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Review

Recent progress in capillary electrophoretic analysis of amino acid enantiomers☆

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ABSTRACT

This review highlights recent progresses in capillary electrophoresis (CE) analysis of amino acid enantiomers in the last decade. Various chiral selectors including cyclodextrins (CDs), bile salts, crown ethers, cinchona alkaloids, metal–chiral amino acid complexes, macrocyclic antibiotics and proteins have been employed to separate amino acid enantiomers. In the CE analysis of amino acids, the selection of the separation mode is one of the most important issues to obtain good resolution of target enantiomers. Among several separation modes, CD-modified capillary zone electrophoresis (CD-CZE), CD electrokinetic chromatography (CDEKC), micellar EKC (MEKC), CD-modified micellar electrokinetic chromatography (CD-MEKC), capillary electrochromatography (CEC), ligand-exchange CE (LE-CE), and nonaqueous CE (NACE) have been employed to the chiral analysis of amino acids. More than 160 published research articles collected from SciFinder Scholar databases from the year 2001 described the enantioseparations of amino acids by capillary-based electrophoresis. This review provides a comprehensive table listing the CE analysis of amino acid enantiomers with categorizing by the separation modes.

Contents

Abbreviations: 18C6H$_4$, 18-crown-6-tetracarboxylic acid; ABA, amino butyric acid; ACA, amino caprylic acid; ACE, affinity CE; ACEC, affinity CEC; AIB, 2-aminoisobutyric acid; APOC, 1-(9-anthryl)-2-propyl chloroformate; AQC, 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate; BGS, background solution; BZ, benzoyl-; C^{4}D, capacitively coupled contactless conductivity detector; CBI, cyanobenz[f]isoindole; CC, counter current; CD-CZE, cyclodextrin-modified capillary zone electrophoresis; CDEKC, CD electrokinetic chromatography; CD-MEKC, CD-modified micellar electrokinetic chromatography; CEC, capillary electrochromatography; CS, chiral selector; DNB, 3,5-dinitrobenzoyl-; DNP, 2,4-dinitrophenyl-; DNS, dansyl-; DNZ, 3,5-dinitorobenzyloxycarbonyl-; DOPA, dihydroxyphenylalanine; EKC, electrokinetic chromatography; FITC, fluorescein isothiocyanate; FMOC, 9-fluorenylmethyl-chloroformate; HP-CD, hydroxypropyl-CD; HS-CDs, highly sulfated CDs; LE-CE, ligand-exchange CE; LIF, laser induced fluorescence; M-CEC, monolithic CEC; MEKC, micellar EKC; MS, mass spectrometry; NACE, nonaqueous CE; NDA, naphthalene-2,3-dicarboxaldehyde; np(amino acid), naphthyl amino acid; OPA, *o*-phthaldialdehyde; p(amino acid), phenyl amino acid; OT-CEC, open tubular CEC; PA-CEC, pressure-assisted CEC; P-CEC, packed CEC; PF, partial filling; PTH, phenylthiohydantoin; SC, sodium cholate; S-CDs, sulfated CDs; SDC, sodium deoxycholate; SDS, sodium dodecyl sulfate; STC, sodium taurocholate; STDC, sodium taurodeoxycholate; tBuC-QN, *tert*-butylcarbamoyl quinine; TGF, temperature gradient focusing.

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1. Introduction

Capillary electrophoresis (CE) has been proven to be a powerful separation technique for chiral compounds since it has the advantages of high resolution, fast separation, and small amount of samples to be analyzed. One of the most successful application areas of CE is the chiral analysis of amino acids. Accompanying with recent progresses in understandings of the function of D-amino acids in mammals, developments of selective and sensitive enantioseparation techniques to allow for the quantification of amino acid enantiomers in real biological samples are still required [1,2]. In the CE analysis of amino acid enantiomers, the selection of the separation mode is one of the most important issues to obtain good resolution of target enantiomers. Among various modes in CE, cyclodextrin-modified capillary zone electrophoresis (CD-CZE), CD electrokinetic chromatography (CDEKC), micellar EKC (MEKC), CD-modified MEKC (CD-MEKC), ligand-exchange CE (LE-CE), affinity CE (ACE) and nonaqueous CE (NACE) have been applied to the separation of racemic amino acids. These separation modes involve only the addition of appropriate chiral selectors (CSs) into a background solution (BGS) for electrophoresis. Hence, CDs, which exhibit low UV absorptivity, are still predominant selectors in CE though various CSs have been developed to separate amino acid racemates.

In employing the CSs with strong UV absorption, e.g., cinchona alkaloids and vancomycin, to the CE analysis with a UV detector, the detection of amino acids is considerably disturbed. To overcome this limitation, two approaches have been developed; counter current (CC) and partial filling (PF) techniques [3–6]. In the CC technique, the CS and analytes which possess opposite charges are selected to migrate opposite directions in the capillary as shown in Fig. 1a. After the sample injection into the capillary filled with a chiral separation solution containing the CS, the chiral resolution occurs in the CS solution zone. During the separation, the CSs migrate toward the inlet side of the capillary, whereas the analytes move toward the outlet. When the outlet vial is filled with a BGS devoid of the CS, therefore, the detection window is cleared from the strong UV background due to the CS. In the PF technique, on the other hand, a separation solution containing the CS is injected as a long plug but shorter than a distance to the detection point into the capillary filled with a BGS devoid of the CS (Fig. 1b). Then, a sample solution is introduced behind the CS plug and the chiral separation is performed with the BGS in both inlet and outlet vials. When the analytes and the CS zone moves in opposite directions as well as the CC technique or the analytes move faster than the CS zone, the detection can be carried out without interference of the UV absorption of the CS. The CC and PF techniques have been often used when CSs having aromatic groups are applied to the EKC and NACE modes. Of course, the application of other detection methods, e.g., laser induced fluorescence (LIF), mass spectrometry (MS) and capacitively coupled contactless conductivity detector (C^4D), is also effective to obtain a stable baseline without the interference of the UV absorption of CSs.

Capillary electrochromatography (CEC), which is the hybrid method of CE and LC, is also considered to be a quite useful technique for the chiral separation since it couples both the high efficiency of capillary electromigration and the high selectivity of chromatography. In CEC, the detection is not affected by the stationary phase, which is compatible especially with the MS detection scheme. Preparation of capillaries for the CEC analysis is mainly categorized by three formats, i.e., immobilization of the stationary phase onto (i) the packing materials (packed CEC (P-CEC)), (ii) the inner surface of the capillary (open tubular CEC (OT-CEC)) and (iii) the monolithic capillary column (monolithic CEC (M-CEC)). In OT-CEC, the preparation of the capillary with the stationary phase is relatively easier than the other two methods, but a lower phase ratio sometimes causes the insufficient separation. For chiral CEC, therefore, packed or monolithic capillaries are generally employed. To achieve the enantioresolution of amino acids, various CSs-bonded packing materials and monolithic structures have been utilized in the CEC mode.

In this article, the chiral separations of amino acids by CE are briefly reviewed because various review papers on the CE separations of enantiomers have been published [7–21]. For detailed mechanism and discussion, these papers should be referred. Since the separation modes strongly affect the chiral resolution, the "capillary"-based electrophoretic analyses of amino acid enantiomers from the year of 2001 are reviewed in the present paper with categorizing by the separation modes. Please refer to Tables 1–9 for an overview of the enantioseparation of amino acids by CE.

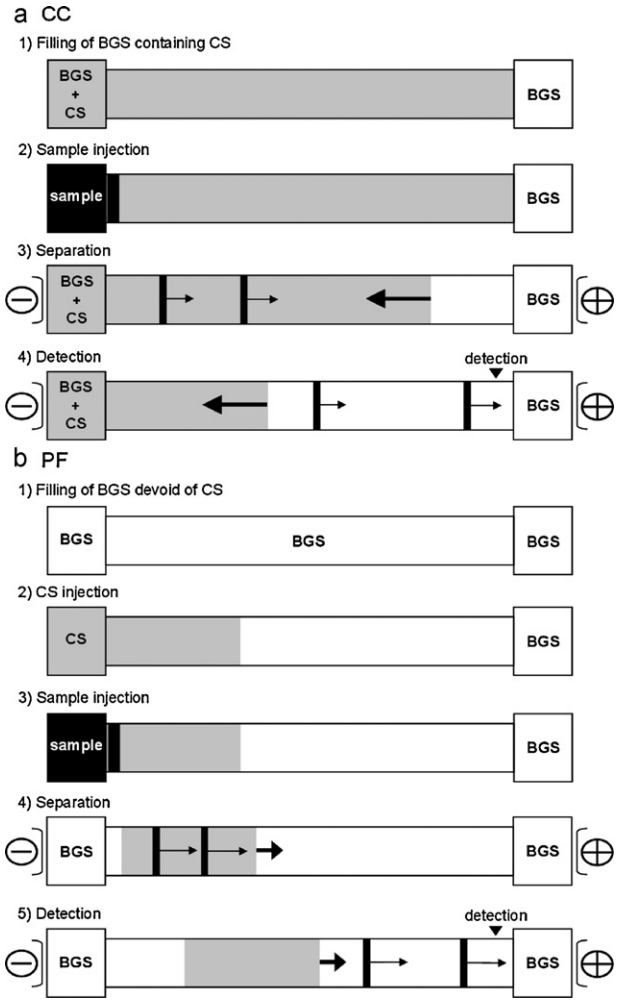

Fig. 1. Schematic illustration of (a) counter-current and (b) partial filling techniques.

Table 1
Chiral separation of amino acids in the CD-CZE mode.

CS	BGS	Sample matrix	Amino acids	Label	Detection	Comments	Ref.
α-CD	50 mM CS/50 mM phosphate (pH 2.2)	BSA	Trp		UV	Binding study	[22]
α-CD	50 mM CD/62 mM phosphate (pH 2.4)		Trp		UV		[23]
β-CD	5 mM CS/100 mM NH_4OAc (pH 6)	Orange juice	Asp, Glu, Ser, Ala, Asn, Pro, Arg	DNS, FITC	MS	Food application	[24]
β-CD	1 mM CS/140 mM borate (pH 9.5)	*E. coli* bacteria culture	Ser, Ala, Glu, Asp	OPA/NAC	UV	SPCD, biological application	[25]
γ-CD	1 mM CS/100 mM borate (pH 10.0)	Food supplements	Arg, Lys, Orn, Asp	FITC	LIF	Food application	[26]
γ-CD	0.75 mM CS/50 mM ammonium carbonate (pH 10.0)	Beer	Orn	FITC	MS/MS	Food application	[27]
HP-α-CD	175 mM HP-α-CD/60 mM phosphoric acid (pH 2.5)	Plant seeds	Phe, Try, Trp, 12-nonprotein amino acids		UV	Biological application	[28]
CDs + tetraoxadiaza-crown ether derivatives	30 mM CSs/phosphate (pH 2.27) or 10 mM Tris–citrate		Trp derivatives		UV		[29]
Tetraoxadiaza-crown ether derivatives, Me-β-CD, DM-β-CD	20–50 mM tetraoxadiaza-crown ether, 20–50 mM CD/15–70 mM phosphate (pH 2.3)		Trp, Tyr-methylester		UV		[30]
DM-β-CD + 18C6H$_4$	5 mM CS/0.5 M acetic acid		Ala, Asn, Glu, Ile, Met, Cys, pGly, Cys-Cys, Phe, Ser, Tyr, Val, Thr		C^4D		[31]
δ-CD	8.8 mM CS/25 mM phosphate-borate (pH 9)		Met, Phe, Ala, Leu, Nle, Nva, Trp, Glu, Asp, Thr	DNS, FMOC	UV		[32]

NAC, *N*-acetyl L-Cys; SPCD, on-line sample preconcentration with chemical derivatization.

2. Separation modes

2.1. CD-CZE

CD-CZE is a separation mode, where "neutral" CDs are added in the BGS as the CSs. CD-CZE is one of the most basic separation modes in the CE analysis of enantiomers. By migrating ionic amino acids in the CDs zone, the chiral separation is obtained. As summarized in Table 1, α-CDs are suitable for the enantioseparation of native Trp [22,23], whereas a wide variety of amino acids labeling with dansyl chloride (DNS), fluorescein isothiocyanate (FITC), *o*-phthaldialdehyde (OPA) and 9-fluorenylmethyl-chloroformate (FMOC) can be resolved by β-CD [24,25] and γ-CD [26,27] by CD-CZE. In addition to natural α-, β-and γ-CDs, derivatized CDs such as hydroxypropyl-α-CD (HP-α-CD), HP-β-CD, methyl-β-CD (Me-β-CD) and dimethyl-β-CD (DM-β-CD) have been also used in CE-CZE, which often give higher selectivity for amino acid racemates than the natural CDs due to the flexibility and asymmetry of the derivatized CDs [28–31]. As a novel CS, δ-CD has been applied to the CD-CZE enantioseparation of labeled amino acids [32]. Since the ring size of is different from α-, β- and γ-CDs, the resolving property of δ-CD is similar or quite different from γ-CD, depending on the structure of the analytes. In the CD-CZE separation, acidic or basic BGSs are generally employed. When an acidic BGS containing neutral CDs is used, a BGS filled capillary is regarded as an unmovable CDs-packed capillary due to almost no migration of the neutral CDs. Hence, positively charged amino acids electrophoretically migrate in the almost unmovable CD zone toward the cathode as well as the CEC mode. Under a basic condition, on the other hand, opposite direction of electrophoretic migrations of negatively charged amino acids to a fast EOF in the bare fused-silica capillary causes slower movements of amino acids toward the cathode. In the CD-CZE analysis, therefore, relatively slower migrations of amino acids are important to gain sufficient retention by neutral CDs, resulting in good resolutions of enantiomers.

In the recent decade, only three reports on employing MS detection to the CE analysis of amino acids have appeared [24,27,33]. Among these papers, two reports describe the CE-MS and CE-MS/MS analysis of amino acid enantiomers in the CD-CZE mode [24,27]. Domínguez-Vega et al. have reported a sensitive MS^n detection of non-protein amino acid ornithine (Orn) [27]. To increase the molecular size for higher MS sensitivity and lower background noise, Orn racemates have been labeled with FITC. Since a low concentration of γ-CD is required for enantioseparation of FITC-Orn, the direct introduction of the BGS into the MS detector is allowed. Under an optimal condition, the LOD of ~1 nM can be obtained, which is 100-fold lower than that of the CE-UV analysis. Such applicability of CD-CZE to CE-MS should be promising for qualitative analysis of amino acid enantiomers in complicated biological matrices.

2.2. CDEKC

In the CDEKC mode, "charged" CDs are added to BGSs as the CS in contradiction to CD-CZE. The ionic CDs work as a pseudostationary phase for the enantioseparation. When the charged CDs interact with racemic amino acids, the analytes migrate at a different velocity from a surrounding aqueous phase due to the electrophoretic migration of the ionic CDs. Although various anionic [34–43] and cationic CDs [44–62] are synthesized for CDEKC as summarized in Table 2, sulfated CDs (S-CDs)[34,35] and highly sulfated CDs (HS-CDs)[36–41] are still the predominant CSs due to their resolution powers and commercial-availability. Since commercially available S-CDs and HS-CDs from Beckmann Coulter are not single isomers but a mixture of sulfated CDs with a different degree of substitution (average; 9 and 12, respectively), a wide range of

Table 2
Chiral separation of amino acids in the CDEKC mode.

CS	BGS	Sample matrix	Amino acids	Label	Detection	Comments	Ref.
HS-γ-CD	2.3 mM HS-γ-CD/60 mM phosphoric acid (pH 2.5)	Plant seeds	Phe, Try, Trp, 12-nonprotein amino acids		UV	Biological application	[28]
β-CDen, NH_2-β-CD, β-CDampy	0.5 mM CS/50 mM ammonium hydrogen carbonate (pH 8.0)	Vinegar, soybeans	Arg, Asn, Ala, Glu, Asp	FITC	LIF, TOF-MS	Food application	[33]
S-β-CD	0.5–3.9 mM CS/0.4–2.4% dextran sulfate, 20 mM phosphate (pH 2.0)		Phe, Tyr, Trp		UV		[34]
S-β-CD	2 or 6% CS/25 mM phosphate (pH 2.0)	Brain microlysate of arctic ground squirrel	Tyr, Thr, Asn, Phe, His, Glu, Met, Ala, Asp, Ile, Ser, Trp, Gln, Val, Leu, Lys	CBI	LIF	FASS + sweeping + dynamic pH junction	[35]
HS-α-CD, HS-β-CD, HS-γ-CD	5% CS/50 mM phosphate (pH 2.5)		Amino acid esters, *N*-protected amino acids		UV	Short end injection	[36]
HS-α-CD, HS-β-CD, HS-γ-CD	3% CS/25 mM phosphate (pH 2.5)		pGly, Phe derivatives,		UV		[37]
HS-α-CD, HS-β-CD, HS-γ-CD	4% CS/35 mM Tris–phosphate (pH 2.5)		Phe, *N*-acetyl-Phe		UV		[38]
HS-β-CD + acetylated γ-CD	5% HS-β-CD, 2% Ac-γ-CD/50 mM phosphate (pH 2.0)	Beer, wine	18-Protein amino acids, Orn	AQC	UV	Food application	[39]
HS-β-CD + acetylated γ-CD	5% HS-β-CD, 2% Ac-γ-CD/50 mM phosphate (pH 2.0)	Dietary supplement, wine	Arg, Lys, Orn	AQC	UV	On-line derivatization	[40]
Sulfopropylated β-CD, HS-β-CD	10–100 mM CS/75 mM borate (pH 9.0), 15 mM phosphate (pH 7.2), 15 mM acetate (pH 4.75)		β-Methyl-Phe, Tyr, Trp, Tic		UV		[41]
HxDAS	10–75 mM CS/25 mM phosphoric acid (pH 2.5)		ABA, Asp, Glu, Leu, Met, Nle, Nva, Phe, Ser, Thr, Trp, Val	DNS	UV		[42]
OS	2.5–25 mM CS/25 mM ethanolamine (pH 9.4)		Asp, Met, Phe, Ser, Trp, Val, pGly, Glu	DNS	UV		[43]
BIMCD	10 mM CS/50 mM acetate (pH 6.0)		Phe, Val, Thr, Glu	DNS	UV		[44]
Mono-(3-alkyl-imidazolium)-β-CD chloride	2.5–20 mM CS/50 mM acetate (pH 5.0, 6.0)		ABA, ACA, Glu, Nle, Nva, Phe, Ser, Thr, Val	DNS	UV		[45]
MA-β-CD	20 mM MA-β-CD, 2 mM γ-CD/100 mM MES, 10 mM His (pH 5.2)		Glu, Nva, Cit, Met, Met-SO_2, Met-SO, Nle, Eth, ABA, ACA	DNP	UV	43 amino acids	[46]
TMA-β-CD	15.3 mM CS/50 mM phosphate (pH 6.8)		Ac-Phe		UV		[47]
per-6-NH_2-β-CD	3 mM CS/50 mM acetate (pH 4.8) or 50 mM phosphate (pH 5.8 or 6.7)		Asn, Leu, Nle, Phe, Trp, Tyr, Asp	CBZ, BOC, BZ	UV		[48]
ALAM-β-CD	7.5 mM CS/20 mM acetate (pH 5.0, 6.0) or 20 mM phosphate (pH 7.0, 8.0)		ABA, ACA, Ala, Asp, Glu, Leu, Met, Nle, Nva, Phe, Ser, Thr, Trp, Val	DNS	UV		[49]
PeAM-β-CD	2.5–20 mM CS/50 mM phosphate (pH 6.0)		Ala, Leu, ABA, Glu, Nle, Phe, Thr, Val	DNS, BZ	UV		[50]
NH_2-β-CD	5–20 mM CS/50 mM phosphate (pH 5.0)		ABA, ACA, Glu, Phe, Thr, Leu	DNS, BZ	UV		[51]
Cl·NH_3-β-CD	5–20 mM CS/50 mM phosphate (pH 6.0)		ABA, ACA, Glu, Phe, Thr, Val	DNS, BZ	UV		[52]
BuAM-β-CD	2.5–20 mM CS/50 mM phosphate (pH 6.0)		ABA, ACA, Glu, Nle, Phe, Thr, Val, Ala	DNS, BZ	UV		[53]
6-Mono(alkylimidazolium)-β-CD chlorides	3–30 mM CS/50 mM acetate (pH 6.0) or 100 mM phosphate (pH 9.6)		ABA, Met, Nle, Nva, Phe, Ser, Trp, Val	DNS	UV		[54]
PrAM-β-CD	2.5–20 mM CS/50 mM phosphate (pH 6.5)		ABA, ACA, Glu, Nle, Phe, Thr, Val, Ala, Leu	DNS, BZ	UV		[55]
Mono-(3-methyl-imidazolium)-β-CD chloride	10 mM CS/50 mM phosphate (pH 8.0)		Ala, ABA, Nva, Nle, Leu, ACA, Phe, Met, Trp, Ser, Thr, Asp, Glu	DNS	UV		[56]
THCMH	0.15–1.8 mM CS/20 mM NH_4OAc (pH 6.8)		Asp, Leu, Glu, Val, Ser, ABA, Thr, Met, Nle, Phe, Nva	DNS	UV		[57]
THALAH	0.25–1.8 mM CS/20 mM NH_4OAc (pH 6.8)		ABA, Asp, Glu, Leu, Met, Nva, Nle, Phe, Ser, Thr, Val	DNS	UV		[58]
3-Deoxy-3-amino-2(S),3(R)-γ-CD	0.05–1.8 mM CS/20 mM NH_4OAc (pH 6.8), 20 mM borate (pH 9.2), 20 mM acetate (pH 3.8)		ABA, Asp, Glu, Leu, Met, Nle, Nva, Phe, Thr, Val	DNS	UV		[59]
Mono-6-deoxy-6-(3R,4R-dihydroxypyrrolidine)-β-CD chloride	5 mM CS/50 mM phosphate (pH 6.0)		Thr, Met, Ser, Val, ABA, Asp, Glu	DNS	UV		[60]
Pyrrolidinium-β-CDs	5 mM CS/50 mM phosphate (pH 7)		ABA, Val, Leu, Ser, Met, Asp, Glu	DNS	UV		[61]
Mono-6-deoxy-6-((2S,3S)-(+)-2,3-*O*-isopropylidene-1,4-tetramethylenediamine)-β-CD	10 mM CS/100 mM phosphate (pH 6.5)		Asp, Cys, Leu, Met, Phe, Ser, Thr, Trp, Tyr, Val	DNS	UV		[62]

β-CDampy, 6-deoxy-6-[*N*-(2-methylamino)pyridine]-β-CD; β-CDen, 6^A-(2-aminoethylamino)-6^A-deoxy-β-CD; HxDAS, hexakis(2,3-di-*O*-acetyl-6-*O*-sulfo)-α-CD; OS, octa(6-*O*-sulfo)-γ-CD; BIMCD, mono-6^A-1-butyl-3-imidazorium-6^A-deoxy-β-CD; MA, 6^A-methylamino; TMA, 6-trimethyl-ammonio-6-deoxy; ALAM, mono-6-*N*-allylammonium-6-deoxy; PeAM, mono-6^A-*N*-pentylammonium-6^A-deoxy-; Cl·NH_3-β-CD, mono-6-ammonium-6-deoxy-β-CD chloride; BuAM, mono-6^A-*N*-butylammonium-6^A-deoxy; PrAM, mono-6^A-*N*-propylammonium-6^A-deoxy; THCMH, 6^A,6^D-dideoxy-6^A,6D-[6,6′-dideoxy-6,6′-di(*S*-cysteamine)-α,α′-trehalose]-β-CD; THALAH, 6^A,6^D-dideoxy-6^A,6^D-*N*-[6,6′-di(β-alanylamido)-6,6′-dideoxy-α,α′-trehalose]-β-CD; BOC, *tert*-butoxycarbonyl.

Table 3
Chiral separation of amino acids in the CD-MEKC mode.

CS	BGS	Sample matrix	Amino acids	Label	Detection	Comments	Ref.
β-CD	20 mM CS/50 mM SDS, 50 mM borate (pH 9.0, 15% MeOH)	Rat brain	Asp	NDA	LIF	Biological application	[63]
β-CD	30 mM CS/30 mM SDS, 50 mM Urea, 20 mM borate (pH 9.0, 10% 2-PrOH)		Ser, Val, Glu	DTDP	UV	Novel labeling reagent	[64]
β-CD	12 mM CS/15 mM SDS, 50 mM phosphate (pH 7.5, 15% 2-PrOH)		Val, Ile, Leu	FMOC	UV		[65]
β-CD	20 mM CS/30 mM SDS, 100 mM borate (pH 9.4)	Orange juice	Arg, Pro, Asn, Ser, Ala, Glu, Asp	FITC	LIF	Food application	[66]
β-CD	20 mM CS/50 mM SDS, 50 mM borate (pH 9.0, 25% MeOH)	Rat brain, Aplysia ganglia, single neurons from Aplysia	Ser, Thr, Val, Leu, Glu, Asp, Lys	NDA	LIF	Biological application	[67]
β-CD	10 mM CS/0.1% SDS, 0.1 M Tris–borate, 2.5 mM EDTA-Na_2, 7 M urea (pH 8.6)	Hydrolyzed protein fertilizers from animal skin, hair, coat, meat	Ala	DNS	UV	Biological application	[68]
β-CD	20 mM CS/30 mM SDS, 100 mM borate (pH 9.4)	Orange juice	Arg, Pro, Asn, Ser, Ala, Glu, Asp	FITC	LIF	Food application	[69]
β-CD	12 mM CS/15 mM SDS, 15 mM borate (pH 9.5)	Compound amino acid injection	Asp	OPA	UV	On-line derivatization	[70]
β-CD	20 mM CS/30 mM SDS, 100 mM borate (pH 9.7)	Vinegar	Arg, Pro, Ala, Glu, Asp	FITC	LIF	Food application	[71]
β-CD	20 mM CS/50 mM SDS, 50 mM borate (pH 9.4, 15% MeOH)	Nerve tissue extract, single neuron	Asp	NDA	LIF	Single-step immunoprecipitation	[72]
β-CD	20 mM CS/30 mM SDS, 100 mM borate (pH 9.7)	Microalgae	Arg, Lys, Ala, Glu, Asp	FITC	LIF		[73]
β-CD	20 mM CS/80 mM SDS, 100 mM borate (pH 10.0)	Transgenic maize	Arg, Ser, Ala, Glu, Asp	FITC	LIF	Food application	[74]
β-CD	20 mM CS/30 mM SDS, 100 mM borate (pH 10.0)	Transgenic yeast	Arg, Asn, Ala, Glu, Asp	FITC	LIF	Food application	[75]
α-CD, β-CD, HP-β-CD, HE-β-CD, Me-β-CD, γ-CD	30 mM CS/50 mM SDS, 80 mM phosphate (pH 8.2)		C- and N-protected Ala, Val, Phe		UV	Amino acid esters	[76]
HP-β-CD	50 mM CS/150 mM SDS, 100 mM Tris–borate (pH 9.0)	Urine, plasma	Val, Ile, Leu	CBI	LED-IF	FASS	[77]
HP-β-CD + DM-β-CD	17.5 mM HP-β-CD, 5 mM DM-β-CD/70 mM SDS, 15 mM borate (pH 10.2, 5% MeOH)	Microdialysate of extracelluar fluid in hypothalamus	Ser	FITC	LIF	Biological application	[78]
HP-γ-CD	15 mM CS/150 mM SDS	Human urine, cerebrospinal fluid, rat brain tissue, *Aplysia* ganglia	Trp	NDA	LIF	Biological samples	[79]
δ-CD	8.8 mM CS/100 mM SDS, 25 mM phosphate-borate (pH 9)		Met, Phe, Ala, Leu, Nle, Nva, Trp, Glu, Asp, Thr	DNS, FMOC	UV		[32]
β-CD + STC	60 mM STC, 30 mM β-CD/40 mM borax (pH 9.0)/15% 2-propanol		Ala, ASp, Glu, Lys, Met, Phe, Ser, Thr, Trp, Tyr, Val	OPA, *N*-acetylated cysteine	UV		[80]
β-CD + STC	30 mM STC, 20 mM β-CD/80 mM borate (pH 9.3)		20-Amino acids including 19 protein amino acids	FITC	LIF		[81]
β-CD + STC	12 mM STC, 8 mM β-CD/5 mM borate (pH 9.2)		Leu, Asp	FITC	LIF	10-s separation in 15-mm capillary	[82]
β-CD + STC	12 mM β-CD, 18 mM STC/80 mM borate (pH 9.3)		Leu, Ala, Glu, Asp	FITC	LIF	On-line preconc. by single drop microextraction	[83]
β-CD + SDC	30 mM β-CD + 60 mM SDC/100 mM borate (pH 9.5)	Aplysia pedal ganglion	Ser	CBI	LIF	Biological application	[84]
β-CD + STDC	30 mM CS/150 mM borate (pH 8.0, 18% 2-propanol)		19-Protein amino acids	FMOC	UV		[85]
β-CD + STDC	30 mM CS/150 mM borate (pH 9.0, 15% 2-propanol)	Human serum	19-Protein amino acids	FMOC	UV	On-line derivatization	[86]
β-CD + OTG	36–90 mM OTG, 10 mM β-CD/25–100 mM SDS, 25 mM Na_2HPO_4, 100 mM H_3BO_3 (pH 6.5)		Val, Nva, Leu, Met, Trp, Phe, Nle, Glu	DNS	UV		[87]

OTG, 1-*S*-octyl-β-D-thioglucopyranoside; DTDP, 3-(4,6-dichloro-1,3,5-triazinylamino)-7-dimethyamino-2-methylphenazine; LED-IF, light-emitting diode-induced fluorescence.

Table 4
Chiral separation of amino acids in the MEKC mode.

CS	BGS	Sample matrix	Amino acids	Label	Detection	Comments	Ref.
SC	80 mM SC/6–10 mM phosphate-borate (pH 9.0)	3% NaCl	Trp	DNS	UV	On-line sample preconc.	[88]
CHAPS	50 mM CS/75 mM SDS, 10 mM β-CD or γ-CD, 25 mM phosphate, 100 mM borate (pH 6.5)		Val, Nva, Leu, Met, Phe, Trp, Nle, Glu	DNS	UV		[89]
3-(*N*-dodecanoyl-L-valylamino)-propyltrimethylammonium bromide, 6-(*N*-nonanoyl-L-valylamino)hexyl-trimethylammonium bromide micelles; 3-(*N*-10-undecenoyl-L-valyl)aminopropyltrimethylammonium bromide polymer-micelle	25 mM CS/50 mM Tris–HCl (pH 7.0)		DNB-Ala, Val, Leu, Phe and their isopropyl esters	DNB	UV		[90]
Poly-L-SULV	10–50 mM CS/275 mM boric acid, 30 mM Na_2HPO_4, 10 mM triethylamine (pH 7.2)		ACA, Arg, Val, Nva, Tyr, Nle, Trp, Phe, His	PTH	UV		[91]
Triton X-102 modified poly-L-SUL	50 mM CS (equivalent monomer concentration)/275 mM boric acid, 20 mM phosphate, 10 mM triethylamine (pH 7.3)		Leu, Trp	PTH	UV		[92]
Poly-L-SULV	50 mM CS (equivalent monomer concentration)/275 mM boric acid, 20 mM phosphate, 10 mM triethylamine (pH 7.0)		Val, Nva, Leu, Trp	PTH	UV	Mw effect	[93]
Poly-*N*-undecenocarbonyl-L-amino acid-sulfate and carboxylate	25 mM CS/25 mM NH_4OAc-25 mM TEA (pH 8.0)		Tyr, Ile, Trp	PTH	UV		[94]
Poly-L-SULV	24 mM CS/50 mM phosphate, 25 mM borate (pH 9.0)		Phe, Leu, Trp, Nor	DNS	UV		[95]
Poly-L-SUCAAS	15 mM CS/25 mM phosphate-acetate (pH 3.0) or 25 mM NH_4OAc-25 mM TEA (pH 8.0)		Tyr, Ile, Trp	PTH	UV		[96]
DHAMAP	25 mM CS/50 mM borate (pH 9.75)		Trp, Phe derivatives, Arg	NDA	UV		[97]
SMA	20 mM CS/100 mM borate (pH 9.5)		Trp, Kyn	NDA	LIF		[98]
n-Dodecyl thioglycopyranoside sulfates	30 mM CS/50 mM phosphate (pH 6.5)		Val, Met, Leu, Phe, Trp	DNS	UV		[99]
n-Dodecyl thioglycopyranoside sulfates	30 mM CS/50 mM phosphate (pH 6.5)		Val, Met, Leu, Phe, Trp	DNS	UV		[100]

CHAPS, 3-[(3-cholamidopropyl)-dimethylammonio]-1-propane sulfonate; poly-L-SUL, poly(sodium undecanoyl-L-leucinate); DHAMAP, 3-[(3-dehydroabietamidopropyl) dimethylammonio]-1-propanesulfonate; SMA, (4α,8α,12α,13*R*,14*R*)-16-(1-methylethyl)-17,19-dinoratis-15-ene-4,13,14-tricarboxylic acid trisodium salt.

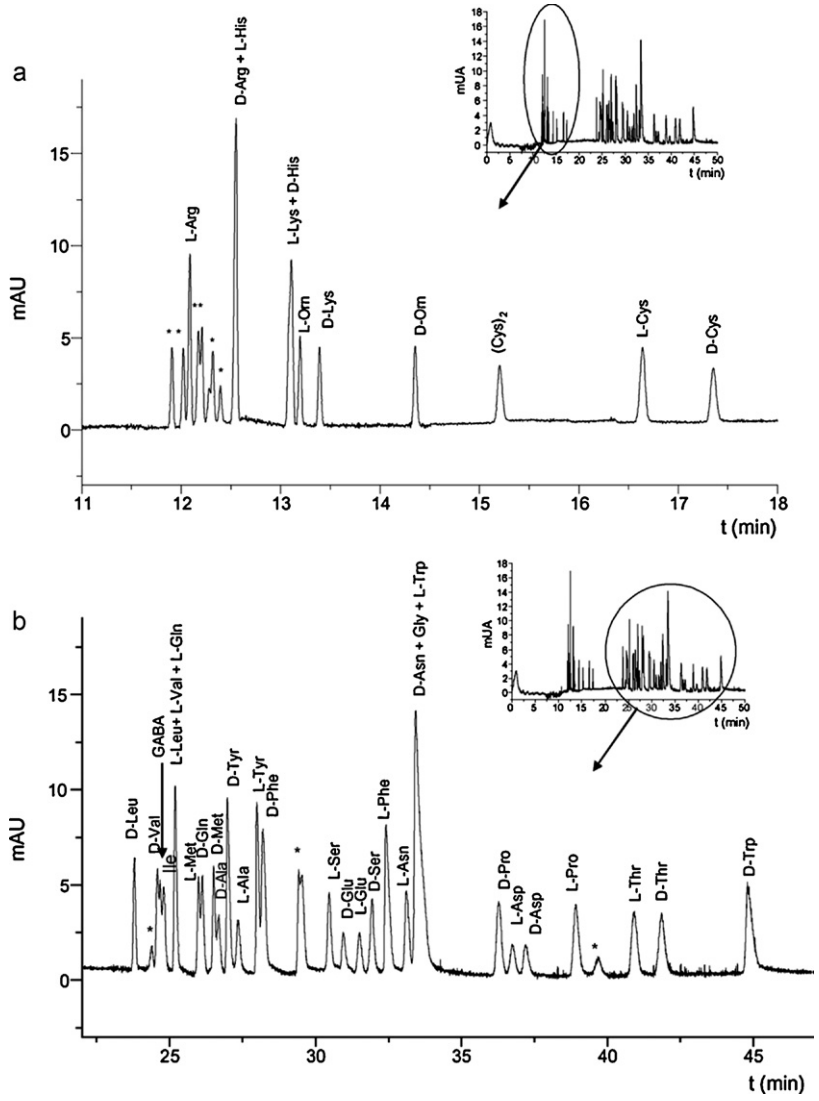

Fig. 2. Electropherograms of a mixture of the 20 protein amino acids, Orn and GABA, divided in two migration zones: (a) first-migrating and (b) second-migrating zone. Experimental conditions: 50 mM phosphate buffer at pH 2.0 containing 5% HS-β-CD and 2% acetylated-γ-CD; applied voltage, −25 kV; temperature, 15 °C; detection wavelength, 260 nm. Asterisks mean unknown peaks.
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analytes, including weak and strong acids and bases as well as zwitterions, can be successfully separated by CDEKC [13]. Actually, these sulfated CDs are significantly effective for the CDEKC separation of racemic amino acids and their derivatives. It should be emphasized that Martínez-Girón et al. have reported a chiral separation of 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC)-labeled 18 protein amino acids and Orn in a single run by employing dual CD system of 5% HS-β-CD and 2% acetylated γ-CD [39]. Among 20 protein amino acids, Ile is not optically resolved even under the optimal condition and Gly is not chiral. This is first demonstration for the simultaneous CE enantioseparation of most protein amino acids (Fig. 2). In the sulfated CD-EKC analysis of amino acid enantiomers, BGSs with pH 2.0–2.5 are generally employed. Since the EOF is suppressed and most of amino acids are positively charged at pH < 4, the cationic analytes, which are well-retained by anionic CDs at a low concentration, are separated and detected at the anodic end.

On the other hand, various types of cationic CDs have been synthesized and applied to the CDEKC analysis of amino acid racemates [44–62]. In contrast to anionic CDs, BGSs with pH 5–8,which is effective for separating neutral amino acids, are applied to EKC using cationic CDs (Table 2). Tang et al. have developed a facile synthetic methodology for cationic single-isomer CDs by replacing a primary hydroxyl group of CD with alkylimidazorium, alkylpyridinium, alkylammonium or ammonium cations [44]. These cationic CDs show good separation abilities toward anionic racemates and amino acids due to attractive electrostatic interactions with analytes. In these cationic CDs, the alkyl chain length of the substituents is important factor to decide the chiral selectivities. For example, the alkyl length of alkylimidazolium substituted β-CD strongly affected the enantioseparation of DNS-amino acids, and as a result that a shorter alkyl chain gives more powerful chiral recognition ability [45]. Although such single-isomer cationic CDs are useful for understanding the chiral separation mechanisms of CDEKC, applications to real sample analyses are still limited. As a successful application of cationic CDs, CDEKC-LIF and CDEKC-TOF-MS using monoamino-β-CD (NH_2-β-CD) have been employed to the enantioseparation of FITC-labeled amino acids in soy beans and vinegar [33]. Because the chiral separation is attained at very low NH_2-β-CD concentration of 0.5 mM, CDEKC can be combined with TOF-MS. As a result, D-Ala and D-Leu are found in the vinegar sample. Comparing wild with transgenic soy beans, D-Arg are found only in the

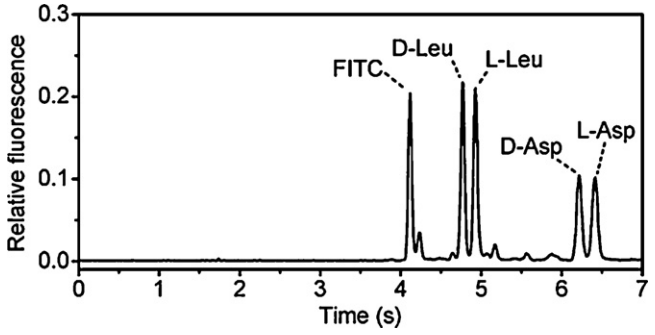

Fig. 3. Electropherograms of achiral and chiral separations of a mixture of 1 μM FITC-labeled amino acids with effective separation length of 15 mm under optimized conditions: BGS, 5 mM borate buffer (pH 9.2) with8 mM β-CD and 12 mM STC. Reprinted with permission from Ref. [82]. Copyright 2009 American Chemical Society.

transgenic sample, which indicate that the inserted transgene can modify some metabolic pathway linked to D/L-Arg.

2.3. CD-MEKC

In the CD-MEKC mode, which is the coupling of MEKC using achiral and/or chiral surfactants with chiral recognition ability of CDs, a micellar solution containing CDs is employed as BGS. Since the MEKC mode provides good resolution of closely related compounds on the basis of minute differences in the partition coefficients to the micelle, it is suitable for resolving amino acid enantiomers in complicated sample matrices. Actually, many applications of CD-MEKC to real sample analyses have been reported in recent years as summarized in Table 3. In CD-MEKC, a neutral or basic buffer (pH 7–10) containing both sodium dodecyl sulfate (SDS) and β-CD is mainly used as the BGS [63–76]. Modified CDs such as HP-β-CD [76–78], DM-β-CD [78] and HP-γ-CD [79] have been also utilized in the CD-MEKC mode. Because the incorporation by the SDS micelle compete with the inclusion into the cavity of CDs, the CD-MEKC separation of racemic amino acids can be easily optimized by tuning the concentrations of SDS and CDs.

Chiral surfactants such as sodium taurocholate (STC) and sodium taurodeoxycholate (STDC) bile salts have been employed in CD-MEKC [80–87]. As a typical example, the use of a BGS of 80 mM borate buffer (pH 9.3) containing 20 mM β-CD and 30 mM STC gives baseline resolutions of20 pairs of FITC-labeled amino acid enantiomers including 19 protein amino acids and a simultaneous separation of 6 pairs of the enantiomers due to a cooperative effect of the dual CS system [81]. Recently, Zhang et al. have developed a high-speed chiral separation by CD-MEKC using both STC and β-CD in a 15-mm long capillary [82]. As shown in Fig. 3, the ultimately rapid separation of racemic FITC-Leu and -Asp within only 7-s clearly reveals the effectiveness of the dual CS system.

2.4. MEKC

In the MEKC mode, various chiral surfactants, including polymerized surfactants, have been developed for the enantioseparation of amino acids as summarized in Table 4. Although bile salts, which are well known as classical chiral surfactants, have been often used as the additive in the CD-MEKC mode, only two reports on MEKC using bile salts (sodium cholate (SC) [88] and 3-[(3-cholamidopropyl)-dimethylammonio]-1-propane sulfonate (CHAPS) [89]) micelle has appeared from the year 2001. In recent 10 years, polymer micelles have been extensively investigated [91–96]. Shamsi et al. have applied poly(sodium *N*-undecanoyl-L-leucylvalinate)(poly-L-SULV) to the MEKC separation of amino

Table 5
Chiral separation of amino acids in the NACE mode.

Mode	CS	BGS	Amino acids	Label	Detection	Comments	Ref.
NACE	Oligo(pentakis(norbornen-5-ylmethylhydroxysiloxyl)β-CD)	20–40 mg/mL CS/35 mM Na_2HCO_3/*N*-methylformamide	Thr, Glu, Asp, Met, Nva, Val	DNS	UV		[101]
CC-NACE	Dimeric forms of carbamoylated QN and QD derivatives	10 mM CS/100 mM octanoic acid, 12.5 mM ammonia/EtOH:MeOH = 6:4	Leu, pGly, Me-Leu, Abu, Phe, Pro, PA	DNB, BZ, DNZ,	UV		[102]
CC-NACE	tBuC-QN	10 mM CS/100 mM octanoic acid, 12.5 mM ammonia/EtOH:MeOH = 6:4	Leu, pGly, Me-Leu, Abu, Phe, Pro, PA	DNB, BZ, DNZ	UV		[103]
CC-NACE	tBuC-QN	20 mM ammonium acetate (pH 6.0) in MeOH/water = 8:2	Leu	DNB	UV	Binding constant	[104]
CC-NACE, PF-NACE	Cinchona alkaloid derivatives	10 mM CS/100 mM octanoic acid, 12.5 mM ammonia/EtOH:MeOH = 6:4	Leu, Me-Leu, Abu, Phg, Phe, Tle, Pro,	DNB, BZ, DNZ, FMOC, DNP	UV		[5]
PF-NACE	Dimetric C_9-carbamates of QN and QD	5 mM CS/100 mM acetic acid, 12.5 mM triethylamine/EtOH:MeOH = 6:4	Abu, Val, Leu, Me-Leu, Ala, Phe, Pro, Asp	DNB, DNZ, BZ, DNP	UV		[105]
PF-NACE	tBuC-QN	10 mM CS/100 mM octanoic acid, 12.5 mM triethylamine/EtOH:MeOH = 6:4	Leu	DNB	UV		[106]

QN, quinine; QD, quinidine.

Table 6
Chiral separation of amino acids in the EKC mode.

Mode	CS	BGS	Sample matrix	Amino acids	Label	Detection	Comments	Ref.
EKC	$18C6H_4$	5 mM CS/20 mM Tris–citric acid (pH 2.5)		Phe, Trp, Tyr, pGly derivatives		UV		[107]
EKC	$18C6H_4$	10 mM CS/10 mM citrate-Tris (pH 2.2)		Arg, Val, Ser, Phe, Tyr, Asp		C^4D		[108]
EKC	$18C6H_4$	4 mM CS/3 mM chrysoidine, 100 mM citric acid, 10 mM Tris (pH 1.9–2.1)		Ala, Asn, Glu, Ile, Met, Cys, pGly, Cys-Cys, Phe, Ser, Tyr, Val, Thr		Indirect-UV		[109]
Heart-cutting 2-D CE	$18C6H_4$	10 mM CS/2.3 M acetic acid (pH 2.1)		Tyr, Trp, Thr		C^4D	1D, CZE; 2D, chiral EKC	[110]
Heart-cutting 2-D CE	$18C6H_4$	10 mM CS/2.3 M acetic acid (pH 2.1)	Ammonium formate (pH 8.56)	Phe, Thr		C^4D	CZE/chiral EKC, dynamic pH junction	[111]
EKC	Vancomycin	5 mM CS/10 mM Tris, 4.4 mM maleic acid, 0.03 mM CTAB		Asp, Glu		C^4D		[112]
EKC	Vancomycin analog A82846B	1–2 mM CS/50 mM phosphate, 0.002% HDB (pH 6)		Val, Glu, Ser, Thr	DNS	UV		[113]
PF-EKC	Vancomycin, teicoplanin	10 mM CS/10 mM sorbic acid, histidine (pH 5)	Teeth dentin, beer	Glu, Asp		Indirect-UV		[114]
PF-EKC	Vancomycin	2.5 mM CS/20 mM ammonium acetate (pH 5)		*N*-acetyl-Glu, Ser, Cys, Try		UV		[115]
PF-EKC	Vancomycin	2 mM CS/0.001% HDB, 50 mM Tris–phosphate (pH 6.2)		Met, Nle, ABA, Glu, Val, Ser, Phe, Leu, Thr	DNS	UV	Short end injection.	[116]
PF-EKC	Vancomycin	0.5–2.5 mM CS/50 mM Tris–phosphate (pH 6.0)		Ala, Leu, Met, Phe, Val, Ile, Ser	FMOC	UV	Short end injection	[117]
PF-EKC	Vancomycin, balhimycin	2 mM CS/0.001% HDB, 50 mM Tris–phosphate (pH 6.0)		ABA, Leu, Met, Nle	DNS	UV		[118]
PF-EKC	Balhimycin, bromobalhimycin	2 mM CS/0.001% HDB, 50 mM Tris–phosphate (pH 6.0)		Nva, Phe, Ser, Leu, Nle, Met, Glu, Val, ABA, Thr, Asp	DNS	UV		[119]
PF-EKC	Balhimycin, dechlorobalhimycin	2.5 mM CS/50 mM Tris–phosphate (pH 6.0), 0.001% HDB		Leu, Val, Thr, Ser, Trp, Glu, Nle, Met, Asp, Phe, Nva	DNS	UV		[120]
PF-EKC	Balhimycin, bromobalhimycin, dechlorobalhimycin	4 mM CS/0.001% HDB, 50 mM Tris–phosphate (pH 6.0)		Ala, Leu, Met, Thr	BZ, NB, DNB, DClB, ClB	UV		[121]
PF-EKC	Cyclohexa-, cyclohepta-peptides	2–5 mM CS/20 mM phosphate (pH 7.0)		Glu, Ala, Gln, Asp, Ser, Norv, Pro, Leu	DNP	UV	NMR study	[122]
PF-EKC	Cyclohexapeptides	2–30 mM CS/20 mM phosphate (pH 7.0)		Glu	DNB, *m*-NB, BZ	UV		[123]
CC-EKC	Terguride derivatives	20 mM CS/200 mM β-Ala-acetate (pH 4.2): MeOH = 1:1		Met, Nle, DABA, Glu, Val, Ser, Phe, Leu, Thr, Asp, Nva		UV		[124]
EKC	Ergoline derivatives	25 mM CS/100 mM β-alanine/acetate (pH 4.2), 40% MeOH, 10% THF		Asp, Glu, Thr, Ser, Val, Nva, Nle, Phe, Met, DAB, Asp-methyl ester	DNS	UV	Effect of steric hindrance	[125]
EKC	Sulfated cyclofructans	10–15 mM CS/4–20 mM acetate-phosphate (pH 3.7–4.7, 5%MeOH)		Trp, Phe, Ala, Tyr, pGly-amides, esters		UV		[126]
EKC	Thiolated β-CD-modified gold nanoparticles	1.0–1.4 mg/mL CS/50 mM borate (pH 8.0, 9.0)		Glu, Asp, Leu, Val	DNP	UV		[127]
EKC	Poly(*N*-acryloyl-L-valine esters)	2% CS/50 mM phosphate-borate (pH 7.0, 20% MeOH)		DNB-Ala, Val, Leu, Phe isopropyl esters	DNB	UV		[128]
EKC	Acrylate copolymer containing *N*-*S*-[1-(1-naphthyl)ethyl]phthalamic acid	1.5% CS/50 mM phosphate (pH 8.0)		Thr, Ile, Nle, Phe, Trp, pGly, Leu	DNB	UV		[129]

HDB, hexadimethrine bromide; CTAB, cetyltrimethylammonium bromide; *m*-NB, *m*-nitrobenzoyl.

Table 7
Chiral separation of amino acids in the CEC mode.

Mode	CS	BGS	Sample matrix	Amino acids	Label	Detection	Comments	Ref.
P-CEC	β-CD, γ-CD-bonded silica particles	10 mM MES (pH* 6.0)/MeOH		Leu, Thr, Nva, Val, Ser, Met, Phe, Nle	DNS	UV		[130]
P-CEC	Teicoplaninaglycone-bonded silica particles	0.2% TEAA (pH 4.1): MeOH = 6: 4		DOPA, Me-DOPA, Phe, pSer, Trp, His, Tyr, Me-Trp		UV	Both P-CEC and PA-P-CEC	[131]
PA-P-CEC	DNB-pGly, DNB-npGly-bonded silica particles	1 mM borate (pH 8.5): ACN = 30:70		naphthylamide derivatives of Phe, Ala, Leu, Met		UV		[132]
P-CEC	(S)-DNB-npGly-bonded silica particles	5 mM phosphate buffer-ACN (30:70)		Ala, Gln, Glu, Gly, Ile, Met, Phe, Pro, Ser, Thr, Val, diaminopropionic acid, ABA	NBD-F	LIF	Photopolymerized sol-gel frit	[133]
P-CEC	(*S*)-*N*-(DNB)leucine-*N*-phenyl-*N*-propylamide-bonded silica particles	37.4% *n*-hexane, 56.1% 2-propanol, 6.5% water		Ala, Leu, Val, pGly, Phe-amide derivatives		UV	Normal phase CEC	[134]
P-CEC	(*S*)-*N*-(DNB)leucine-*N*-phenyl-*N*-propylamide-bonded silica particles	90–100% ACN		Ala, Leu, Val, pGly, Phe-amide derivatives		UV	Polar organic mode	[135]
P-CEC	$18C6H_4$-bonded silica particles	20 mM Bis-Tris (pH 3.0–4.5)/MeOH = 8:2		Phe, Trp, pGly, Tyr		UV		[136]
M-CEC	γ-CD-bonded silica monolith	50 mM MES-Tris (pH 8.0): MeOH = 60:40		Glu, Thr, ABA, Met, Nva, Nle, Leu, Phe	DNS	UV		[137]
M-CEC	(+)-1-Allyl-(5*R*,8*S*,10*R*)-terguride-bonded silica monolith	20 mM NH4OAc (pH 3.6)/ACN = 1:1		Ser, Thr, Val, Leu, Ile, Phe, Met, Glu, Asp, Trp	DNS	UV		[138]
PA-M-CEC	Teicoplaninaglycone-bonded silica particles-loaded silica monolith	40% TEAA (pH 4.1)/40% MeOH/20% ACN		Phe derivatives, Trp, Tyr derivatives, Ala, Leu, Leu, Met derivatives, Nle, Nva, Ser, Val		UV	Both M-CEC and PA-M-CEC	[139]
M-CEC	*O*-9-(Tert-butylcarbamoyl)-11-[2-(methacryloyloxy)ethylthio]-10,11-dihydroquinine-bonded acrylate polymer monolith	0.4 M AcOH, 4 mM NEt_3/ACN-MeOH (80:20)		Leu, Ser, Ala	FMOC, DNS, DBD, CBZC	UV, Flu		[140]
M-CEC	L-4-Hydroxy-Pro-bonded slilica particles-loaded polymethacrylamide monolith	1 mM Cu(II)/50 mM phosphate (pH 4.5)		Trp, Phe, Kyn, Tyr derivatives, pGly, pSer		UV		[141]
M-CEC	β-CD derivatives-bonded acrylate monolith	5 mM phosphate (pH 6.5)	Plasma	Phe, Tyr, Trp, Ala, Lys, His, Arg		UV	Biological application	[142]
M-CEC	Maleopimaric acid-bonded silica monolith	10 mM phosphate (pH 7.5)/ACN = 7:3		Arg, Val, Leu, Trp, Kyn, Phe derivatives	PTC	UV		[143]
PA-MIP-M-CEC	L-Tyr, Phe, Trp, CBZ-Tyr template MIP	50 mM acetate (pH 4.0)/ACN = 2:8		Tyr, Phe, Trp, CBZ-Tyr		UV		[144]

NBD-F, 4-fluoro-7-nitro-2,1,3-benzoxadiazole; DBD, 7-dimethylaminosulfonyl-1,3,2-benzoxadiazol-4-yl; CBZC, carbazole-9-carbonyl; PTC, phenylthiocarbamyl.

Table 8
Chiral separation of amino acids in the LE-CE mode.

Mode	CS (metal–CS ligands complex)	BGS	Sample matrix	Amino acids	Label	Detection	Comments	Ref.
LE-EKC	Zn(II)-L-Lys complex	3 mM CS/5 mM acetate, 100 mM boric acid (pH 7.6)		Trp, Tyr, Phe		UV		[145]
LE-EKC	Zn(II)-L-Arg complex	3 mM CS/5 mM acetate, 100 mM boric acid (pH 8.2)	Rice-brewed suspensions	Trp, Phe, Tyr, Met, Val	FMOC	UV		[146]
LE-EKC	Zn(II)-L-Arg complex	3 mM CS/100 mM borate-acetate (pH 8.0)	Rice vinegar	Tyr, Phe, Trp, Asn, His, Ser, Thr, Met, Cys, Asp, Glu, Ala, Ile, Lys, Orn, Leu, Val	DNS	UV		[147]
LE-EKC	Zn(II)-L-Arg complex	3 mM CS/100 mM borate-acetate (pH 8.4)	Serum	Ala, Asn, Asp, Cys, Glu, Ile, Leu, Lys, Met, Orn, Phe, Pro, Ser, Thr, Tyr, Val	DNS	UV	On-line derivatization	[148]
LE-EKC	Zn(II)-L-Orn complex	3 mM CS/100 mM borate-acetate (pH 8.2)		Trp, Phe, Tyr, Asn, Met, Phe, Ser	DNS	UV	Determination of DAAO kinetic constant	[149]
LE-EKC	Cu(II)-L-Pro, Cu(II)-*trans*-4-hydroxy-L-Pro complexes	10–50 mM CS/5 mM acetate (pH 4.0)		Trp, Tyr, Phe, pSer, His, Thr, Ile, Ser, Val		UV		[150]
LE-EKC	Cu(II)-L-His, L-hydroxy-Pro complexes	10 mM CS/5 mM phosphoric acid (pH 4.5)		Halogenated Phe, Trp		UV		[151]
LE-EKC	Cu(II)-L-hydroxy-Pro complexes	10 mM CS/5 mM phosphoric acid (pH 4.3)		β-methyl-Phe, Trp, Tyr, Tic		UV		[152]
LE-EKC	Cu(II)-(*S*)-3-aminopyrrolydine, -L-His complexes	5 mM CS/20 mM NH_4OAc (pH 6.5), 0.2–0.5% PVA		Phe, Trp		UV		[153]
LE-EKC	Cu(II)-L-phenylalaniamide, L-prolinamide, L-alaniamide complexes	10 mM CS/20 mM NH_4Ac (pH 5.0–6.0)		Leu, Glu, Nle, Nva, Met, Asp, Phe, Ser, Thr, Trp, Val, ABA	DNS	UV		[64]
LE-EKC	Zn(II)-L-phenylalaninamide complex	4 mM CS/100 mM borate-acetate (pH 8.2)	Serum	Asn, Asp, Ile, Met, Phe, Ser, Thr, Trp, Tyr	DNS	UV	Biological application	[154]
LE-EKC	Cu(II), Co(II), Ni(II), Zn(II) complexes of D-gluconic acid, D-saccharic acid, L-threonic acid	20–30 mM CS/pH 5–12		Phe, Trp, Tyr derivatives, DOPA, His				[155]
LE-EKC	Cu(II)-L-alkyl-3-methylimidazolium L-Pro complexes	15 mM CS/30% MeOH (pH 4.0)		Phe, Trp, Tyr, His		UV		[156]
LE-CD-EKC	CDmh-Cu(II) complex	0.25 mM CS/20 mM ammonium acetate (pH 6.8)		Trp		UV		[157]
LE-CD-EKC	β-CDen-Cu(II) complex	20 mM CS/5.0 mg/mL PEG20000, 1.0% tert-BuOH (pH 5.8)		His, Phe, Tyr, Trp		UV		[158]
LE-CD-EKC	β-CD derivatives-Cu(II) complexes	0.6–1.8 mM CS/20 mM AcONH4 (pH 6.8)		Phe, Trp, Tyr		UV		[159]
LE-CD-EKC	β-CDen-Cu(II) complex	10 mM CS/5.0 mg/mL PEG20000, 1.5% tert-BuOH (pH 4.2)		Phe, Ala, Leu, Ile, Ser	DNS	UV		[160]
LE-CD-EKC	β-CD-Zn(II)-L-Val complex	4 mM complex + 4 mM β-CD/100 mM borate-acetate (pH 8.1)	Serum	Ala, Arg, Asn, Cys, Ile, Leu, Met, Phe, Pro, Thr, Trp, Tyr, Val	DNS	UV	On-line incubation for determination of LAAO kinetic constant	[161]
LE-EKC, LE-MEKC	Cu(II)-L-hydroxy-Pro complexes	10 mM CS/0–25 mM SDS, 5 mM phosphate (pH 4.4)		Phe, Trp, DOPA		UV		[162]
LE-EKC, LE-MEKC	Cu(II)-L-prolinamides complexes	10 mM CS/0 or 8 mM SDS, 20 mM NH_4Ac (pH 6.0)		Leu, Nva, ABA, Met, Val, Ser, Thr, Phe, Trp, Nle, Glu, Asp	DNS	UV		[163]
LE-EKC, LE-MEKC	Cu(II)-L-Orn complex	1 mM CS/0 or 30 mM SDS, 20 mM NH_4OAc (pH 8.0)		Phe, Trp, Thr, Asp, Met, Nbu, Nva, Nle, Val, Glu	DNS	UV		[164]
LE-MEKC	Cu(II)-4-hydroxy-L-Pro complex	25 mM CS/0–20 mM SDS (pH 4.5)		fluoro-Phe, Tyr		UV		[165]
LE-MEKC	Cu(II)-L-Lys complex	3.33 mM CS/50 mM SDS, 10 mM NH_4Ac (pH 6.0)		Try, pSer, Phe, Trp		UV		[166]
LE-MEKC	Cu(II)-L-Val complex	4 mM CS/20 mM SDS, 20 mM NH_3-NH_4OAc (pH 9.0)		Val, Thr, Nva, Nbu, Asp, Met, Leu, Glu, Nle, Phe, Trp	DNS	UV		[167]
LE-MEKC	Cu(II)-D-penicillamine complex	4 mM CS/20 mM SDS, 20 mM NH_4OAc (pH 6.5)		ABA, Asp, Met, Nva, Leu, Val, Nle, Phe	DNS	UV		[168]
LE-EKC, LE-M-CEC	Cu(II)-L-phenylalaniamide, L-prolinamide, L-alaniamide complexes-bonded silica monolith	LE-EKC: 10 mM CS/20 mM NH_4Ac (pH 5.0) LE-M-CEC: 0.25–0.5 mM $Cu(Ac)_2$, 50–100 mM NH_4Ac (pH 6.5–7.6)/ACN = 3:7		Leu, Nva, ABA, Met, Val, Ser, Thr, Phe, Trp, Nle, Glu, Asp	DNS	UV		[169]
LE-M-CEC	Cu(II)-L-prolinamide complex-bonded silica monolith	0.5 mM $Cu(Ac)_2$/50 mM NH_4Ac (7:3, pH 6.5)/ACN		Leu, Met, Nle, Nva, Phe, Ser, Thr, Trp, Val, ABA	DNS	UV		[170]
LE-M-CEC	Cu(II)-L-phenylalaniamide complex-bonded silica monolith	0.5 mM $Cu(Ac)_2$/50 mM NH_4Ac (7:3, pH 5.5)/ACN		Leu, Met, Nle, Nva, Phe, Ser, Thr, Trp, Val, ABA	DNS	UV		[171]
LE-M-CEC	Cu(II)-L-hydroxy-Pro complex-bonded silica monolith	0.5 mM $Cu(Ac)_2$, 50 mM NH_4Ac (pH 6.5)/ACN = 7:3		Nva, Met, Val, Ser, Thr, Phe, Trp, Nle	DNS	UV		[172]
LE-M-CEC	Cu(II)-D-Val complex-bonded gel monolith	5 mM CS/10 mM NH_4OAc (pH 3.5)		Ser, Ala, Phe, Trp, Tyr	DNS	UV	Trans-(1S,2S)-1,2-bis-(dodecylamido) cyclohexane gel monolith	[173]
LE-M-CEC	Cu(II)-L-4-hydroxy-Pro, Cu(II)-L-prolinamide complex-bonded acrylate monolith	0.1 mM $Cu(Ac)_2$, 50 mM NH_4OAc (pH 6.5, 4.5)		DOPA, Phe, Tyr, Trp derivatives		UV		[174]
LE-P-CEC	Cu(II)-L-hydroxy-Pro complex-coated silica particles	0.5 mM Cu(II)/25 mM NH_4OAc (pH 4.5)		Phe, Trp, Tyr derivatives, DOPA		UV		[175]

CDmh, β-CD substituted by an imidazole-bound histamine; β-CDen, 6^A-(2-aminoethylamino)-6^A-deoxy-β-CD; PVA, poly(vinylalcohol); DAAO, D-amino acid oxidase; LAAO, L-amino acid oxidase.

Table 9
Chiral separation of amino acids in other modes.

Mode	CS	BGS	Sample matrix	Amino acids	Label	Detection	Comments	Ref.
ACE/MEKC	SC + HSA	12 mM SC, 0.8–1.6% HSA/8–10 mM borate (pH 8.9–9.1, 5–10% MeOH)	Compound amino acid injection	Asp, Glu	DTAF	LIF	Dual CS	[176]
M-ACEC	BSA-encapsulated hydrogel monolith	10 mM phosphate buffer (pH 7.0)		Trp		UV	Sol–gel	[177]
OT-ACEC	Avidin physically coated capillary	10 mM phosphate (pH 5.95)		Ser, Met, Thr, Val, Nle, Leu, Trp, ABA	DNS	UV		[178]
M-ACEC	Avidin physically coated silica monolithic capillary	10 mM phosphate (pH 5.95, 15% MeOH)		Ser, Trp, Thr	DNS	UV		[179]
OT-ACEC	Avidin-phospholipid coated capillary	20 mM Tris (pH 7.4)		Trp, Ser, Thr	PTH			[180]
TGF	γ-CD	10 mM CS/1 M Tris–borate (pH 8.3)		Glu	DNS	LIF	×600 enrichment in 3-cm capillary	[181]
TGF	HP-β-CD	1.5 mM CS/0.5 M Tris–borate (pH 8.3)		Asp, Glu, Gly, Ala, Ser, Val, AIB	5-FAM SE	LIF	Scanning TGF	[182]
Achiral MEKC		7.5 mM SDC, 20 mM SDS/20 mM borate (pH 9.8)	β-Amyloid fibrils, human senile plaque	Hydrolysate of peptides	(+)-APOC, (−)-APOC	LIF	Diastereoisomer separation	[183]
Achiral MEKC		40 mM SDS/20 mM borate (pH 10, 10% ACN)		Asp, Glu, Ala, Ser, Val, Leu, Ile, Phe, Gly	(+)-APOC	LIF	Diastereoisomer separation	[184]
Achiral MEKC		0–80 mM SDS/20 mM borate (pH 9.2)		20-Amino acids	FMOC-L-Ala-NCA		Diastereoisomer separation	[185]
Achiral MEKC		17 mM SDS/50 mM phosphate (pH 9.0, 25% ACN)	Human plasma	Vigabatrin	DHAIC	UV	Diastereoisomer separation	[186]
Achiral MEKC		5 mM SDS/50 mM borate (pH 9.5, 20% ACN)		Ser, Ala, Val, Cys, Thr	DHAIC	UV	Diastereoisomer separation	[187]
Achiral MEKC		18 mM SDS/50 mM phosphate (pH 9.0, 25% ACN)	Human plasma	Asn, Met, Leu, Phe, Trp, Ser, Val, Ala, Thr, vigabatrin	DDHAIC	UV	Diastereoisomer separation	[188]
Achiral CZE		15 mM borate (pH 10.5)	Amino acid oral solution	Tyr	DBD-PyNCS	LED-IF	Diastereoisomer separation	[189]

DTAF, 5-(4,6-dichloro-s-triazin-2-ylamino)fluorescein; 5-FAM SE, carboxyfluorescein succinimidyl ester; LED-IF, light-emitting diode-induced fluorescence.

acid enantiomers [91]. By using the polymerized surfactant in MEKC, a wide variety of racemates can be resolved in neutral and basic pH BGSs. Among various racemates, cationic and neutral analytes are well separated by poly-L-SULV, and thus the chiral separations of PTH-amino acids are achieved at pH 7.2. Poly-*N*-undecenoyl-L-amino acid-sulfate (poly-L-SUCAAS) is also effective for separating amino acid enantiomers [96]. At pH 3.0 and 8.0, PTH-Tyr, -Ile and -Trp can be resolved within 23 min in the MEKC mode. Although the polydispersity of polymeric surfactants may reduce the enantioseparation efficiencies [93], the broad applicabilities of the poly-L-SULV and poly-L-SUCAAS are very attractive for not only amino acids but pharmaceutical applications.

Recently, novel Rosin- [97,98] and sugar-based chiral surfactants [99,100] have been synthesized and applied to the MEKC enantioseparation of amino acids. In the sugar-based chiral surfactants, it has been revealed that the surfactant structures of the carbohydrate head groups including their anomeric configurations have significant effects on the enantiomeric separation and the migration behavior in MEKC [99].

2.5. NACE

Nonaqueous BGS containing hydrophobic CSs is used in the NACE mode (Table 5) [5,101–106]. In the NACE chiral separation of amino acid enantiomers, the applications of cinchona alkaloids have been extensively investigated. Among several cinchona alkaloid derivatives, *tert*-butylcarbamoyl quinine (tBuC-QN) is regarded as a standard CS in NACE since the carbamate modification of native QN provides significant improvement of enantioselectivities for acid racemates [5]. This may be due to more rigid CS structure with defined binding pocket and favorable hydrogen donor–acceptor properties of the carbamate group. As mentioned in the Introduction Section, cinchona alkaloids exhibit strong absorption in the UV region, so that the CC and PF techniques are often employed to reduce the background noise. Although complicated injection procedures are required in these techniques, the run-to-run RSD of the NACE analysis using cinchona alkaloids is acceptable, typically less than 1%. In NACE, furthermore, evaporation of nonaqueous solvent such as MeOH and EtOH often causes the changes in the concentration of electrolytes, resulting in reduced repeatabilities. Therefore, frequent exchanges of the BGS vial are effective to obtain precise and reproducible analysis of enantiomers in NACE.

2.6. EKC

In this section, the chiral separation using a BGS containing ionic CSs except for CDs and chiral surfactants is classified into the EKC mode (Table 6) [107–129]. To date, (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid (18C6H$_4$) [107–111], vancomysin [112–118] and balhimycin [118–121] have been mainly employed as the CSs in the EKC mode. Since 18C6H$_4$ forms inclusion complex with primary ammonium cations, it is effective to separate racemic amino acids in acidic BGSs (pH 1.9–2.5). At pH 1.9–2.5, 18C6H$_4$ is slightly negatively charged due to deprotonation of one carboxylate group with pK_a of 2.1. Hence, the migration velocity of the complex should be different from that of free amino acid, resulting in the EKC separation of the racemates.

Multi-dimensional separations in CE have attracted much attention due to the high-peak capacity. So far, different interfaces combining two separation modes such as isoelectric focusing-gel electrophoresis and CZE–MEKC have been developed. Anouti et al. have developed a heart cutting second dimensional (2-D) CE separation technique, which is combining normal CZE with chiral EKC using 18C6H$_4$ in a single capillary [110,111]. In this technique, the first dimensional separation is carried out in an achiral BGS for the CZE separation, and then a target fraction separated in the first dimension is mobilized toward the inlet end by hydrodynamic pressure with a BGS containing 18C6H$_4$. Finally, the chiral EKC separation of target amino acid is performed by applying constant voltage. As can be seen in Fig. 4, the trace of C^4D_2 clearly shows the enantioseparation of the fraction of Trp and Gln in the second dimension. The coupling of higher peak capacity in the 2D-CE separation with chiral discrimination ability of the 18C6H$_4$-EKC mode should be effective for the analysis of closely resembled amino acid racemates.

Two glycopeptide antibiotics, vancomycin and balhimycin, are employed to the enantioseparation of amino acids [112–121]. Since these macrocyclic antibiotics show strong UV absorption according to aromatic rings, the PF technique has been often combined with the EKC separation. For vancomycin, an optimal enantioselectivity for amino acids appears at pH ~6.0, while for balhimycin higher enantioselectivity than that of vancomycin is obtained at a pH less than 6 [118]. This higher selectivity can be explained by higher dimerization constant of balhimycin. The dimer conformation may be structurally favorable for the chiral recognition of amino acids.

2.7. CEC

CEC is a hybrid technique that combines the partitioning effect of HPLC with the electrophoretic one of the CE in the capillary. In CEC, the mobile phase is driven by only the EOF or both EOF and pressurized flow (pressure-assisted CEC; PA-CEC) in the capillary column consisting of CSs-bonded stationary phase. For the chiral separation of amino acids, packed CEC (P-CEC) and monolithic CEC (M-CEC) modes have been employed as summarized in Table 7. In the P-CEC mode, CSs bonded silica particles are packed into the capillary. To obtain the chiral discrimination of amino acids, the capillaries packed with β- and γ-CDs [130], teicoplanin aglycone [131], 3,5-dinitrobenzoyl (DNB)-phenyl Gly [132], DNB-npGly [133], (*S*)-*N*-(DNB)leucine-*N*-phenyl-*N*-propylamide [134,135] and 18C6H$_4$ [136] bonded silica particles have been prepared. By using nonaqueous BGSs in the (*S*)-*N*-(DNB)leucine-*N*-phenyl-*N*-propylamide bonded silica capillaries, the CEC separation of aromatic amides of amino acid can be achieved through enantioselective π–π donor–acceptor interactions between the CSs and the analytes in the normal phase [134] and polar organic modes [135]. In P-CEC, the difficulty in the preparation of frits, which require higher heat treatment for sintering silicate or silica, is often problematic. To overcome this limitation, Kato et al. have reported a preparation of photopolymerized sol–gel frits for the packed capillary and an application to the CEC separation of amino acid racemates [133]. This technique provides easy and fast preparation of packed capillary. It should be emphasized that the separation performance of racemic amino acids in the packed capillary is superior to that in the monolithic capillary with embedded chiral particles. Since various chiral packing materials developed in LC is available for P-CEC, such sophisticated packing technique is indispensable to obtain versatile enantioselectivity for amino acids with sufficient phase ratio.

In the last decade, developments of monolithic capillaries have progressed rapidly for not only capillary-LC but CEC. In the chiral M-CEC separation of amino acids with silica-based monolith, γ-CD [137], (+)-1-allyl-(5*R*,8*S*,10*R*)-terguride [138] and maleopimaric acid-bonded, and silica particles binding teicoplaninaglycone-loaded silica monoliths [139] have been employed. On the other hand, acrylate monolithic capillaries have been also applied to CEC with binding several CSs such as QN derivative [140], L-4-hydroxy-Pro [141],β-CD derivatives [142] and maleopimaric acid [143]. Recently, molecularly imprinted polymer (MIP) monolith capillary has been prepared for chiral separation of Tyr, Phe and Trp racemates [144]. However, the resolution has been apparently insufficient due to serious peak tailings. Hence, further investiga-

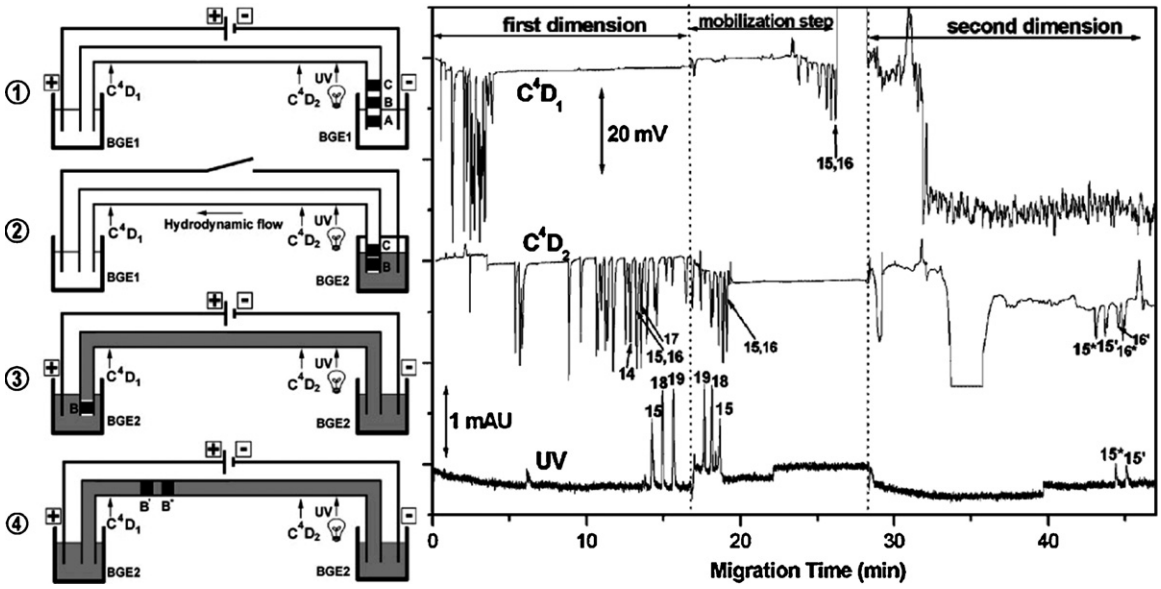

Fig. 4. (a) Principle of heart-cutting 2-D CE for the achiral (first dimension) and chiral separation (second dimension) of complex mixtures in a single capillary. 1, Achiral separation and evacuation of fraction A in the first dimension; 2, introduction of the second-dimension electrolyte (BGE2) by hydrodynamic flow; 3,isolation of fraction B in the capillary after evacuation of fraction C by the inlet end of the capillary; 4, chiral resolution of the fraction B in the second dimension of the separation. (b) Heart-cutting 2-D CE in a single capillary for the chiral separation of D,L-Trp and D,L-Gln. Background electrolyte (BGE) 1: 2.3 M acetic acid, pH 2.1; BGE 2: BGE 1 + 10mM18C6H$_4$. Peak identification: 14: D,L-Met, 15: D,L-Trp, 16: D,L-Gln, 17:D,L-Glu, 18: D,L-Phe, 19: D,L-Tyr.
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tions should be required for the enantioseparation of amino acids by using the MIP monolithic capillaries.

2.8. LE-CE

Enantiodiscrimination by the ligand-exchange mechanism is applied to several separation modes in CE (Table 8). In LE-CE, the formation of diastereomeric ternary mixed metal complexes between CS ligands and amino acid analytes. Generally, Cu(II) and Zn(II) cations are used as the central metal; L-amino acids and L-hydroxy-Pro are employed as the CS ligand. Since the stability constants of the diastereomeric mixed complexes with the analytes enantiomers are different, the migration velocities are also different between the target racemates. As the most simple method in LE-CE, both metal ions and CS ligand are added into a BGS; LE-EKC mode. In addition to L-amino acids [145–151] and L-hydroxy-Pro [150–153], (*S*)-3-aminopyrrolydine [153], L-amino acid amides [64,154], D-sugar acids [155] and1-alkyl-3-methylimidazolium L-Pro [156] have been used as the CS ligands in the LE-EKC separation of native, DNS-, FMOC-labeled amino acid racemates.

To enhance the regio- and stereo-specificity toward amino acid enantiomers, several CDs have been added to a BGS containing the metal–CS ligands complexes, which is called as the LE-CD-EKC mode in this review [157–161]. As a typical example, Cucinotta et al. have reported an application of β-CD substituted by an imidazole bound His (CDmh) to the LE-CD-EKC separation of racemic Trp [157]. As shown in Fig. 5, only CDmh–Cu(II)–L-Trp complex has the right geometry to permit indole inclusion, resulting in higher formation constant. The higher stability of the complex causes faster migration of L-Trp than D-Trp in a neutral pH BGS.

When SDS is added to the metal–CS ligands complex system, both chiral selectivity by the LE principle and high resolution ability for closely resembled analytes by MEKC can be obtained; LE-MEKC mode [162–168]. Chen et al. have clearly demonstrated the effect of SDS on the enantioresolution of DNS-Nva, -Asp and -Val [163]. In a Cu(II)-L-prolinamide system, the peaks of DNS-L-Asp and DNS-D-Val are overlapped, while by adding SDS these amino acids are resolved well to six peaks. It should be noted that the migration order is often changed by adding SDS, which is due to the retention effect by the pseudostationary phase.

The combination of LE and CEC is also employed to the chiral separation of amino acids. In the LE-CEC mode, CS ligands are immobilized onto the surface of silica particles or monolithic structures [169–175]. By using a BGS containing metal ions, the LE separation according to the metal–CS ligand complex stationary phase can be performed. Mizrahi et al. have reported an application of a low molecular weight organogel filled monolithic capillary to LE-CEC [173]. The organogel, which forms stable, fibrillar, thermoresponsible and thixotropic gel, provides very high enantioresolution for DNS-Trp in the presence of Cu(II) and D-Val. The optimal pH is 3.5, which differs from normal LE-CE separation of DNS-amino acids at pH 5–8. Such specific LE separation performance in the organogel filled capillary can be explained by the difference in axial ligation of the amide carbonyl backbone of the gel to DNS-D- or L-amino acid–D-Val–Cu(II) ternary complexes.

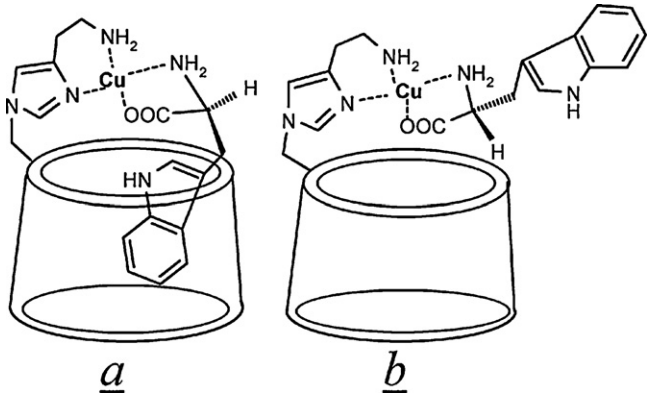

Fig. 5. Cu(II)–CDmh ternary complexes with each of the Trp enantiomers: hypothesized structure of the complex with: (a) L-enantiomer; (b) D-enantiomer.
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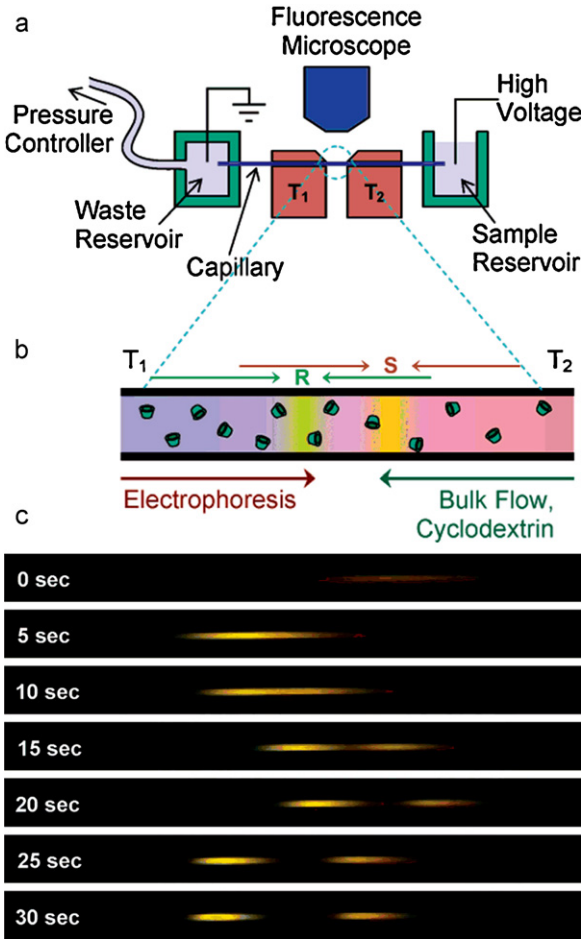

Fig. 6. (a) Schematic illustration of the TGF apparatus. A linear temperature gradient is formed along the capillary in the 2-mmspace between the copper blocks regulated at temperatures T_1 and T_2. (b) Schematic of chiral TGF separations. (c) Real-time chiral TGF of DNS-D,L-Glu. T_1, 13 °C (left side in image); T_2, 40 °C; +1000 V/cm;BGS, 10 mM γ-CD in 1 M Tris–borate (pH 8.3). The D-enantiomer peak is to the left in the figure; the L-enantiomer is to the right.

2.9. Other separation modes

In this section, ACE, temperature gradient focusing (TGF), and indirect separation based on diastereoisomer formation of amino acid enantiomers are summarized (Table 9). Wang et al. have reported chiral separation of Glu and Asp using human serum albumin (HSA) and SC as the dual CSs. Since HSA and SC work as affinity ligand and pseudostationary phase, for enantioresolution, respectively, the amino acids are optically separated by both ACE and MEKC separation mechanisms [176]. Other attempts to use protein affinity ligands (BSA and avidin) have also demonstrated in the M-CEC and OT-CEC format, where native, PTH- and DNS-labeled amino acid racemates are successfully separated [177–180].

Ross and co-workers have developed a novel TGF technique, which is applied to the enantioseparation of amino acids by the addition of CDs to BGS [181,182]. In TGF, the capillary is mounted to span two controlled temperature blocks as shown in Fig. 6a. A linear temperature gradient is formed along the capillary in the 2-mm space between the copper blocks regulated at temperatures T_1 and T_2. With temperature gradient focusing, a combination of a temperature gradient, an applied electric field, and a buffer with a temperature-dependent ionic strength is used to cause analytes to move to equilibrium, zero-velocity points along a capillary (Fig. 6b). Chiral separations are accomplished by adding γ-CD to the BGS. Theγ-CD interacts preferentially with one enantiomer, shifting its focusing location so that it is resolved from the other enantiomer (Fig. 6c). When low concentration sample solution is continuously injected into the capillary for 30 min, racemic DNS-Glu is focused to a peak concentration 600-fold higher than the sample input concentration. Although the long sample injection time is required for high focusing efficiency in the present stage, TGF will become the effective focusing and separation technique for chiral analysis of amino acids through further investigations.

Coupling of the derivatization of target enantiomers to form diastereoisomer with achiral MEKC or CZE separation have been utilized in the chiral analysis of amino acids. To form diastereoisomer, several chiral reagents such as (+)- or (−)-1-(9-anthryl)-2-propyl chloroformate (APOC) [183,184], FMOC-L-alanyl *N*-carboxyanhydride (FMOC-L-Ala-NCA) [185], dehydroabietylisothiocyanate (DHAIC) [186,187], degrading dehydroabietylisothiocyanate (DDHAIC) [188] and *R*(−)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(*N*,*N*-dimethylaminosulfonyl)-2,1,3-benzoxadiazole (DBD-PyNCS) [189] have been employed in CE.

3. Applications

3.1. Biological

CE analysis of amino acid enantiomers has been successfully applied to many types of biological matrices, mainly brain [35,63,67,79], neuron [67,72,79,84], serum [86,148,154,161], plasma [77,142,186,188] and urine [77,79]. By employing the CD-MEKC mode, naphthalene-2,3-dicarboxaldehyde (NDA)-labeled Asp [63], Glu [67], Trp [79] enantiomers in rat brain tissue can be quantified without special sample pretreatments; frozen brain is grinded, then obtained homogenate is ultrasonicated, and finally the supernatant is collected for the NDA labeling. Kirschner et al. have reported a simultaneous quantification of cyanobenz[f]isoindole (CBI)-labeled L-Glu, L-Asp and D-Ser in brain microlysate of arctic ground squirrel by CDEKC using S-β-CD [35]. In the report, the combination of three on-line sample preconcentration techniques, i.e., field amplified sample stacking, sweeping by S-β-CD and dynamic pH junction, has improved the LODs for CBI-Ser and -Glu to 0.20 and 0.30 nM, respectively. The CD-MEKC mode is also effective for the analysis of amino acid enantiomers in neural samples. NDA-Glu [67], NDA-Trp [79] and CBI-Ser [84] enantiomers in ganglia and single neurons of *Aplysia californica* are analyzed by CD-MEKC. Miao et al. have combined single-step immunoprecipitation with CD-MEKC to achieve accurate peak identification of D-Asp in electropherograms for nerve tissue extracts and single neurons [72]. The addition of anti-D-Asp serum to the sample allows confirmation of D-Asp peak assignment since the antibody eliminates free D-Asp signal. Such single-step immunoprecipitation is useful for analyzing complex biological samples.

The LE-EKC and LE-CD-EKC modes using Zn(II)–L-amino acid [148,161] and Zn(II)–L-phenylalaninamide complexes [154] have been employed to the quantification of amino acid racemates in serum samples. Qi et al. have developed an online incubation assay to determine enzyme kinetic constant between L-amino acid oxidase (LAAO) and L-Trp in LE-CD-EKC mode [161]. In the online incubation assay, sample solution containing racemic Trp is injected between LAAO plugs, and then the sandwich sections are mixed, finally the enzymatic reactions occur in the capillary. By quantifying L-Trp separated by LE-CD-EKC, the catalytic rate constant can be estimated. Indirect separation based on the diastereoisomer formation is also effective for biological sample including human plasma [186,188], β-amyloid and senile plaque core [183]. High separation efficiency in MEKC provides a simul-

taneous chiral discrimination of eleven APOC-labeled amino acids from hydrolysate of aggregated β-amyloid fibrils and senile plaque core [183].

Other attempts to analyze amino acid enantiomers in biological matrices such as *Escherichia coli* bacteria culture [25], plant seeds [28], hydrolyzed protein fertilizers [68], microalgae [73] and extracelluar fluid in hypothalamus [78] have appeared in the CD-CZE, CD-MEKC and CDEKC modes.

3.2. Food analysis

CDs based chiral separation modes have been employed to identify and quantify amino acid enantiomers in beverage and food matrices including beer [27,39], wine [39,40], orange juice [24,66,69], vinegar [33,71], soy beans [33], and supplements [26,40]. Cifuentes and co-workers have studied CD-MEKC using β-CD for the analysis of chiral amino acids in transgenic maize [74]. By combining CD-MEKC and LIF detection, D- and L-forms of Arg, Ser, Ala, Glu and Asp, corresponding to the majority amino acids usually found in maize, are separated in less than 25 min with the efficiencies up to 890,000 plates/m and high sensitivity, i.e., LODs as low as 160 nM are obtained for D-Arg. Using CD-MEKC–LIF, different D-amino acids are detected in maize samples, providing the reproducible quantification of the enantiomeric excess for each amino acid in the transgenic maize. They have applied the developed method to the chiral analysis of amino acids in transgenic yeast [75].

The LE-EKC using Zn(II)-L-Arg complex has also applied to the enantioresolution of amino acids in rice-brewed suspension [146] and rice vinegar [147]. As a typical example, seven amino acids, i.e., L-Asp, L-Tyr, L-Met, D-Ser, L-Ser, D-His, L-His, in commercial rice vinegars can be separated and detected only with centrifugation and DNS-labeling of the sample when a BGS of 100 mM boric acid, 5 mM ammonium acetate, 3 mM Zn(II) and 6 mM L-Arg at pH 8.0 is employed [147].

3.3. Pharmaceuticals

In the pharmaceutical applications, the CZE separation of Tyr enantiomers labeled with a chiral fluorescent tag in an amino acid oral solution [189], the CD-MEKC separation of Asp enantiomers [70] and the ACE/MEKC dual separation of Asp and Glu enantiomers in compound amino acid injections [176] have been reported. Cheng et al. have developed a unique chiral analysis system for pharmaceuticals application by the coupling of fully automated flow injection with CD-MEKC for on-line derivatization and separation of amino acid enantiomers, respectively [70]. The developed flow injection-CD-MEKC system allows the determination of L-Asp in a compound amino acid injection, which agrees well with the claimed value.

4. Conclusion

In this review, chiral analyses of amino acids in CE are reviewed with categorizing by the separation modes. A number of techniques for CE enantioseparations has been developed, including CD-CZE, CDEKC, CD-MEKC, MEKC with chiral surfactants, EKC with ionic pseudostationary phases, NACE, CEC, LE-CE, ACE and TGF modes. By optimizing the separation conditions, most of protein amino acids can be optically separated in a single run. Although developing novel CSs adaptable to CE are continuously progressed, CDs used in the CDEKC and CD-MEKC modes are the predominant CSs for the enantioresolution of amino acids according to their resolution powers and commercial-availability. Coupling of derivatization to form diastereoisomers with MEKC is also effective for the simultaneous chiral analysis of amino acids, especially for biological samples.

Many publications have appeared dealing with biological, food and pharmaceutical applications of the CE chiral separations for amino acids. It has been demonstrated that CE is a suitable technique for quantification of amino acids enantiomers even in complicated sample matrices. To overcome several problems in employing the UV detector, alternative detection schemes such as LIF, C^4D and MS-detection have been applied. Among them, the importance of MS detection will be growing continuously for biological applications despite the number of publications on the CE-MS analysis of amino acid enantiomers is still limited in the present stage. Considerable developments for combining chiral electrophoretic separation with MS detection are expected in this field as well as progresses of miniaturized and integrated devices [190–196] for high-speed and high-throughput chiral analysis systems.

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