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Review

Analysis of biologically active amines by CE

This paper provides an overview on the current status of the analysis of biogenic amines by CE. The basic CE separation and detection strategies for the analysis of biogenic amines are briefly described. CZE and MEKC that provide highly efficient and reproducible analysis of biogenic amines are particularly surveyed. With respect to the detection of biogenic amines, we focus on LIF, UV-visible absorption, electrochemiluminescence, and MS. Derivatization strategies, indirect methods, and on-line concentration techniques such as field-amplified sample stacking, sweeping, and use of polymer solution are described. To show the practicality of CE, we highlight currently developed techniques for the determinations of biogenic amines in biological samples, including foods, beverages, cerebrospinal fluids, urine, and single cells.

Keywords: Biogenic amines / CZE / MEKC / Polymer solution / Stacking

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1 Introduction

Biogenic amines that are found in various organisms, cells, foods, and beverages are a group of low-molecular-mass organic bases, including aliphatic amines (mono-, di-, and polyamines), catecholamines, indolyl amines, and imidazolyl amines [1]. They are formed and metabolized in living organisms commonly through the decarboxylation of amino acids or the amination and transamination of aldehydes and ketones. The importance of biologically active amines is reflected by the fact that they are widely found in diverse biological systems such as

neuron circuits and are associated with many cell processes, including cell proliferation and differentiation, synthesis of nucleic acids and proteins, membrane stability, and signal transduction [2–7]. Dopamine (DA), norepinephrine (NE), epinephrine (E), serotonin (5-HT), and 3-(3,4-dihydroxyphenyl)alanine (DOPA) that are neurotransmitters in central and peripheral nervous systems are some of the most important biogenic amines [8–10]. Despite their important roles in biological systems, putrescine (PUT), cadaverine (CAD), and histamine (HIS) have been implicated in human poisoning when present at considerable concentrations [11]. Reported symptoms associated with excess uptake of biogenic amines include headaches, nausea, hypo- or hypertension, and cardiac palpitations. In addition, some biogenic amines such as PUT may react with nitrites to produce potential carcinogenic nitrosamines that induce certain types of cancers like colon carcinogenesis [12].

CE is one of the most powerful separation techniques for the analysis of biogenic amines because of its speed, high resolving power, and extremely small sample requirement [13–16]. However, its concentration detection limit is higher when compared with HPLC and ion chromatography, mainly because of lower amounts of injected sample and shorter optical path length. In addition, reproducibility of CE is usually not as great as the other two techniques, mainly due to variation of EOF as a result of adsorption of solutes on the capillary wall. Because most biogenic amines are protonated ($R-NH_3^+$)

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Abbreviations: ACCQ, 6-aminoquinoyl-*N*-hydroxysuccinimidyl-carbamate; CAD, cadaverine; CBQCA, 3-(4-carboxybenzoyl)-2-quinolinecarboxaldehyde; CL, chemiluminescence; CSF, cerebrospinal fluid; DA, dopamine; DEA, diethylamine; DOPA, 3-(3,4-dihydroxyphenyl)alanine; E, epinephrine; ELCL, electrochemiluminescence; FASS, field-amplified sample stacking; FQ, 3-(2-furoyl)quinoline-2-carboxaldehyde; HIS, histamine; 5-HT, serotonin; ILIF, indirect laser-induced fluorescence; LINF, laser-induced native fluorescence; MEA, methylamine; NDA, naphthalene-2,3-dicarboxaldehyde; NE, norepinephrine; OPA, o-phthalaldehyde; PEO, poly(ethylene oxide); PUT, putrescine; SPD, spermidine; SPM, spermine; TA, tryptamine; TB, Tris-borate; TBA, tributylamine; TEA, triethylamine; TPA, tripropylamine; TRP, tryptophan; TYR, tyramine; Vis, visible

under physiological conditions, they are cations and thus their separations by CZE are commonly carried out under acidic conditions in the presence of small EOF [17, 18]. However, the separations of biogenic amines by CZE are sometimes unsuccessful, especially because the biogenic amines have low hydrophilic properties, similar dissociation constant (K_a) values, and the tendency to be adsorbed on the capillary wall. To overcome the solubility problem and improve resolving power, MEKC is selected. Detergents such as SDS and CTAB that form micelles at concentrations greater than their critical micelle concentrations are added to the BGEs to provide pseudo-stationary phases for resolving of neutral solutes in MEKC [19, 20].

The detection of biogenic amines in CE is usually difficult, mainly because either the analytes of interest lack strong responses to the detection systems or their amounts are extremely low in biological samples. The sensitivity of UV-visible (Vis) absorption is poor for most biogenic amines [21–27], especially aliphatic amines because of their low molar absorptivity [22]. Alternatively, fluorescence [28–33], electrochemical detection such as amperometry [34–39], conductometry [39–41], contactless conductometry [42–44], electrochemiluminescence (ELCL) [45, 46], chemiluminescence (CL) [47–49], and MS [50–55] are employed for detecting low concentrations of biogenic amines. Although these detection methods provide better sensitivity than UV-Vis absorption detection, they (besides MS) commonly suffer from high selectivity. For example, native (intrinsic) fluorescence detection is only useful for some biogenic amines such as catecholamines, while ELCL detection is only applicable to tertiary amines. To improve the sensitivity and/or applicability, derivatization of biogenic amines with suitable agents is usually conducted in CE with the aforementioned detection modes. To prevent problems such as long reaction time, tedious processes, or side products associated with derivatization, a number of indirect detection methods have been proposed [56–60]. Indirect UV-Vis absorption and indirect LIF (ILIF) are the two most common indirect detection methods in CE. Although they are convenient and simple, sample pretreatment is usually required to reduce matrix interference.

Many reviews dealing with new trends in CE and bioapplications of CE have been published in several journals such as *Analytical Chemistry*, *Electrophoresis*, and *Journals of Chromatography A and B* [61–66]. In this review, some advanced CE and MEKC techniques for the analyses of biogenic amines that are either of importance or for our own interest will be described in more detail. We particularly focus on the basic concepts and new developed techniques for analysis of biogenic amines in CE with dif-

ferent detection modes, such as LIF, indirect UV-Vis absorption, ILIF, ELCL, and MS. Owing to the popularity and importance of CZE and MEKC for the separation of biogenic amines, recently developed separation and/or stacking (concentration) techniques based on these two separation modes are emphasized. To highlight the potential of CE for life science, the analyses of biogenic amines in a variety of samples such as foods, beverages, and body fluids such as urines, cerebrospinal fluids (CSFs), and single cells are discussed.

2 Detection

2.1 LIF and UV-Vis absorption

CE in conjunction with UV-Vis absorption or fluorescence detection is convenient for the analysis of biogenic amines [21–33]. Having LODs at the μM or sub- μM level for aromatic and heterocyclic amines, CE with UV-Vis absorption is widely used for the analysis of samples such as foods and beverages that contain high concentrations of amines of interest [67, 68]. Because many solutes absorb light in the UV region, CE separation has to provide extremely high resolving power in order to minimize matrix interferences. When compared with UV-Vis absorption detection, laser-induced native fluorescence (LINF) detection provides selectivity and at least ten times greater sensitivity for aromatic and heterocyclic amines [22]. Generally, the CE-LINF electropherograms of biological samples are relatively simple when compared to those of CE with UV-Vis absorption detection, mainly because of fewer analytes having intrinsic fluorescence properties. CE-LINF techniques using UV lasers such as argon ion laser at 275 nm and Nd:YAG laser at 266 nm have been developed for the determination of catecholamines at the nM level [69]. With its high sensitivity and efficiency, CE-LINF has been applied to the analysis of catecholamines in single cells [70]. However, CE-LINF systems using UV lasers are highly selective and limited to most laboratories mainly because of high costs of the UV laser and the requirement of regular maintenance of the optical systems.

For biogenic amines, such as aliphatic amines that have low absorption coefficients and/or quantum yields, derivatization is required when conducting UV-Vis absorption and fluorescence detection in CE [22–24, 31–33, 71]. Derivatization can be applied either prior to, during, or after CE separation, depending on the reaction speed and conditions, as well as whether the labeling agents and side products have detrimental effects on the separation resolution and detection sensitivity [72–74]. It is preferable to choose labeling agents that are pure, have low

optical backgrounds, are stable, and react quickly and selectively with the analytes under mild conditions. In addition, the derivatives should have high absorption coefficients and/or fluorescence quantum yields. Matrix interference is minimized when the derivatives of biogenic amines with the labeling agents have strong absorbance or fluorescence at the wavelengths in the near IR region [75]. Alternatively, ELCL is a good choice for the analysis of tertiary amines, mainly because of its sensitivity and selectivity [39, 76].

In addition to the aforementioned requirements for choosing a labeling agent, the charge/mass ratio and hydrophilicity of the derivatives must be carefully considered. When applying CZE, a labeling agent that forms charged derivatives with amines of interest is preferable. Good candidates are those containing heteroatoms like 6-aminoquinoyl-*N*-hydroxysuccinimidyl-carbamate (ACCQ) [32]. When applying MEKC, great differences in the partition coefficients among the derivatives in micelles are a major concern. In this case, labeling agents such as naphthalene-2,3-dicarboxaldehyde (NDA) and *o*-phthalaldehyde (OPA) are favorable because their derivatives with amines usually have greater partition coefficients than the amines themselves, between the pseudostationary phase and aqueous phase [22, 28, 32]. A wide variety of labeling reagents for amino compounds have been hitherto employed in CE, including ACCQ, fluorescein isothiocyanate (FITC), NDA, OPA, 3-(4-carboxybenzoyl)-2-quinolinecarboxaldehyde (CBQCA), dansyl chloride, 3-(2-furoyl)quinoline-2-carboxaldehyde (FQ), and fluorescamine [14, 77–90]. Most of the labeling agents can be used in LIF and UV-Vis absorption detection, depending on the availability of the CE systems and sensitivity requirement. When choosing a labeling agent, in addition to available light sources, particular attention must be paid to what types of amines are of interest. For example, NDA, OPA, and FQ are only suitable for primary amines; ACCQ, FITC, dansyl chloride, and 5-(4,6-dichloro-*s*-triazin-2-ylamino)fluorescein are good for primary and secondary amino compounds. Representative reactions for different types of amines with fluorophores are presented in Scheme 1, including ACCQ, OPA, NDA, CBQCA, FQ, FITC, dansyl chloride, and 5-(4,6-dichloro-*s*-triazin-2-ylamino)fluorescein. Table 1 briefly summarizes reaction conditions and speed, optical properties, and sensitivity of amines using different labeling agents that are commonly used in CE.

2.2 Indirect LIF and indirect UV-Vis absorption

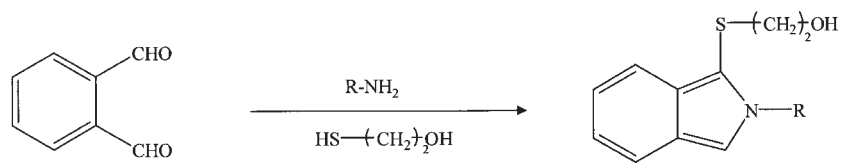
Indirect optical detection methods in conjunction with CE have been successfully demonstrated for the analysis of

amines. To optimize sensitivity, an ionic dye with a great absorption coefficient and/or fluorescent quantum yield and a very stable laser are both required according to Eq. (1):

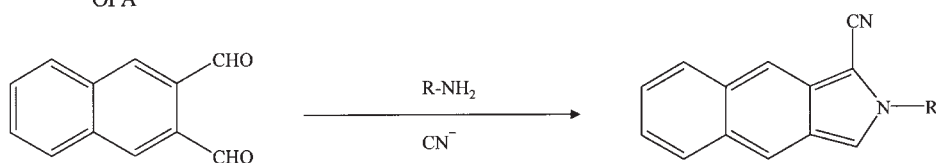
$$C_{\text{LOD}} = C_m / (\text{DR} \times \text{TR}) \quad (1)$$

where C_{LOD} is the concentration LOD; C_m is the concentration of the relevant mobile-phase component; DR is the ability to measure a small change on top of a large signal and is equal to an S/N of the background signal; and TR refers to transfer ratio that is the degree of displacement of the probe (co-ion) by the analyte. According to Eq. (1), successful applications of indirect measurements depend on having an instruments that provides a large DR, a displacement mechanism that allows efficient transfer of the probe by the analytes (*i.e.*, a large TR), and situations where the other mobile-phase component does not respond to the signal [91]. Although the LOD of an analyte is inversely proportional to the probe concentration (C_m), one cannot use concentrations of the probe that are too low because of baseline instability. The prediction of the LOD of a solute according to Eq. (1) may also not be possible if there are complicated sample matrixes.

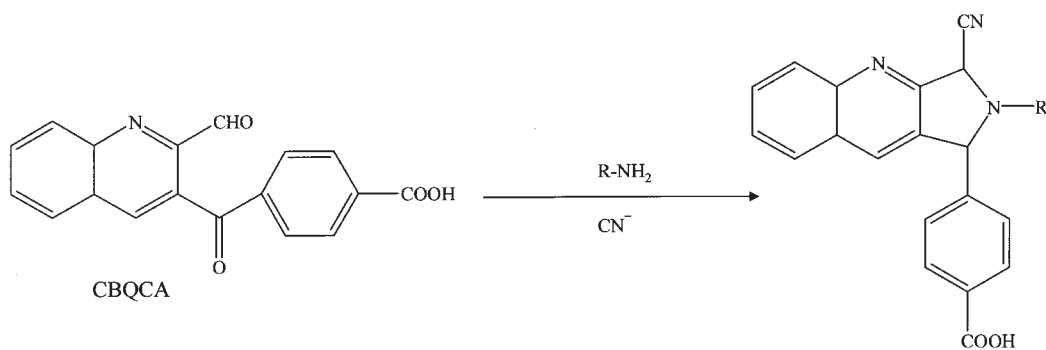
Indirect UV absorbance detection is characterized as a monitoring of the light absorption level of the BGE where the analyte zones cause transient decreases in light absorption as a result of replacing the light absorbing coions in the BGE by nonabsorbing analytes [92]. A series of theoretical and experimental studies in CZE with indirect UV detection have been presented by Boček's group [92–100]. According to their models, it is possible to predict the profiles (positive or negative, fronting, symmetric, or tailing) of the peaks corresponding to the analytes. Their models also allow explanation of the occurrence of system peaks. Quantitative determination of cations such as triethylamine (TEA), K^+ , Li^+ , and Tris ions by indirect UV absorbance detection in CZE without conducting calibration curves has been proposed [92]. The determination is based on the concept of the conversion factor of an analyte that is defined as the ratio of the measured temporal peak area to the product of its migration time and transfer ratio. Thus defined, the conversion factor is of general validity for all analytes separated and detected in a given BGE since it has the same value for the same amounts of various analytes. If a sample is enriched with a known concentration of a standard component, the conversion factor of this standard can be calculated and then the concentrations of all other analytes can be determined, without the use of any calibration graph.



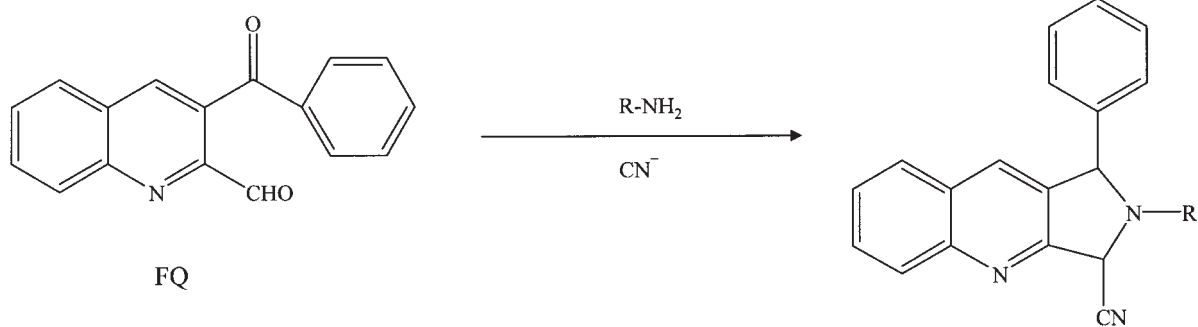
OPA



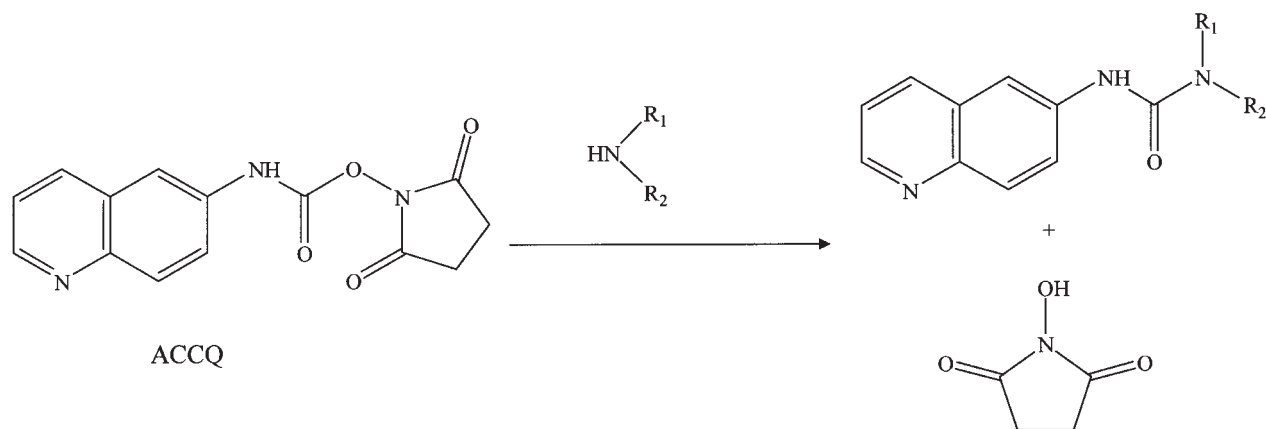
NDA



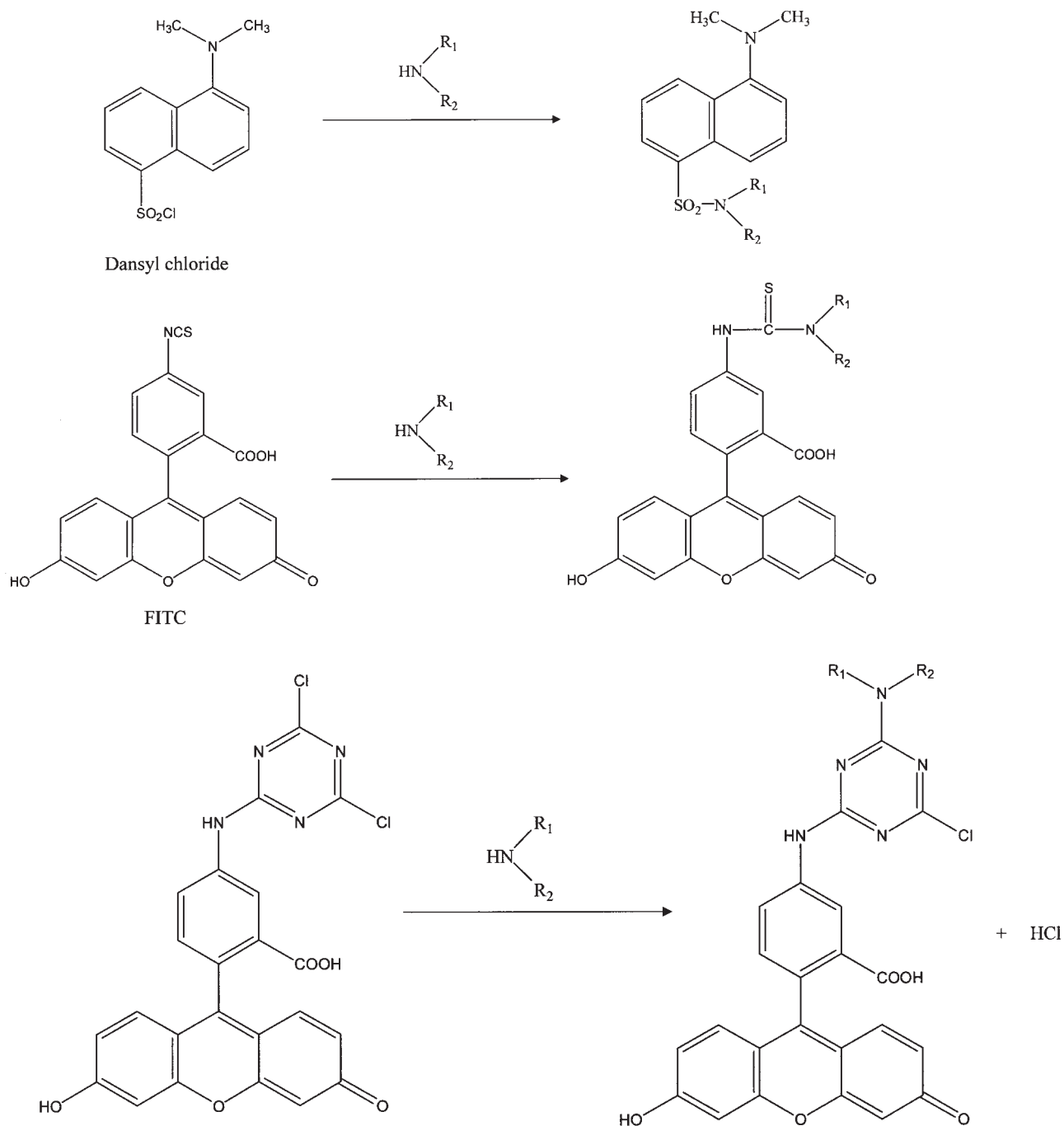
CBQCA



FQ



ACCQ



5-(4,6-dichloro-*s*-triazin-2-ylamino)fluorescein

Scheme 1. Representative derivatization reactions of biogenic amines with different reagents.

CE with indirect UV-Vis absorption detection is useful for the determination of ionic analytes such as amines, metal ions, and organic acids [96–108]. However, we have learned that there are some limitations of indirect detection methods according to Eq. (1). In order to achieve high

sensitivity, the concentrations of charged ions in BGEs relative to that of the ionic chromophores/fluorophores have to be kept as low as possible. In other words, the pH of BGEs can not be too high or too low and the ionic strength of BGEs must be low. When conducting CE with

Table 1. Derivatizing reagents for amino compounds derivatization used in CE (N.G., not given; for abbreviations see title page)

Reagent	CE mode	Reaction conditions	Optical properties	Analytes	LODs	Ref.
4-Fluor-7-nitro-1,2,3-benzaxadiazole	CZE-LIF	5 mM borate buffer at pH 7.2, 65°C for 15 min	Ex: 400–490 nm Em: 515 nm	diaminopropane, diaminoethane, CAD, HIS, PUT, SPD, SPM	21~51 nM	[17]
<i>N</i> -(4-Aminobutyl)- <i>N</i> -ethylisoluminol	MEKC-CL	<i>N</i> -(4-Aminobutyl)- <i>N</i> -ethylisoluminol and <i>M,N</i> -disuccinimidyl carbonate were mixed for 2 h and amines were then added to the above mixture and reacted for 2 h	N.G.	diaminopropane, diaminoethane, CAD, PUT	35~120 nM	[48]
ACCC	MEKC-LIF	Borate buffer, 55°C for 10 min	Ex: 254 nm Em: 395 nm	CAD, HIS, PUT, SPD, SPM, TA, TYR	1~40 μM	[32]
Dansyl chloride	MEKC-LIF	10 mM borate buffer, 70°C for 30 min	Abs: 254 nm	1-Amino-2-methylpropane, 1-amino-3-methylbutane, 2-aminoethanol, 2-aminopropane, <i>n</i> -butylamine, <i>n</i> -pentylamine, <i>n</i> -hexylamine, ethylamine, morpholine, DEA, MEA	N.G.	[77]
DTAF	MEKC-LIF CZE-LIF	0.5 M carbonate buffer at pH 9.5, 35–40°C for 1 h	Ex: 488 nm Em: 520 nm	CAD, PUT, SPM	17~43 μg/L	[78]
FITC	MEKC-LIF	20 mM borate buffer at pH 10.0, room temperature for 5–12 h	Ex: 488 nm Em: 516 nm	1,6-Diaminoethane, 2-phenylethylamine, CAD, HIS, PUT, SPD, SPM, TA, TYR	0.72~35 nM	[79]
FITC	CZE-LIF	20 mM borate buffer at pH 9.2, room temperature for 12 h	Ex: 488 nm Em: 516 nm	5-HT, DA, DOPA, E, NE	0.90~1.36 nM	[80]
FITC	CZE-LIF	0.2 M bicarbonate at pH 8.8, room temperature for overnight	Ex: 488 nm Em: 520 nm	Dimethylamine, dipropylamine, morpholine, piperidine, pyrrolidine, DEA, MEA	0.9~5.0 nM	[81]
FQ	MEKC-LIF	Excess of FQ and KCN, 40°C for 40 min	Ex: 488 nm Em: 560 nm	2-Phenylethylamine, CAD, HIS, PUT, SPD, SPM, TA, TYR	0.5~10 nM	[82]
FQ	CZE-LIF	Excess of FQ and KCN, 65°C for 16 min	Ex: 448 nm Em: 630 nm	5-HT, DA, NE	0.1~80 nM	[83]
NDA	CZE-EC	20 mM phosphate-borate buffer at pH 7.7, room temperature for 20 s	N.G.	Diaminopropane, diaminoethane, CAD, PUT	51~380 nM	[84]
NDA	CZE-LIF	20 mM borate buffer at pH 9.1, room temperature for 15 min	Ex: 400–490 nm Em: 515 nm	Histidine, CAD, HIS, PUT	3.8~5.5 nM	[85]
OPA	MEKC-LIF	20 mM phosphate-borate buffer at pH 10.0	Ex: 340 nm Em: 450 nm	CAD, HIS, SPD, TYR	1.0~1000 μM	[86]
OPA	MEKC-LIF	20 mM phosphate-borate buffer at pH 10.0	Ex: 340 nm Em: 450 nm	CAD, HIS, SPD, TYR	0.2 μM	[87]
OPA	CEC-UV	10 mM borate buffer at pH 10.0, room temperature for 5 min	Abs: 340 nm	5-HT, CAD, HIS, PUT, TYR	50~100 μM	[88]
OPA	MEKC-LIF	0.1 M tetraborate buffer, room temperature for 2–3 min	Abs: 254 nm	3-Methoxypropylamine, benzylamine, butylamine, cyclohexylamine, ethylamine, hexylamine, isopropylamine, pentylamine, propylamine, MEA	0.32~2.2 μM	[22]
OPA	MEKC-LIF	0.1 M tetraborate buffer, room temperature for 2–3 min	Ex: 325 nm	3-Methoxypropylamine, benzylamine, butylamine, cyclohexylamine, ethylamine, hexylamine, isopropylamine, pentylamine, propylamine, MEA	0.089~0.46 μM	[22]

an indirect detection system, sample pretreatment is usually required to suppress the detrimental effect on sensitivity due to high concentrations of salts in the sample matrixes. Because the displacement ratio is suppressed in high-conductivity media, MEKC is usually not performed in CE with indirect detection systems.

Imidazole and other chromophoric amines such as histidine and HIS as background ions are sensitive for indirect detection of aliphatic amines in CE, with the LODs at an S/N of 3 of around 0.1 ppm (corresponding to 10 fmol in 10 nL sample volume) [101]. BGE containing copper(II) as the primary electrolyte constituent was used for the analysis of amines by CE with indirect UV-Vis absorption [102]. Copper(II) is well suited for this application because its electrophoretic mobility is closely matched to the analytes of interest and it has the required spectral characteristics for indirect UV detection. Ma and Zhang [103, 104] demonstrated CZE with indirect UV-Vis absorption detection for the determination of polyamines in tumor cells. After releasing the amines from proteins by treating the sample with TCA, improved sensitivity (sevenfold), greater quantitative reproducibility, and quantization of both bound and free underivatized polyamines in PC-12 tumor cells were achieved. A rapid separation of 21 amines by CZE with indirect UV-Vis absorption using a BGE consisting of 4 mM copper(II) sulfate, 4 mM formic acid, and 4 mM 18-crown-6 at pH 3.0 was demonstrated [106]. Adding 18-crown-6 to the BGE dramatically increased the resolving power as a result of the formation of stable inclusion complexes with the protonated primary amines, while it did not cause significant loss in sensitivity. The method provided LODs of 0.05 $\mu\text{g}/\text{mL}$ for most of the amines.

CE in conjunction with ILIF detection was first reported by Yeung and Kuhr [109, 110]. The concept of indirect fluorescence measurements is essentially the same as that in indirect UV-Vis absorbance measurements, except background fluorescence instead of absorbance is monitored. Gross and Yeung [58] described a CZE-ILIF method for separating seven amines (ammonium, dimethylamine, trimethylamine, propylamine, tetraethylamine, DEA, and Tris). The separation was completed within 6 min using a 0.96-mM quinine sulfate buffer at pH 5.7. Huang *et al.* [51] conducted the analysis of amines under acidic conditions by CZE-ILIF using cresyl violet as a probe. The method showed that the analysis of aliphatic amines at the level of sub- μM by CE-ILIF is realistic.

2.3 ELCL

ELCL involves the production of light near the electrode surface by generation of species that can undergo highly energetic electron transfer reactions, and it has proved to

be sensitive and selective for biogenic amines. In ELCL, chemicals such as Tris(2,2'-bipyridyl)ruthenium(III) ($\text{Ru}(\text{bpy})_3^{2+}$) that possess excellent chemical stability, favorable electrochemical properties, and high emission quantum yields are used for generating ELCL signals through reactions with tertiary amines [45, 46, 111–116]. The analysis of DA and E by their quenching ability of the ELCL signal of the $\text{Ru}(\text{bpy})_3^{2+}$ /tripropylamine (TPA) was demonstrated in CE, with the LOD values of 10 and 30 nM for DA for E, respectively [113]. In that system, $\text{Ru}(\text{bpy})_3^{2+}$ was added in the ELCL cell, while TPA was as an additive of the running buffer. Wang's group [46] developed a new end-column ELCL detector coupled to a CE system for the analysis of amines. A 300 μm in diameter of platinum working electrode was directly coupled with a 75- μm id capillary without an electric field decoupler. Under the optimum conditions, the method provided a linear range for TPA between 10^{-10} and 10^{-5} M with correlation coefficient of 0.998. The practicability of that method was validated in the determination of lidocaine spiked in a urine sample, with an LOD of 20 nM. A similar technique was applied to the determination of four polyamines such as PUT, CAD, spermidine (SPD), and spermine (SPM) [114]. The LODs were 190 nM for PUT and CAD and 7.6 nM for SPD and SPM, respectively. A simple ELCL system that was fabricated on a PDMS chip was used for the analyses of TPA and proline, with their LODs of 5.2 and 13 nM, respectively [115]. Using indium/tin oxide working electrodes in CE-ELCL systems, the LOD for praline was 1.0 μM and its theoretical plate number was 4000 [45]. The detector response is analyte-dependent, *e.g.*, tryptophan (TRP) gives no response and the response for histidine was about 13-fold lower than that of proline. A flow cell that was made from a mixture of Sylgard 184 silicone elastomer, a curing agent, and aluminum oxide was fabricated and used in a CE-ELCL system. Using $\text{Ru}(\text{bpy})_3^{2+}$ prepared in 15 mM sodium borate (pH 8.0), the system allowed the analysis of three trialkyl amines: TEA, TPA, and tributylamine (TBA) [116]. When compared with a poly-(dimethylsiloxane) (PDMS) flow cell, the new flow cell provided about fourfold sensitivity improvement as a result of higher light collection efficiencies.

2.4 MS

MS detection is popular for the analysis of biogenic amines in CE, with the advantages of sensitivity and capability for structure identification [50–55, 117–121]. With the capability for structure identification, comigrated peaks from CE can be further differentiated by their differences in the mass/charge ratios. Thus, the requirement for providing high-resolving power in CE is not as high as that for CE with optical and ELCL detections. However,

MS detection systems are much more expensive than the optical and ELCL detection systems discussed above. Another disadvantage of using MS detection is the requirement of highly efficient interface between the CE and MS detection systems. Among different interfaces used for CE-MS coupling, sheath flow ESI sources are the most popular, whereby the sheath liquid circumflows the end of the capillary, closes the electrical circuit, and provides a constant flow rate [122–125]. It is important to select a suitable supplement fluid because a difference between BGE and supplemental fluid results in a moving ionic boundary that complicates the separation process and causes poor reproducibility. To overcome those shortages, ESI interfaces that do not require supplemental fluid have been developed [126–132]. A device combining a flow injection unit with CE-ESI-MS for the direct automatic determination of biogenic amines (CAD, HIS, tryptamine (TA), ethanolamine, isopropylamine, isoamylamine, 2-pentylamine, phenylethylamine) in wine sample was developed by Santos *et al.* [117]. Using the optimized parameters, nine biogenic amines were analyzed by CE-MS, with the LODs from 0.018 to 0.09 $\mu\text{g/mL}$. The method was validated by the determination of nine biogenic amines in red and white wine samples, with the RSD values of their concentrations ranging from 2.4 to 5.0%. Peterson *et al.* [118] presented a CE-MS method for the analysis of 5-HT and its precursors, tryptophan (TRP), and 5-hydroxytryptophan, in human plasma. To improve the quality of the quantitative results, deuterated analogs of the analytes were employed as internal standard. The LODs in the sub- μM to low- μM range for these analytes were obtained in platelet-rich plasma after enrichment using SPE. Vuorensola *et al.* [119] analyzed catecholamines in urine by CE-MS and the results were compared with LC with electrochemical detection [120]. Although the LC method provided greater sensitivity for standard analytes, CE-MS allowed the detection of the selected analytes even in the urine of healthy patients without conducting sample pretreatment. A similar set of analytes was investigated by using polyvinyl alcohol-coated capillaries. Eight similar analytes in urine samples after SPE clean-up were analyzed by CE-MS, with improved resolution [121]. The recoveries of all analytes, with the exception of epinephrine (75%), were over 80%. The method was applied to quantitate catecholamines (DA, NE, and E) and metanephrine in urine samples, showing that their concentrations are all in the sub- μM level.

3 Stacking

Owing to the extremely small sample volumes injected (nL) and the small size of the capillary (*e.g.*, 50–100 μm diameter), CE provides lower concentration sensitivity than does

HPLC. In order to improve the sensitivity, a number of stacking techniques, including field-amplified sample stacking (FASS), large-volume sample stacking, pH-mediated stacking, ITP, and sweeping have been proposed in CE. Readers that are interested in the principles, advantages, and limitations of these techniques should refer to several excellent reviewed papers [133–141]. Herein, we only focus on three stacking methods in CE for the analysis of amines, which are field amplification [142–144], sweeping [145–147], and use of polymer solutions [69].

The simplest and most common technique used for sample stacking is FASS [136]. It is based on the concept that ions electrophoretically migrating through a low-conductivity solution (usually a diluted buffer or water) into a high-conductivity solution slow down dramatically at the boundary of the two solutions. We point out that the electric field strength is higher over the low-conductivity sample zone than the BGE. Main limitations of FASS are that the ionic strength of the sample zone must be significantly lower than the BGE and the analytes must possess charges. Sensitivity improvements for amines greater than 1000-fold in CE have been reported [143]. Sreedhar *et al.* [116] demonstrated a stacking approach (FASS and pH junction) for determining TEA, tripropylamine (TPA), and tributylamine (TBA) by applying a CE-ELCL approach. Once amines (cations) prepared in citric acid solution ($\text{pH} < 4.0$) migrate towards the BGE (15 mM sodium borate at $\text{pH} 8.0$), they slow down and are stacked at the boundary as a result of deprotonation and decreases in the electric field. This method provided concentration LODs of 24, 20, and 32 nM for TEA, TPA, and tributylamine (TBA), respectively. A stacking approach based on the changes in pH, viscosity, and electric field is effective for the determination of amines in CE [144]. Representative conditions for simultaneous stacking and separation of amines and acids are: samples are prepared in a solution ($\text{pH} 3.1$) consisting of 10 mM citric acid, 89% ACN, and water; a capillary is filled with 1.5 M Tris-borate (TB) buffer ($\text{pH} 10.0$); and the anodic vial contains a BGE consisting of 50 mM propionic acid, Tris, 10% glycerol, and water ($\text{pH} 9.0$). The system allowed the analysis of large-volume samples that were injected at 15 kV for 360 s, and the LODs for 5-HT and 5-hydroxyindole-3-acetic acid were 0.27 and 0.31 nM, respectively, representing about 400- and 800-fold sensitivity improvements when compared to those injected at 1 kV for 10 s.

Sweeping is a technique particularly for on-column sample concentration of neutral analytes based on the partition coefficient of the analytes between the pseudostationary phase and aqueous phase in MEKC [148, 149]. After filling the capillary with a micellar BGE, a large plug of sample zone is injected. When the voltage is applied, micelles

migrate in the capillary and sweep the analytes. As a result, narrow analyte bands are formed, leading to improved sensitivity. Sweeping MEKC using a BGE consisting of 75 mM SDS, 10 mM triethanolamine, 20% methanol, and 30 mM phosphoric acid (pH 2.4) allowed up to ~500- and 400-fold increases of peak heights of 1-naphthylamine and 1-phenylethylamine, respectively [145]. When simultaneously conducting FASS and sweeping, the sensitivity improvements for amines and cationic analytes were very profound [146]. The analytes were first focused by FASS and then the focused sample zones were subjected to a second focusing based on sweeping. The LODs were 4.1 and 8.0 ppt for laudanosine and naphthylamine, respectively, with the improvements approaching a million-fold when compared with those by applying a usual sample injection. A similar method was conducted for determining environmentally relevant aromatic amines (*N*-1-naphthylethylenediamine, 3,4-dichloroaniline, 3,5-dimethylaniline, 2,4-dichloroaniline, 2,3-dichloroaniline, 2,5-dichloroaniline, 3-chloroaniline, 1-phenylethylamine, *N*-ethylaniline, 2-methylaniline, 4-methoxyaniline, 2-nitroaniline, and 4-nitroaniline) [147]. The LODs and sensitivity improvements for the analytes were at the ppt levels and were 10^4 - to 10^5 -fold, respectively.

CE using polymer solutions is an alternative approach for improved sensitivity of amines, with the advantages of high stacking efficiency and simplicity [69]. Prior to sample injection, a capillary is filled with a high concentration (>400 mM) of TB buffer (pH 10.0) to generate a high and reproducible EOF. After injecting a large-volume sample from the anodic end, neutral polymers such as poly(ethylene oxide) (PEO) enter the capillary *via* EOF from the anodic end. Owing to increases in the viscosity and decreases in electric field, the anionic analytes slow down and are stacked at the boundary between the sample zone and PEO solution. This approach allows stacking of large and small solutes that are in either high- or low-conductivity media, but it is only good for anions. Hsieh *et al.* [69] used this stacking technique for the analysis of small solutes using a 2.0% PEO solution prepared in 200 mM TB buffer (pH 9.0). The LOD value for L-TRP was 70 pM when conducting CE-LIF for large-volume sample analysis. The practicality of the method was validated by the analysis of urine samples (2.80 μ L). The electropherogram depicted in Fig. 1 clearly exhibits the peak-height enhancements for the metabolites of biogenic amines, such as 5-hydroxyindole-3-acetic acid, homovanillic acid, and DL-vanillic mandelic acid.

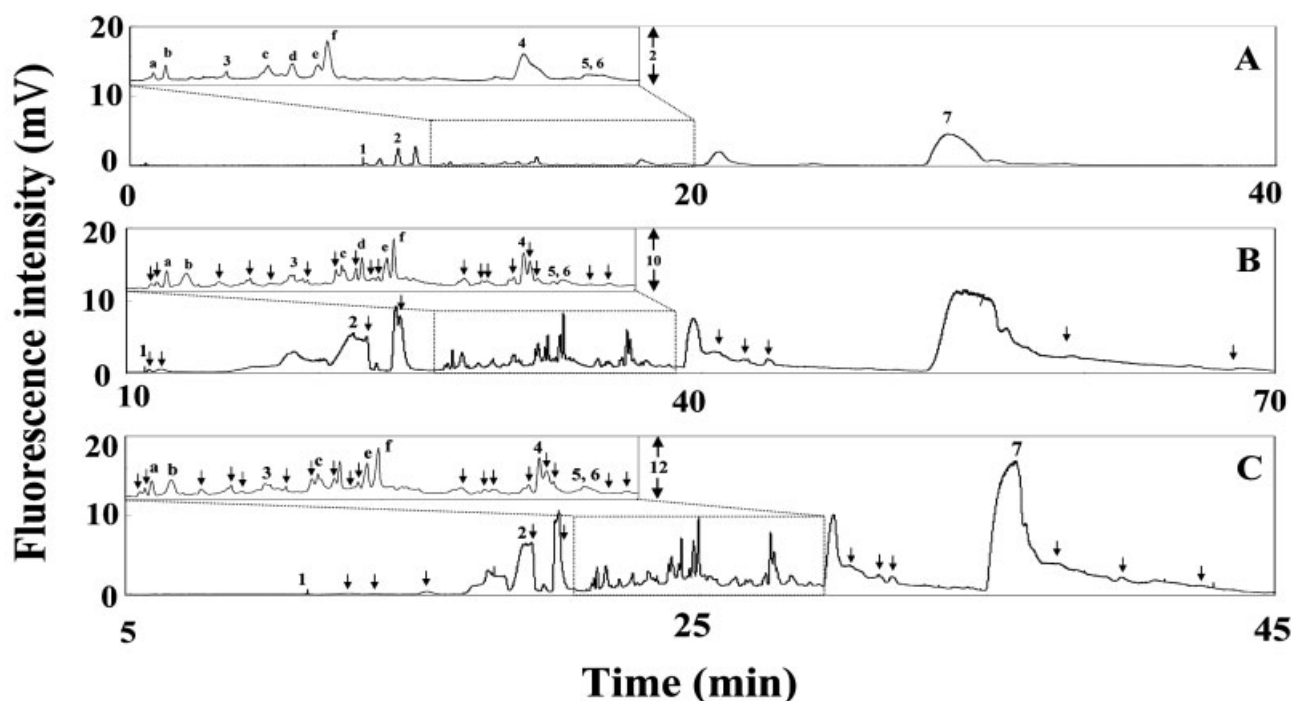


Figure 1. Analysis of a urine sample in the presence of EOF at 15 kV using 1.5% PEO prepared in 200 mM TB (pH 9.0). Peak identities: (1) 5-HT, (2) TRP, (3) human serum albumin, (4) 5-hydroxyindole-3-acetic acid, (5) homovanillic acid, (6) DL-vanillic mandelic acid, (7) 3-indoxyl sulfate, and (a)–(f) represent unidentified peaks. A 60-cm capillary was filled with 1.5 M TB, pH 10.0. The injections of a urine sample were conducted at 30 cm height for 10 s in (A), and 180 s in (B) and (C). A short plug of Tris-propanate buffer was injected at 30 cm height for 10 s after sample injection in (C). Reprinted from [69], with permission.

4 Applications

The analysis of biogenic amines is of interest and importance as addressed in Section 1. A number of CE-based techniques have been developed for the determinations of biogenic amines from extremely complicated biological samples such as foods, beverages, and body fluids [150–160]. With their sensitivity and capability of handling extremely small sample volumes, several CE-LIF techniques have been shown to be powerful for the analysis of single cells, CSF, urine, retinas, and brain tissues [161–169]. In this section, we focus on the applications of CZE- and MEKC-based techniques for the determinations of biogenic amines in biological samples, including food, beverages, CSF, urine, as well as single cells.

Studies focusing on biogenic amines in foods and beverages usually involve two aspects: toxicology and/or their quality [67]. The samples were usually blended with water that was adjusted to pH 5.2 by addition of HCl and heated at high temperature such as 70°C. After the sample was cooled down, the precipitate was removed. A CZE method was employed for simultaneous determination of ammonia, dimethylamine, trimethylamine, and trimethylamine-*N*-oxide in foods, and the result is depicted in Fig. 2 [170]. With the advantages of simplicity and sensitivity (the LODs for the analytes were lower than 0.04 mM, corresponding to 2 mg nitrogen *per* 100 g fish), the method has a great potential to replace the laborious and time-consuming Conway micro-diffusion method for determining amines in meats. The determinations of HIS and tyramine (TYR) in foods (fish, cheese, meat products, vegetarian products, tomatoes, and olives) are also quite straightforward when performing CZE. After conducting derivatization of amines with OPA, the OPA-amine derivatives were analyzed by CZE-LIF within 9 min, with LODs of 0.5 mg/kg and 6 mg/kg for HIS and TYR, respectively [171]. Simultaneous determination of multiple constituents (alcohols, amines, amino acids, and flavonoids) in beer samples by CZE was demonstrated by Cortacero-Ramírez *et al.* [68]. By using 70 mM borate solution (pH 10.25) as the BGE, at least 18 compounds were identified. A flow-injection manifold was used in CZE for automatic determination of biogenic amines in wine from different Spanish regions within 15 min [172]. Prior to CE analysis, the wine samples were subjected to clean-up and concentration of the target analytes by SPE using a weak cation exchanger. Because the sample eluates from SPE contained large amounts of acids, a big unknown peak and relatively unstable baseline were problematic for detection. By using a BGE consisting of 4 mM copper(II) sulfate, 4 mM formic acid and 4 mM 18-crown-6 (pH 3.0), the LODs for TYR and MEA were 0.05 and 0.1 µg/mL, respectively. Seven amines (TA, HIS, TYR,

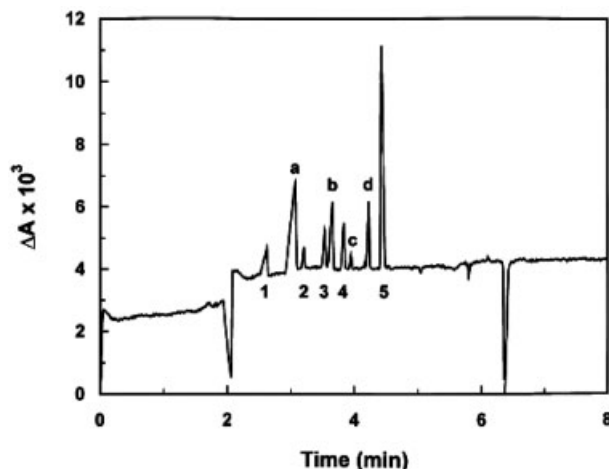


Figure 2. Electropherogram of a fish extract with amines added. A sample was prepared by mixing 50 µL cod fillet extract with 450 µL amine solution containing 0.5 mM ammonium chloride, 0.5 mM dimethylammonium chloride, 0.5 mM trimethylamine-*N*-oxide, and 0.24 mM methylammonium chloride. Peak identities: (1) ammonium, (2) methylammonium chloride, (3) dimethylammonium chloride, (4) trimethylamine, (5) trimethylamine-*N*-oxide, (a) K⁺, (b) Na⁺, (c) Ca²⁺, (d) Mg²⁺. Reprinted from [170], with permission.

SPM, SPD, CAD, and PUT) were found in food samples (wine, salami, and chive) by MEKC when using 100 mM boric acid buffer (pH 8.9) containing 50 mM SDS and 10% ACN as the BGE [31]. After treatment of the samples with 6% perchloric acid and enrichment using an ion-exchange resin, the analytes were derivatized with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate. Although the fluorophore used is selective to amines, there were still many unidentified peaks detected in the wine samples, somehow causing trouble in identification and quantification. Two CE methods (CZE-EC and MEKC-LIF) were compared for the determination of aromatic amines in water samples [173]. By using a buffer (pH 9.0) consisting of 5 mM sodium tetraborate, 4.5 mM boric acid, and 20 mM SDS, the separation of six aromatic amines (1,3-phenylenediamine, 2-methoxyaniline, 4-ethoxyaniline, 4,4'-diaminobiphenyl, 2-methylaniline, and 2,4-dimethylaniline) by MEKC was completed within 4 min. When compared with CZE, MEKC provided the advantages of rapidity (5 min), high separation efficiency (200 000 plate numbers), and repeatability (1%).

Catecholamines and polyamines that are found in central nervous system, tissue cells, and body fluids such as serum, urines, and CSF are considered to play important roles in biological function [174]. Screening of catecholamines and polyamines in these biological samples is usually conducted for diagnosis of heart diseases, var-

ious mental diseases, stress, and cancers [174]. By using 25 mM tetraborate buffer (pH 10.82) containing 65 mM SDS, MEKC with UV-Vis absorption detection was used to determine catecholamines and its metabolites, with the LOD and the LOQ values at around 0.05 and 0.1 $\mu\text{g}/\text{mL}$ for most of the analytes, except for DA. Prior to MEKC analysis, the urine samples were subjected to clean-up and concentration by SPE. One representative electropherogram of the patient's urine is depicted in Fig. 3. The study suggested that the concentration ranges of catecholamine metabolites (5-hydroxyindole-3-acetic acid, DOPA, DL-vanillic mandelic acid, and homovanillic acid) in patients' urine samples were from 0.186 to 76.4 $\mu\text{g}/\text{mL}$. MEKC-LINF with a KrF excimer laser is an alternative for exploring the electrophoretic profiles of human samples such as urine, saliva, and serum [175]. When using a UV-laser, sample derivatization is not required for the determination of catecholamines. By using a buffer (pH 9.3) consisting of 6 mM sodium tetraborate, 10 mM sodium phosphate, and 75 mM SDS, six analytes (TRP, uric acid,

5-hydroxyindole-3-acetic acid, 3-indoxyl sulfate, homovanillic acid, and dl-vanillic mandelic acid) were identified in human urine samples by MEKC-laser-induced native fluorescence (LINF). A simple, efficient, and rapid method for simultaneous analysis of ten biological amines and acids by CZE-LINF under discontinuous conditions was demonstrated [176]. The analysis was completed within 12 min when using a capillary treated with 0.5 M NaOH and then immediately filled with 10.0 mM formate-Tris solutions (pH 4.0) containing 10.0 mM KCl. Treating the capillary with 0.5 M NaOH after each run ensured generation of high bulk EOF and reproducibility ($\text{RSD} < 3.1\%$) for the analysis of urine samples without conducting sample pretreatment. Silica nanoparticles that were prepared in formic acid solutions (pH < 4.0) were considered as an EOF modifier for simultaneous analysis of amines and acids by CE-LINF at pH 3.0 [177]. Adsorption of silica nanoparticles on the capillary wall as well as the interactions with analytes reduced analytes adsorption, leading to highly efficient and reproducible separation results.

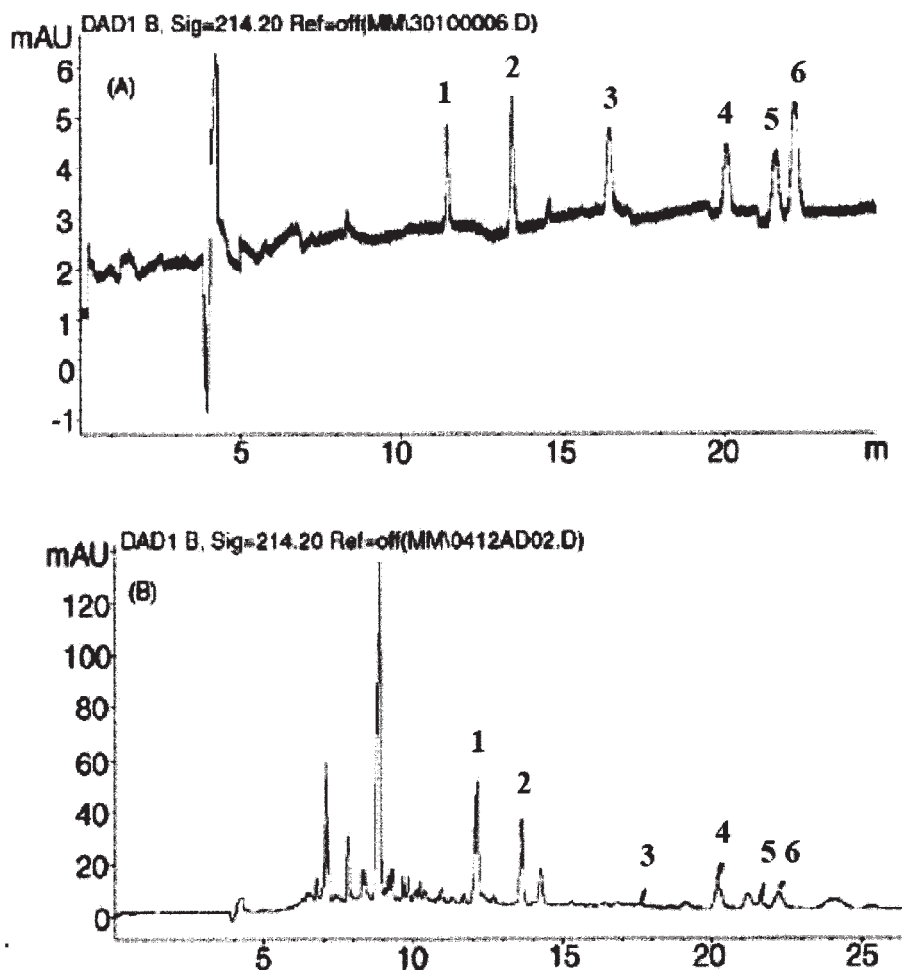


Figure 3. Electropherogram of anionic catecholamine metabolites: (A) standard mixture and (B) patient urine sample after SPE cleaning. Peak identities: (A) (1) 5-hydroxyindole-3-acetic acid, (2) trichloromethiazide, (3) DOPA, (4) homovanillic acid, (5) vanillic mandelic acid, (6) isovanillic mandelic acid; Peak identities: (B) (1) 5-hydroxyindole-3-acetic acid, (2) DOPA, (3) homovanillic acid. Electrolyte solution: 25 mM sodium tetraborate-65 mM SDS (pH 10.82), current, 98 μA , voltage, +18 kV, injection with 0.5 psi pressure for 3 min, detection at 214 nm (UV-Vis absorption), capillary, 40/48 cm ($L_{\text{det}}/L_{\text{tot}}$). Reprinted from [174], with permission.

The LODs for TA and 5-HT were 0.09 and 0.15 nM, respectively. Although CE-LINF is selective to heteroaromatic amines, there are many peaks present in the electropherograms of the urine samples. The unidentified peaks are probably due to proteins (e.g., albumin) and peptides containing TRP, tyrosine, and phenylalanine residues, nucleotides, oligonucleotides, benzoic compounds, polycyclic aromatic hydrocarbon, heterocyclic compounds, and so on. In order to minimize the salt effect (fluorescence quenching), dilution of urine samples with BGEs is highly suggested when conducting CE-LINF. CE-LIF is efficient for the analysis of CSF samples from leukemic children treated with high doses of methotrexate [178]. The electropherogram depicted in Fig. 4 shows that more than 50 peaks of FITC-derived amino acids and primary amines were found in a 50- μ L CSF sample in less than 22 min without sample purification prior to CE analysis. Twenty-one peaks were identified from these peaks, and quantitative determinations of 19 analytes were carried out. The study observed differences in individual amino acid levels in the CSF samples from healthy people and patients. We note that when conducting derivatiza-

tion, particular care must be taken so as to reach high derivatization efficiency and minimize undesired side products.

The determination of biogenic amines in cells by CE is usually conducted after sample pretreatment. The cell medium was removed by centrifugation, a high concentration of TCA (e.g., 15%) was used to release protein and DNA-bound biogenic amines, and the amines were then enriched by liquid extraction with organic solvent such as diethyl ether and SPE. After being derivatized with FITC, polyamines (PUT, SPD, SPM) and 5-HT, DA, E, NE were separated by CE-LIF. When conducting derivatization, pH, percentage of organic solvent, temperature, buffer composition, buffer concentration, and reaction time must be carefully evaluated because they affect the efficiency of the derivatization reaction. Under optimized conditions, the linearity of the analytes ranged from 0.05 to 1.0 μ M and their LODs (S/N = 3) ranged from 0.03 to 2.50 nM. The method allowed simultaneous determination of SPM, SPD, PUT, 5-HT, E, NE, and DA in PC-12 tumor cell extracts within 18 min [179]. One representative

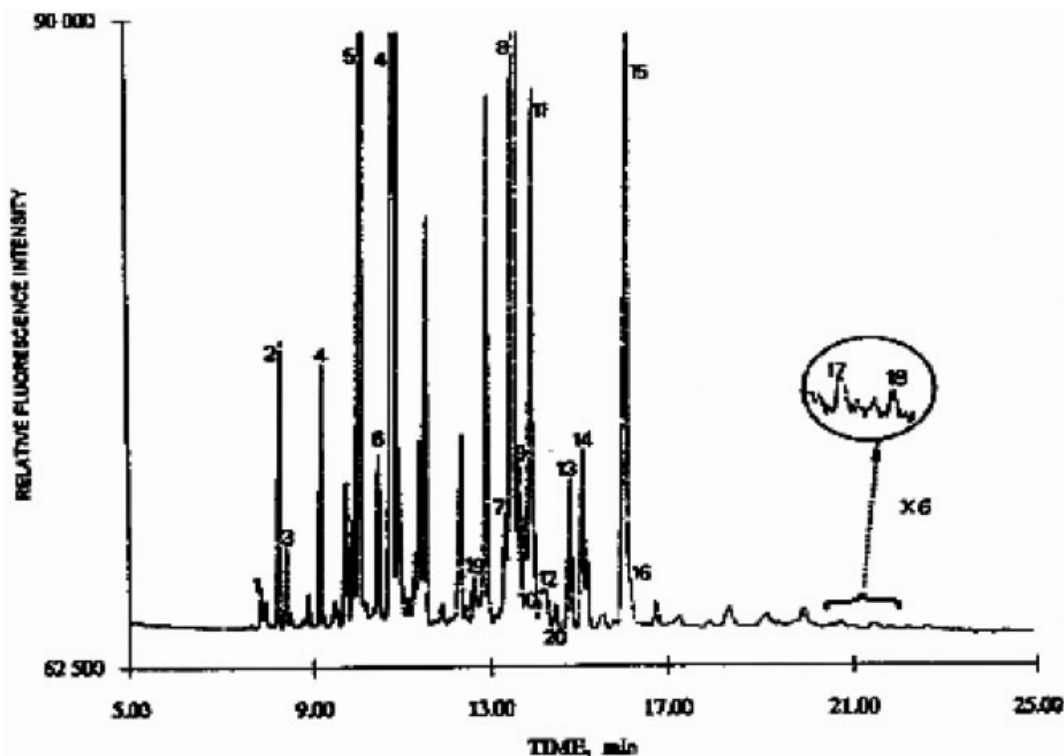


Figure 4. Separation of fluorescein thiocarbamyl-amino acids and -amines from the acute lymphoblastic leukemic patient J. Peak identities: (1) lysine, (2) arginine, (3) ornithine, (4) blank, (5) ammonia, (6) TYR, (7) leucine, (8) glutamine, (9) tyrosine, (10) valine, (11) threonine, (12) phenylalanine, (13) serine, (14) alanine, (15) taurine, (16) glycine, (17) glutamic acid, (18) aspartic acid, (19) citrulline, (20) asparagine. Conditions: buffer, 100 mM boric acid (pH 9.3), 100 mM SDS. Analysis: +20 kV, 42 μ A, hydrodynamic injection 2 s. Reprinted from [178], with permission.

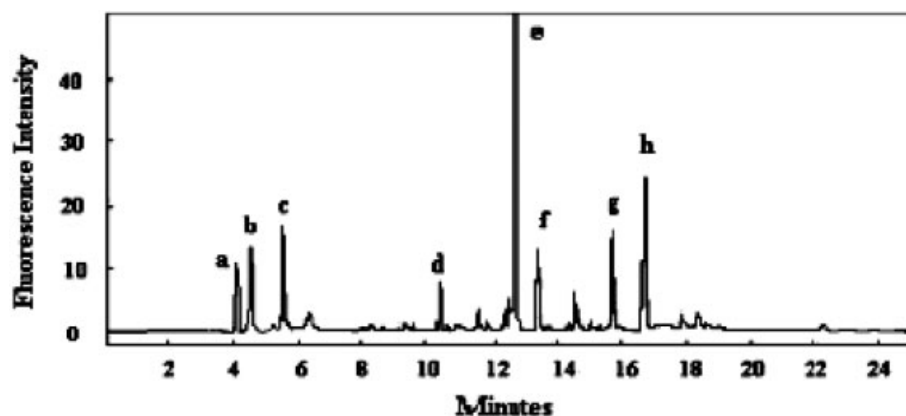


Figure 5. Electropherogram of FITC-polyamines and FITC-catecholamines in PC-12 tumor cell extracts. Peak identities: (a) SPM, (b) SPD, (c) PUT, (d) 5-HT, (e) FITC, (f) E, (g) NE, (h) DA. A 50- μ m-id \times 40-cm fused-silica capillary was used for separation; separation voltage, 25 kV, pressure injection, 0.5 psi/20 s, separation buffer, 40 mM sodium tetraborate and 60 mM boric acid, pH 9.0. Reprinted from [179], with permission.

electropherogram is exhibited in Fig. 5. Boric acid buffer (100 mM, pH 9.5) containing 10 mM SDS and 10 mM 18-crown-6 is a useful BGE for the determination of catecholamines (5-HT, DA, E, NE) and polyamines (PUT, SPD, SPM) in PC-12 cell extracts by MEKC with UV-Vis absorption detection [180]. In the presence of SDS and 18-crown-6, the analytes were well resolved within 20 min.

5 Conclusions

This review has focused on recent progress in the analyses of biogenic amines by CZE and MEKC, which offer the advantage of speed, efficiency, and reproducibility. The incorporation of numerous detection methods with on-line concentration has enabled CE to be quite versatile and amenable to the sensitive analysis of a variety of trace amines. With these advantages, CE is powerful for the determination of biogenic amines in biological samples, including foods, beverages, CSF, urine, and single cells. In order to have a wider coverage of the analytes, techniques that provide great efficiency, sensitivity, and peak capacity are highly demanded. It is thus our strong belief that an advanced CE-based multidimensional technique such as CZE-MEKC will become one of the most important techniques for the analysis of solutes including amines, amino acids, organic solutes, and inorganic solutes. The identification of numerous small solutes of interest in cells, tissues, and organs is essential for metabolomics. To achieve this goal, CE-based multidimensional techniques in conjunction with multiple detection systems such as LIF and MS are essential.

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