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Review

Optical resolution of drugs by capillary electrophoretic techniques

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Abstract

This review surveys the optical resolution of drugs by capillary electrophoretic (CE) techniques. The CE techniques involve capillary zone electrophoresis, electrokinetic chromatography, capillary gel electrophoresis, capillary isotachophoresis and electrochromatography. Each section is arranged according to species of chiral selectors used in CE from the viewpoint of practical use. Optical resolution of enantiomers by CE techniques is mainly achieved through the modification of the separation solution with chiral additives such as cyclodextrins or proteins. A brief separation theory or separation procedure for each CE technique and some typical applications are also described for practical guidelines.

Contents

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1. Introduction

Capillary electrophoresis (CE) has become a powerful and a popular separation technique because of the rapid separation and high resolution achieved [1–3] and fully automated instrumentation. Several different separation modes, from capillary gel electrophoresis (CGE) to micellar electrokinetic chromatography (MEKC) [4,5], have been developed and consequently a wide variety of substances such as ions, drugs and biopolymers can be analysed by CE, according to the physico-chemical properties of the analyte. In particular, for small molecules such as pharmaceuticals, CE offers adequate separation, detection, simplicity, etc., for a method of quality control. CE is now recognized as an important option among separation methods in pharmaceutical analysis, because its separation principle differs from those of other separation methods such as high-performance liquid chromatography (HPLC). Some monographs [6–12] and reviews [13–16] that provide overviews of CE techniques are available. Some review papers that have focused on the qualitative aspects of CE techniques have been published [17–23].

Concerning the separation of enantiomers, CE techniques can take advantage of an ultra-high separation efficiency, easy exchanges of separation media, extremely small volumes of the sample and the media, etc., in comparison with HPLC. This means that, in the development of a CE chiral separation method, one can easily alter the separation solution to find the optimum separation medium and can also use an expensive chiral selector. This is a great advantage of CE as a quality control method from an economic viewpoint. Some papers reviewing chiral separations by CE have recently become available [24–27].

In CE chiral separations, much work has been reported on the direct resolution of enantiomers by capillary zone electrophoresis (CZE) employing cyclodextrins (CDs) (CD-CZE) [28–73]. Differential inclusion-complex formations of CD with the solute provide differential solute migrations and chiral recognition. CDs have also been successfully used in the chiral separation by CGE [74,75], MEKC (CD-MEKC) [76–87] or capillary isotachophoresis (ITP) [88–94]. Chiral separation by CE using a CD immobilized capillary tube [commercially available as chiral stationary phases for gas chromatography (GC)] has been also reported [95–98]. CD derivatives having a charge have been used in CE (CD-EKC) to separate enantiomers [69–73,97,99].

Other than CDs, proteins have been vigorously investigated as chiral selectors in CE chiral separations [100–109]. In the addition of proteins, the separation mode can be classified as one of EKC, i.e., affinity EKC. These chiral selectors have already been successfully applied to HPLC and been demonstrated to be effective in chiral separations [110]. The chiral selector used in HPLC such as polysaccharides should be applicable in CE apart from CDs or proteins. Further investigations of adopting chiral selectors known from HPLC or GC in CE will be made to extend the application range.

In this paper, chiral separations of drugs by CE techniques are described mainly on the basis of practical use, with the structures of the drugs that are enantioresolved by the method. Various CE techniques for optical resolution are also overviewed briefly. A detailed separation theory of each CE chiral separation has been described previously [27].

2. Capillary electrophoretic techniques and approach to chiral separation

CE or high-performance capillary electrophoresis, which was first introduced by Mikker et al. [1], Jorgenson and Lukacs [2] and Hjerten [3], has been demonstrated to be highly efficient. Several modes, which are used routinely in conventional electrophoretic methods, have been exploited in capillary systems to obtain a highly efficient separation. The most successful and popular operational mode is zone electrophoresis. In zone electrophoresis, background electrolytes or gels are filled in the capillary to maintain a constant electric field along the tube. The former is CZE and the latter method is

called CGE. The results obtained are recorded as electropherograms, which are similar to chromatograms in HPLC, and can be easily handled with a data processor as used in HPLC. ITP separation has been performed in the capillary system. Capillary isoelectric focusing (IEF) has also been reported with some detection systems, specially developed for IEF.

Although CE has a high resolving power, it can separate only ionic or charged compounds, because its separation principle is based on the difference in electrophoretic mobilities. To overcome this problem, Terabe and co-workers [4,5,99] combined CE and chromatographic separation principles. This method is called electrokinetic chromatography (EKC). EKC can be considered as a branch of CE and performed with the same apparatus as CZE based on chromatographic principles. A homogeneous solution that contains an ionic pseudo-stationary phase is used to separate electrically neutral solutes. Various compounds are available as pseudo-stationary phases for EKC. Among many modes of EKC, micellar EKC (MEKC), which uses micellar solutions of ionic surfactants such as sodium dodecyl sulphate (SDS), has become the most popular method. Besides MEKC, CD-EKC, in which charged CD derivatives are employed as a carrier, can be applied to the chiral separation.

Other than CZE, CGE, ITP, IEF and EKC, electrochromatography (EC) can be also classified as a branch of CE, although the separation principle is identical with that of conventional liquid chromatography. EC uses electroosmosis for mobile phase delivery instead of a high-pressure pump as in HPLC. Many wall-coated or packed capillary columns can be used in the EC mode. The operational formats in the CE separations mentioned above are summarized in Table 1. The detailed separation principle of each CE mode has been described in other reviews and monographs [6–16] and is not given here.

In CE chiral separations, the same strategies as have been exploited in chiral separations in HPLC can be used. There are two possible techniques to resolve enantiomers in CE: (1) direct chiral separation and (2) separation of enantiomers derivatized to diastereomers with chiral reagents. Direct enantiomeric separation by CE can be achieved through modification of the buffer solutions with chiral additives to form a diastereomeric pair. The difference in the stability of diastereomeric complexes leads to a successful enantioseparation. CDs or CD derivatives [29–99], proteins [100–109], crown ethers [25,62,111,112] and polysaccharides [113,114] can be applied in CE as chiral selectors. The addition of chiral ligands and metals can be

Table 1
Modes of capillary electrophoretic techniques and applicability to chiral separation

Mode	Applicability[a]	Chiral selector
Capillary zone electrophoresis (CZE)	VG	CD, crown ether
Cyclodextrin-modified CZE (CD-CZE)		
Capillary gel electrophoresis (CGE)	G	CD
Isoelectric focusing (IEP)	×	
Capillary isotachophoresis (ITP)	NVG	CD
Electrokinetic chromatography (EKC)	VG	
Micellar EKC (MEKC) using chiral surfactants		Chiral surfactant
Cyclodextrin-modified MEKC (CD-MEKC)		CD
EKC with charged cyclodextrins (CD-EKC)		Charged CD
Affinity EKC		Protein, polysaccharide
Electrochromatography (EC)	G	Chiral column (for GC)

[a] VG, very good; G, good; NVG, not very good; ×, out of adaptability.

effective for the enantiomeric separation of amino acids [115,116]. These chiral additives have been successfully demonstrated to be effective for chiral separations in HPLC [110]. In MEKC, besides the addition of chiral compounds mentioned above, direct chiral separation can be obtained with the use of chiral surfactants [117–130]. The basic theory of each CE mode as a chiral separation method has been described previously [27] and in this review recent applications of CE chiral separations are reported according to the chiral selector. Most chiral compounds developed are pharmaceuticals, pesticides, herbicides, etc., and these are all small molecular compounds. Therefore, the CZE or EKC mode is the most successful and easy to adopt as a chiral separation method among the many CE modes. IEF is not suitable for enantiomeric separation.

3. Chiral separation by host–guest interaction

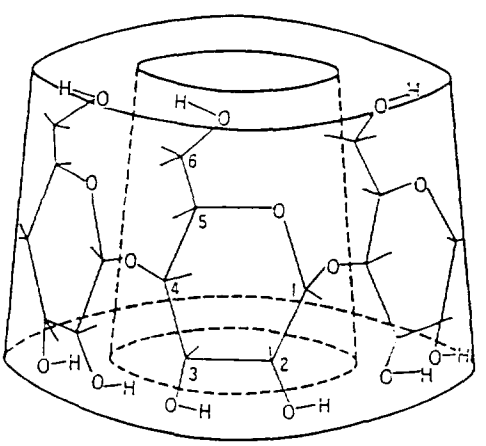
3.1. Use of cyclodextrins (CDs)

3.1.1. Addition of neutral CDs

CDs are cyclic oligosaccharides consisting of six, seven or eight glucopyranose units corresponding to the particular names, α-, β- or γ-CD. The structure of CDs is that of a truncated cone with a hydrophobic cavity, whose size differs significantly among the CDs. Some properties of CDs and a schematic structure are given in Table 2. CDs tend to include compounds that fit their cavities by hydrophobic interaction. The host–guest complexation constants depend on the suitability between the guest molecule and the CD, where the cavity diameter and the geometry of the CD are important factors. Successful enantioresolution results from the

Table 2
Physico-chemical properties and structure of α-, β- and γ-cyclodextrin [110]

Parameter	Type of CD		
	α-	β-	γ-
Number of glucose units	6	7	8
Molecular mass	972	1135	1297
Inner diameter of cavity (Å)	5.7	7.8	9.5
Outer diameter of cavity (Å)	13.7	15.3	16.9
Cavity volume (Å^3)	174	262	427
Solubility in water (25°C) (%, w/v)	14.5	1.8	23.2



differences in the inclusion-complex formation constants. Other than natural α-, β- and γ-CDs, many CD derivatives have been developed for increased solubility in water or to modify the cavity shape or to add a special function. Table 3 summarizes enantiomeric separations by CZE through the addition of neutral CDs (CD-CZE). For some drugs, unmodified β- and γ-CD were successful, but for many enantiomeric drugs derivatized CDs such as heptakis-(2,6-di-O-methyl)-β-CD (DM-β-CD) or hydroxypropyl-β-CD (HP-β-CD) were effective. CD-CZE is the most successful technique for enantiomeric separation by CE and a variety of applications have been reported [28–73]. As shown in Table 3, typically acidic buffer solutions are employed in CD-CZE, because suppressed electroosmosis yields a higher enantiomeric resolution.

Optical resolution by CZE with CD as the chiral selector (CD-CZE) was first introduced by Fanali's group [28]. They successfully separated enantiomers of ephedrine alkaloids [28,29], isoproterenol [28], terbutaline [30], propranolol [30], tryptophan derivatives [29,31], ketamine [32], octopamine [32] and folinic acids [33] by CZE employing CDs. Wren and Rowe [34–37] described a separation model in CD-CZE and derived an equation describing the differential mobility between an enantiomeric pair (1 and 2), $\Delta\mu$, as follows:

$$\Delta\mu = \frac{[C](\mu_f - \mu_c)(K_1 - K_2)}{1 + [C](K_1 + K_2) + K_1K_2[C]^2} \quad (1)$$

where μ_f is the electrophoretic mobility of the enantiomers in free solution, μ_c is the electrophoretic mobility of the CD-complexed enantiomers, K_1 and K_2 are the formation constants of inclusion complexes of the two enantiomers and [C] is the concentration of the CD. The mobility of each complex, μ_c, is assumed to be equal to the mobility of the chiral selector. Eq. 1 shows the dependence of $\Delta\mu$ on the difference in the mobilities between the free and the CD-complexed enantiomer, $\mu_f - \mu_c$, the formation constants, K_1 and K_2, and the concentration of CD. It is obvious that the larger difference $\mu_f - \mu_c$ the greater is the $\Delta\mu$ value. It was also reported that there is an optimum concentration of CD giving the maximum enantioselectivity. Successful applications of this model to the enantiomeric separation of propranolol [34,35], oxprenolol, metoprolol, atenolol [36], practolol and ephedrine [37] were reported by Wren and Rowe. Rawjee and co-workers [38–40] also described a more complex model of CD-CZE and applied it to the enantiomeric separation of fenoprofen, ibuprofen [38], naproxen [39] and homatropine [40].

Snopek et al. [41] used hydroxyalkylcelluloses such as methylhydroxyethylcellulose (MHEC) and hydroxyethylcellulose (HEC) in CD-CZE with uncoated capillaries under acidic conditions to suppress the electroosmotic flow (EOF). The addition of HEC or MHEC was found to be effective in improving the resolution of enantiomers of chloramphenicol, thioridazine and ketotifen. They also investigated the ITP chiral separation of the same solutes employing CDs. Soini et al. [42] used cationic surfactants such as cetyltrimethylammonium bromide (CTAB) and cetylpyridinium chloride (CPC) together with MHEC in CD-CZE with uncoated or polyacrylamide-coated capillaries at pH 2.7–3.0. The presence of CTAB or CPC at around their critical micelle concentration improved the reproducibility of the migration times and peak shapes of basic solutes such as verapamil and fluoxetine with uncoated capillaries. They also determined verapamil and bupivacaine extracted from serum and reported that the CE chiral separation was better than HPLC or GC chiral separations because of the easy development and optimization of the method. Belder and Schomburg [43] added poly(vinyl alcohols) and HEC to the buffers in CD-CZE with uncoated capillaries. Besides the effects of the reduction of the EOF, i.e., increase in the migration times, both the peak symmetry and peak width were considerably improved and good enantiomeric separations were achieved for four tocainide compounds. This can be ascribed to the suppression of the solute adsorption to the wall through the dynamic coating with the additives. Quang and Khaledi [44,45] employed tetraalkylammonium

Table 3
Chiral separation by capillary zone electrophoresis with neutral CDs (CD-CZE)

Compound[a]	CD	pH	Additive	Ref.
Amino acids (AAs)				
Ala-β-naphthylamide	β, γ, diacetyl-β	2.0		60
DNS-AAs (Asp, Glu, Leu, Nle, Nva,	α, β, γ	9.0		59,61
Phe, Val, Met, Ser, Thr, Trp,	DM-α, DM-β			
α-amino-*n*-butyric acid)	TM-α, TM-β			
DNS-AAs (Leu, Thr, Met)	γ	9.0		25
DNS-AAs (Trp)	α	2.2		25
DNS-AAs (Phe)	HP-β	9.0, 6.0		57,69
Phe, Tyr, Trp	α	2.5		29,62
Trp derivatives	α	2.5		31
Antidepressants				
Fluoxetine[1]	TM-β	2.7	MHEC, CTAB	42
Mianserin[2]	HP-β	3.3		53
Nefopam[3]	HP-β	3.3		53
Nomifensine[4]	HP-β	3.3		53
Antipsychotics				
Promethazine[5]	γ	3.0		66
Thioridazine[6]	γ	2.5		41
Hypnotics				
Metomidate[7]	HP-β	3.3		53
Zopiclone[8]	HP-β	3.3		53
Barbiturates				
Hexobarbital[9]	DM-β, α, β	9.0		59
	DM-β, β, HP-β	8.3	EDTA	69
Pentobarbital[10]	TM-α, TM-β	9.0		59
Secobarbital[11]	TM-α, α	9.0		59
Anaesthetics				
Bupivacaine[12]	DM-β	2.9	MHEC, CTAB	42
Ketamine[13]	DM-β	2.5		32
	β	3.3		53
Mepivacaine[14]	DM-β	2.9	MHEC, CTAB	42
Adrenergic drugs				
Epinephrine[15]	DM-β	2.4, 2.5		20,28,29,32,46,65
	β	2.5	TMA	44
Norepinephrine	DM-β	2.4, 2.5		28,32
	β	2.5	TMA	44
Ephedrine (*SR*, *RS*)[16]	DM-β	2.4, 2.5, 3.3		28,37,53,56
	β	2.5	TBA	44
	DM-β	2.5	MeOH	58
	DM-β	2.5	HPC, TBA	73
Norephedrine	DM-β	2.4, 2.5, 3.3		28,53,56
	DM-β	2.5	HPC, TBA	73
Pseudoephedrine (*SS*, *RR*)	β	2.5	TBA	44
N-Methylephedrine	DM-β	2.5, 3.3		53,56
	DM-β	2.5	HPC, TBA	73
N-Methylpseudoephedrine	DM-β	2.5	HPC, TBA	73
Etilefrine[17]	HP-β	3.3		53
	DM-β	3.0		66
Norfenefrine[18]	HP-β	3.3		53
Octopamine[19]	DM-β	2.5		32
	HP-β	3.3		53
Pholedrine[20]	HP-β	3.3		53
Synephrine[21]	DM-β	3.3		53
Cardiotonic				
Denopamine[22]	DM-β	2.2	Urea	52
Dopamine agonist				
Quinagolide[23]	β	2.5		62

Table 3 *(continued)*

Compound[a]	CD	pH	Additive	Ref.
Bronchodilators				
Clenbuterol[24]	HP-β	2.2, 3.3		47,53
	β	4.0		49
Isoproterenol[25]	DM-β	2.4, 2.5, 3.3		28,32,53
	β	2.5	TBA, TMA	44
	DM-β	2.5	TMA	45
Picumeterol[26]	DM-β	2.2, 2.3		23, 50
	β	4.0		49
Salbutamol[27]	DM-β	3.3		53
Terbutaline[28]	DM-β, β	2.5		30
Trimetoquinol[29]	DM-β, β	2.2–2.5		66
Trimetoquinol, analogues	β-CD polymer	2.7, 6.5		51
β-Blockers				
Alprenolol[30]	HP-β	2.5	TMA	45
Atenolol[31]	DM-β	3.0, 2.5, 2.4		36,37,65
	DM-β	2.5	TMA	45
Carvedilol[32]	DM-β	2.9	MHEC, CTAB	42
	β	3.3		53
Labetalol[33]	HP-β	2.5	TMA	45
Metoprolol[34]	DM-β	3.0		36
Nadolol[35]	HP-β	2.5	TMA	45
Oxprenolol[36]	DM-β	3.0		36
	DM-β	2.5	TMA	45
Pindolol[37]	DM-β	2.5	TMA	45
	DM-β	3.0	MHEC, CTAB	42
Practolol[38]	DM-β	2.5		37
Propranolol[39]	DM-β, β	3.1, 2.5	MeOH, urea	30,34
	DM-β	3.0	MeOH, ACN	35,36
	DM-β, TM-β, β	2.5	TBA, TMA	44,45
	HE-β, HP-β	2.4		65
Ca-channel blocker				
Verapamil[40]	TM-β	2.7	MHEC, CTAB	42
Antihypertensive				
Lofexidine[41]	HP-β	3.3		53
Antiarrhythmics				
Sotalol[42]	HP-β	3.3		53
Tocainide,[43], analogues	γ	3.0	HEC	43
Anticholinergics				
Ambucetamide[44]	HP-β	3.3		53
Benzetimide[45]	HP-β	4.0		19
Homatropine[46]	β	6.25	HEC	40
Timepidium[47]	γ	2.7	Urea	52
Antihistaminics				
Carbinoxamine[48]	β	2.5	Urea	20
Chlorpheniramine[49]	β	2.5	MeOH, urea	64
Dimethindene[50], metabolites	β, HP-β, HE-β	3.3		53
Doxylamine[51]	β	2.5	(CTAB)	44
Antiasthmatics				
Ketotifen[52]	β, γ	3.5, 3.75	MHEC	41
Ketotifen intermediate, analogue	γ	3.75, 3.5		41
Ketotifen, N-oxide form	β	2.5		41
Anti-inflammatories				
Fenoprofen[53]	β, HP-β	4.50, 4.41	HEC	38,39
Ibuprofen[54]	β	4.50	HEC	38
Naproxen[55]	HP-β	4.86	HEC	39
Anticoagulant				
Warfarin[56]	DM-β-MA	9.2		54
Antifungal				
Tioconazole[57]	HP-β	4.3	MeOH	63

(Continued on p. 252)

Table 3 *(continued)*

Compound[a]	CD	pH	Additive	Ref.
Antibacterial				
Chloramphenicol[58]	DM-β	3.5	MHEC	41
Antimalarials				
Mefloquine[59]	HP-β	3.3		53
Primaquine[60]	γ	3.0		66
Anticancer agents				
Leucovorin[61] (6*S*, 6*R*)	DM-β	6.0		33
Leucovorin, metabolite (6*S*, 6*R*)	γ	7.0	Urea	67
Aldose reductase inhibitor				
AL03152[62], AL03363	β, DM-β, HE-β, HP-β	11		65
Aromatase inhibitors				
Aminoglutethimide[63]	α, β, γ	2.5		68
Aminoglutethimide analogues	β, DM-β	2.5		68
Fadrozole[64]	β, DM-β	2.5		68
Others				
Benzoin[65]	γ	3.0	Urea	66
1,1′-Binaphthyl-2,2′-diyl hydrogenphosphate[66]	β, glycosylated α	9.0		57
1,1′-Binaphthyl-2,2′-dicarboxylic acid[67]	α, glycosylated α	9.0		57
Fluparoxan[68], analogues	β	2.5, 2.7	IPA, urea	48,71
Phenoxy acid herbicides[69]	α, DM-β	4.8		55
Troger's base	β	2.5		25
Mandelic acid	HP-β	6.0		72
m-Hydroxymandelic acid	HP-β	6.0		72
p-Hydroxymandelic acid	HP-β	6.0		72
3,4-Dihydromandelic acid	HP-β	6.0		72
2-Phenyllactic acid	β, HP-β	5.0, 6.0		72
3-Phenyllactic acid	β, HP-β	5.0, 6.0		72
Precursor of drug ENX 792	β	2.5		62
CNS-active compounds	α, β, DM-β	2.5		56
Imafen	HP-β	3.3		53

[a] Superscript numbers refer to structures of the solutes shown in Fig. 13.

(TAA) cations in CD-CZE with uncoated capillaries for the enantiomeric separation of basic compounds such as ephedrine alkaloids, doxylamine, isoproterenol, metaphrine [44] and some β-blockers [45]. TAA cations added to the buffer covered the capillary wall, leading to the suppression of solute adsorption and the reduction of the EOF.

Peterson and Trowbridge [46] applied the CE technique for the determination of *d*-epinephrine in a pharmaceutical formulation containing *l*-epinephrine. The enantiomers of epinephrine were resolved by CD-CZE employing DM-β-CD at pH 2.4. Quantification using an internal standard method showed good reproducibility and small amounts of *l*-epinephrine (around 1%) could be determined. Altria et al. [47] examined inter-company cross-validation on CE chiral separations of clenbuterol racemates to check the repeatability of the CE method. Three different CE instruments were employed among seven pharmaceutical companies for this exercise. The good repeatability obtained from the seven companies showed that a CE method is transferable between different companies. Altria and coworkers also successfully employed a CE chiral separation method for the enantiomeric purity testing of fluparoxan [48] and picumeterol [23,49,50]. They reported that the detection limits of the undesired enantiomers of fluparoxan and picumeterol were 0.3% and 0.1%, respectively. In CE chiral analysis, peak area normalization is performed because the later migrating enantiomer migrates more slowly through the detector and gives an overestimated peak area [23].

Recently, Nishi and co-workers [51,52] used β-CD polymer as a chiral selector in CD-CZE. The structure of the β-CD polymer, which is synthesized by condensation of β-CD molecules with epichlorohydrin, is shown in Fig. 1. The enantiomers of trimetoquinol and related compounds except for laudanosine were successfully resolved by CD-CZE with β-CD polymer. An electropherogram of five racemates is shown in Fig. 2, where one of the enantiomers of norlaudanosoline was co-eluted with one of the enantiomers of laudanosoline. They also applied the method to the optical purity testing of the drug and reported that about 0.1% of the minor enantiomer was detectable by the method. A typical electropherogram for ca. 0.2% of the *R*-form (inactive) added to the standard trimetoquinol (*S*-form) is shown in Fig. 3 [52]. β-CD polymer will be more favourable than the usual CDs because the high molecular mass of the polymer cause a reduced mobility, leading to a larger difference in $\Delta\mu$ in Eq. 1.

Heuermann and Blaschke [53] reported the enantiomeric separation of 23 basic drugs by CD-CZE. Many of them were successfully enantioresolved by employing HP-β-CD. An example is shown in Fig. 4, where six chiral drugs with different structures were separated in a single run. Gareil et al. [54] reported the chiral separation of warfarin by CZE with DM-β-CD.

As in CD-CZE, introduction of CD in the MEKC mode (CD-MEKC) is also successful for enantiomeric separations [76–87] because CD has chirality. Apparent distribution coefficients of the solutes between the micelle and the nonmicellar phase are changed through the addition of CD, hence the CD-MEKC mode has been also effective for the separation of highly hydrophobic solutes, which are almost totally incorporated in the micelle and cannot be separated in simple MEKC [131–133]. Miyashita and Terabe [76,77] employed CD-MEKC for the separation of dansylated (DNS) DL-amino acids (DL-AAs) using γ-CD and SDS. A more successful separation of eleven DNS-DL-AAs was achieved by using a mixture of β-CD and γ-CD with SDS [78].

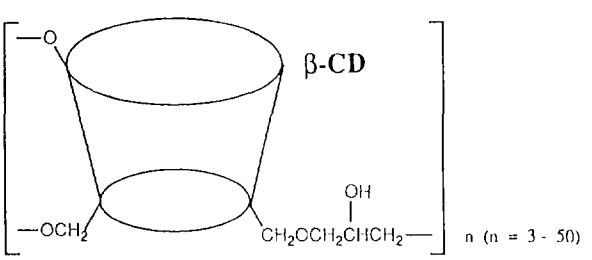


Fig. 1. Structure of β-CD polymer.

Nishi et al. [79] examined the enantiomeric separation of various compounds by CD-MEKC with four CDs. Enantiomers of binaphthyl compounds, thiopental, pentobarbital, etc., were successfully resolved with γ-CD and SDS. They also reported that some other chiral additives such as *l*-menthoxyacetic acid (*l*-MEN) or *d*-camphor-10-sulphonate (*d*-CAM) to a solution of SDS and γ-CD considerably improved the enantioresolution of pentobarbital and thiopental. Ueda and co-workers [80,81] applied CD-MEKC to the separation of naphthalene-2,3-dicarboxaldehyde (CBI)-labelled DL-AAs using β-CD or γ-CD with SDS. CBI-DL-AAs were more effectively enantioresolved using γ-CD and SDS. Generally, γ-CD showed a higher enantioselectivity than β-CD in CD-MEKC chiral separations with SDS as mentioned above. In the MEKC system, a surfactant monomer exists in the aqueous phase and can be included by the CD. The presence of a surfactant monomer will be related to the above-observed results. Prunonosa and co-workers [82,83] successfully resolved the enantiomers of cicletanine by using CD-MEKC with β- or γ-CD. They also applied this CD-MEKC method to the determination of cicletanine in human plasma and reported a detection limit of 10 ng/ml for each enantiomer [83]. Recently enantiomers of diniconazol and uniconazol were successfully resolved by MEKC using SDS and γ-CD [84].

In chiral separations by CD-CZE, the most effective parameter for the enantioselectivity, other than the cavity size and the concentration of CD, is the buffer pH. This is also the case for CD-MEKC. However, selectivity manipulation and separation optimization for solutes in a more complex matrix seem to be more easily achieved using CD-MEKC than CD-CZE, because

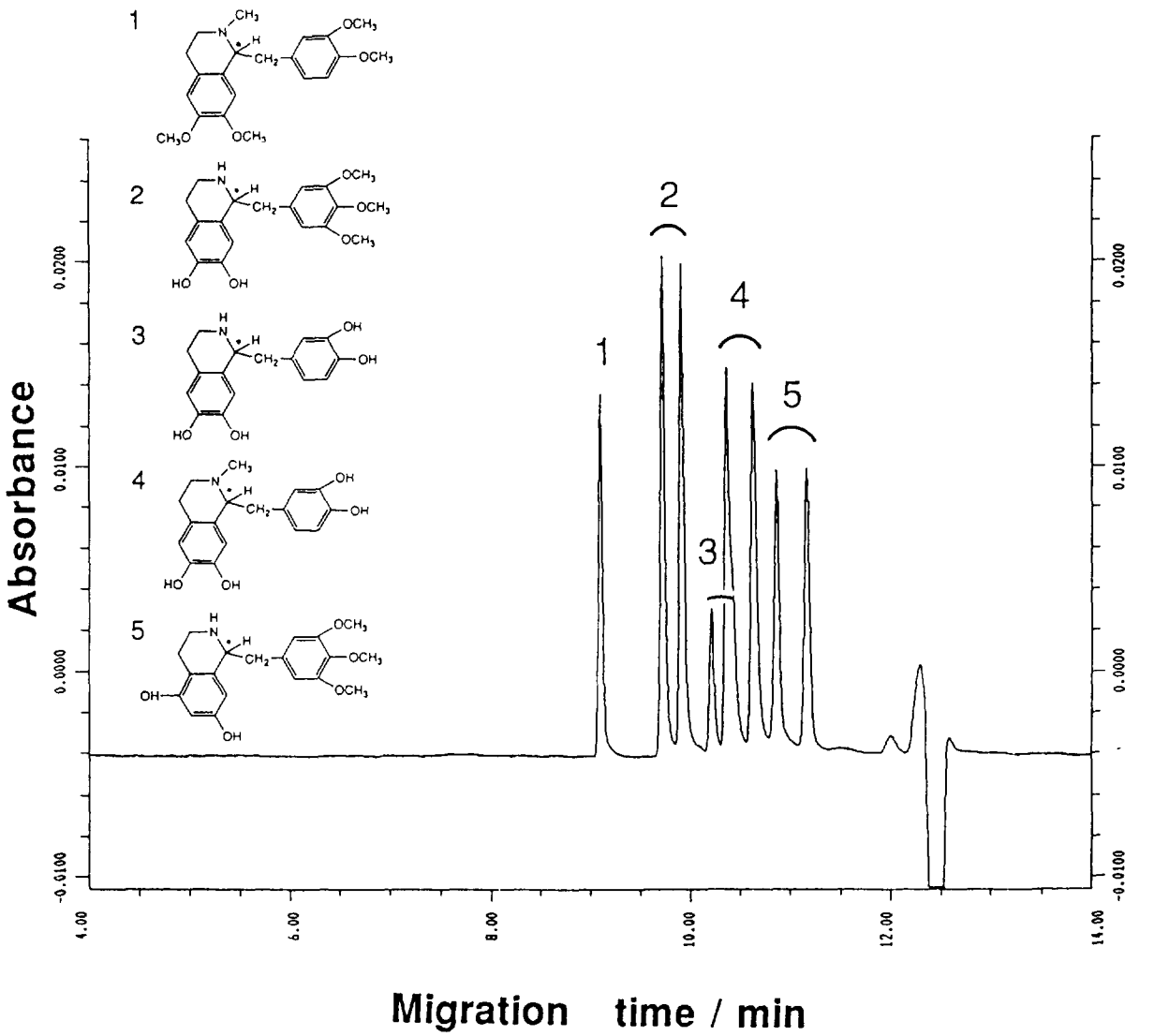


Fig. 2. Separation of enantiomers of trimetoquinol and related compounds by CD-CZE using β-CD polymer. Solutes: 1 = laudanosine; 2 = trimetoquinol; 3 = norlaudanosoline; 4 = laudanosoline; 5 = trimetoquinol isomer. Buffer, 7% β-CD polymer in 25 m*M* phosphate buffer of pH 6.5. Separation tube, fused-silica capillary, 57 cm (effective length 50 cm) × 75 μm I.D.; 15 kV; 214 nm; 23°C.

another interaction mechanism such as solubilization is operational in CD-MEKC. This is one of great advantages of CD-MEKC as in simple MEKC.

Other than CD-MEKC with SDS, Okafo et al. [85] used a mixture of sodium taurodeoxycholate (STDC) with β-CD for the separation of enantiomers of DNS-DL-AAs, methenytoin and fenoldopam. As discussed below, MEKC with STDC is effective for the enantiomeric separation of DNS-DL-AAs, diltiazem, trimetoquinol, etc. The resolution of DNS-DL-AAs in a mixture of STDC and β-CD was considerably improved compared with those obtained with either of the two chiral additives. Good enantiomeric separation of methenytoin and its principle

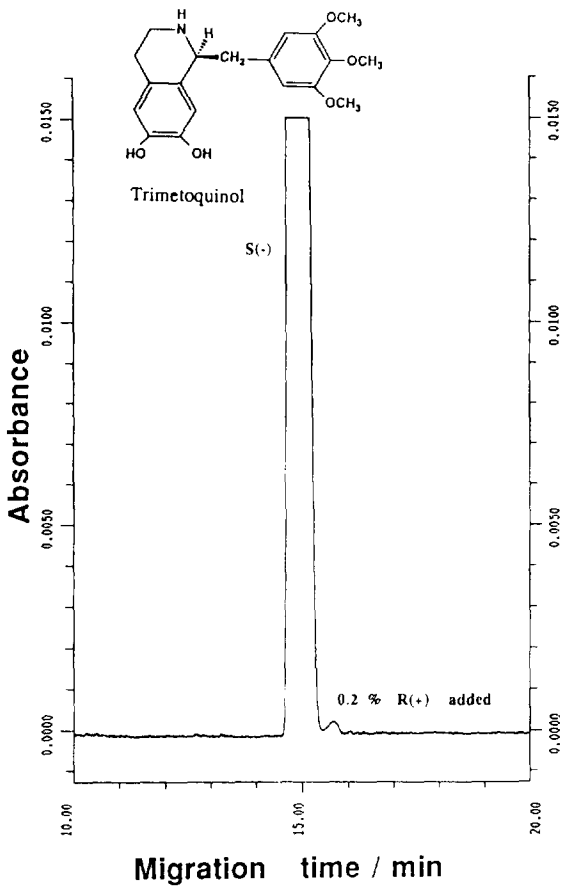


Fig. 3. Optical purity testing of trimetoquinol hydrochloride by CD-CZE with β-CD polymer. Buffer, 5% β-CD polymer in 25 m*M* phosphate buffer of pH 2.7. Other conditions as in Fig. 2 (from Ref. [52]).

metabolite 4-hydroxymethenytoin was only achieved in a mixture of STDC and β-CD.

Okafo and Camilleri [86] also applied CD-MEKC with STDC with β-CD to separate CBI-DL-AAs, CBI derivatives of baclofen and CBI derivatives of three aminophosphates. CBI derivatives of glutamic acid and histidine, which were not resolved by CD-MEKC using SDS and β-CD or γ-CD [80], were successfully resolved by using STDC and β-CD. They used this method for the determination of the chirality of amino acids, which are obtained by hydrolysis of D-Phe^7-bradykinin. Lin et al. [87] reported the separation of some DNS-DL-AAs by MEKC with STDC and β-CD. Chiral separation using a CD-bile salt system showed great enantioselectivity and can be recommended for one of starting conditions in CE chiral separations. Successful chiral separations by CD-MEKC are summarized in Table 4.

CDs have also been successfully used in ITP for the separation of enantiomers. Snopek and co-workers [24,88–94] extensively studied the separation of enantiomers by ITP and have reviewed the field [24].

3.1.2. Use of charged CDs (CD-EKC)

Other than neutral CDs or CD derivatives mentioned above, charged CDs have also been applied to CE as chiral selectors based on the concept of EKC [69–73,97,99]. Charged CDs used in CE enantiomeric separations are summarized in Table 5. This type of CD was first used by Terabe et al. for the separation of positional isomers of aromatic compounds [134] and the enantiomeric separation of DNS-AAs [99]. In CD-CZE or CD-MEKC, CDs added to the buffer solution are electrically neutral and move under the described conditions with the velocity of the EOF. In case of the charged CDs, they migrate with their own electrophoretic mobilities according to the polarity, and consequently this mode can be classified as one branch of EKC (CD-EKC). In CD-EKC, neutral compounds can be enantioresolved as well as ionic compounds. The migration order between the enantiomer (i.e., D or L) also can be manipulated by selecting natural CDs or charged CDs as described below. These are great advantages of CD-EKC as a quality control method for optical purity testing.

Terabe and co-workers prepared two β-CD derivatives to be used in EKC: mono(6-β-aminoethylamino-6-deoxy)-β-CD (CDen) [99] and 2-O-carboxymethyl-β-CD [134]. Enantiomeric separation of six DNS-AAs was successfully achieved by CD-EKC using a 25 m*M* CDen solution at pH 3.0. Hydroxypropylcellulose (HPC) (0.1%) was added to the buffer to suppress the EOF. Under these conditions CDen migrates to the negative electrode, and therefore solutes strongly included by the CDen have short

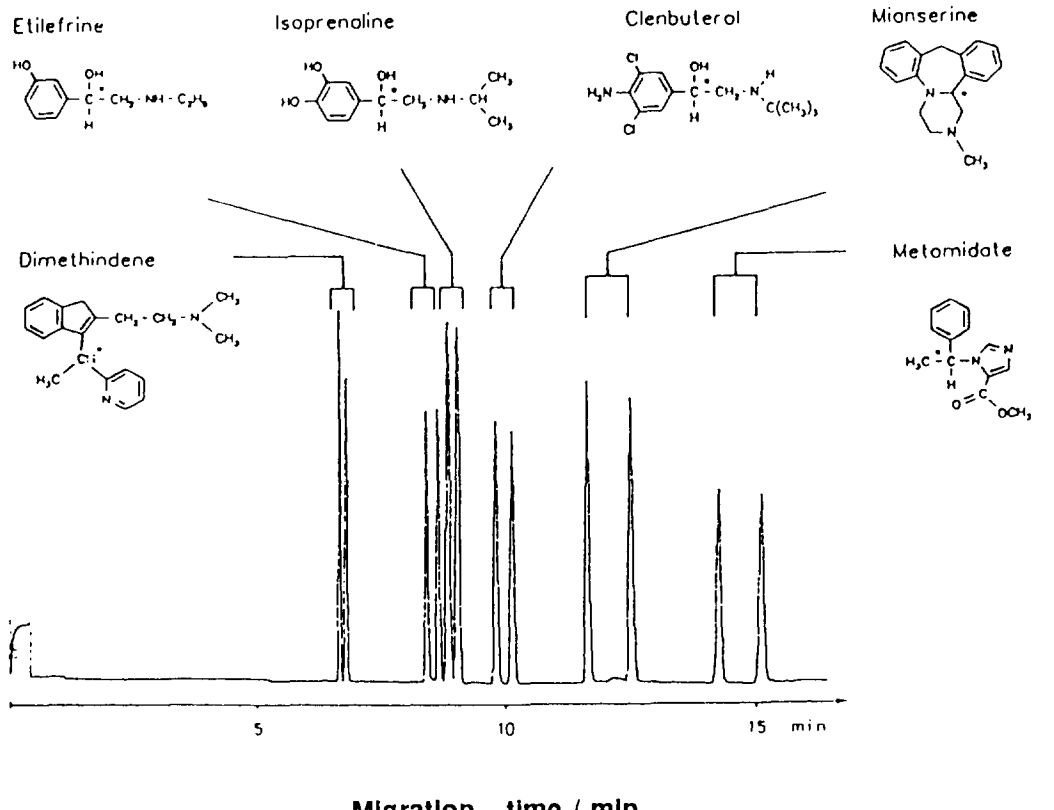


Fig. 4. Separation of enantiomers of some basic drugs by CD-CZE with HP-β-CD. Buffer, 0.3% HP-β-CD in 50 m*M* phosphate buffer of pH 3.3. Separation tube, fused-silica capillary, 40 cm effective length × 50 μm I.D.; 400 V/cm; 200–205 nm; 21°C (from Ref. [53]).

migration times and the weakly included solutes have long migration times.

Schmitt and Engelhardt employed carboxymethylated β-CD [69,70], carboxyethylated β-CD [70] and succinylated β-CD [70], which all have carboxyl groups, for the separation of enantiomers of oxazolidine, doxylamine, ephedrine, dimetinden and propranolol at pH 5.5–6.0. At pH < 4 all carboxylic functions on the CD are protonated, and hence it behaves like an uncharged CD (CD-CZE mode). At high pH (>5), deprotonation of the carboxyl groups leads to mobility of the negatively charged chiral selector as a micelle-like system (CD-EKC mode). Under such conditions (pH > 5), enantiomers of electrically neutral solutes such as hexobarbital, binaphthyl and oxazolidinone were successfully resolved as well as charged solutes. These solutes were also resolved under acidic conditions, i.e., with uncharged CDs. Smith [71] used a carboxymethylethyl β-CD for the CE chiral separation of a neutral drug GR57888X (under development) at pH 12.4.

To determine the enantiomeric excess of an enantiomeric drug, it is advantageous to determine an enantiomer migrating faster than the main component. In order to change the migration order of enantiomers in HPLC, another column with the complementary chiral selector must be used. However, this can be obtained by the reversal of the EOF with the same chiral selector in CE. Reversal of EOF is usually achieved by coating the capillary wall with a cationic surfactant, etc. Other than this, by employing these modified CDs, which can be used in the charged or uncharged mode under

Table 4
Chiral separation by cyclodextrin-modified MEKC (CD-MEKC)

Compound[a]	CD	Surfactant	pH	Additive	Ref.
Amino acids (AAs)					
DNS-AAs	γ, β	SDS	8.3, 8.6	MeOH	76,77,78
	β	STDC	7.2, 7.8		85,87
CBI-AAs	γ, β	SDS	9.0		80,81
	β	STDC	7.0		86
Antiepileptic					
Mephenytoin[70], metabolite	β	STDC	7.2		85
Hypnotic					
Glutethimide[71]	β	SDS	7.0	Urea, MeOH	68
Barbiturates					
Hexobarbital[9]	β, DM-β	SDS	7.0	Urea, MeOH	68
Mephobarbital[72]	β	SDS	7.0	Urea, MeOH	68
Pentobarbital[10]	γ	SDS	9.0	*l*-MEN, *d*-CAM	79
Secobarbital[11]	γ	SDS	7.0	Urea, MeOH	68
Thiopental[73]	γ	SDS	9.0	*l*-MEN, *d*-CAM	79
Antihypertensives					
Cicletanine[74]	β, γ	SDS	8.6		82,83
Fenoldopam[75], analogue	β	STDC	7.2		85
Aromatase inhibitors					
Aminoglutethimide analogue	DM-β	SDS	7.0	Urea, MeOH	68
Fadrozole[63]	β, DM-β	SDS	9.0	Urea, MeOH	68
Muscle relaxant					
Baclofen[76] (CBI derivative)	β	STDC	7.0		86
Antifungals					
Diniconazole[77]	γ	SDS	9.0	Urea	84
Uniconazole[78]	γ	SDS	9.0	Urea	84
Others					
Aminophosphates (CBI derivative)	β	STDC	7.0		86
1,1′-Binaphthyl-2,2′-diyl hydrogenphosphate[66]	β, DM-β TM-β, γ	SDS	9.0	*l*-MEN, *d*-CAM	79
2,2′-Dihydroxy-1,1′-dinaphthyl[79]	TM-β, γ	SDS	9.0	*l*-MEN, *d*-CAM	79
2,2,2-Trifluoro-1-(9-anthryl)ethanol[80]	β, γ	SDS	9.0	*l*-MEN, *d*-CAM	79

[a] Superscript numbers refer to structures of the solutes shown in Fig. 13.

the low EOF conditions, reversal of the migration order of the enantiomers was achieved for the enantiomeric separation of ephedrine [70].

Nardi et al. [72] employed 6^A-methylamino and 6^A,6^D-dimethylamino-β-CD for the enantiomeric separation of some 2-hydroxy acids including mandelic acid. These CD derivatives having positive charges were effective for the optical resolution of these acidic solutes. Dette et al. [73] used tetrakis [6-O-(4-sulphobutyl)]-β-CD (Na^+ salt) as an anionic carrier in CD-EKC for the separation of enantiomers of four ephedrine alkaloids. This CD has the benefit of a wide range of basic pH available for the optical resolution to manipulate selectivity for both enantiomeric separation and different solutes.

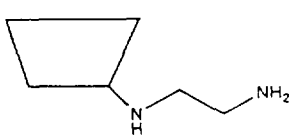
3.1.3. Immobilization of CDs (EC and CGE)

CDs have been used in CE enantiomeric separations through immobilization other than addition. Mayer and co-workers [95–98] reported the application to CE of CD derivative-

Table 5
Charged cyclodextrins used in chiral separations by capillary electrophoresis (CD-EKC)

Charged cyclodextrin	Solute[d]	pH	Ref.
Cationic CD derivatives			
Mono-(6-β-aminoethylamino-6-deoxy)-β-CD[a]	DNS-AAs	3.0	99
6^A-Methylamino-β-CD	Mandelic Acid	5.0	72
6^A,6^D-Dimethylamino-β-CD	*m*-Hydroxymandelic acid		
	p-Hydroxymandelic acid		
	3,4-Dihydroxymandelic acid		
	2-Phenllactic acid		
	3-Phenllactic acid		
Anionic CD derivatives			
2-O-Carboxymethyl-β-CD[b]	(Aromatic isomers)		134
Carboxymethylated β-CD (3.6 per CD ring)	Doxylamine[51]	2.5	70
Carboxyethylated β-CD (ca. 6 per CD ring)	Ephedrine[16]	5.8 (charged)	
Carboxysuccinated β-CD (3.5 per CD ring)	Dimenthinden[50]		
	2,2-Dihydroxy-1,1′-dinaphthyl[79]		
	Hexobarbital[9]		
	Oxazolidine[81]		
	Propranolol[39]		
Carboxymethylated β-CD (3.6 per CD ring)	Ephedrine[16]	2.5	69
	Dimenthinden[50]	6.0 (charged)	
	Doxylamine[51]		
Carboxymethylated β-CD (3.6 per CD ring)	GR57888X[82]	12.4	71
Tetrakis[6-O-(4-sulphobutyl)]-β-CD (Na^+ salt)[c]	Ephedrine[16]	10.0	73
	Norephedrine		
	Pseudoephedrine		
	Methylephedrine		
	Methylpseudoephedrine		
6-O-Trimethylenesulphonic acid β-CD (Na^+ salt) (mixture of mono-, bis- and tri-)	1,1′-Binaphthyl-2,2′-diyl hydrogenphosphate[66]		97

a



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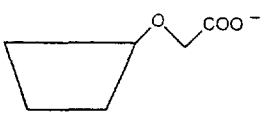

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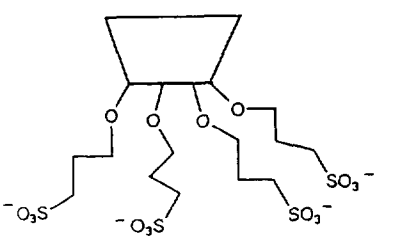


[d] Superscript numbers refer to structures of the solutes shown in Fig. 13.

coated capillary tubes, which are commercially available as a chiral capillary column for GC (Chiralsil-Dex). This mode can be classified as electrochromatography (EC), in which the mobile phase is driven by the EOF instead of a high-pressure pump as used in HPLC.

Chiralsil-Dex with permethylated β-CD has been found to be effective for the enantiomeric separation of 1,1′-dinaphthyl-2,2′-diyl hydrogenphosphate, 1-phenylethanol [95], ibuprofen, flurbiprofen, cicloprofen [96] and hexobarbital [97], and also has numerous applications in GC and supercritical fluid chromatography (SFC). The optical resolution of etodolac, which was not enantioresolved by the β-CD-type Chiralsil-Dex, was also achieved by employing Chiralsil-Dex

with permethylated γ-CD [96]. These highly hydrophobic and polar compounds, which cannot be analysed with Chiralsil-Dex in the GC mode, were successfully analysed and enantioresolved by using the CE technique. In the enantiomeric separation of 1,1′-dinaphthyl-2,2′-diyl hydrogenphosphate by Chiralsil-Dex, reversal of the elution order was obtained through the addition of sulphonated β-CD to the buffer solution [97]. This is explained by the opposite enantioselectivity of the CD selectors employed in the mobile and stationary phases. Armstrong et al. [98] synthesized another type CD derivative, permethylated allyl-substituted β-CD, and used a capillary coated with this derivatized CD and organosilane copolyer in CE analysis and also in GC and SFC. Racemic mephobarbitol was baseline resolved with a 50 m*M* phosphate buffer of pH 7.8.

Besides the wall coating method, CDs have been immobilized by using a gel. A capillary filled with a gel containing CD was employed for the enantiomeric separation of DNS-AAs by Guttman et al. [74]. The mode of separation is CGE. They prepared a cross-linked polyacrylamide gel incorporating a CD by simply adding the CD to the solution prior to polymerization. They reported that β-CD was the most effective and the addition of methanol (MeOH) (10%) enhanced the enantioselectivity. Cruzado and Vigh [75] synthesized allyl carbamoylated β-CD, and prepared both cross-linked (solid-type) and linear (liquid-type) gels after copolymerization with acrylamide for use in CGE to separate enantiomers. The cross-linked gel was not stable enough to operate for a long time because of bubble formation, etc., although good enantioselectivity of DNS-DL-Phe was obtained. In contrast, the liquid gel showed much better stability and reproducibility although the enantioselectivity was slightly lower than with the solid gel. Nine DNS-AAs and homatropine were successfully enantioresolved using the liquid gel-filled capillary.

3.2. Use of crown ethers

Crown ethers are known to form stable complexes with alkali metals, alkaline earth metals and primary amine ions. Recently the use of a crown ether as a chiral selector in CE has been reported by Kuhn and co-workers [25,62,111]. Only one crown ether, 18-crown-6-tetracarboxylic acid (18C6) (see Fig. 5), is known to have been successfully used for the separation of enantiomers of primary amines. A separation of three DL-AAs by CZE with 18C6 is shown in Fig. 6 [25]. To form host–guest complexes, the amino groups must be protonated, and therefore acidic conditions are essential. They reported the separation of the enantiomers of 22 different amines such as some AAs, dopa, noradrenaline, norephedrine and naphthylethylamine by using 10 m*M* Tris–citrate buffer solution of pH 2.2. In that case, the ionization of carboxyl groups is suppressed, and the solutes migrate to the negative electrode owing to the positive charge on the ammonium group. That means solutes separated by CZE. When the chiral centre of the guest molecule is adjacent to the amine functionality, the best enantioresolutions are achieved. Hohne

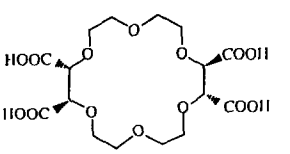


Fig. 5. Structure of 18-crown-6-tetracarboxylic acid (18C6).

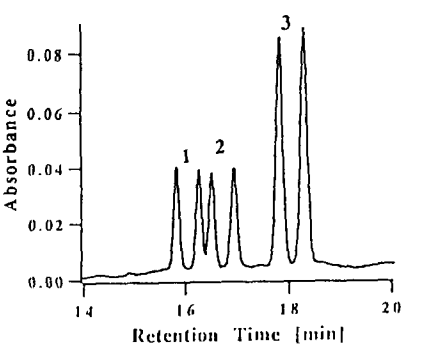


Fig. 6. Separation of three DL-amino acids by CZE with 18C6. Solutes: 1 = DL-Trp, 2 = DL-Phe, 3 = DL-Dopa. Buffer, 10 m*M* 18C6 in 10 m*M* Tris–citric acid buffer of pH 2.2. Separation tube, fused-silica capillary, 50 cm effective length × 75 μm I.D.; 260 V/cm; 214 nm (from Ref. [25]).

et al. [112] also applied the same crown ether to CE enantiomeric separations at pH 2.07 and reported the successful enantiomeric separation of six amines such as octopamine, methoxamine and norephedrine. The synergistic effect of CDs and crown ethers on the enantiomeric separation has also been reported [25]. By using 10 m*M* 18C6 and 20 m*M* α-CD, the resolution of DL-Trp improved to 7.37, compared with those obtained from either used alone (1.29 and 5.67, respectively).

4. Chiral separation by affinity interaction

4.1. Use of proteins (affinity EKC)

To separate enantiomers, the idea of using proteins as the chiral selector is natural from their great success in HPLC. Some of protein-coated stationary phases [ovomucoid (OVM), avidin, α_1-acid glycoprotein (AGP) and bovine serum albumin (BSA)] are commercially available and have been demonstrated to be effective for a wide variety of enantiomers. A protein has charge and migrates according to its electrophoretic mobility in CE. Therefore, the separation mode can be classified as a branch of EKC (affinity EKC). The solutes, both ionic and neutral, form diastereomeric complexes with the protein in dynamic equilibrium. The difference in the formation constant between the diastereomeric pair causes the difference in effective electrophoretic mobilities between the enantiomers. Assuming a similar model to that reported by Wren and Rowe for CD-CZE, the difference in apparent mobilities, $\Delta\mu_{app}$, between the enantiomers can be described as follows [106]:

$$\Delta\mu_{app} = \frac{(\mu_s - \mu_p)(K_2 - K_1)[P]}{(1 + K_1[P])(1 + K_2[P])} \quad (2)$$

where P is a protein employed as a pseudo-stationary phase, K_1 and K_2 are binding constants of the enantiomeric pair 1 and 2 and μ_s and μ_p are the electrophoretic mobilities of the free analyte and of the protein, respectively. The mobility of each complex is assumed to be equal to μ_p. It is obvious in Eq. 2 that K_1 and K_2, and also μ_s and μ_p, must be different. It is clear that the larger the difference between μ_s and μ_p, the greater is the resolution (the larger is $\Delta\mu_{app}$). Therefore, the optimization of the pH will be important to improving the enantiomeric separation.

Various proteins have been employed for the separation of enantiomers in affinity EKC: BSA [100–102], AGP [102], OVM [102,103], fungal cellulase [102], human serum albumin (HSA) [104], cellulase [cellobiohydrolase I (CBH I)] [105] and avidin [106]. Some characteristics of the protein used in affinity EKC are summarized in Table 6 [135]. In the practical use of proteins as chiral selectors, some problems such as protein adsorption on the capillary wall, absorption of UV light in the shorter wavelength region and relatively low purity of the proteins occur. These effects cause irreproducible separations and band broadening. However, affinity EKC with proteins is an attractive mode, particularly for investigating the interaction of drugs with specific proteins. Wall-coated capillaries and some additives such as organic modifiers or surfactants have been used to prevent adsorption or to improve the peak shape.

Barker et al. [100] used BSA (0.1% solution) to separate 6*S*- and 6*R*-stereoisomers of leucovorin with a bare capillary at pH 7.0 or a PEG-coated capillary at pH 7.2. It was found that the 6*R*-isomer has a greater affinity for BSA. The migration orders are reversed between the two capillaries because of the difference in the EOF velocities. The detection wavelength was 280 nm, where the difference between leucovorin absorption and BSA absorption is large. They also used BSA in addition to dextran for the optical resolution of leucovorin and some drugs in a linear polyacrylamide-coated capillary [101].

Busch et al. [102] applied four proteins, AGP, OVM, fungal cellulase and BSA, to affinity EKC using an untreated fused-silica capillary under neutral conditions (pH 6.8 or pH 7.4). These problems have been demonstrated to be effective in chiral HPLC. Detection was carried out at 280 or 260 nm. BSA was effective for the enantio-

Table 6
Some characteristics of proteins used in CE as a chiral selectors and their application

Property	BSA	OVM	AGP	HSA	Fungal cellulase	CBH I	Avidin
Molecular mass	67 000	28 000	44 000	68 000	60 000–70 000	60 000–70 000	70 000
Isoelectric point	4.7	4.5	2.7	4.7	3.9	3.9	10
Sialic acid residues	–	0.3	14	–	–	–	–
Disulphide bridges	17	8	2	–	12		–
Carbohydrate (%)	–	30	45		6	6	20.5
CE mode [ref.]	EKC [100–102] CGE [107,108]	EKC [103]	EKC [102] EC [109]	EKC [104]	EKC [102]	EKC [105]	EKC [106]
Separated solutes[a]	Benzoin[65] DNS-Nva, DNS-Leu Ibuprofen[54] Leucovorin[61] Promethazine[5] Trp Warfarin[56]	Benzoin Chlorpheniramine[49] Eperisone[83] Tolperisone[84]	Alprenolol[30] Benzoin Cyclophosphamide[85] Disopyramide[86] Hexobarbital[9] Metoprolol[34] Oxprenolol[36] Pentobarbital[10] Promethazine	2,3-Dibenzoyl tartaric acid N-2,4-Dinitrophenyl glutamic acid 3-Indolelactic acid Kynurenine[87] Trp	Pindolol[37]	Alprenolol[30] Labetolol[33] Metoprolol[34] Pindolol Propranolol[39]	Flurbiprofen[88] Ibuprofen[54] Ketoprofen[89] Leucovorin[61] Vanilmandelic acid Warfarin[56]

[a] Superscript numbers refer to structures of the solutes shown in Fig. 13.

meric separation of Trp, benzoin and warfarin. The addition of 1-propanol (3–6%) improved the enantioselectivity through the improvement of peak shapes. The enantiomers of promethazine, which were not resolved with BSA alone, were successfully resolved by addition of 3% 1-propanol to 0.33% BSA solution. However, too large an addition (found 10%) of 1-propanol reduced the enantiomeric resolution, contrary to expectation. AGP (ca. 0.1% solution) recognized enantiomers of promethazine, and fungal cellulase (ca. 0.1% solution) was effective for the separation of enantiomers of pindolol. OVM was unsuccessful with the test analytes. However, Ishihama et al. [103] recently employed OVM in affinity EKC and a successful enantioseparation of benzoin, tolperisone, eperisone and chlorpheniramine was obtained.

Vespalec et al. [104] used 1% HSA in 10 m*M* acetic acid–Tris buffer solution of pH 9.6 with a bare capillary and pH 8 for a linear polyacrylamide-coated capillary to separate enantiomers of Trp, kynurenine and some other compounds. They heated alkaline HSA solutions mildly to achieve stabilization of the enantioselectivity of HSA. Valtcheva et al. [105] employed CBH I (0.4%) to separate enantiomers of five β-blockers: (*R*,*S*)-propranolol, (*R*,*S*)-pindolol, (*R*,*S*)-metoprolol, (*SS*,*RR*)- and (*RS*,*SR*)-labetolol and (*R*,*S*)-alprenolol, with non-cross-linked polyacrylamide-coated capillaries at pH 5.1. They also introduced a 2–3 mm long agarose plug at one end of the capillary to prevent any hydrodynamic flow. By using a high concentration (25–30%) of 2-propanol (IPA), a satisfactory peak shape was obtained, leading to good enantioresolution.

Tanaka et al. [106] employed avidin, which is a basic protein having p*I* ≈ 10, to separate enantiomers of acidic compounds such as ibuprofen, ketoprofen, flurbioprofen and leucovorin. Avidin is positively charged under neutral or acidic conditions, hence a wall-coated capillary was used to prevent the adsorption of avidin. Peak shapes were improved through the addition of alcohols such as ethanol, 1-propanol and (IPA). The enantiomeric separation of racemic ibuprofen and ketoprofen by affinity EKC with avidin is shown in Fig. 7, where 10% IPA was added to the protein solution [106].

Immobilized proteins have been also successfully used in CE as chiral selectors other than by addition. Birnbaum and Nilsson [107] prepared a capillary filled with gels consisting of BSA (ca. 1.7%) cross-linked with glutaraldehyde for the separation of DL-Trp. Sun et al. [108] immobilized BSA on dextran by using cyanogen bromide. The linear polyacrylamide-coated capillary was filled with the BSA–dextran polymer and used for the separation of diastereomers of leucovorin. Li and Lloyd [109] investigated the applicability of capillaries packed with an im-

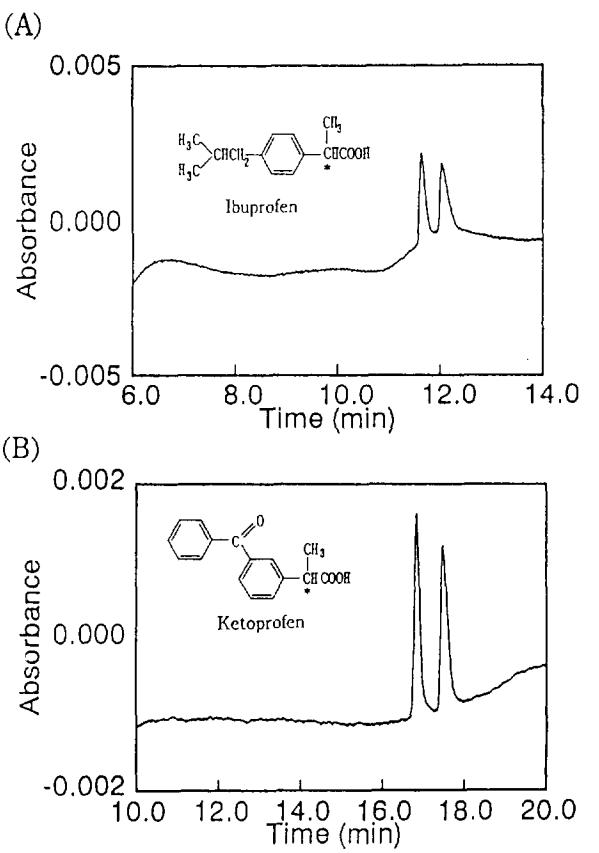


Fig. 7. Enantiomeric separation of racemic (A) ibuprofen and (B) ketoprofen by affinity EKC with avidin. Buffer, 25 μ*M* avidin in 50 m*M* phosphate buffer of pH 6.0 containing 10% IPA. Separation tube, coated capillary tube cartridge, 31.5 cm effective length × 50 μm I.D. (Bio-Rad); −12 kV; (A) 230 nm, (B) 270 nm; 25°C (from Ref. [106]).

mobilized AGP stationary phase to direct chiral separations. The separation mode can be classified as EC. The AGP stationary phase has been found to be effective for chiral separations in HPLC [110]. Ten compounds including hexobarbital, pentobarbital, benzoin, ifosfamide, cyclophosphamide and β-blockers were successfully optically resolved by EC with a capillary packed with AGP stationary phase. A variety of applications using proteins as chiral selectors in CE are also summarized in Table 6. Most proteins are considered to recognize chirality, and therefore enantiomer separations by CE using proteins will increase.

4.2. Use of polysaccharides

Besides proteins, various natural products can be applied to separate enantiomers for CE. Polysaccharides such as cellulose and amylose are the most readily available from successful enatioseparations in HPLC. Many cellulose derivatives have already been found to be effective in HPLC chiral separations and most of them are now commercially available [110].

Concerning enantiomeric separation, only two papers have reported the addition of saccharides as a chiral selector in CE. D'Hulst and Verbeke [113] used maltodextrins and corn syrups as chiral additives in CZE. The former are mixtures of linear α-(1,4)-linked D-glucose polymers and the latter are maltooligosaccharide mixtures. The enantiomers of non-steroidal anti-inflammatory drugs such as flurbiprofen and ibuprofen, and warfarin and some other drugs, were successfully optically resolved with 10 m*M* phosphate buffer solution of pH 7.0–7.5 through the addition of saccharides. The separation mechanism is not clear; however, some affinity interaction may cause the chiral recognition.

Nishi et al. [114] used dextran sulphate as a chiral selector in CE for the enantiomeric separation of trimetoquinol and clentiazem. Dextran sulphate (Na^+ salt), which is a mixture of linear α-(1,6)-linked D-glucose polymers having a sulphate group in the molecule, has three sulphate groups per D-glucose unit, as shown in Fig. 8.

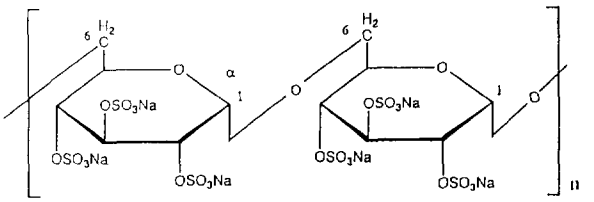


Fig. 8. Unit structure of dextran sulphate (Na^+ salt).

Therefore, it can be used as a chiral pseudo-stationary phase in affinity EKC like proteins. As expected from affinity EKC with proteins, pH values in addition to the concentration of the chiral selector are most critical for the enantioselectivity. A chromatogram of racemates of trimetoquinol and its hydroxy positional isomer obtained by affinity EKC with dextran sulphate is shown in Fig. 9. With both maltodextrin and dextran sulphate, trials to improve the enantioselectivity by combination with another additive

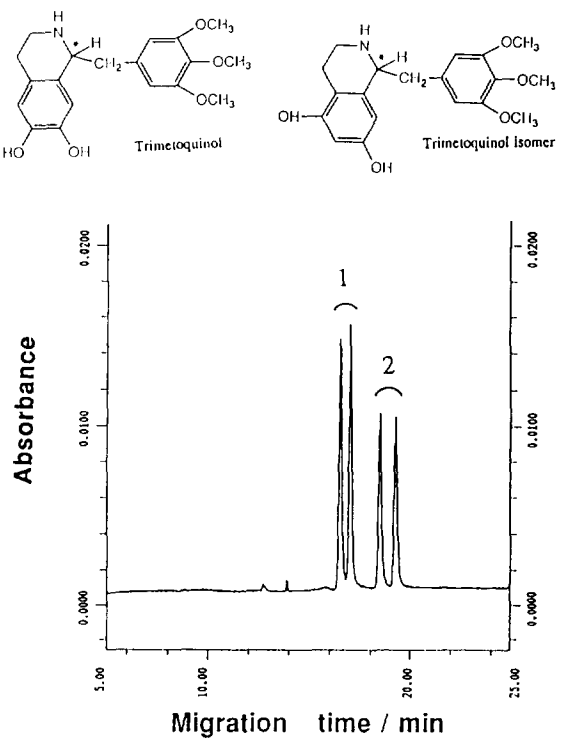


Fig. 9. Separation of enantiomers of trimetoquinol and its isomer by affinity EKC with dextran sulphate. Buffer, 3% sulphate in 25 m*M* phosphate buffer of pH 5.5. Other conditions as in Fig. 2 (from Ref. [114]).

such as an organic modifier or a surfactant were not successful.

Besides the addition of saccharides as chiral selectors, Sun et al. [101] used dextran as an additive to improve the resolution of enantiomers in affinity EKC with protein. By adding dextran (ca. 5%) to a 10 m*M* phosphate buffer solution of pH 7.12 containing 0.1% BSA, enantiomers of ibuprofen, leucovorin, DNS-DL-Leu, DNS-DL-Nva and mandelic acid, which are all difficult to separate using BSA alone as the chiral selector in CE, were successfully resolved. They reported that the effect is ascribable to the decrease in migration velocity of BAS, leading to an enhancement of enantioselectivity. Large species such as proteins have a much larger effect on the mobility in comparison with small species (i.e., samples) with the polymer network of dextran. It is reasonable to expect that dextran will also influence the enantioselectivity of the solute.

5. Chiral separation by solubilization

5.1. Use of chiral micelles (micellar EKC)

In MEKC, enantiomeric separations have been successfully achieved by employing chiral surfactants [26,84–86,117–130]. The chiral surfactants used in CE chiral separations are summarized in Table 7. Although CZE is only applicable to ionic compounds, MEKC can separate both ionic and non-ionic enantiomers, as in CD-EKC mentioned above. In MEKC chiral separations, a chiral surfactant solution or a mixed micelle solution of a chiral and an achiral surfactant can be employed. Two classes of chiral surfactants have been successfully employed for enantiomeric separations by MEKC: optically active amino acid-derived synthetic surfactants [117–122] and natural surfactants such as bile salts [123,125–129], digitonin [119], glycyrrhizic acid and β-escin [130]. Non-ionic

Table 7
Chiral surfactants used in MEKC chiral separations

Surfactant	Solute[a]	Additive	Ref
Amino acid derivatives			
SDVal, SDAla	Amino acid derivatives N-(3,5-Dinitrobenzoyl)-O-isopropyl N-(4-Ditrobenzoyl)-O-isopropyl N-Benzoyl-O-isopropyl	MeOH	117,118
SDVal	PTH-AAs	MeOH, urea	119–121
	Benzoin[65]	MeOH, urea	120
	Warfarin[56]	MeOH, urea	120
SDGlu	PTH-AAs	MeOH, urea	122
	Benzoin	MeOH, urea	122
Bile salts analogues			
SC, STC, SDC, STDC	DNS-AAs	SDS	123
	Carboline derivatives[90]		125
	2,2′-Dihydroxy-1,1′-dinaphthyl[79]		125,128,129
	Diltiazem[91], analogues		125,126
	1-Naphthylethylamine		125
	Trimetoquinol[29], analogues		125–127
	1,1′-Binaphthyl-2,2′-diyl hydrogenphosphate[66]		128
	1,1′-Binaphthyl-2,2′-dicarboxylic acid[67]		128
Digitonin	PTH-AAs	SDS	119
Glycyrrhizic acid	PTH-AAs, DNS-AAs	SDS	130
β-Escin	PTH-AAs, DNS-AAs	SDS	130

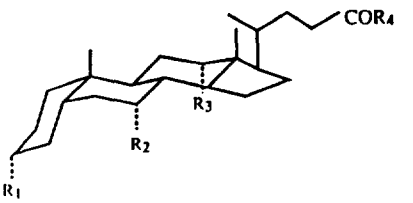
[a] Superscript numbers refer to structures of the solutes shown in Fig. 13.

surfactants such as digitonin must be used together with an ionic surfactant such as SDS to form a mixed micelle having electrophoretic mobility. Most analytes are adsorbed on the surface of the micelle and interact with the polar groups of the surfactants, and therefore surfactants having chiral polar groups will be effective.

The first chiral separation by MEKC was reported by Cohen et al. [136]. They used N,N-didecyl-L-alanine as a chelating surfactant together with SDS and metal ions (Cu^{2+}) to form mixed micelles having a chiral ligand. The difference in the formation constants led to the successful chiral separation of DNS-DL-AAs.

Dobashi et al. [117,118] used sodium N-dodecanoyl-L-valinate (SDVal) and sodium N-dodecanoyl-L-alaninate to separate N-(3,5-dinitrobenzoyl) derivatives of DL-AAs, etc. Otsuka and Terabe [119–121] also used SDVal for the separation of phenylthiohydantoin (PTH)-DL-AAs. They reported that the addition of MeOH and urea improved peak shapes, hence improving the resolution [120,121]. Beozoin and warfarin were also enantioresolved under the same conditions. Addition of SDS or a high concentration of urea was effective for the enhancement of the selectivity. Recently, Otsuka et al. [122] employed N-dodecanoyl-L-glutamate (SDGlu) for the MEKC enantiomeric separation of PTH-DL-AAs. Through the addition of MeOH and urea, three PTH-DL-AAs, Nva, Val and Trp, were successfully separated. By using mixed micelles of SDGlu and SDS, five PTH-DL-AAs were enantioseparated.

Terabe et al. [123] first used bile salts for the separation of enantiomers by MEKC. By using sodium taurocholate (STC) or sodium taurodeoxycholate (STDC) under acidic conditions, some DNS-DL-AAs were enantioresolved, although a long separation time was required. Various bile salts are well known as natural anionic surfactants. The structures of some bile salts are shown in Fig. 10. Bile salts are assumed to form helical-structured micelles with a reversed micelle conformation [124], leading to unique characteristics compared with long-chain alkyl-type surfactants. Although not many applications have been published on enantiomeric



Bile salt	R_1	R_2	R_3	R_4
Sodium cholate	OH	OH	OH	ONa
Sodium taurocholate	OH	OH	OH	$NHCH_2CH_2SO_3Na$
Sodium deoxycholate	OH	H	OH	ONa
Sodium taurodeoxycholate	OH	H	OH	$NHCH_2CH_2SO_3Na$

Fig. 10. Structures of some bile salts.

separations with bile salts, the enantioselectivity of bile salt micelles seems to be greater for relatively flat and rigid compounds, expected from its micelle structure.

Nishi et al. [125–127] successfully used four bile salts for the separation of enantiomers of diltiazem, trimetoquinol and some other drugs under neutral conditions. Enantiomers of carboline derivatives and 2,2′-dihydroxy-1,1′-dinaphthyl were well separated with four bile salts [125]. However, enantiomers of diltiazem and trimetoquinol were separated with only STDC at pH 7.0 [126,127]. Enantiomers of diltiazem, which were not enantioresolved by CD-CZE or CD-MEKC, were only resolved by MEKC with STDC. A chromatogram of racemic diltiazem and related compounds is shown in Fig. 11, where 50 m*M* STDC was added to the 20 m*M* phosphate–borate buffer solution of pH 7.0. Cole and co-workers [128,129] also separated enantiomers of three binaphthyl derivatives by MEKC with bile salts. They reported that the addition of MeOH improved the resolution owing to the expansion of elution range. Okafo and co-workers [85,86] and Lin et al. [87] used STDC with or without β-CD for the separation of DNS-DL-AAs.

Otsuka and Terabe [119] used a non-ionic surfactant, digitonin, which is a natural product, the glycoside of digitogenin, for the optical separation of PTH-DL-AAs. To form a charged micelle, SDS was added to the digitonin solutions. Six PTH-DL-AAs were optically separated

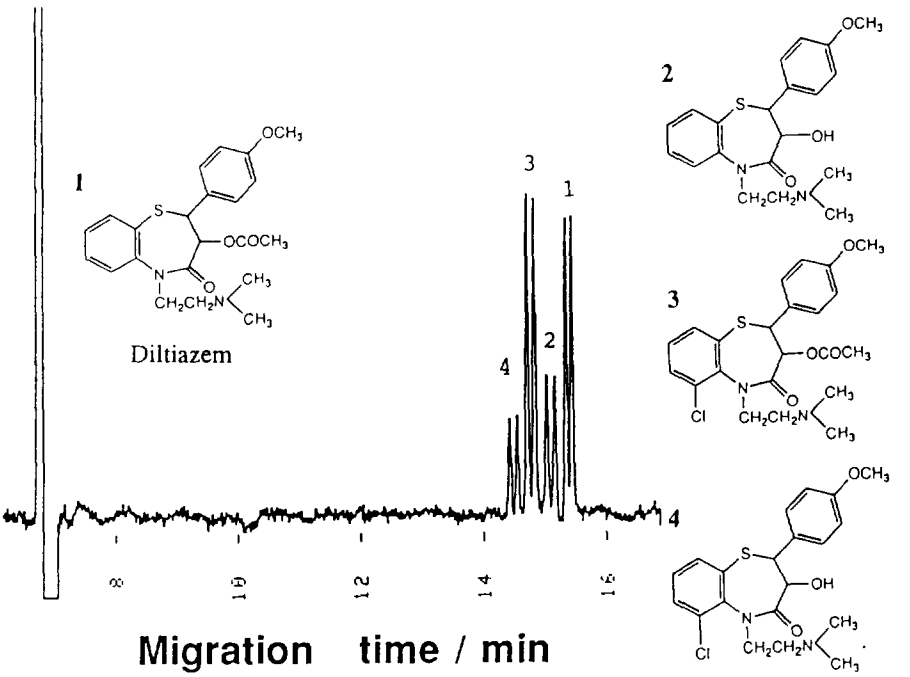


Fig. 11. Separation of enantiomers of diltiazem and related compounds by MEKC with bile salt. Solutes: 1 = diltiazem; 2 = desacetyldiltiazem; 3 = 6-chlorodiltiazem; 4 = desacetyl-6-chlorodiltiazem. Buffer, 50 mM STDC in 20 m*M* phosphate–borate buffer of pH 7.0. Separation tube, fused-silica capillary, 65 cm (effective length 50 cm) × 50 μm I.D.; 20 kV; 210 nm; ambient temperature (from Ref. [126]).

under acidic conditions, although a long analysis time was required. No enantiomeric separation was achieved under neutral conditions. Two other natural surfactants, glycyrrhizic acid and β-escin, were employed by Ishihama and Terabe [130] in MEKC for the optical resolution of DNS-DL-AAs and PTH-DL-AAs. Single micelles of these surfactants were not effective for enantiorecognition. Glycyrrhizic acid was then used with octyl-β-glucoside and SDS at pH 7.0. In this instance, fluorescence detector should be used because of the strong UV absorption of glycyrrhizic acid due to the carbonyl group conjugated to a carbon–carbon double bond. Some DNS-DL-AAs were successfully resolved under neutral conditions with a long analysis time. As for β-escin, nine PTH-DL-amino acids were optically resolved by adding SDS under acidic conditions.

Besides the applicability of MEKC to the optical resolution of neutral compounds, another advantage of MEKC is the easy manipulation of selectivity [137]. The selectivity can be changed by changing surfactants or adding various additives. The MEKC mode is suitable for separating solutes in complicated matrices, as in the case of CD-MEKC. It is desirable that many other chiral surfactants become available as separation carriers for enatiomeric separations by MEKC.

5.2. Use of microemulsions (microemulsion EKC)

Microemulsion EKC uses a microemulsion, which is prepared by mixing oil, water, a surfactant and a cosurfactant such as a medium alkyl-chain alcohol, as a pseudo-stationary phase instead of a micelle as in MEKC [138,139]. A microemulsion works similarly to ionic micelles for the separation of neutral compounds. Solutes are incorporated into the microemulsion according to their hydrophobicity. In chiral separations by microemulsion EKC, a lipophilic chiral selector must be used. Aiken and Huie [140] used a microemulsion consisting (2*R*,3*R*)-di-*n*-butyl tar-

trate–SDS–butanol–buffer (pH 8.1) (0.5:0.6:1.2:97.7) for the chiral separation of ephedrine. A selectivity value of 2.6 was obtained.

6. Chiral separation by ligand-exchange complexation

CE optical resolution by ligand exchange was first reported by Gassman et al. [115] in 1985. They employed a Cu(II)–histidine complex as an additive to buffer solutions for the optical resolution of DNS-DL-AAs. DNS-DL-AAs will form ternary complexes, which consist of Cu(II), L-histidine and DNS-L-AA or DNS-D-AA. These diastereomeric complexes are electrically identical and probably have close electrophoretic mobilities; however, the formation (or stability) constants of the complexes are different and hence enantiomeric separation can be achieved. They also used an Cu(II)–aspartame complex system for the separation of the same class of compounds [116]. Fourteen of eighteen DNS-DL-AAs were successfully optical resolved by employing aspartame. This can be ascribed to the instability of the six-membered chelate rings of Cu(II)–aspartame compared with the five-membered ring of Cu(II)–histidine. Fanali et al. [141] separated enantiomers and diastereomers of Co(III) complexes with ethylenediamine, *o*-phenanthroline, etc., by using sodium L-(+)-tartrate at pH 5.25. Cohen et al. [136] used ligand-exchange complexation with N,N-didecyl-L-alanine in MEKC as mentioned above.

7. Chiral separation by derivatization to diastereomers

When a direct chiral separation by CE is not successful, chiral derivatization techniques can be adopted, as in HPLC. Schutzner et al. [142] derivatized DL-Trp with (+)-diacetyl-L-tartaric anhydride to diastereomeric pairs and separated these diastereomers by CZE with a polyacrylamide-coated capillary at pH 6.35. They added polyvinylpyrrolidone to the CZE buffer as a polymer network additive. The diastereomers, which have identical electrophoretic mobilities at 0% polyvinylpyrrolidone and pH 6.35, were separated through the addition of the polymer. An increase in the concentration of the polymer to 6% improved the optical resolution. Some kind of interaction as in a reversed-phase HPLC system probably caused the separation. It was essential to add the polymer to CZE buffer for the diastereomeric separation of the solutes.

As already mentioned, the separation of diastereomers by a simple CZE system is usually difficult, and therefore an EKC system such as MEKC must be employed for this purpose. Nishi et al. [143] derivatized DL-AAs with 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate (GITC) and separated the GITC-derivatized DL-AAs by MEKC using SDS at pH 7.0 or 9.0 with high efficiency. An example is shown in Fig. 12. Lurie [144] also used GITC to derivatize six phenethylamines including amphetamine, ephedrine and norephedrine and separated the GITC derivatives by MEKC with SDS and MeOH at pH 9.0.

Tran et al. [145] used 1-fluoro-2,4-dinitrophenyl-5-D- (or -L-)alaninamide (Marfey reagent) to derivatize DL-AAs and some peptides. The separation of the derivatized compounds was carried out by MEKC with SDS at pH 8.5. The migration order between D- and L-AAs was reversed by changing the reagent chirality (D- or L-Marfey reagent). These results show that the separation of diastereomers by MEKC is far superior to that by CZE with respect to high efficiency and easy manipulation of selectivity.

Kang and Buck [146] separated OPA-derivatized DL-AAs by MEKC with SDS and MeOH at pH 9.5. They used both N-acetyl-L-cysteine and N-*tert.*-butyloxycarbonyl-L-cysteine as a chiral reagent together with OPA. Some diastereomers were separated by a simple CZE with some additives such as TAA salts. However, MEKC gave a better resolution than CZE.

8. Conclusions

Most of the separation principles are the same as those applied in HPLC. Naturally, most

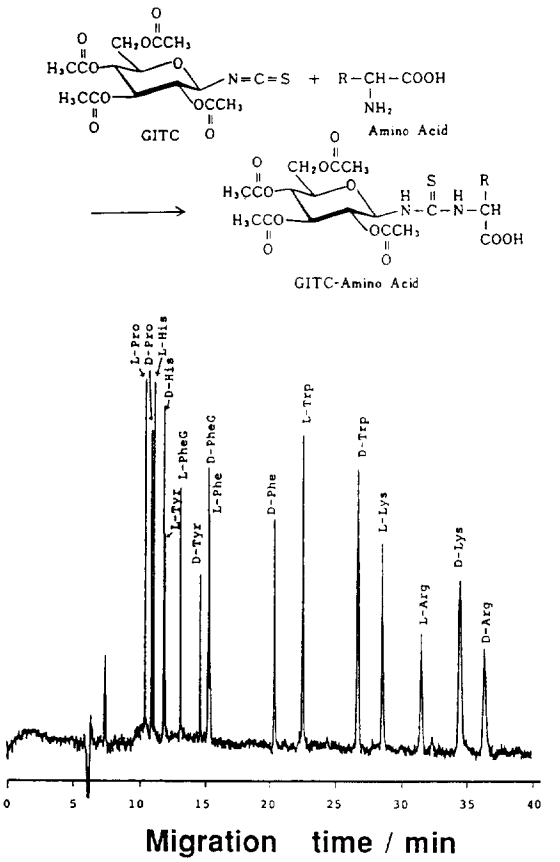


Fig. 12. Separation of eight GITC-derivatized DL-amino acids by MEKC with SDS. Buffer, 0.2 *M* SDS in 20 m*M* phosphate–borate buffer of pH 9.0. Other conditions as in Fig. 11 (from Ref. [143]).

additives or chiral selectors used in CE are borrowed from that used in HPLC, except for the chiral surfactants in MEKC. However, CE chiral separation has many advantages over HPLC chiral separation, such as its high separation efficiency and easy manipulation of selectivity. As discussed in CD-EKC, the migration order between D- and L-forms in CE also can be selected without changing the column and chiral selector, which is often required in HPLC chiral analysis, to obtain a favourable migration order. Another is related to economic aspects. To find the optimum separation media, one can simply alter the separation solution, which has an extremely small volume. This means that the use of several kinds of chiral columns, which are relatively expensive, and an organic solvent is not necessary in CE chiral separations. Extremely small volumes of media also allow the use of expensive chiral additives in CE. Although the relative standard deviation (R.S.D.) in the migration times and peak areas in CE analysis are inferior to those given by fully automated modern HPLC instruments, in which usually R.S.D. values not more than 0.5% are obtained without difficulty, CE chiral separations are suitable as a quality control method of optical purity testing, where R.S.D. values of around 1% and the sensitivity of UV detection are satisfactory for the purpose. Other than CDs or proteins, some specially designed chiral selectors will become

Table 8
Recommended starting conditions for a first attempt at CE chiral separation

Solute	Chiral additive	Buffer
Cationic solutes	i 20 m*M* DM-*β*-CD	Phosphate buffer of pH 2.5
	ii 20 m*M* HP-*β*-CD	Phosphate buffer of pH 2.5
	iii 5% *β*-CD polymer	Phosphate buffer of pH 2.5
	iv 20 m*M* *γ*-CD	Phosphate buffer of pH 2.5
Anionic solutes	i 20 m*M* DM-*β*-CD	Phosphate buffer of pH 7.0
	ii 20 m*M* HP-*β*-CD	Phosphate buffer of pH 7.0
	iii 5% *β*-CD polymer	Phosphate buffer of pH 7.0
	iv 20 m*M* *γ*-CD	Phosphate buffer of pH 7.0
	v (i, ii, iii, or iv)–100 m*M* STDC	Phosphate buffer of pH 7.0
Neutral solutes	i 50 m*M* STDC	Phosphate buffer of pH 9.0
	ii 20 m*M* *β*-CD–50 m*M* STDC	Phosphate buffer of pH 9.0
Primary amine	10 m*M* 118C6	Tris–citrate buffer of pH 2.2

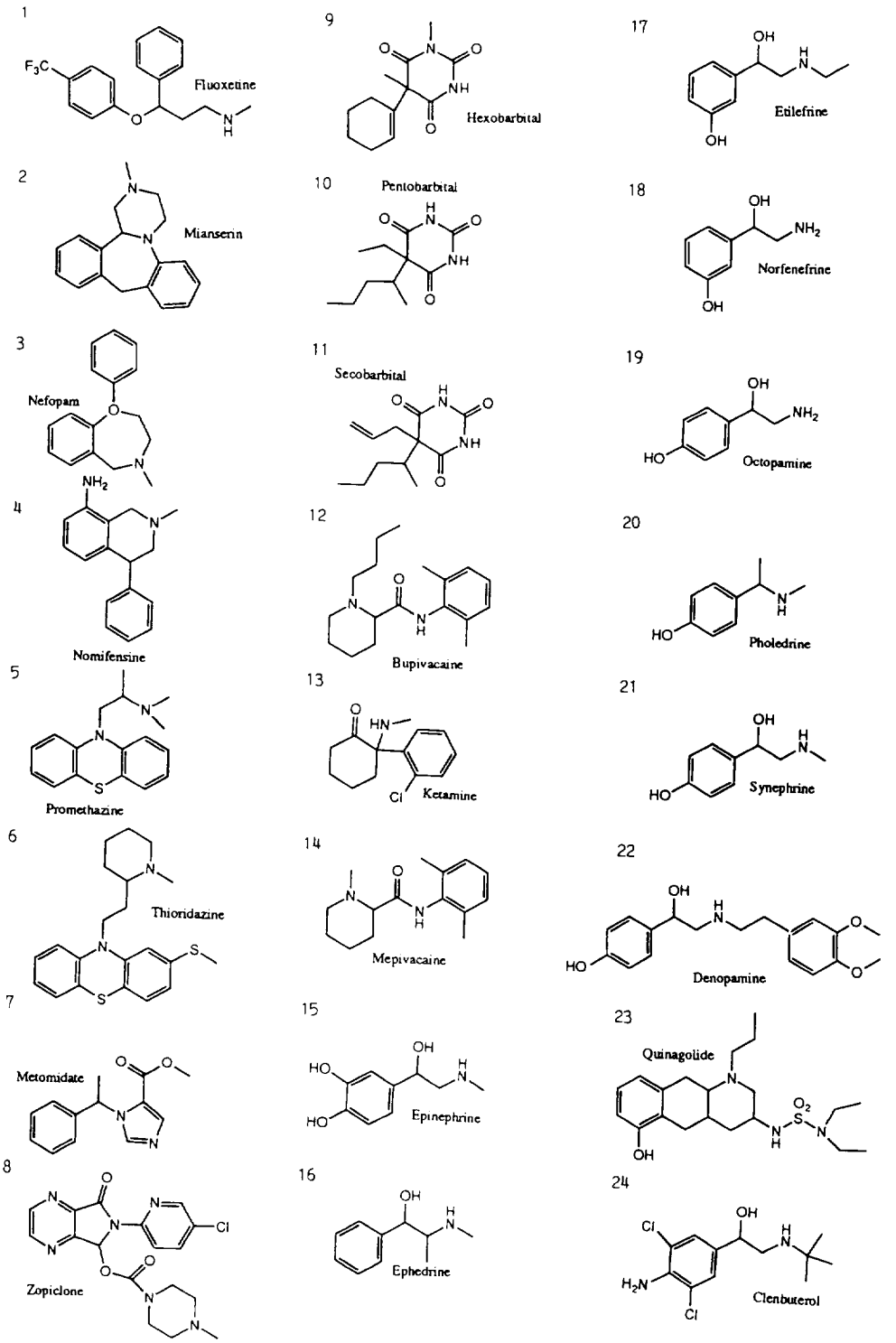
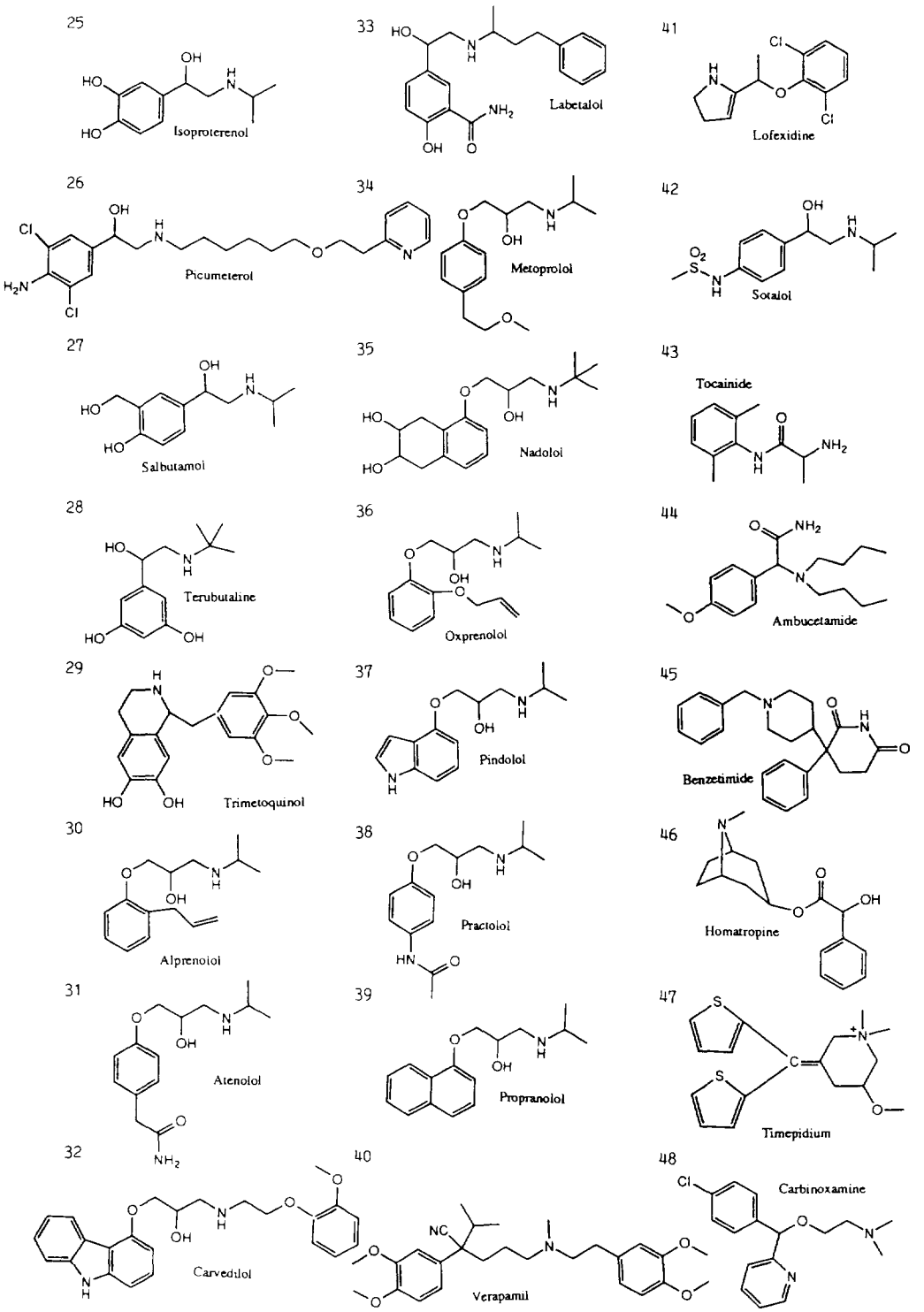


Fig. 13. (*Continued on p. 270*)


25
Isoproterenol
26
Picumeterol
27
Salbutamol
28
Terubutaline
29
Trimetoquinol
30
Alprenolol
31
Atenolol
32
Carvedilol
33
Labetalol
34
Metoprolol
35
Nadolol
36
Oxprenolol
37
Pindolol
38
Practolol
39
Propranolol
40
Verapamil
41
Lofexidine
42
Sotalol
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Tocainide
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Ambucetamide
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Benzetimide
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Homatropine
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Timepidium
48
Carbinoxamine

Fig. 13.

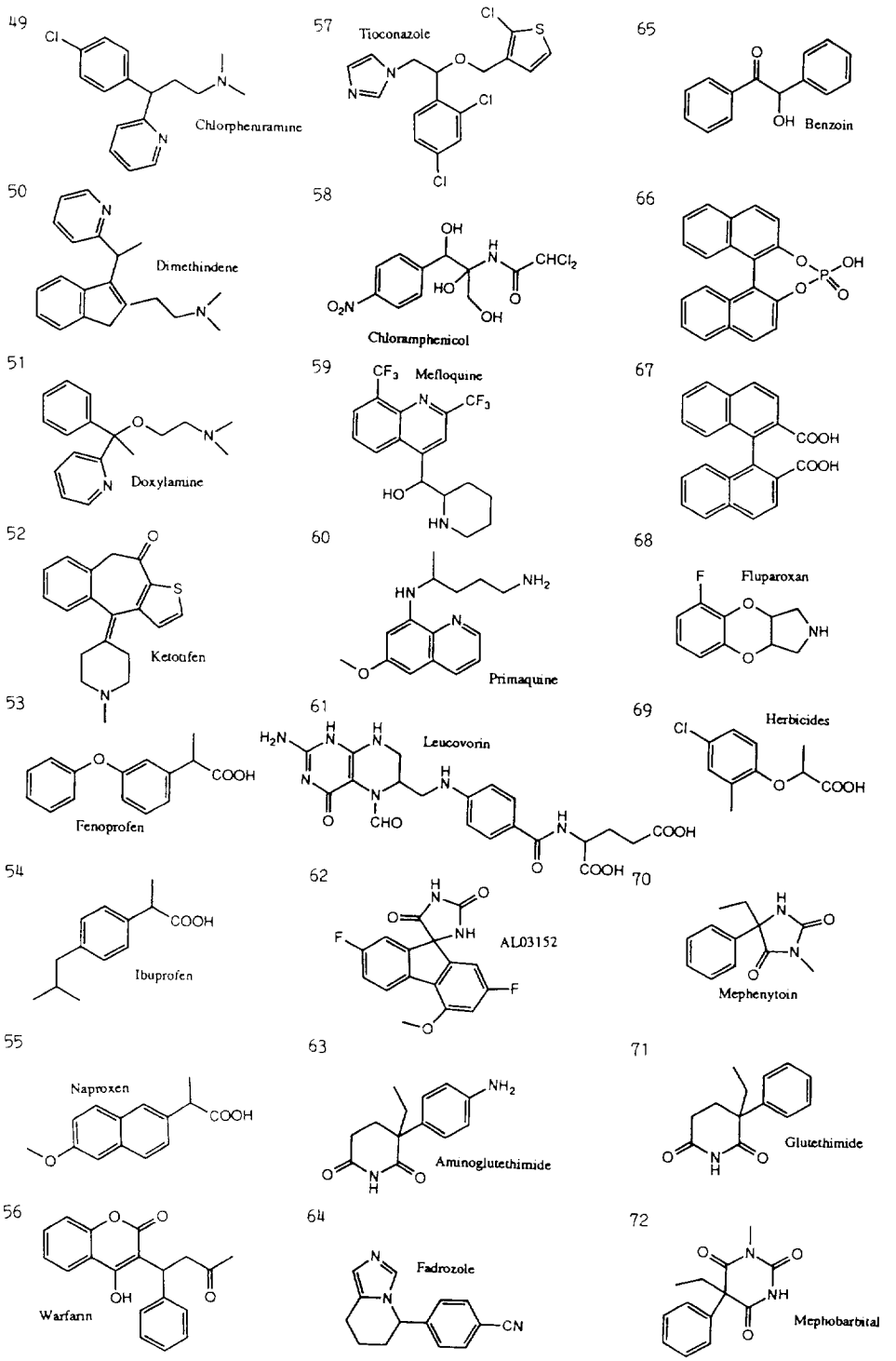

Fig. 13. (*Continued on p. 272*)

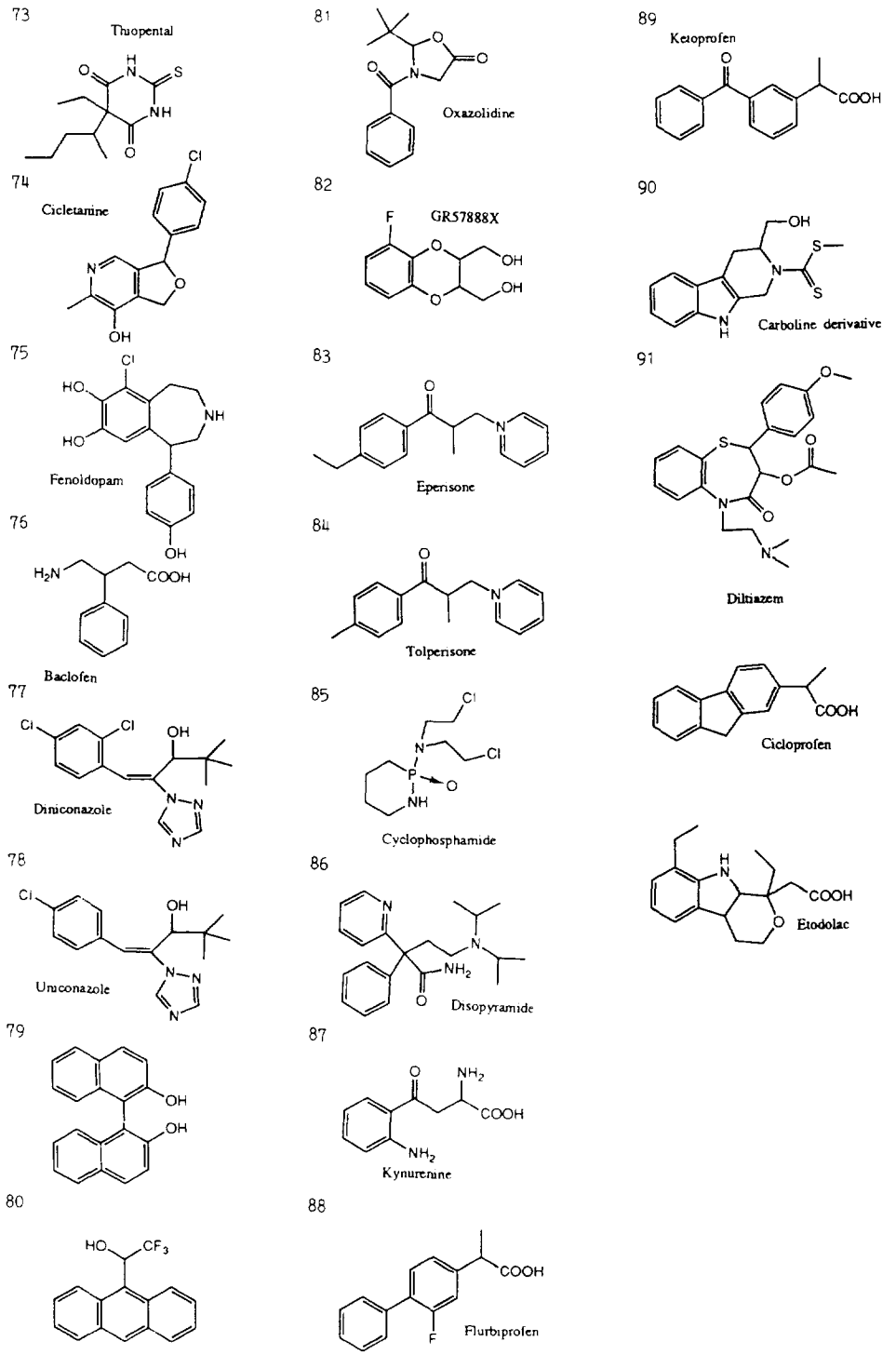

Fig. 13. Structures of the solutes.

available for CE chiral separations. Recently a chiral method development kit, which consists of several buffer solutions containing some chiral additives, was made commercially available. According to the brochure, the kit consists of four CDs. From the results summarized in Tables 3 and 4, and the wide enantioselectivity of DM-β-CD or HP-β-CD in CD-CZE, some starting buffer conditions to be attempted can be described in Table 8. Affinity EKC with proteins is also included in Table 8. The structure of the drugs summarized in Tables 3–7 with identification numbers and some other compounds described in the text are shown in Fig. 13.

Abbreviations

AAs	Amino acids
ACN	Acetonitrile
AGP	Orosomucoid (α_1-acid glycoprotein)
BSA	Bovine serum albumin
18C6	18-Crown-6-tetracarboxylic acid
d-CAM	Sodium *d*-camphor-10-sulphonate
CBH I	Cellobiohydrolase I
CBI	Naphthalene-2,3-dicarboxaldehyde
CD-CZE	Cyclodextrin-modified capillary zone electrophoresis
CD-EKC	Cyclodextrin-modified electrokinetic chromatography
CD-MEKC	Cyclodextrin-modified micellar electrokinetic chromatography
CDen	Mono-(6-β-aminoethylamino-6-deoxy)-β-cyclodextrin
CD(s)	Cyclodextrin(s)
CE	Capillary electrophoresis
CGE	Capillary gel electrophoresis
CPC	Cetylpyridinium chloride
CTAB	Cetyltrimethylammonium chloride
CZE	Capillary zone electrophoresis
DM-β-CD	Heptakis(2,6-di-O-methyl)-β-cyclodextrin
DM-β-MA	Dimethylcyclomaltoheptaose
DNS	Dansyl
EC	Electrochromatography
EDTA	Ethylenediaminetetraacetic acid
EKC	Electrokinetic chromatography
EOF	Electroosmotic flow
GC	Gas chromatography
GITC	2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate
HEC	Hydroxyethylcellulose
HPC	Hydroxypropylcellulose
HP-β-CD	Hydroxypropyl-β-cyclodextrin
HPLC	High-performance liquid chromatography
HSA	Human serum albumin
IEF	Isoelectric focusing
IPA	2-Propanol
ITP	Isotachophoresis
MEKC	Micellar electrokinetic chromatography
l-MEN	*l*-Menthoxyacetic acid
MeOH	Methanol
MHEC	Methylhydroxyethylcellulose
OVM	Ovomucoid
PTH	Phenylthiohydantoin
R.S.D.	Relative standard deviation
SC	Sodium cholate
SDC	Sodium deoxycholate
SDS	Sodium dodecyl sulphate
SDAla	Sodium N-dodecanoyl-L-alaninate
SDGlu	Sodium N-dodecanoyl-L-glutamate
SDVal	Sodium N-dodecanoyl-L-valinate
SFC	Supercritical fluid chromatography
STC	Sodium taurochlote
STDC	Sodium taurodeoxycholate
TAA	Tetraalkylammonium
TBA	Tetrabutylammonium
TMA	Tetramethylammonium
TM-β-CD	Heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin

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