromatogr., 593 (1992) 265–274.
[44] H. Wan, P.E. Andersson, A. Engstrom and L.G. Blomberg, J. Chromatogr. A, 704 (1995) 179–193.
[45] J. Prunonosa, A.D. Gascon and L. Gouesclou, Application Data DS-798, Beckman, Fullerton, CA, 1991.
[46] J. Prunonosa, R. Obach, A. Diez-Cascon and L. Gouesclou, J. Chromatogr., 574 (1992) 127–133.
[47] H. Nishi, T. Fukuyama and S. Terabe, J. Chromatogr., 553 (1991) 503–516.
[48] R. Furuta and T. Doi, J. Chromatogr., 676 (1994) 431–436.
[49] R. Furuta and T. Doi, Electrophoresis, 15 (1994) 1322–1325.
[50] H. Siren, J.H. Jumppanen, K. Manninen and M.-L. Riekkola, Electrophoresis, 15 (1994) 779–784.
[51] E. Francotte, S. Cherkaoui and M. Faupel, Chirality, 5 (1993) 516–526.
[52] J.E. Noroski, D.J. Mayo and M. Moran, J. Pharm. Biomed. Anal., 13 (1995) 45-52.
[53] R. Furuta and T. Doi, J. Chromatogr. A, 708 (1995) 245–251.
[54] V.C. Anigbogu, C.L. Copper and M.J. Sepaniak, J. Chromatogr. A, 705 (1995) 343–349.
[55] H. Watarai, Chem. Lett., (1991) 391–394.
[56] S. Terabe, N. Matsubara, Y. Ishihama and Y. Okada, J. Chromatogr., 608 (1992) 23–29.
[57] J.H. Aiken and C.W. Huie, Chromatographie, 35 (1993) 448–450.
[58] Ishihama, Y. Oda, K. Uchikawa and N. Asakawa, Anal. Chem., 67 (1995) 1588–1595.
[59] A. Nardi, A. Eliseev, P. Bocek and S. Fanali, J. Chromatogr., 638 (1993) 247–253.
[60] S. Terabe, H. Ozaki, K. Otsuka and T. Ando, J. Chromatogr., 332 (1985) 211–217.
[61] N.W. Smith, J. Chromatogr. A, 652 (1993) 259–262.
[62] T. Schmit and H. Engelhardt, J. High Resolut. Chromatogr., 16 (1993) 525–529.
[63] T. Schmit and H. Engelhardt, Chromatographia, 37 (1993) 475–481.
[64] T. Schmit and H. Engelhardt, J. Chromatogr. A, 697 (1995) 561–570.
[65] J. Szeman and K. Ganzler, J. Chromatogr. A, 668 (1994) 509–517.
[66] R.J. Tait, D.O. Thompson, V.J. Stella and J.F. Stobaugh, Anal. Chem., 66 (1994) 4013–4018.
[67] I.S. Lurie, R.F.X. Klein, T.A.D. Cason, M.J. LeBelle, R. Brenneisen and R.E. Weinberger, Anal. Chem., 66 (1994) 4019-4026.
[68] C. Dette, S.Ebel and S. Terabe, Electrophoresis, 15 (1994) 799–803.
[69] B. Chankvetadze, G. Endresz and G. Blaschke, Electrophoresis, 15 (1994) 804–807.
[70] B. Chankvetadze, G. Endresz and G. Blaschke, J. Chromatogr. A, 700 (1995) 43–49.
[71] A. Aumatell, R.J. Wells and D.K.Y. Wong, J. Chromatogr. A, 686 (1994) 293–307.
[72] B. Chankvetadze, G. Endresz and G. Blaschke, J. Chromatogr.A, 704 (1995) 234–237.
[73] Y. Tanaka, M. Yanagawa and S. Terabe, J. High Resolut. Chromatogr., submitted for publication.
[74] W. Wu and A.M. Stalcup, J. Liq. Chromatogr., 18 (1995) 1289–1315.
[75] H. Nishi, unpublished data.
[76] G.E. Barker, P. Russo and R.A. Hartwick, Anal. Chem., 64 (1992) 3024–3028.
[77] P. Sun, N. Wu, G.E. Barker and R.A. Hartwick, J. Chromatogr., 648 (1993) 475–480.
[78] S. Busch, J.C. Kraak and H. Poppe, J. Chromatogr., 635 (1993) 119–126.
[79] Y. Tanaka and S. Terabe, J. Chromatogr. A, 694 (1995) 277–284.
[80] T. Arai, M. Ichinose, H. Kuroda, N. Nimura and T. Kinoshita, Anal. Biochem., 217 (1994) 7–11.
[81] D. Wistuba, H. Diebold and V. Schrig, J. Microcol. Sep., 7 (1995) 17–22.
[82] R. Vespalec, V. Sustacek and P. Bocek, J. Chromatogr., 638 (1993) 255–261.
[83] L. Valtcheva, J. Mohammad, G. Pettersson and S. Hjerten, J. Chromatogr., 638 (1993) 263–267.
[84] Y. Tanaka, N. Matsubara and S. Terabe, Electrophoresis, 15 (1994) 848–853.
[85] Y. Ishihama, Y. Oda, N. Asakawa, Y. Yoshida and T. Sato, J. Chromatogr. A, 666 (1994) 193–201.
[86] J. Haginaka and N. Kanasugi, unpublished data.
[87] D.K. Lloyd, S. Li and P. Ryan, J. Chromatogr. A, 694 (1995) 285–296.
[88] D.K. Lloyd, S. Li and P. Ryan, Chirality, 6 (1994) 230–238.
[89] H. Nishi, K. Nakamura, H. Nakai, T. Sato and S. Terabe, Electrophoresis, 15 (1994) 1335–1340.
[90] H. Nishi and S. Terabe, J. Chromatogr. Sci., 33 (1995) 698–703.
[91] H. Nishi, K. Nakamura, H. Nakai and T. Sato, Anal. Chem., 67 (1995) 2334–2341.
[92] A.M. Stalcup and N.M. Agyei, Anal. Chem., 66 (1994) 3054–3059.
[93] A.M. Abushoffa and B.J. Clark, J. Chromatogr. A, 700 (1995) 51–58.
[94] S. Terabe and T. Isemura, Anal. Chem., 62 (1990) 650–652.

[95] S. Terabe and T. Isemura, J. Chromatogr., 515 (1990) 667–676.
[96] D.W. Armstrong, K.L. Rundlett and J.-R. Chen, Chirality, 6 (1994) 496–509.
[97] K.L. Rundlett and D.W. Armstrong, Anal. Chem., 67 (1995) 2088–2095.
[98] D.W. Armstrong, K.L. Rundlett and G.L. Reid, III, Anal. Chem., 66 (1994) 1690–1695.
[99] D.W. Armstrong, M.P. Gasper and K.L. Rundlett, J. Chromatogr. A, 689 (1995) 285–304.
[100] K. Ghowsi, J.P. Foly and R.J. Gale, Anal. Chem., 62 (1990) 2714–2721.
[101] M.G. Khaledi, S.C. Smith and J. K. Straaters, Anal. Chem., 63 (1991) 1820–1830.
[102] K. Otsuka, S. Terabe and T. Ando, J. Chromatogr., 348 (1985) 39–47.
[103] J.K. Straaters and M.G. Khaledi, Anal. Chem., 63 (1991) 2503–2508.
[104] J.W. Jorgenson and K.D. Lukacs, Anal. Chem., 53 (1981) 1298–1302.
[105] H. Nishi and S. Terabe, Electrophoresis, 11 (1990) 691–701.
[106] S. Terabe, J. Pharm. Biomed. Anal., 10 (1992) 705–715.
[107] J.P. Foly, Anal. Chem., 62 (1990) 1302–1308.
[108] H. Nishi, T. Fukuyama and M. Matsuo, J. Microcol. Sep., 2 (1990) 234–240.
[109] S.A.C. Wren and R.C. Rowe, J. Chromatogr., 603 (1992) 235–241.
[110] S.A.C. Wren and R.C. Rowe, J. Chromatogr., 609 (1992) 363–367.
[111] A.R. Campanelli, S.C. De Sanctis, E. Chiessi, M. D'Alagni, E. Giglio and L. Scaramuzza, J. Phys. Chem., 93 (1989) 1536–1542.
[112] R.O. Cole, M. Sepaniak, W.L. Hinze, J. Gorse and K. Oldiges, J. Chromatogr., 557 (1991) 113–123.
[113] H. Nishi, T. Fukuyama, M. Matsuo and S. Terabe, J. Chromatogr., 513 (1990) 279–295.
[114] C. Bjergegaard, L. Ingvardsen and H. Sorensen, J. Chromatogr. A, 653 (1993) 99–108.
[115] A.D. Harman, R.G. Kibbey, M.A. Sablik, Y. Fintschenko, W.E. Kurtin and M.M. Bushey, J. Chromatogr. A, 652 (1993) 525–533.
[116] S. Takeda, S. Wakida, M. Yamane and K. Higashi, Anal. Sci. 7 (1991) 1113–1114.
[117] S. Boonkerd, M.R. Detaevernier, Y. Michotte and J. Vindevogel, J. Chromatogr. A, 704 (1995) 238–241.
[118] J. Cai and Z.E. Rassi, J. Chromatogr., 608 (1992) 31–45.
[119] J.T. Smith and Z.E. Rassi, J. Chromatogr. A, 685 (1994) 131–143.
[120] J.T. Smith, W. Nashabeh and Z.E. Rassi, Anal. Chem., 66 (1994) 1119–1133.
[121] N. Tanaka, T. Tanigawa, K. Hosoya, K. Kimata, T. Arai and S. Terabe, Chem. Lett., (1992) 959–962.
[122] H. Ozaki, S. Terabe and A. Ichikawa, J. Chromatogr. A, 680 (1994) 117–123.
[123] C.P. Palmer, M.Y. Khaled and H.M. McNair, J. High. Resolut. Chromatogr., 15 (1992) 756–762.
[124] N. Tanaka, T. Fukutome, T. Tanigawa, K. Hosoya, K. Kimata, T. Araki and K.K. Unger, J. Chromatogr. A, 699 (1995) 331–341.
[125] D.N. Heiger and R.E. Majors, LC·GC, 13 (1995) 12–23.
[126] H. Hjerten, J. Chromatogr., 347 (1985) 191–198.
[127] K.A. Kobb, V. Dolnik and M. Novotny, Anal. Chem., 62 (1990) 2478–2483.
[128] L.Z. Avila, Y.-H. Chu, C. Blossey and G.M. Whitesides, J. Med. Chem., 36 (1993) 126–133.
[129] F.A. Gomez, L.Z. Avila, Y.-H. Chu and G.M. Whitesides, Anal. Chem., 66 (1994) 1785–1791.
[130] J. Liu, K.J. Volk, M.S. Lee, E.H. Kerns and I.E. Rosenberg, J. Chromatogr. A, 680 (1994) 395–403.
[131] A.M. Krstulovic (Editor) Chiral Separation by HPLC, Ellis Horwood, Chichester, 1989.
[132] G. Subramanian (Editor) A Practical Approach to Chiral Separations by Liquid Chromatography, VCH, Weinheim, 1994.
[133] A. D'Hulst and N. Verbeke, J. Chromatogr., 608 (1992) 275–287.
[134] A. D'Hulst and N. Verbeke, Chirality, 6 (1994) 225–229.
[135] H. Soini, M. Stefansson, M.-L. Liekkola and M.V. Novotny, Anal. Chem., 66 (1994) 3477–3484.
[136] Japanese Society for Biochemistry, Data Book on Biochemistry, Tokyo Kagaku Dojin, Tokyo, 1979, pp. 491–492; Merck Index, Merck, Rahway, NJ, 11th ed., 1983, p. 427.
[137] H. Nishi, S. Izumoto, K. Nakamura, H. Nakai and T. Sato, Chromatographia, submitted for publication.
[138] H. Nishi, J. Chromatogr. A, 735 (1996) 345.
[139] Reagent Brochure, Cyclolab, Budapest.
[140] M.J. Sepaniak, R.O. Cole and B.K. Clark, J. Liq. Chromatogr., 15 (1992) 1023–1040.
[141] H. Nishi, Y. Kokusenya, T. Miyamoto and T. Sato, J. Chromatogr. A, 659 (1994) 449–457.
[142] H. Nishi, K. Ishibuchi, K. Nakamura, H. Nakai and T. Sato, J. Pharm. Biomed. Anal., in press.
[143] A.D. Tran, T. Blanc and E.J. Leopold, J. Chromatogr., 516 (1990) 241–249.
[144] L. Kang and R.H. Buck, Amino Acids, 2 (1992) 103–109.
[145] A. Tivesten and S. Folestad, J. Chromatogr. A, 708 (1995) 323–337.
[146] G.N. Okafo, K.K. Rana and P. Camilleri, Chromatographia, 39 (1994) 627–630.
[147] I.S. Lurie, J. Chromatogr., 605 (1992) 269–275.
[148] P. Leroy, L. Bellucci and A. Nicolas, Chirality, 7 (1995) 235–242.
[149] W. Schutzner, S. Fanali, A. Rizzi and E. Kenndler, J. Chromatogr., 639 (1993) 375–378.
[150] W.Schutzner, G. Caponecchi, S. Fanali, A. Rizzi and E. Kenndler, Electrophoresis, 15 (1994) 769–773.
[151] C. Dette and H. Watzig, Electrophoresis, 15 (1994) 763–768.