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Review

Electromigration methods for amino acids, biogenic amines and aromatic amines

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Abstract

Methods of electromigration in laboratory apparatus of small-bore size have recently undergone development at a remarkably rapid pace, leading to a variety of new analytical techniques. One such technique is called ''capillary electrophoresis'' (CE), which is further classified on the basis of electromigration mode, viz., ''capillary zone electrophoresis'' (CZE), which, in turn, has several variations. This review aims to give a short overview of the various electromigration methods for amino compounds by using CE. Firstly, this review briefly summarizes the detection methods employed for detection of monoamines and polyamines by CE for both native and derivative forms. Next, current CE methods are described, and their applications to detection of amino acids, biogenic amines, aromatic amines, including heteroaromatic amines and their enantiomers, are introduced from representative papers. Finally, new methods for single-cell analysis and microchip CE techniques are focused on.

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1. Introduction

Beginning in the early 1950s, scientists have made great efforts to develop new analytical methods for detection of chemical substances in living tissues, in order to better understand and to elucidate the roles such substances play in biological systems. Many chemists have concentrated their energies on developing analytical techniques that were both highly sensitive and highly specific. Among the many modern procedures for this kind of analysis based on the field of separation technology, one such method uses electromigration within a fused-silica capillary and is called ''capillary electrophoresis'' (CE) [1–3]. The CE method meets the requirements of high sensitivity and high specificity, and has proved itself to be a suitable microseparation technique for the analysis of a wide variety of substances.

Amines and polyamines appear to have a number of important functions in the control mechanisms of living system, and their activities are interesting not only when viewed as nutrients, but also when viewed from the standpoint of overall biological activity. Therefore, the development and use of the CE methods for the detection of amines and polyamines, including aromatic and heterocyclic aromatic amines, in food and other biological samples, is deemed to be very important.

The aims of this paper are: (1) to provide the reader with the latest breakthrough and improvements in detection techniques for amino compounds with CE; (2) to discuss electromigration methods for amino compounds in various modes, e.g., capillary zone electrophoresis (CZE) and its modifications, including the method using a support material in a fixed stationary phase or in a moving pseudo-stationary phase in a separation capillary; (3) to introduce the application of these methods to the detection of amino acids, biogenic amines, environmental pollutants and enantiomers of amino compounds as described in published papers and, finally; (4) to introduce the latest uses of these techniques for single-cell analysis and fabricated microchip CE analysis, both of which are examples of the latest micro-analytical methods.

2. Detections for amines and polyamines

Because amines and polyamines (except for aromatic and heterocyclic aromatic amines) generally show little, if any, ultraviolet (UV) or visible light absorption, fluorescence, or electrochemical activity, the detection limits are relatively low. Further, it is well known that CE methods, while having some attractive merits, also have some demerits in comparison with modern chromatographic methods such as gas chromatography (GC) or liquid chromatography (LC). One of the main problems which hinders the widespread application of CE is the low sensitivity of detection inherent due to the short pathlength of light which passes through the capillary. In order to overcome this problem, derivatization methods [4,5] is a way to increase the sensitivity of the CE methods. The other methods were also investigated such as indirect detection and in-capillary derivatization instead of the normal derivatization procedures.

In addition, CE determinations of amino compounds are usually performed by employing either native or derivative forms. It is sometimes the essential factor to choice the electromigration method which chemical form of analyte to employ. The chemical form depends on the choice of electromigration method. Therefore, it is necessary to discuss the current available detection methods such as optical spectroscopic methods for CE prior to go to the next chapter on electromigration methods.

2.1. Derivatization

The determination of amines and polyamines including amino acids and biogenic amines in a complex matrix is very difficult because the compounds are usually present at very low concen-

trations. That they also absorb in the low-wavelength region as do many other compounds is a source of interference. This problem has been solved by using derivatization to enable monitoring of a higher absorbance wavelength, or fluorescence. Ideally, a derivatization reaction proceeds rapidly under mild conditions, forming a single suitable and highly absorbant or fluorescent derivative, whereas the reagent itself does not trigger the detector, nor interrupt separation or detection of the derivatives. A wide variety of derivatization reagents for amino compounds have been hitherto employed in the high-sensitivity and high-selectivity analytical methods of LC and CE [6–8]. Labeling reagents having hetero-atoms, such as nitrogen as part of their molecular structure, sometimes possess the merit of putting an additional charge into native analytes, as this electric charge sometimes leads to the differences in the mobility of the analytes.

The structures of the most representative reagents used in CE for tagging amino compounds and their derivatives are summarized in Table 1.

2.2. Indirect detection

Unfortunately, chemical modifications can be time-consuming and unreliable, resulting in the dilution of the sample, affecting the separation process, and difficult to implement with very small sample volumes. To solve this problem, in 1988, Kuhr and Yeung [24] first demonstrated an indirect detection technique that can be used as a universal detection scheme for CZE, without the need for time-consuming pre-capillary derivatization or experimentally complicated post-capillary derivatization procedures. Using a run buffer containing a background electrolyte (BKE) in which a chromophore or fluorophore is present, the native analytes can be indirectly detected in the capillary with a high degree of sensitivity. They demonstrated the electromigration of native amino acids in a run buffer consisting of 1 m*M* sodium salicylate as the BGE and 0.2 m*M* sodium carbonate adjusted to pH at 9.7, and detected analytes using laser-induced fluorescence detector (LIF).

Subsequently, Lee and Lin [25,26] demonstrated the applicability of this technique for amino acid separation using a conventional UV detector. A 20-amino-acid mixture electromigrated in a solution (at pH 11) containing a BGE of 10 m*M* *p*-amino salicylic acid (PAS) or 4-(*N*,*N*′-dimethylamino)-benzoic acid (DMBA) in the presence of 20 m*M* alpha-cyclodextrin (CD). The key point in this method was the kind of BGE chosen. In the published reports, this technique is generally employed in analyses of biogenic amines in food or biological materials [27–32].

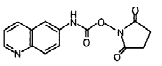
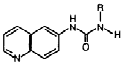
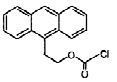
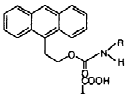
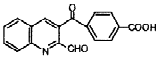
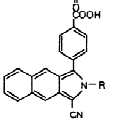
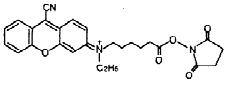
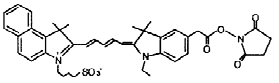
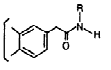
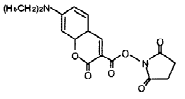
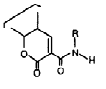
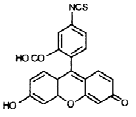
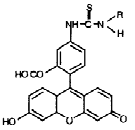
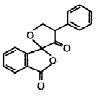
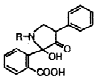
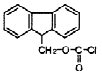
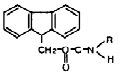
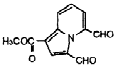
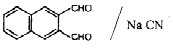
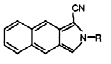
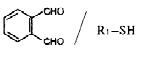
2.3. In-capillary derivatization (ICD)

Former days when developed the indirect detection method, the most currently available detection method of amino compounds in high-sensitive was the derivatization method that was performed by reacting the molecules of amino groups with a derivatizing reagent. This derivatizing reaction is carried out just before (pre-capillary derivatization) or just after (post-capillary derivatization) electromigrations. Although post-capillary derivatization is preferred in both CE and LC, one unavoidable problem in this derivatization is peak broadening, because the separation capillary is connected to the reagent capillary (reactor) and because one or more reagents are added. Usually, either a pressure or a voltage driven device is used for reagent addition, and in either case, the residence time of the analyte in the reactor determines the amount of band broadening; this parameter is influenced by laminar flow and differences in the mobility of the free and derivatized analyte. Pre-capillary derivatization, on the other hand, requires a rather complicated batch-wise procedure to react a free amino group with a derivatizing reagent in an offline chamber. Further, if small amounts of sample can be directly analyzed without having to be derivatized prior to electromigration, CE analysis at a nano-liter level becomes a possibility.

Another idea which contrasts with indirect detection methods is that the inlet of the capillary tube of CE (which is used for separation) can also be used as a small reaction chamber; this approach is named the ‘‘in-capillary’’ or ‘‘on-column’’ derivatization technique. Fischman et al. [33] first pointed out the possibility, and demonstrated this technique by performing derivatization and separation of amino acids.

Taga’s group [34] also studied this method in

Table 1
Chemical structures of derivatizing reagents and derivatives of amino compound ($R–NH_2$)[a]

Name	Chemical structure		Ref.
	Reagent	Derivative	
AccQ			[9]
AEOC			[10]
CBQCA			[11]
CTSP			[12]
DCC			[13]
DCCS			[14]
Dns-Cl			[15]
FITC			[16]
Fluorescamine			[17]
FMOC			[18]
IDA		Not identified	[19]
NDA			[20]
OPA			[21]
PITC			[22]

[a] See Refs. [9–22].

detail by introducing of an OPA reagent solution and sample solution containing free amino acids to the inlet of the capillary by ''tandem mode'' (i.e., reagent solution-sample solution) or by ''sandwich mode'' (i.e., reagent solution-sample solution-reagent solution) via hydrostatic injection. After a specified time required to complete the derivatization reaction, a high voltage was applied to the capillary. The derivatives were separated due to migration and then were detected on the capillary. The migration orders obtained from this method were almost the same as those from the pre-capillary method.

In 1996, Oguri and his group [35] first reported an on line mode in-capillary derivatization capillary electrophoresis (ICD-CE) method, and they demonstrated the usefulness of this technique for the determination of amines and polyamines. This method is quite different from the method just mentioned above, which is based on the idea that the separations and derivatizations of analytes are performed simultaneously during the electromigration of native analytes in a separation capillary tube filled with a run buffer containing derivatization reagent. The ICD-CE method offers the same advantages as the indirect detection method.

OPA/thiol reagent [21] is a suitable label reagent for amino compounds since it reacts within 1 min at room temperature under basic conditions in aqueous environment. Although OPA/thiol derivatives are intensely florescent and show large Stokes-shifts, the reagent is very hard to adapt to chromatographic techniques because the derivatives are normally not very stable. However, when the OPA reagent was used with the ICD-CE method, the problem of instability of the derivatives did not arise.

3. Electromigration of amines and polyamines in CZE

In the beginning of the 19th century, Reuss, the Russian scientist first observed the phenomenon of electromigration when he noticed that particles in a clay-suspension evidenced movement when a voltage was applied to the suspension [1]. In the 20th century, the electromigration method was developed as separation tool for high-order polymer compounds such as protein and DNA. However, this method received no attention from analytical chemists. In 1974, however, Virtanen [36] originally introduced one of the variations of the electromigration method using an electrophoretic medium inside of a narrow-bore capillary (less than 0.1 mm, I.D.), hence the name of ''capillary electrophoresis'' (CE). Further development of CE led to the method known as ''capillary zone electrophoresis'' (CZE) [37]. In this section, electromigration brought about using CZE are reviewed and applications of this method are listed in Table 2.

3.1. Open tubular uncoated fused-silica capillary

Numerous researchers have achieved very efficient and rapid separations of amino compounds by electromigration in a capillary tube filled with a free solution, or in CZE. CZE is the most commonly applied technique, because it allows for the separation of molecules based on differential migrations of their charge species in an electric field as the main driving force. Another driving force of the analyte in CZE is widely known as ''electro-osmotic flow'' (EOF). The walls of untreated fused-silica capillaries are negatively charged above a pH of 2.5 because the silanol groups are partly deprotonated. The EOF arises from cations, which are attracted by the capillary wall. A concentration gradient of positive charge close to the capillary walls results from the competition of electrostatic attraction and diffusion. The velocity of EOF (v_{eo}) is expressed as following equation:

$$v_{eo} = \epsilon\zeta\eta^{-1}E$$

where ϵ, η and E are the permittivity of run buffer solution, the viscosity of run buffer solution and the electrical field strength in the run buffer, respectively, and ζ is the potential voltage between the capillary wall and the surface of run buffer solution.

The solvated cations migrate towards the cathode taking along the buffer solution by frictional forces. The migration of analytes depends on both the driving force of the electric charge and on EOF. The migration time (ts) of analyte or solute in CZE mode is usually given by the equation:

$$ts = L^2/[(\mu_E + \mu_{EO}) \cdot V],$$

Table 2
Electromigration of amino compounds in CZE

Analyte	Derivative	Run buffer	Capillary size (length×I.D.)	Applied voltage	Ref.
Amino acid	AEOC	17.5 m*M* acetate buffer (pH 4.8)	74 cm×50 μm	30 kV	[38]
	CBQCA	100 m*M* TES buffer (pH 7.05)	40 cm×50 μm	10 kV	[39]
	CTSP	15 m*M* phosphate buffer (pH 5.0)	50 cm×50 μm	30 kV	[12]
	DnS-Cl	50 m*M* phosphate buffer (pH 7)	100 cm×75 μm	30 kV	[40]
	DCC	75 m*M* borate buffer (pH 9.0): methanol (72.5:27.5, v/v)	75 cm×75 μm	30 kV	[13]
	DCCS	5 m*M* carbonate buffer (pH 9.0)	60 cm×50 μm	20 kV	[14]
	Fluorescamine	50 m*M* borate buffer (pH 8.3)–50 m*M* lithium chloride	57 cm×75 μm	18 kV	[41]
	FITC	5 m*M* carbonate buffer (pH 10)	99 cm×50 μm	25 kV	[16]
	OPA	50 m*M* sodium acetate buffer (pH 9.5)–15% methanol–1% THF	100 cm×50 μm	30 kV	[42]
	NDA	20 m*M* borate buffer (pH 9)	100 cm×50 μm	30 kV	[43]
	PTC	50 m*M* borate buffer (pH 9.6)	60 cm×75 μm	10 kV	[44]
	ND	1 m*M* salicylate–0.2 m*M* sodium-carbonate (pH 9.7)	100 cm×50 μm	45 kV	[24]
	ND	10 m*M* PAS or DMBA-20 m*M* alpha-CD (pH 11.0)	90 cm×75 μm	20 kV	[26]
	ND	15 m*M* carbonate–5 m*M* luminol–25 m*M* hydrogen peroxide (pH 10)	50 cm×50 μm	20 kV	[45]
Biogenic amine	FITC	20 m*M* borate–20% acetone–5 m*M* DM-CD	47 cm×75 μm	25 kV	[46]
	ND	4 m*M* cupric sulfate–4 m*M* formic acid–4 m*M* 18-crown-6 (pH 3.0)	57 cm×75 μm	15 kV	[27]
	ND	4 m*M* cupric sulfate–4 m*M* formic acid–4 m*M* 18-crown-6 (pH 4.5)	63 cm×75 μm	10 kV	[28]
	ND	50 m*M* phosphate–7 m*M* 1,3-diaminopropane (pH 2.35)	65 cm×50 μm	30 kV	[29]
	ND	100 m*M* Tris-borate (pH 8.0)–0.05 m*M* CTAB-0.005% PAV	57 cm×75 μm	20 kV	[30]
	ND	5 m*M* quinine sulfate (pH 3.0)	60 cm×25 μm	30 kV	[31]
	ND	5 m*M* quinine sulfate–2% ethanol (pH 3.0)	63 cm×75 μm (polyacrylamide coated)	8 kV	[32]
Aromatic amine	ND	50 m*M* phosphate–7 m*M* 1,3-diamino-propane (pH 2.35)–38% acetonitrile	65 cm×10 μm	30 kV	[47]
Heterocyclics amine	ND	31% methanol–20 m*M* phosphate (pH 2.0)	46.4 cm×50 μm	18 kV	[48]
	ND	2 m*M* ammonium acetate (pH 3.0)–20% methanol	54 cm×50 μm	22 kV	[49]
	ND	30 m*M* sodium chlorie–50 m*M* phosphate (pH 2.1)–26% methanol	51 cm×50 μm	25 kV	[50]
Alkyl amines	Fluorescamine	50 m*M* phosphate buffer (pH 7)	100 cm×75 mm	30 kV	[51]

where L is the capillary length, μ_E is the analyte's electrophoretic mobility, μ_{EO} is the electro-osmotic mobility, and V is the applied voltage [52].

An additional advantage occurs in CE, the shape of the EOF profile is position-like. The flow velocity is constant over most of the capillary tube cross the section and drop to zero only near the tube walls. This is fortunate as the flat flow profile of electroosmosis will add the same velocity component to all solutes, regardless of their radial position, and

will thus not cause any significant dispersion of the zone. The more familiar parabolic laminar flow profile, such as occurs in capillaries in ordinary hydraulic flow, would lead to serious zone spreading. Therefore, separation efficiency is usually better in CE as compared to LC, where the flow profile is parabolic.

As for amino compounds, amino groups usually possess a positive charge, and dissociate into cations in an acidic medium. Amino compounds usually migrate from anodic site to cathodic site through a separation capillary depending on both the driving forces of the electric charge of the amino compound and on EOF. In pH mediums of pH less than 2.5, the driving force depends entirely on the electric charge of amino compound, because the latter force, i.e. EOF, becomes negligible.

3.2. Open tubularcoated fused-silica capillary

The silanol groups on the surface of a silica or glass capillary wall are masked with alkyl reagents, because EOF sometimes interferes with the separations of analytes. Using a capillary coated with trimethylchlorosilane, Jorgenson and Lukacs [40] examined the effects of EOF on zone resolution and analysis time of seven dansylated amino acids. Recently, Zhao et al. [49] demonstrated the detection of 14 heterocyclic amines by using polyvinyl alcohol (PAV)-coated capillary electrophoresis equipped with electrospray-mass spectroscopy (ES-MS).

4. Electromigration in pseudo-chromatographic CE

The principle of CZE is that molecules migrate under the influence of an electric field at various rates depending on their charge to mass ratio. Although CZE is a well-established analytical technique, as mentioned above, for the analysis of charged spaces of native free amino compounds, the electric charge of the molecules sometimes decreases due to masking of a functional amino group by the derivatizing reagent. Sometimes, it is very difficult to separate and determine the numerous derivatized amino compounds by using only CZE mode. In order to accomplish a high-resolution separation of these compounds with CE, a number of methods derived from both principles of CZE and LC, viz., capillary electrochromatography (CEC), capillary gel electrophoresis (CGE), micellar electrokinetic chromatography (MEKC) and electrokinetic capillary chromatography (EKC), have been demonstrated as a pseudo-chromatographic CE technique. The former two modes employ a support material in a fixed stationary phase, and the support material in the latter two is one of a pseudo-stationary phase. Although, historically, MEKC has been generally classified as a type of EKC, MEKC is, in this review, distinguished from EKC. Only the one method using micelle-forming reagent in a moving pseudo-stationary phase is referred to as MEKC and the other methods using a non-micelle moving pseudo-stationary phase are referred to as EKC, and are separately discussed in this section, and these applications are listed in Table 3.

4.1. Capillary electrochromatography (CEC)

The analytical method of electromigration in a capillary tube packed with particulate as a fixed stationary phase named capillary electrochromatography (CEC) was first demonstrated by Pretorious et al. [53] in 1974. They examined EOF as a method to pump solvents in micro glass columns (5 cm of length$\times$0.1 mm of I.D.) packed with micro-particulate silica. The CEC method is a new and emerging technique with great potential and is a microseparation technique that combines the best features of LC and CE. The separation of compounds can be achieved by differential partition between two phases, usually a stationary and mobile phase, differential electromigration, or a combination of both. In addition, the mobile phase in CEC is driven by EOF or by a combination of EOF and pressure. The latter allows easy hyphenation with high-tech analytical instrumentation, such as mass spectroscopy (MS) or nuclear magnetic resonance spectroscopy (NMR). Because this technology is still quite new, there are few reports as to its application to amines and polyamines in the literature. One application is the separation of dansylated 3-amino-acid (glycine, glutamic acid, leucine) mixture as reported by Alicea-Maldonado and Colón [54]. Three amino acid derivatives effectively migrated in a capillary tube

Table 3
Electromigration of amino compounds in pseudo-chromatographic CE

Analyte	Derivative	Run buffer	Capillary size (length×I.D.)	Applied voltage	Ref.
Amino acid	AEOC	7 m*M* borax–15 m*M* phosphate (7.5)–35 m*M* SDS–7 m*M* urea	50 cm×50 μm	30 kV	[38]
	CBQCA	10 m*M*–borate buffer (pH 9.3)–10 m*M* SDS	49.3 cm×10 μm	20 kV	[65]
	CBQCA	50 m*M* borate buffer (pH 9.0)–20% DMSO–30 m*M* SDS	50 cm×50 μ	30 kV	[66]
	CBQCA	50 m*M* TES (pH 7.0)–50 m*M* SDS	104 cm×50 μm	25 kV	[11]
	Dns-Cl	100 m*M* Tween 20–25 m*M* phosphate (pH 2.4)	34 cm×25 μm	16 kV	[62]
	Dns-Cl	5% isopropanol–200 m*M* 1,2-hexanediol–100 m*M* SDS–20 m*M* tris (pH 8.0)	47 cm×50 μm	20 kV	[67]
	Dns-Cl	1 *M* borate–50 m*M* phosphate buffer (pH 7.56)–49 m*M* SDS	50 cm×50 μm	15 kV	[68]
	DCC	20 m*M* borate buffer (pH 9.0)–27.5% methanol–17.5 m*M* SDS	75 cm×33 μm	30 kV	[14]
	Fluorescamine	20 m*M* phosphate buffer (pH 9.5)–100 m*M* SDS	40 cm×50 μm	11.1 kV	[69]
	FTC	20 m*M* phosphate buffer (pH 7.5)–75 m*M* SDS	40 cm×50 μm	11.1 kV	[70]
	FMOC	20 m*M* phosphate buffer (pH 9.5)–25 m*M* SDS	40 cm×50 μm	16.6 kV	[69]
	FMOC	50 m*M* borate buffer (pH 9.5)–15% methanol–2% THF–50 m*M* SDS	70 cm×50 μm	20 kV	[71]
	IDA	20 m*M* borate–phosphate buffer (pH 7)–3% methanol–2% THF–50 m*M* SDS	50 cm×50 μm	20 kV	[19]
	PTH	4.3 *M* urea–100 m*M* SDS–100 m*M* borate–50 m*M* phosphate (pH 7.0)	50 cm×50 μm	10 kV	[72]
	PTH	12.5 m*M* borate–50 m*M* phosphate buffer (pH 7.0)–40 m*M* SDS	65 cm×50 μm	10 kV	[73]
	PTH	12.5 m*M* borate–12.5 m*M* phosphate buffer (pH 7.0)–35 m*M* SDS	39 cm×33 μm	8 kV	[74]
	TRITC	5 m*M* boric acid buffer (pH 9)–10 m*M* SDS	92 cm×50 μm	30 kV	[23]
Biogenic amine	AccQ	10%–acetonitrile–100 m*M* borate (pH 8.9)–50 m*M* SDS	55 cm×50 μm	15 kV	[75]
	FITC	20 m*M* borate–20% acetone–5 m*M* DM-cylodextrine	47 cm×75 μm	25 kV	[46]
	OPA/NAC	20 m*M* phosphate–borate–2 m*M* OPA–16 m*M* NAC–20 *M* SDS (pH 10)	100 cm×50 μm	25 kV	[76]
Heterocyclic amine	ND	15 m*M* borax (pH 9.1)–5 m*M* CTAB	60 cm×20 μm	−25 kV	[63]

packed with ethylene chlorotrifluoroethyene (ECTFE) particles using acetonitrile–water (9:1, v/v) containing 0.15% trifluoroacetic acid as a mobile phase. As the result of their examinations, it was shown that EOF can be effectively controlled with trifluoroacetic acid (TFA) in a run buffer and can also play an active role in the separation process. The highest resolution was achieved in the ECTFE-packed capillary column when using 20 m*M* TFA.

4.2. Capillary gel electrophoresis (CGE)

The method of electromigration in a gel packed capillary, referred to as ‘‘capillary gel electropho-

resis'' (CGE), was introduced by Hjertén [55] in 1983. Traditional electrophoresis has become a basic separation technique employed to analyze biopolymers such as protein, polysaccharide and DNA. The principle of electromigration mechanism of CGE is the same as the classical electrophoresis. The gel, which is packed in a separation capillary, acts as a molecular sieving matrix leading to separation based on analyte size. However, there is little mention made in the literature of the application of CGE as an analytical method for amines and polyamines, because the molecular-weight differences of amino compounds, which have the molecular weight less than 1000, are very small. Rather CGE is more suitable for high-molecular-mass substances such as peptides [56], proteins [57], DNA [58] and polysaccharides [59] than that for low-molecular-mass substances such as amino compounds dealt with this review.

Liu et al. [59] examined the effect on migration orders of four amino acids (arginine, glycine, histidine and leucine) of different types of capillaries. In untreated open tubular capillaries, electro-osmotic flow serves as the major force for the migration of solutes. Elution order in this case was arginine, histidine, leucine and glycine. When the capillary was treated to deactivate the capillary wall or filled with polyacrylamide gel, electro-osmotic flow was eliminated and the polarity of high voltage had to be reversed for continuing experiments. Charged solutes migrated in terms of their electrophoretic mobilities. The migration orders were thus totally reversed.

4.3. Micellar electrokinetic capillary chromatography (MEKC)

In 1984, the idea of using micelle instead of a stational phase in CEC was first introduced by Terabe et al. [60,61]. This technique was named micellar electrokinetic capillary chromatography (MEKC) and widely extends the applicability of the technique to the analysis of neutral substances. When amino compounds or electrically neutral molecules are placed in the electric field of a capillary tube filled with an electrolyte including a surfactant, such as sodium dodecyl sulfate (SDS), analytes electromigrate depending on the differences in distribution of analytes between an aqueous eluent and an micellar pseudo-stationary phase. The mechanism of this mode is almost the same as that of a reversed-phased LC mode or EKC. The migration time (t_R) in MEKC is given by

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

where k' is the capacity factor, and t_0 and t_{mc} are the migration times of the aqueous and micellar phases, respectively.

Other surfactants, e.g., Tween 20 [62] as a non-ionic surfactant, cetyltrimethylammonium bromide (CTAB) [63] and dodesylteimethylammonium bromide (DTAB) [64] were examined for amino compounds in MEKC. Results of these investigations showed that MEKC is one of best methods for detection of amines, polyamines and their derivatives using electromigration analysis.

4.4. Electrokinetic capillary chromatography (EKC)

After introducing MEKC methods, another idea was to add a substance to the run buffer to act as a pseudo-stationary phase, as with EKC. In addition, the separation and transport mechanism of EKC is almost the same as that of CEC as mentioned before chapter 4.1. But, the cases of a capillary tube packed with particulate as a fixed stationary phase and filled with free solution as a pseudo-stationary phase used are named EKC and CEC, respectively, in this review.

In an application of this technique to amino compounds, Bazzanella et al. [77] demonstrated the separation of 13 *n*-alkylamine derivatives of OPA/2-mercaptoethanol (2-ME) with EKC mode using resorcarene-octacarboxylic acids, macrocyclic molecules built up by four alkylidene-bridged resorcinol units as pseudo-stationary phases. Other molecule-forming reagents, e.g., oligomerized sodium 10-undecylenate [78], CD [79], starbust dendrimers (poly(amidoamines)) [80] and ionic polymers [81,82], have been used for electromigration with EKC.

5. Applications of CE

In this section, we briefly exsamine the applications of the electromigration methods to the amino

compounds to a field as follows: (1) amino acid; (2) biogenic amine which is included mono and polyamines; (3) pollutant which is included aromatic and hetero-cyclic amines and (4) enantiomers.

5.1. Amino acids

A considerable number of investigations have appeared over the past few years dealing with the separation of amino acids by CE as well as by the other chromatographic separation methods because amino acids are such important components in living bodies. Most of the reported electromigration methods were optimized with MEKC, because simultaneous separation of more than 20 kinds of amino acids whose chemical structures are very close to each other has been very difficult with other methods.

In 1991, Liu and his co-workers [11] demonstrated the MEKC separation of a 17-component amino acid solution after derivatizing with 3-(4-carboxybenzol)-2-quinolinecarboxaldehyde (CBQCA) equipped with a laser-induced fluorescence detector. The amino acid derivatives migrated and effectively separated in a fused-silica capillary tube (73 cm effective length×50 mm I.D.) field with a 50 m*M* SDS-50 m*M* 2-[[*N*-[tris(hydroxymethyl)methyl]amino]-ethanesulfonic acid (TES) buffer at pH 7.02 under an electric field of 25 kV. Their successful electropherogram is shown in Fig. 1.

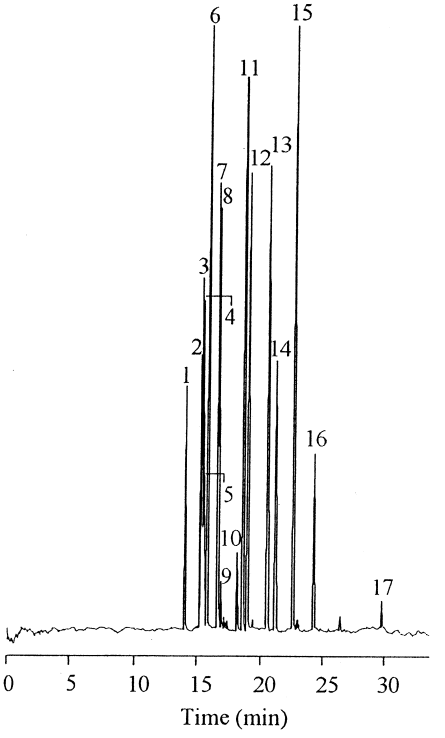


Fig. 1. Electromigration profile of CBQCA-amino acids in MEKC. Peak identification: 1, arginine; 2, tryptophan; 3, tyrosine; 4, histidine; 5, methionine; 6, isoleucine; 7, glutamine; 8, aspartate; 9, threonine; 10, phenylalanine; 11, leucine; 12, valine; 13, serine; 14, alanine; 15, glycine; 16, glutamic acid; 17, aspartic acid. Capillary: 50 μm (I.D.)×104 cm (length). Run buffer: 50 m*M* SDS-50 m*M* TES buffer (pH 7.02). Operating voltage: 25 kV. Reproduced from [11].

Currently, protein sequence is determined by repetitive application of the Edman degradation reaction sequence [22]. In these reactions, an isothiocyanate such as phenylisothiocyanate (PITC) is coupled to the N-terminal amino group of the protein under basic conditions to form the thiocarbamoyl derivative. The thiocarbamoyl is treated with acid to produce the cyclic thiazolinone amino acid derivative, the thiazolinone intermediate is subsequently converted to the stable phenylthiohydantoin (PTH)-amino acid derivative by exposing with aqueous acid. In order to determine the amino acid sequence in a protein, a high-sensitivity separation of the final products, PTH-amino acid derivatives is essential. Terabe and his research group [72,73] originally studied in detail the electromigration of PTH-amino acids with MEKC. Further, Waldron and Dovichi [74] reported a very powerful technique for the separation and sub-femtomole determination of 20 PTH-amino acid derivatives equipped with thermo-optical detector.

Albin et al. [69] also studied methods for separation of six amino acids using four different fluorophors, viz., fluoresceinisothiocyanate (FTC), fluorescamine, 9-fluorenylmethylchloroformate (FMOC) and OPA. Each derivative was migrated in 20 m*M* of sodium tetraborate (pH 9.5) containing varying amount of surfactant (SDS), and was detected by the designated fluorescence detector.

In 1997, Oguri et al. [83] also reported a convenient method for determination of 16-amino acids by using ICD-CE method. The migration profile of the compounds in ICD-CE is shown in Fig. 2.

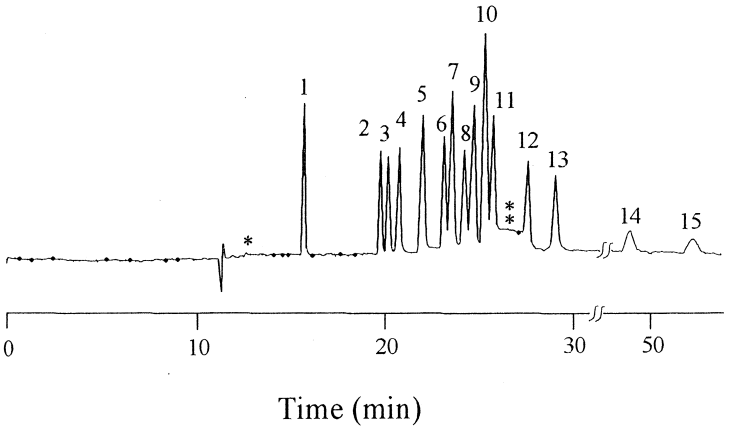


Fig. 2. Electromigration profile of OPA/NAC-amino acids in ICD-CE. Peak identification: 1, arginine; 2, phenylalanine; 3, tyrosine; 4, leucine; 5, isoleucine; 6, methionine; 7, valine; 8, lysine; 9, alanine; 10, glycine; 11, histidine; 12, serine; 13, threonine; 14, glutamic acid; 15, aspartic acid. The symbols * and ** represent ammonia and cystine, respectively. Capillary: 50 μm (I.D.)×100 cm (length). Run buffer: 20 m*M* β-CD-2 m*M* OPA/NAC-100 m*M* phosphate-borate buffer (pH 10). Operating voltage: 22 kV. Reproduced from [83].

5.2. Biogenic amines

Bioactive amino compounds with molecular weights less than 500 are generally called by the name of ''biogenic amines'' examples of which are histamine (His), tryptamine (Trp), tyramine (Tyr), cadaverine (Cad), ormithine (Orm), putrescine (Put), spermidine (Spd), spermine (Spm), neuroactive amino acids and other mono- and polyamino compounds. These amines are synthesized from amino acids by enzymatic decarboxylation and widely exist in nature, for example, in the mammalian body in connection with nerve transmission substances, or in spoiled foods, especially those which are capable of inducing allergic reactions.

In general, biogenic amines, especially polyamines, such as Spd, Spm, and Put, are more difficult to completely derivatize than monoamines. Therefore, electromigration for biogenic amines have hitherto been performed only with the native form without any derivatizations by CZE, rather than by MEKC. For example, Arce and his co-workers [27] demonstrated the electromigrated separation of 21 amino compounds containing biogenic amines by CZE with indirect UV detection as shown in Fig. 3.

Tumor cells contain levels much higher than in normal cells of common polyamines such as Put, Spd, and Spm. Zhang et al. [31] demonstrated that

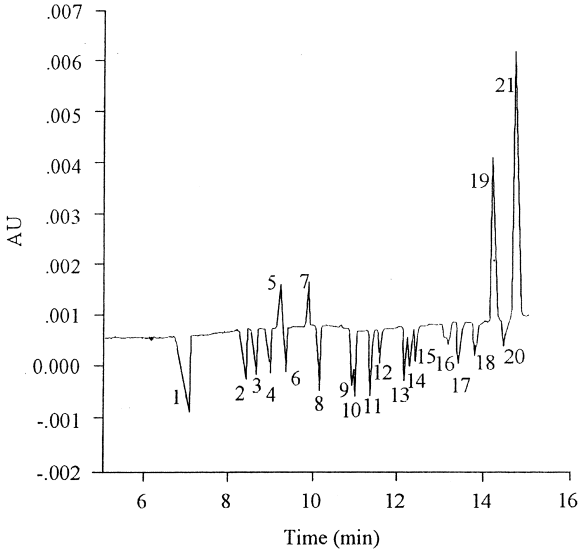


Fig. 3. Electromigration profile of free 21-biogenic amines in CZE with indirect photometric detection. Peak identification: 1, ammonia; 2, methylamine; 3, 1,3-diaminopropane; 4, putrescine; 5, histamine; 6, cadaverine; 7, agmatine; 8, ethylamine; 9, ethanolamine; 10, spermidine; 11, propylamine and morpholine; 12, isopropylamine; 13, diethylamine; 14, butylamine; 15, spermine and isobutylamine; 16, amylamine and isoamylamine; 17, 1-methylbutylamine; 18, hexylamine; 19, phenethylamine; 20, heptylamine; 21, tyramine. Capillary: 75 μm (I.D.)×63 cm (length). Run buffer: 4 m*M* cupric sulphate-4 m*M* formic acid-4 m*M* 18-crown-6 (pH 3.0). Operating voltage: 10 kV. Reproduced from [27].

CZE with indirect photometric detection using quinine sulfate as a BGE was capable of determining underivatized free polyamines in tumor cells (PC 12 cell line). Zhou et al. [32] also established a CE method with indirect UV detection using the same BGE in a run buffer (pH 3) for separation of three polyamines (Put, Spd and Put) in serum. Thus, the use of the CZE method and its variations with indirect detection became established as a general scheme for electromigration and the detection of biogenic amines.

More sensitive detection of biogenic amines can be accomplished by MEKC with derivatization methods as reported by the several groups [76,84–86]. Nouadji et al. [86] used a ball-lens LIF to determine florescein thiocarbamyl biogenic amine in dairy products using MEKC. More recently, Kovács et al. [75] reported a high-sensitivity electromigration method using 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AccQ) as the fluorescence derivatization reagent. Seven biogenic amine-AccQ derivatives (histamine, tyramine, tryptamine, spermidine, spermine, cadaverine, putrescine) were distinctly separated and detected with high sensitivity by MEKC using SDS as surfactant.

5.3. Pollutants

A number of aromatic or heteroaromatic amino compounds exist widely as environmental pollutants, thereby touching very closely on the daily lives of people everywhere in the world. Therefore, analytical methods to detect such compounds were highly sought after and subsequently developed in order to help preserve human health [87]. The applications of CE to the analysis of pollutants such as aromatic or heteroaromatic amino compounds have been reviewed by Dabek-Zlotorzynska [88].

Cavallaro et al. [29] examined the separation of a 21-component aromatic amine solution using CZE with UV detection, and showed a good separation profile of these amines as shown in Fig. 4. The analytes migrated in an uncoated fused-silica capillary filled with a 50 m*M* phosphate buffer (pH 2.35) in the presence of 1,3-diaminopropane as a surface modifier.

Brumley and Jones [89] demonstrated the separation of four kinds of benzidines and of aniline and 15 kinds of its analogues by MEKC using cholic acid as the micellar-forming reagent in a 50 m*M* borate buffer (pH 8.3).

Because pollutants are present in the environment at levels of only a few parts per billion or less, highly sensitive and highly selective detection methods are required to identify them. Takeda and his group [90] also presented the separation and identification of aniline, *p*-nitroaniline and naphthylamine by MEKS using SDS in a 20 m*M* phosphate buffer (pH 6.2) equipped with an ES-chemical ionization (CI)-MS as a detector.

Wu and et al. [48] reported an optimized electropherogram of 13 heterocyclic amines by using CZE. The heterocyclic amines migrated at 18 kV in a 20 m*M* phosphate buffer (pH 2.0) containing methanol (31%) as organic modifier. Recently, Y. Zhao et al. [49] analyzed 14 heterocyclic amines by CZE in a POV-coated capillary tube equipped with CE-ES-MS, which enabled it to perform highly sensitive and highly selective detection. The optimized electrolyte was composed of 20 m*M* ammonium acetate buffer (pH 3.0) and methanol (20%).

5.4. Enantiomers

Due to the tragic events linked to the use of thalidomide occurring in the early 1960s in Japan and other countries, the importance of enantiomeric separation analysis was recognized, and analytic methods for such have been developed which use chromatographic techniques, including CE [91].

To date, there is generally four different way of doing chiral separation of amino compounds by CE: (1) ligand exchange complexation, (2) host–guest complexation, (3) solubilization of enantiomers by optically active pseudo-stationary phase and (4) solubilization of diastereomers by pseudo-stationary phase.

The first method mentioned above is based on a multicomponent chelate complex consisting of Cu^{2+} as the central ion and two chiral bifunctional ligands, L-histidine [92–94]. The analyte such as Dns-D,L-amino acid mixture replaces one chelator by forming a ternary diastereomers complex (Cu-L-histidine-Dns-D-amino acid and Cu-L-histidine-Dns-L-amino acid). The differences in the complex-forming constants were resulted to the different migration pro-

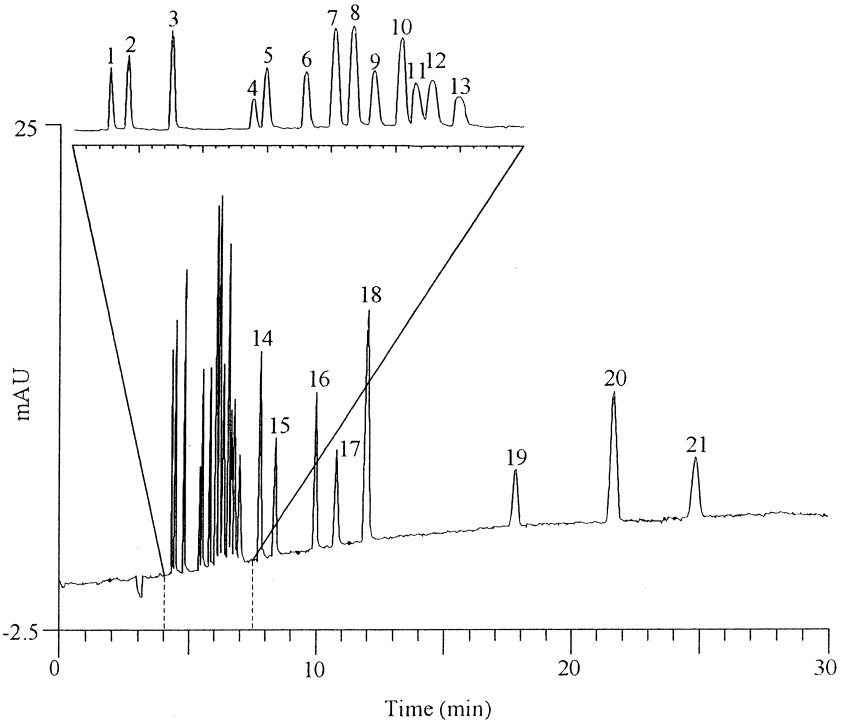


Fig. 4. Electromigration profile of 21 aromatic amines in CZE. Peak identification: 1, pyridine; 2, *p*-phenylenediamine; 3, benzidine; 4, *o*-toluidine; 5, aniline; 6, *N,N*-dimethylaniline; 7, *p*-anisidine; 8, *p*-chloroaniline; 9, *m*-chloroaniline; 10, ethylaniline; 11, α-naphthylamine; 12, diethylaniline; 13, *N*-(1-naphthyl)ethylenediamine; 14, 4-aminophenazone; 15, *o*-chloroaniline; 16, 3,4-dichloroaniline; 17, 3,3′-dichlorobenzidine; 18, 2-methyl-3-nitroaniline; 19, 2,4-dichloroaniline; 20, 2,3-dichloroaniline; 21, 2,5-dichloroaniline. Capillary: 50 μm (I.D.)×65 cm (length). Run buffer: 7 m*M* diaminopropane-50 m*M* sodium dihydrogenphosphate buffer (pH 2.35). Operating voltage: 30 kV. Reproduced from [29].

files in CZE. By using this method, Zare and his group [92] first described the successful separation of enantiomers of Dns-amino acids with CZE.

The second category is that chiral separation can be performed on the basis of the host–guest complexation between enantiomer and chiral selector such as crown ether or CD [108–116]. Host-guest complexation is mostly accomplished by CD and alkylated CD derivatives. It is also well known that crown ether forms its stable chelate complex with ammonium ion, alkaline metals and alkaline earth metals as just like a host–guest complexation. There are some examples of enantiomeric separation of amino acids by using optical active crown ethers in CZE. For example, Ma and Horváth [117] investigated a successful chiral separation biogenic amines at subzero temperature by CZE using 2,6-dimethyl-β-cyclodextrin (DMBCD) as a chiral selector and using both neat aqueous and hydro-organic electrophoretic media. Each amine of five enantiomer pairs electromigrated at 30 kV in a fused-silica capillary tube (37 cm×50 μm, I. D.) filed with a pH 2.5 buffer solution consisting of 20 m*M* DMBCD, 5 *M* urea and 150 m*M* sodium phosphate in water–methanol (90:10, v/v) as shown in Fig. 5.

The third is based on the differences in solubilization of enantiomers between an aqueous phase and optically active pseudo-stationary phase in MEKC or EKC. To perform a chiral separations of amino acid enantiomers by MEKC, a number of chiral surfactants such as sodium *N*-dodecanoyl-L-valinate (SDVal) [95–98], sodium *N*-dodecanoyl-L-glutamate (SDGlu) [99], sodium taurocholate (STC), sodium taurodeoxycholate (STDC) [100,101], digitonin [97], glycyrrhizinic acid (GRA) [102], antibiotics [104,105] and polysaccharide [106] were examined.

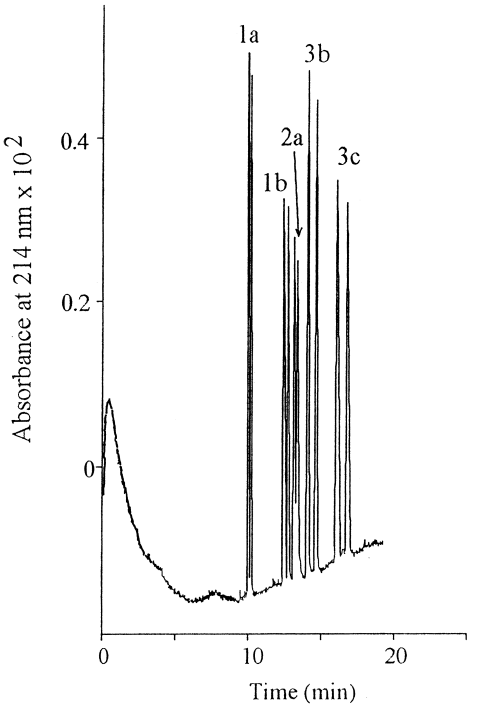


Fig. 5. Electromigration profile of five enantiomer pairs of biogenic amines in CZE. Peak identification: 1a, β-hydroxyphenethylamine; 1b, norephedrine; 2a, octopamine; 3b, epinephrine; 3c, isoproterenol. Capillary: 50 μm (I.D.)×37 cm (length). Run buffer: 50 m*M* DMBCD-5 *M* urea-10% methanol in 150 m*M* sodium phosphate buffer (pH 2.5). Operating voltage: 30 kV. Reproduced from [117].

Finally, the enantiomeric separation can be performed based on the differences in solubilization of diastereomers between an aqueous phase and pseudo-stationary phase. In order to convert a chiral amino compound to its diastereoisomer, amino compounds were tagged with chiral derivatization reagent such as OPA/chiral thiol. The following chiral thiol compounds, *N*-acetylcysteine (NAC) [118], *N*-boc-cysteine [119] and tetra-*O*-acetyl-1-thio-β-D-glucopyranse [120] were employed for the separations of D,L-amino acid enantiomers. The other chiral reagents, 1-fluoro-2,4-dinitrophenyl-5-L-alanine (Marfey's reagent) [107], tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate (GITC) [121], and (+)- and (−)-1-(9-fluorenyl)-ethyl chloroformate (FLEC) [122] were also examined in CE.

Although a number of enantiomeric separations of D,L-amino acids in CE based on the above-mentioned theories were also demonstrated [92–102,104–107,113–122], the methods were rather complicated. Recently, a very simple method of separation of D,L-amino acid enantiomers was developed using an ICD-CE technique [83]. Using OPA/*N*-acetylcysteine (NAC) as a derivatizing reagent in a run buffer of ICD-CE, D and L-amino acids were converted into their diastereoisomers, that migrated and were detected in a separation capillary without any complicated procedures. Electromigration methods for enantiomeric amino acids and amines are listed in Table 4.

6. Micro-separation techniques

Although CE has come to be a useful and powerful microseparation technique with many potential advantages as an analytical method, CE still does not hold the position as the method of first choice of among analytical separation techniques. Beginning in the early 1990s, new applications of CE to molecular science have been presented by several groups [123]. In this section, several important works treating amino compound analyses using electromigration method are briefly reviewed.

6.1. Single cell analysis in CE

It has long been a goal of biochemists to study the chemistry of single cells. However, measurements of a large number of compounds in a single cell are very difficult because cell contents occur in such small amount and at such low levels. The development of assay methods for single cells is very important to elucidate biological functions, because individual cells in biological samples are not equivalent with each other. Although bioassay [124], fluorocolorimetry [125], enzyme immunoassay [126], and LC [127–129] have been and continue to be used, these methods can only supply an averaged figure based on a group of cells being analyzed, and are incapable of analyzing single cells. In 1990, Gilman and Ewing [130] reported the analysis of amino compounds, including amino acids and peptides, in a single mammalian cell performed by CZE with an at-inlet in-capillary derivatization technique and LIF. The front end of the separation capillary

Table 4
Electromigration methods for enantiomeric amino compounds

Chiral selector	Analyte	CE mode	Ref.
Cu(II)/L-Histidine	Dns-D,L-amino acids	CZE	[92]
Cu(II)/Aspartame	Dns-D,L-amino acids	CZE	[93]
Cu(II)/Alanine	Dns-D,L-amino acids	MEKC	[94]
SDVal	DNB-D,L-amino acids	MEKC	[95]
SDVal	PTH-D,L-amino acids	MEKC	[96]
SDVal	PTH-D,L-amino acids	MEKC	[97]
SDVal	PTH-D,L-amino acids	MEKC	[98]
SDGluu	PTH-D,L-amino acids	MEKC	[99]
Tauroyl cohoic acid?	Dns-D,L-amino acids	MEKC	[100]
Tauroyl cohoic acid?	Dns-D,L-amino acids	MEKC	[101]
Digitonin	PTH-D,L-amino acids	MEKC	[97]
Glycyrrhizinic acid	PTH-D,L-amino acids	MEKC	[102]
(*R*)- and (*S*)-*N*-dodecoxycarbonylvaline	ephedrine enantiomers	MEKC	[103]
Vancomycin	AccQ-D,L-amino acids	CZE	[104]
Vancomycin	AccQ-D,L-amino acid	CZE	[105]
Polysaccharieds	trans-2-phenylcyclopropylamine	CZE	[106]
Marfey's reagent	D,L-amino acids	MEKC	[107]
beta-CD	Dns-*N*-methyl-taurine	MEKC	[108]
beta- and gamma-CD	trimipramine	nonaqueous CE	[109]
Quaternary ammonium beta-CD	tri-, dicyclicamine	CZE	[110]
18-crown-6+CD	primary amine enantiomers	CZE	[111]
18-crown-6+CD	primary amine enantiomers	CZE	[112]
18-crown-6-tetracarboxylic acid	norephedrine, D,L-amino acids	CZE	[113]
18-crown-6-tetracarboxylic acid	D,L-amino acids	CZE	[115]
18-crown-6-tetracarboxylic acid	D,L-amino acids	CZE	[116]

was used as the derivatization chamber, where the individual rat pheochromocytoma (PC12) cell and lysing/derivatizing buffer, containing digitonin, naphthalene-2,3-dicarboxaldehyde (NDA) and sodium acetonitrile, were electrophoretically introduced and mixed. After completion of the reaction in the same place, the derivatized analytes were subsequently migrated, separated and detected.

By using CE technology, Ewing's group also investigated neurotransmitter compartmentalization within the cell body of neurons in the hope to gain greater understanding of the role of the cell body in metabolism, uptake and storage of neurotransmitters. They demonstrated, for the first time, the direct determination of the cytoplasmic concentration of dopamine or biogenic amine in single, intact neurons of the pond snail, *Planorbis corneus*. The employment of this technique need not be limited to the study of neurotransmitter concentrations alone. Picoliter samples from cytoplasma, coupled with other electromigration schemes, should be applicable to the investigation of hormone peptidic levels, intracellular messenger concentrations and general metabolism, all at the single cell level [131].

Oguri et al. [132] presented a simple method for determination of histamine in a rat peritoneal mast cell by on-line mode ICD-CE. The direct detection of histamine in rat peritoneal mast cells was achieved by incorporating SDS in the run buffer as the cell-lysing reagent. This method induced automatic cell

lysis, labeling of histamine with fluorescence reagent OPA/NAC), electromigration, and detection following direct injection of cell suspension into the separation capillary of the ICD-CE.

6.2. Electromigration on microfabricated chip CE

In 1992, Manz et al. [133] first presented an electromigration of FTH-amino acids in glass microchannels that were fabricated using standard photolithographic and chemical etching techniques. Adapting it to CE pumping action is easily achieved due to the electrokinetic effect, and fluid flow is precisely controlled by regulating the applied potentials at the terminals of the microchannel. Therefore, it is possible to eliminate the need for moving micro-parts such as pumps and valves by controlling the electric fields in the micro-channel. Micro-fabrication offers the possibility to miniaturize traditional analytical instrumentation with the advantages of speed, automation and volumetric reduction of samples, reagent and waste. A further advantage is that diffusion velocity of a solution in a micro-channel increases by a factor of 100 for a 1/10th reduction in channel size [134].

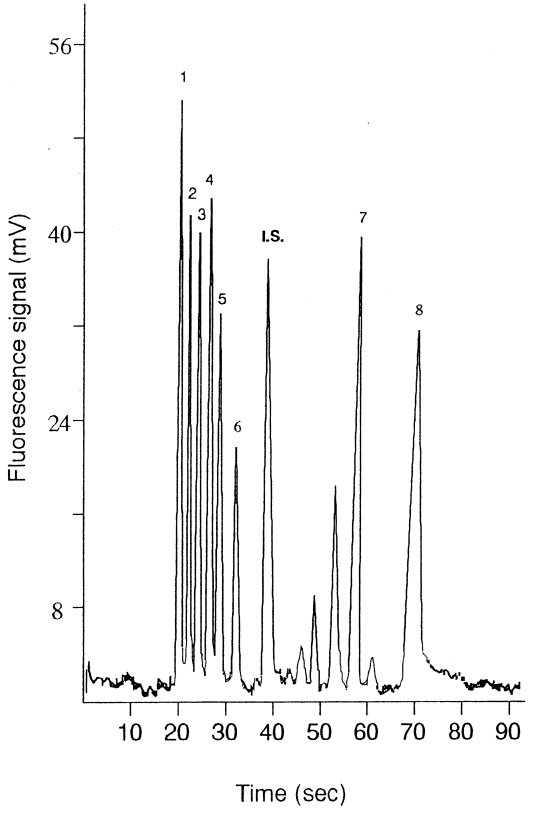


Fig. 6. Electromigration profile of FITC-amino acids in microchip-CE. Peak identification: 1, putrescine; 2, histamine; 3, tyramine; 4, cadaverine; 5, phenylethylamine; 6, tryptamine; 7, spermidine; 8, spermine; I.S., internal standard (1,6-diaminohexane). Separation channel: 50 μm (wide)×8 μm (deep)×4.5 cm (length). Run buffer: 40 m*M* SDS-120 m*M* borate buffer (pH 9.4). Operating voltage: 3 kV. Reproduced from [136].

Following the pioneering work of Manz et al., CE has been expanded throughout the world to the microfabricated environment, the so called ''lab-on-a-chip'' or ''micro total analysis system'' (μ-TAS). Such advanced techniques are mainly employed in the field of DNA sequencing and diagnosis [135]. In an application to amino compounds, Rodriguez and co-workers [136] demonstrated the efficient separation of 8-FTC-biogenic amines as shown in Fig. 6. Further evolution and improvements of this technique are expected to take place in the next century.

7. Conclusions

Although many variations of CE have been developed, and have been applied to electromigrations of amino compounds having low-molecular weight, CZE and MEKC have standardly employed electromigration methods for amino compounds. The recommended electromigration method for free amines and polyamines or native free amino compounds is CZE in a low pH environment, and indirect detection method can be recommended for these compounds at present. Although MEKC or EKC is a suitable method for analyses of derivatized amino compounds using surfactants, for either stationary phase or pseudo stationary phase modes, further advances in electromigration methods are necessary for application to a much wider range of natural samples, including numerous amines and polyamines, and analyses of single cells. In the next generation, development of electromigration methods in combination with micro-separation techniques is seen as a having paramount priority.

8. Abbreviations

AccQ	6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate
AEOC	2-(9-anthryl)ethylchloroformate
BKE	background electrolyte
CBQCA	3-(4-carboxybenzol)-2-quinolinecarboxaldehyde
CD	cyclodextrin
CE	capillary electrophoresis
CEC	capillary electrochromatography
CGE	capillary gel electrophoresis
CTAB	cetyltrimethylammonium bromide
CTSP	9-cyano-*N*,*N*,*N′*-triethyl-*N′*-(5-succinimidyloxycarbonylpentyl)pyronin
CZE	capillary zone electrophoresis
DCC	dicarbocyanine reagent
DCCS	7-(diethylamino)coumarin-3-carboxylicacid succinimidyl ester
DM-CD	dimethyl-β-cyclodextrin
DMBA	4-(*N*,*N′*-dimethylamino)-benzoic acid
Dns-Cl	dansyl chloride
DTBA	dodecyltrimethylamonium bromide
ECTFE	ethylene chlorotrifluoroethyene
EKC	electrokinetic chromatography
EOF	electroosmotic flow
ES-MS	electrospray-mass spectroscopy
FMOC	9-fluorenylmethylchloroformate
FITC	fluoresceinisothiocyanate
FTH	fluorescein thiohydantoin
GC	gas chromatography
GITC	tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate
GRA	glycyrrhizinic acid
ICD	in-capillary derivatization
IDA	1-methoxycarbonylindolizine-3,5-dicarbaldehyde
LC	liquid chromatography
LIF	laser induced fluorescence detector
2-ME	2-mercaptoethanol
MEKC	micellar electrokinetic capillary chromatography
NAC	*N*-acetylcysteine
ND	not derivatized
NDA	naphthalene-2,3-dicarbo
OPA	*o*-phthalaldehyde
PAV	polyvinyl alcohol
PAS	*p*-amino salicylic acid
PITC	phenylisothiocyanate
PTH	phenythiohydantoin
SDGlu	sodium *N*-dodecanoyl-L-glutamate
SDS	sodium dodesyl sulfate
SDVal	sodium *N*-dodecanoyl-L-valinate
STC	sodium taurocholate
STDC	sodium taurodeoxycholate
TES	2-[[*N*-[tris(hydroxymethyl)methyl]-amino]ethanesulfonic acid
TFA	trifluoroacetic acid
TRIC	tetramethylrhodamine isothiocyanate
UV	ultraviolet

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