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Review

Recent advances in enrichment techniques for trace analysis in capillary electrophoresis

CE is gaining great popularity as a well-established separation technique for many fields such as pharmaceutical research, clinical application, environmental monitoring, and food analysis, owing to its high resolving power, rapidity, and small amount of samples and reagents required. However, the sensitivity in CE analysis is still considered as being inferior to that in HPLC analysis. Diverse enrichment methods and techniques have been increasingly developed for overcoming this issue. In this review, we summarize the recent advances in enrichment techniques containing off-line preconcentration (sample preparation) and on-line concentration (sample stacking) to enhancing sensitivity in CE for trace analysis over the last 5 years. Some relatively new cleanup and preconcentration methods involving the use of dispersive liquid–liquid microextraction, supercritical fluid extraction, matrix solid-phase dispersion, etc., and the continued use and improvement of conventional SPE, have been comprehensively reviewed and proved effective preconcentration alternatives for liquid, semisolid, and solid samples. As for CE on-line stacking, we give an overview of field amplication, sweeping, pH regulation, and transient isotachophoresis, and the coupling of multiple modes. Moreover, some limitations and comparisons related to such methods/techniques are also discussed. Finally, the combined use of various enrichment techniques and some significant attempts are proposed to further promote analytical merits in CE.

Keywords:
Capillary electrophoresis / Microextraction / On-line stacking / Sample preparation / Trace analysis
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Abbreviations: **BPA**, bisphenol A; **CPE**, cloud point extraction; **DLLME**, dispersive liquid–liquid microextraction; **EF**, enrichment factor; **FA**, field amplication; **FESI**, field-enhanced sample injection; **HBP**, 2,4-dihydroxy-benzophenone; **HCB**, high-conductivity buffer; **HF**, hollow fiber; **HLB**, hydrophilic–lipophilic balance; **HMBP**, 2-hydroxy-4-methoxy-benzophenone; **LD**, liquid desorption; **LE**, leading electrolyte; **LLE**, liquid–liquid extraction; **LLME**, liquid-liquid microextraction; **LLLME**, liquid-liquid-liquid microextraction; **LPME**, liquid-phase microextraction; **LVSS**, large-volume sample stacking; **MAE**, microwave-assisted extraction; **MCX**, mixed-mode/cationic-exchange; **MIP**, molecularly imprinted polymer; **MSPD**, matrix solid-phase dispersion; **MSS**, micelle to solvent stacking; **MWCNT**, multi-walled carbon nanotube; **NSM**, normal stacking mode; **PAEKI**, pressure-assisted electrokinetic injection; **PCA**, phenazine-1-carboxylic acid; **PLE**, pressurized liquid extraction; **SAs**, sulfonamides; **SAE**, sonication-assisted extraction; **SBSE**, stir bar sorptive extraction; **SDME**, single-drop microextraction; **SFE**, supercritical fluid extraction; **SPME**, solid-phase microextraction; **TE**, terminating electrolyte; **tITP**, transient ITP; **W/O**, water-in-oil

1 Introduction

Trace and ultra-trace analysis has received wide concerns with increasing significance and popularity. The determination of trace targets in different matrix samples is becoming more and more common. According to the definition of the term "trace component" proposed by the International Union of Pure and Applied Chemistry, the limit for trace analysis means the concentration of 100 ppm (100 μg/g) [1]. Table 1 presents a classification of analytical methods and techniques by analyte concentration in the tested samples. Interestingly, the current determination of trace components, evencmd in samples with complex matrices, does not pose major problems and is usually carried out routinely in many laboratories. This is mainly attributed to various effective pretreatment and enrichment methods/techniques as well as rapidly developed instrumental technologies such chromatographic separation techniques as HPLC and CE.

CE is currently a very important technique for the separation of analytes and is a well-established alternative to HPLC since it is characterized by some important features such as different selectivity from HPLC, high separation

Colour Online: See the article online to view Figs. 1, 5, 6, 8–12 in colour.

Table 1. Classification of analytical methods and techniques by analyte concentration in a sample

	Classification	Analyte concentration
Trace analysis	Submicrotrace	$<10^{-12}$ μg/g ($<10^{-10}$%)
	Ultra-microtrace	$<10^{-9}$ μg/g ($<10^{-7}$%)
	Microtrace	$<10^{-6}$ μg/g ($<10^{-4}$%)
	Trace	$<10^{-4}$ μg/g (<0.01%)
Semi-microanalysis	Secondary	<1%
Macroanalysis	Primary	1–100%

efficiency, small sample size, low solvent consumption, and rapid analysis [2, 3]. However, the determination of trace analytes in samples by CE-based analytical techniques usually requires their prior extraction from the matrix and preconcentration [3, 4]. Sample pretreatment and preconcentration is a crucial part of chemical analysis and in a sense has become the bottleneck of the whole analytical process [4]. Although some traditional extraction methods continue to be the most widely used concentration techniques, such as SPE and liquid–liquid extraction (LLE) which often contain a large number of matrix components that may co-elute with the analytes and disturb the quantitative analysis, there is a growing search for time and labor saving sample pretreatment methods which aim at the reduction of the matrix content and the enrichment of the target analytes. Also, they are expected more eco-friendly capable of using smaller amounts of solvents and sample as well as ideally involving as few operations as possible in order to minimize potential errors and shorten analysis times. Thus, some cleanup/concentration methodologies such as solid-phase microextraction (SPME), stir bar sorptive extraction (SBSE), matrix solid-phase dispersion (MSPD), hollow fiber (HF) extraction, supercritical fluid extraction (SFE), pressurized liquid extraction (PLE), cloud point extraction (CPE), and dispersive liquid–liquid microextraction (DLLME) have demonstrated effective for preconcentration purposes and thereby allow significant improvement of the detection sensitivity in CE.

Recently, some excellent reviews on this topic have appeared in special journal issues [3, 5–7] based on the importance of sample preparation for CE. Almeda et al. [3] reviewed available preconcentration techniques such as SPE (including off-line, at-line, on-line, and in-line), LLE with a membrane, electrophoretic preconcentration, SPME, SBSE, CPE, etc. for use in CE analysis (mainly with UV-Vis detection). Hernández-Borges et al. [5] summarized the different strategies that have been developed for sample preparation prior to CE-MS. Breadmore et al. [6] reviewed in detail insight into the field of on-line concentration, in which different strategies were mainly summarized that have been developed for sample preparation prior to CE and supplements the information contained in previously published reviews about this emerging field of analysis. Frost et al. [7] reviewed some of the off-line sample preconcentration procedure and on-line enrichment methods for various analytes. All the review works mainly summarize some specific, single technologies such as SPE and CE-MS, and some of them do not include new techniques such as DLLME and so on.

Meanwhile, the methodologies of the on-column concentration techniques have also been specially reviewed in some publications [8–11]. Lin and Kaneta [8] summarized velocity gradient-based on-line concentration techniques in CE for biomolecules, in which the electrophoretic velocities of the analyte molecules are manipulated by field amplification, sweeping, and isotachophoretic migration. Mala et al. [9] reviewed sample stacking techniques in CZE, including concentration adjustment, application of a pH step, MEKC and sweeping, and transient ITP (tITP). Aranas et al. [10] gave a most comprehensive summary on sweeping in CE, especially about the mechanism study, and such relatively new modes of sweeping as transient trapping (tr-trapping) and analyte focusing by micelle collapse are also discussed, as wells as the combination of sweeping with other on-line preconcentration techniques are described. Mala et al. [11] provided a thorough survey of related work on sample stacking in CE, focusing on Kohlrausch adjustment of concentrations, pH step, micellar systems, and combined techniques. All the review works pay more attentions on some specific techniques, mode, or analytes such as sweeping, CZE, and biomolecules, and a wider and more fundamental covering of various on-column concentration techniques is not given, as well as some latest techniques are not included.

In this review, we focus on the current enrichment techniques involving off-line preconcentration (sample preparation) and on-line concentration (sample stacking) to increase analyte amounts into capillary and therefore enhance sensitivity in CE analysis, presenting a wide and fundamental covering of enrichment techniques and methods developed and applied since 2008. Some new techniques related to the sample preparation such as DLLME, SBSE, CPE, etc., and related to the sample stacking such as extremely large volume electrokinetic stacking, featuring sweeping, micelle to solvent stacking (MSS), etc., as well as some combined enrichment techniques, are specially summarized. Finally, we also make some attempts to explore the future development trends of the enrichment techniques for elevating CE-based detectability.

2 Sample preparation

Typically, sample preparation includes such steps as homogenization or sampling, extraction, preconcentration (if needed), cleanup, and final concentration prior to analysis. Figure 1 shows the most common analytical procedures for liquid, semisolid, and solid samples by CE. Sample preparation is generally purposed to the following objectives: (i) use of smaller sample sizes; (ii) reduction or elimination of organic solvents; (iii) generic extraction procedures for multiclass compounds; (iv) integration of several preparation steps into one (e.g., application of passive samplers for simultaneous sampling, extraction, and enrichment of pollutants from liquid, semisolid, and solid samples); (v) potential for

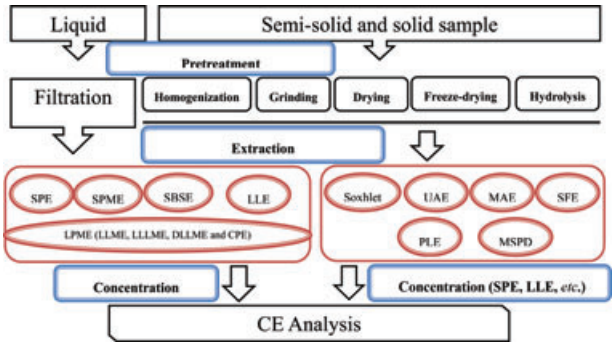

Figure 1. Sample preparation procedures prior to CE.

automation and/or high-throughput determination. The choice of extraction methods largely depends on the matrix, that is, different methods are required for liquid, solid, or semisolid samples.

2.1 Liquid sample

Liquid samples (e.g., water samples) are generally easier to handle than semisolid and solid samples (e.g., sediment and soil samples). For liquid samples preparation, conventional extraction techniques (e.g., SPE and LLE) have been in service, and meanwhile, various recent extraction techniques including SPME, SBSE, and liquid-phase microextraction (LPME) are gaining popularity.

2.1.1 SPE

Sample preparation using SPE was first introduced in the mid-1970s. SPE is a widely used technique in analytical sciences to carry out the preconcentration of analytes and the cleanup of samples. As a classic sample preparation method, SPE is still being used and developed including off-line and at/on/in-line SPE. An overview of SPE-CE system has been given in several reviews [12–16]. The present review is focused on application-driven studies in special sorbents of the SPE coupled with CE.

2.1.1.1 Off-line SPE

Concerning the use of off-line SPE, it is probably the most widely used sample pretreatment procedure prior to CE. So far, different sorbents, such as C_8 [17], C_{18} [18–21], hydrophilic–lipophilic balance (HLB) [22–27], mixed-mode/cationic-exchange (MCX) [28, 29], mixed-mode/anion-exchange [30], weak anion exchange [31], multiwalled carbon nanotubes (MWCNTs) [32, 33], and molecularly imprinted polymers (MIPs) [34–37] are commonly used for SPE.

In [17], C_1, C_2, C_8, cyanopropyl, HLB, and MCX were tested as sorbents, and cyanopropyl was selected as the optimum SPE sorbent for the determination of antipsychotic aripiprazole in human plasma finally, prior to CE. In Ref. [26], an Oasis HLB 96-well format SPE for quantification of ulifloxacin in plasma sample was developed, followed by CZE. The combination of CE with 96-well format SPE greatly simplified the preparation process and decreased the time required for the biological sample preparation. Rodríguez-Gonzalo et al. [27] used restricted-access materials to limit the access of macromolecules in the honey sample before SPE. Bunz et al. [31] compared the extraction efficiencies of four sorbents including Strata X-AW, Oasis weak anion exchange, mixed-mode/anion-exchange, and BondElute NH_2 for the determination of sulfates, sulfonates, and phosphates in urine sample prior to CZE-MS. Strata X-AW was finally selected as the best sorbent of SPE. Performance comparisons of different sorbents have been performed, such as C_{18}, Strata X-AW, HLB, and MCX, etc. [38–41]. Wu et al. [42] used a microcolumn packed with coal cinders as solid-phase extractant of *para*-, *meta*-, and *ortho*-phenylenediamines isomers in hair dyes, and results indicated the coal cinders had great prospects in SPE.

Springer et al. [32] developed an SPE procedure using MWCNTs as sorbent in combination with CZE determination of metsulfuron-methyl and chlorsulfuron in different water samples. In the research, a flow injection system was employed and allowed using small amount of MWCNTs packed in a minicolumn to carry out the preconcentration step. More applications of CNT in SPE-CE have been comprehensively reviewed in [33].

Recently, MIPs and magnetic nanoparticles (e.g., Fe_3O_4) have aroused wide concerns. They can be used as the selective sorbents after modified. The MIPs-SPE and magnetic nanoparticles based SPE have been demonstrated to be highly selective preconcentration procedures for sensitive determination. Mei et al. [34] prepared bisphenol A (BPA)-MIPs via precipitation polymerization as the sorbent of SPE. Then, the BPA and its analogues were determined by CE-UV. Under the optimal protocol, molecularly imprinted SPE presented higher selectivity and recovery for BPA than that of conventional C_{18}-SPE. And commercial molecularly imprinted SPE column have already been used popularly [35, 36]. A magnetic SPE method coupled to CE was developed for the determination of tetracycline, oxytetracycline, chlortetracycline, and doxycycline in milk samples, and five different magnetic phenyl silica adsorbents covered with magnetite were synthesized [43]. Hsu et al. [44] reported a microscale SPE method using alumina-coated iron oxide nanoparticles (Fe_3O_4@Al_2O_3 NPs) as the affinity adsorbent for glyphosate and its major metabolite aminomethylphosphonic acid in aqueous solution. Liu [45] described a preconcentration procedure using a gold nanoparticles coated silica gel as SPE sorbent for the determination of three neutral steroids of testosterone, progesterone, and testosterone propionate. A commercial C_{18}-bonded silica gel was also tested as sorbent. However, the gold nanoparticles coated SPE sorbent displayed superior cleanup efficiency toward the urine sample matrix.

2.1.1.2 At/on/in-line SPE

For at/on/in-line SPE-CE, the column is integrated into the CE system. Generally, the system can be divided into three main set-ups, that is, the SPE column can be an open tubular capillary coated with SPE sorbent, a packed bed or monolithic material in a capillary, or a thin impregnated membrane positioned between two capillaries. This section has already been described in detail [12]. Two attractive reviews about the coupling between at/on/in-SPE and CE have been published by Ramautar et al. [12] and Puig et al. [46], respectively. No further examples are thus discussed here.

We just discussed some applications of the in-line SPE prior to CE. Table 2 summarizes the relevant studies reported [47–62] about at/on/in-line SPE applications in CE, including descriptions of analytes, sample, SPE sorbents, sample volume, concentration factor and LOD, etc. Ramautar et al. [61] developed a CE-MS method by using a monolithic sol–gel concentrator for in-line SPE to evaluate, for the analysis of methionine enkephalin in biological samples. Commercial sol–gel capillaries had a total length of 60 cm and effective length of 50 cm. Operational SPE parameters such as sample pH, loading volume, elution volume, and composition have been studied. Under the optimum conditions, a 40-fold preconcentration was demonstrated for a methionine enkephalin test solution by using a loading volume of 3200 nL. Also self-prepared monoliths were popular right now. Thabano et al. [48] prepared the monoliths in DuPont Teflon AF Fluoropolymer coated fused silica capillaries derivatized with γ-MAPS. Then some vital characterization of them must be carried out, such as SEM and the total ion-exchange capacity. One advantage of the self-prepared monoliths is that they obtained the special use that is adapted to special requirement. Recently, the use of magnetic particles as solid supports for extraction in the in-line SPE-CE format has become quite attractive. Silica-coated iron oxide particles were synthesized and used as the solid support by Tennico and Remcho [54]. Figure 2 shows the process for performing in-line magnetic extraction coupled to CE [54]. The self-synthesized functionalized iron oxide particles showed high attraction to the fluorescent dyes and the reuse of the sorbents was also very attractive as it led to coat saving and waste reduction. A multisyringe flow injection analysis-CE system was developed by Horstkotte et al. [60]. The proposed hyphenated multi-syringe flow injection analysis-CE system was used to automate in-line sample acidification, analyte preconcentration, elution, hydrodynamic injection, electrophoretic separation, and detection as well as the maintenance and reconditioning of the SPE column and the separation capillary. Satisfyingly high repeatability and low limits of detection were achieved with concentration factors of about 120 for all analytes.

2.1.2 SPME

SPME is a microextraction technique, which means that the amount of extraction solvent is very small compared to the sample volume. SPME was developed to address the need for rapid sampling and sample preparation, both in the laboratory and in the field. It is a solvent-free sample preparation technique and combines sampling, isolation, and enrichment into one step [63]. The first commercial SPME device was made by Supelco, as shown in Fig. 3 [64]. In SPME, microquantities of the solid sorbent or liquid polymer or a combination of both in appropriate format were exposed in the headspace of sample or to sample solution. Under appropriate conditions, the analytes were extracted to the fiber or polymer from sample. SPME has several important advantages compared to the traditional sample preparation techniques, including rapid, simple, effective, solvent free and sensitive, compatible with such separation and detection modes of HPLC/GC/CE, wide quantitative range, convenient for designing portable devices for field sampling, and highly accurate and reliable for trace analysis.

Nowadays, various implementations of SPME have been developed, including fibers, vessels, stirrers, agitation mechanism disks, and in-tube [63]. Among them, only fiber and in-tube SPME are the most widely used modes. Puig et al. [46] mainly reviewed some applications of on-line SPME and in-tube SPME, coupled with CE. Kumar and Malik [65] reviewed the hyphenation of SPME with CE-MS.

Commercially commonly used SPME fibers mainly include PDMS, polyacrylate, carbowax/divinylbenzene, polydimethylsiloxane/divinylbenzene, polydimethylsiloxane/carboxen, carbowax/template resins, and divinylbenzene/carboxen/polydimethylsiloxane [66]. But there are several disadvantages about these sorbents, such as relatively low recommended operating temperature, instability and swelling in organic solvents, breakage of the fiber, and so on. Kumar et al. [66] reviewed various sol–gel methods for the development of SPME fibers and their applications to CE.

Similar to SPE, SPME is also used as off- and on/in/at-line mode. Kataoka and Saito [67] reviewed some applications of SPME in CE, including off-line and in-line SPME in biomedical analysis. When SPME was used as off-line preconcentration procedure prior to CE, it usually need a solvent desorption procedure. Ravelo-Pérez et al. [68] developed an off-line SPME method for the analysis of seven pesticides, with fiber coatings of polydimethylsiloxane/divinylbenzene. And tomato samples were first extracted with acetone and then evaporated to dryness and reconstructed in water. After SPME, desorption of the pesticides from the fiber was carried out with methanol. The extract obtained was evaporated to dryness and reconstituted with CE buffer. Similar research was also performed for the determination of pesticides in red wines samples [69].

As an alternative to conventional SPME, a methodology of polymer monolith microextraction based on the use of a capillary monolithic column was developed for in-line SPME. Compared with conventional SPME, the monolithic structure has a larger surface area and emerges as a more popular alternative to packed columns because of the simplicity of preparation. The in-tube configuration is better at protecting the extraction material from physical damage. In addition, the convective mass transfer procedure and low pressure-drop

Table 2. Overview of in-line SPE-CE applications

Analyte	Sample	SPE sorbent	Sample volume (μL)	Concentration factor (fold)	Final volume of the concentrated sample (nL)	Limit detection[a)]	Remark	Ref.
Opioid peptides	Human plasma	C_{18}	60	1200	50	0.01–0.1 ng/mL	Combined with pH gradient step	[47]
Neurotransmitters	Urine, cola drink, wastewater	Weak anion-exchange monolith	–	–	–	0.5–0.7 ng/mL	–	[48]
Lanthanum and gadolinium	Tap water	C_{18}	–	–	–	20 and 80 pg/L	–	[49]
Parabens	Cosmetic products	C_8	600	4	150 000	0.07–0.1 μg/mL	FIA[b)] and MEKC	[50]
Thalidomide racemates	Human urine	C_8, octyl, and norvancomycin-bonded monolith	–	–	–	90–94 μg/L	CEC	[51]
Terbutaline and 4-hydroxy-3-methoxymethamphetamine	Aqueous solution	Synthesized methacrylate-based monolith and C_{18}	–	333 and 1000	–	2.5–15 ng/mL	NACE and CZE	[52]
Opioid peptides	Human plasma	Sep-packs C_{18}, $^tC_{18}$, C_8, and $^tC_2$, Oasis® HLB, isolutes ENV+, Strata™-X, and Oasis® MCX	75 and 60	–	–	1–10 ng/mL	–	[53]
Fluorescent dyes	Aqueous solution	Functionalized magnetic particles	–	–	–	1 μg/mL[c)]	–	[54]
Neuropeptides	Human plasma	C_{18}	270	5400	50	1–5 μg/mL	–	[55]
Triazine herbicides	Urine samples	Molecularly imprinted polymers (MIPs)	2.7	45	60	0.2–0.6 μg/mL		[56]
Sulfonamide residues	Natural waters	Hydrophilic–lipophilic balance (HLB)	20	417	48	0.23–0.56 μg/mL	–	[57]
Tryptic peptides	Protein standards	C_{18}	20	100	200	n.s.	–	[58]
Ochratoxin A	River water	C_{18}	640	4741	135	1 ng/L	–	[59]
Nitrophenols	Aqueous solution	C_{18}	800	4	200 000	0.03–0.35 μmol/L	Multisyringe FIA[b)]	[60]
Methionine enkephalin	Cerebrospinal fluid	Sol–gel monolith	–	–	50	1 ng/mL	–	[61]
Benzoic and sorbic acid	Food products	C_8	1800	10	180 000	0.8–3.3 μg/mL[c)]	–	[62]

a) Limit of detection: S/N = 3; n.s., not specified.
b) Flow injection analysis.
c) Limit of quantification: S/N = 10.

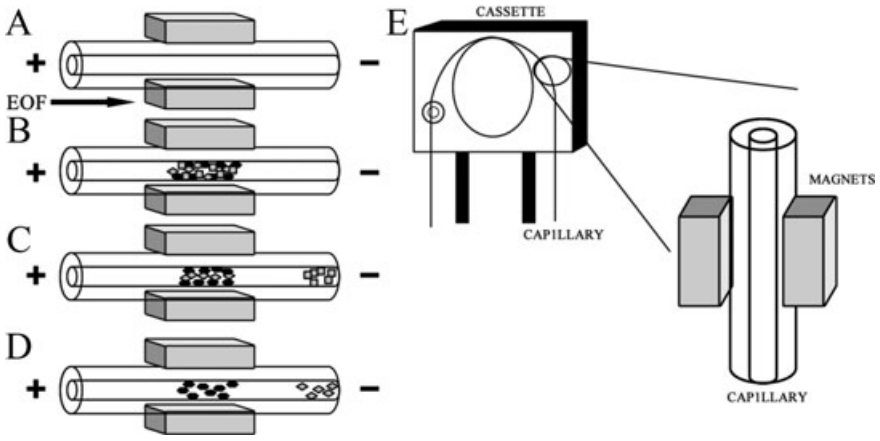

Figure 2. Schematic diagram of the process for performing in-line magnetic extraction with CE. (A) Conditioning step: capillary is conditioned and NdFeB permanent magnets are placed around the capillary; (B) sample loading: sample mixture containing magnetic particles is introduced into the capillary and retained by the magnets; (C) washing step: analytes of interest will interact with the sorbents, whereas interfering components are eluted; (D) elution step: retained analytes are eluted by applying a stronger eluent; and (E) a diagram showing how the magnets sandwich the capillary in the cassette. Reprinted with permission from [54] Wiley-Blackwell.

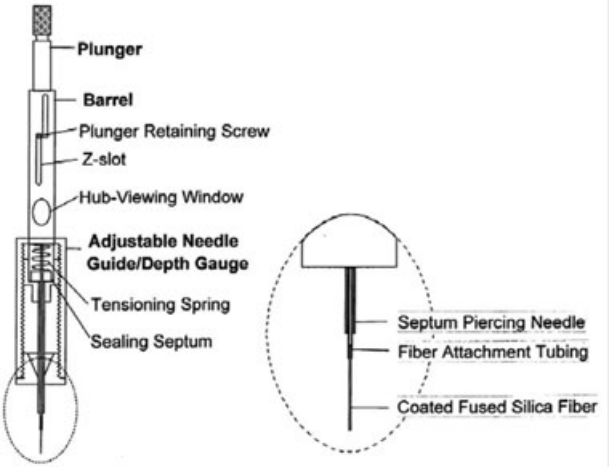

Figure 3. Design of the first commercial SPME device made by Supelco. Reprinted with permission from [64]. Copyright (2000), Elsevier.

offered by the porous structure can facilitate the extraction process [69]. So far, poly-(methacrylic acid-ethylene glycol dimethacrylate), a commonly used material, has been employed for the preparation of polymer monolith microextraction monolithic column prior to CE [69–72]. Jarmalavičienė et al. [73] adopted continuous restricted-access media (RAM) as the SPME monolith material for analysis of caffeine in biological fluids. RAM is a class of tailor-made stationary phase that is increasingly used in clinical research instead of the elaborative and often nonreproducible sample cleanup steps required for total analytical process in pharmacokinetics studies. The adsorption centers for small analytes are localized exclusively at the inner pore surface while the outer surface, which is in contact with macromolecules, carries hydrophilic functional groups [73]. The detection sensitivity for caffeine by SPME-CZE was corresponding to a 1000-fold improvement compared to that by CZE only. Moreover, the polymeric monolithic material can be easily synthesized in situ and can provide notable monolithic structures and tailored functional groups for specific purposes [74–78]. In this mode, polymeric monolithic column is usually synthesized in a fused silica capillary as needed, such as phenyl-functionalized hybrid silica monolithic column [74], butyl methacrylate monolithic column [75, 76], and so on. A mercapto groups incorporated hybrid silica-based monolith, which consists of a continuous porous silica backbone, was successfully synthesized by sol–gel technology [77]. The results showed that the monolith contained high sulfur content (up to 3.05%) with a hierarchical porous structure (through pores and mesopores) and large specific surface area (467 m^2/g). Due to the favorable chemical reactivity of mercapto pendant moieties, the hybrid monolith could be facilely derivatized to yield various functional groups. They were then oxidized by using hydrogen peroxide to yield sulfonic acid groups, which exhibited excellent cation-exchange capability and was then applied to in-tube microextraction of anesthetics in human urine. MIPs are also very popular material for the in-line SPME. They also can be synthesized in situ which made the extraction highly sensitive and efficient. Zhang et al. [78] synthesized a MIPs monolith for the microextraction of urinary 8-hydroxy-2′-deoxyguanosine prior to CE. The MIPs monolith was prepared inside a fused silica capillary by in situ polymerization method. The prepolymers used guanosine as dummy template, 4-vinylpyridineand acrylamide as functional monomers, and *N*,*N*-methylene bisacrylamide as crosslinker. Then, the pretreated fused silica capillary was filled with the prepolymerization mixture and the polymerization reaction was allowed to run at 60°C for 18 h. After the treatment, the monolith was used for the microextraction, followed by CE.

2.1.3 SBSE

SBSE is also based on sorption extraction like SPME. In contrast with the coated fiber for SPME, SBSE uses a coated magnetic stir bar to capture analytes during stirring. In SBSE, the sorbent is coated on a magnetic stirring bar, and liquid samples are simply stirred with this bar. After extraction, the stir bar is removed and dried with a soft tissue. The analytes are desorbed either thermally or with liquid. There is

one commonly used stir bar coated with PDMS and it is also the only commercially available at present. Although the PDMS stir bar can effectively extract nonpolar and weakly polar compounds, it fails to extract the strongly polar compounds because of the nonpolar characteristic of PDMS. In recent years, various techniques for preparing stir bar have been developed. Guan and coworkers [79,80] adopted sol–gel technique to prepare unique stir bar and successfully applied it for the extraction of polycyclic aromatic hydrocarbons and organophosphorus pesticides. To improve the polarity of the coating on stir bars, some new sorbents have been prepared. A PDMS/β-CD extraction phase was prepared by sol–gel and it showed higher selectivity to medium polar compounds of estrogen and BPA compared with that of the PDMS stir bar [81]. Recently, molecular imprinted technique has also been employed to prepare various stir bars to improve extraction selectivity and efficiency and thereby detection sensitivity [82–84].

Most SBSE applications involve the use of thermal desorption followed by GC and liquid desorption (LD) followed by HPLC. During LD mode, the polymer-coated stir bar is immersed in a stripping solvent or solvent mixture for the chemical desorption of the extracted solutes. Therefore, the LD mode can also be used prior to CE [85]. In this research, SBSE in combination with MEKC was developed for the simultaneous extraction and determination of acrinathrin, bitertanol, cyproconazole, fludioxonil, flutriafol, myclobutanil, pyriproxyfen, and tebuconazole in lettuce, tomato, grape, and strawberry samples. And the SBSE was also compared with SPE. The recoveries obtained by SPE ranged 40–106% whereas 12–47% by the SBSE. The LOQs were much lower by SPE (0.2–0.5 mg/kg depending on the processed sample amount) than those obtained by SBSE (1 mg/kg for each compound). Although the performance of the SBSE is lower than the SPE, this research has also provided a potential sample preparation procedure prior to CE. Still, few researches refer to SBSE-CE, and its further exploration is very essential in future sample treatment development.

2.1.4 LLE

LLE is a classic sample preparation method and it remains a popular choice. Several parameters may influence the extraction performance, including extraction solvent type, sodium chloride content, volume of extraction solvent, vortex time, and sample pH. Liu et al. [86] used salting-out LLE for the extraction of sulfonamides (SAs) from different matrices of honey, river water, and human urine. The parameters that influenced the extraction efficiency mentioned above were investigated. The optimum extraction conditions were obtained as follows: 150 μL of ACN was added to the 500 μL of sample solution containing 300 mg/mL sodium chloride at a pH of 6.5. This procedure afforded a convenient, fast, and cost-saving operation with high cleanup ability for the SAs determination in various matrices. Also, LLE was used for sample preparation of amphetamines in urine [87] and polyphenols in wine samples [88]. Although LLE is a classic sample preparation method, it is time-consuming and tedious, and requires large amounts of potentially toxic organic solvents [86]. The demand to reduce the consumption of organic solvent is more and more essential, so LPME, such as single-drop microextraction (SDME), LLME (liquid-liquid microextraction), LLLME (liquid-liquid-liquid microextraction), DLLME, etc., becomes popular in recent years. LPME is a simple, inexpensive sample pretreatment procedure and is compatible with GC, HPLC, and CE. Additionally, two- and three-phase systems are possible in LPME.

2.1.4.1 SDME

The simplest mode of LPME is the SDME, in which analytes are extracted from a stirred aqueous sample into a drop of organic solvent (approximately 1–3 μL) suspended from the needle of a microsyringe. After the extraction completed, the organic solvent drop is retracted into the syringe for analysis, as shown in Fig. 4, that is, direct immersion, headspace, or three-phase SDME. Meanwhile, SDME faces a main

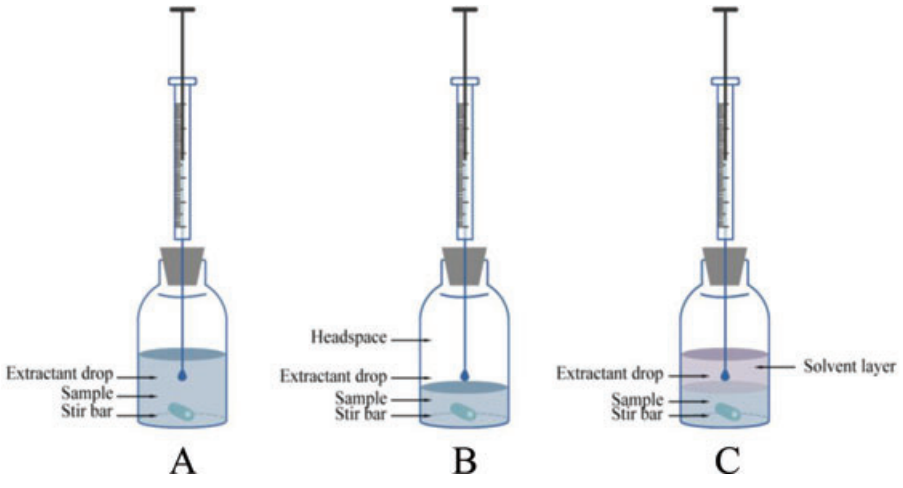

Figure 4. SDME modes. (A) Direct immersion SDME; (B) headspace SDME; and (C) three-phase SDME.

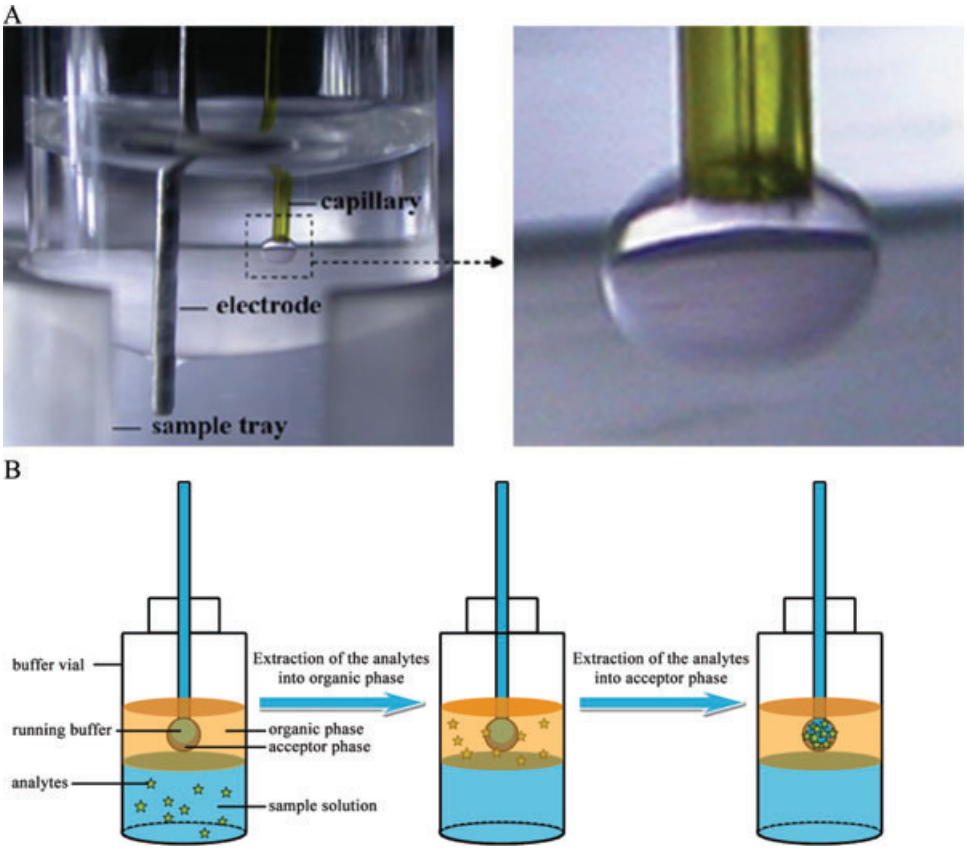

Figure 5. Image of a droplet in the organic phase (A) and schematic illustration showing what occurs in the droplet (B). (A) Reprinted with permission from [91]. Copyright (2000), Elsevier.

challenge, that is, drop instability. Gao et al. [89] developed a SDME coupled with CE method for the determination of six fluoroquinolones in urine samples. Aqueous sample solution was used as donor phase, mixture of dichloromethane and toluene was used as middle phase floating on the top of aqueous sample, and NaOH solution was used as acceptor phase. Under optimized SDME conditions besides a stable droplet, good recoveries were obtained at two concentration levels ranging from 81.8% to 105.9%. The LODs varied from 7.4 to 31.5 μg/L (S/N = 3).

Prior to CE, SDME can also be used in commercial CE instruments [90, 91]. The whole process combined SDME with CE on-line consisted of the following four steps: (i) the acceptor solution was injected into the capillary, which was first filled with the running buffer; (ii) the drop formation was realized when a backward pressure was applied in this step; (iii) the sample in the donor phase was extracted into the organic phase and subsequently back-extracted into the acceptor phase; and (iv) the enriched extractant was injected into the capillary with a forward pressure. The photographic image of a droplet in the organic phase was shown in Fig. 5A [91]. And a schematic illustration which shows what occurs in the droplet is shown in Fig. 5B. The on-line SDME-LLLME-CE [90, 91] not only allowed commercial CE instruments to directly handle complex matrices, but also facilitated the automation and miniaturization of CE methodologies.

2.1.4.2 HF-LPME and LLLME

In order to further improve drop stability of SDME, HF-LPME method is developed. The hollow fiber is not only a support for the extracting solvent, enabling the use of larger volumes, but also a physical barrier between the phases. In HF-LPME, the hollow fiber membrane saturated with acceptor phase is usually set at the needle tip of the microsyringe. As the fiber together with the syringe is fixed on the stirred sample vial, the extraction starts. After an optimum period of time, the plunger is withdrawn and the acceptor solution is retracted back into the syringe. The needle is removed and its content is introduced to a CE microvial for subsequent analysis. Several researches have been performed based on HF-LPME prior to CE [92–97]. The developed HF-LPME methods proved to be applicable for various matrices, such as orange juice [92], human urine [93, 94], plasma [95], and water samples [96, 97].

2.1.4.3 DLLME

DLLME was introduced as a new mode of LPME by Assadi and co-workers in 2006 [98]. Figure 6 shows the basic procedure of DLLME [99]. As seen, in DLLME, the appropriate mixture of extraction and disperser solvents is rapidly injected by syringe into an aqueous sample containing the analytes of interest. The fine particle of extracting solvent that is dispersed into aqueous phase allows its interaction with the

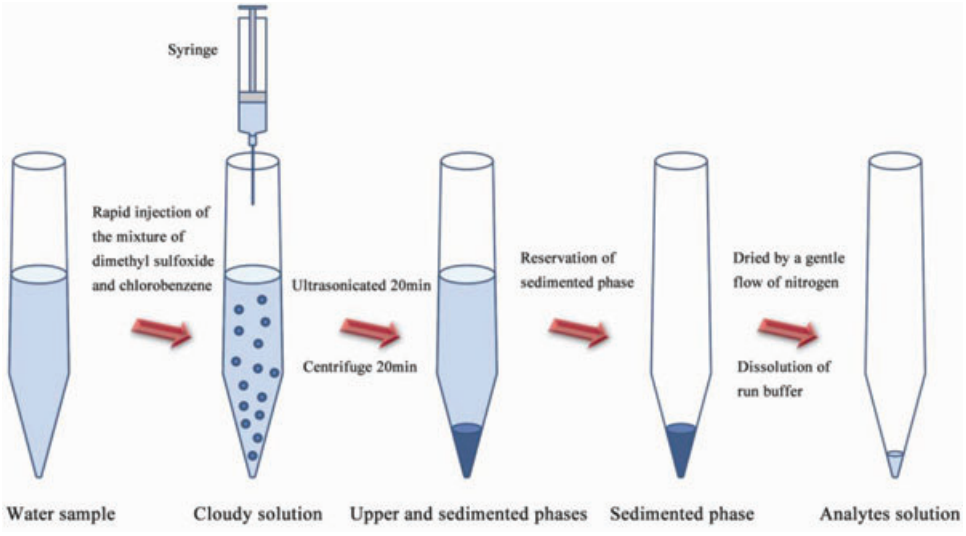

Figure 6. Scheme of the DLLME procedure. Reprinted with permission from [99] Wiley-Blackwell.

analyte. The extraction solvent must be a high-density water-immiscible solvent, such as chlorobenzene, carbon tetrachloride, and tetrachloroethylene, whereas the disperser solvent must be a water miscible, polar solvent, such as acetone, methanol, and ACN. After centrifugation, extraction solvent is normally sedimented at the bottom of the tube and is taken with a microsyringe for its later analysis [100]. There are several important conditions to be optimized, such as types and volumes of extraction and disperser solvents, ionic strength and sample pH, and extraction and centrifugation time, all of which can significantly affect the extraction efficiency. Our group had developed a DLLME-MEKC method for the determination of SAs [99]. In this research, orthogonal design was used to assist to find the major extraction factors as well as Box–Behnken design for the optimum extraction conditions of DLLME for five SAs. We also developed a DLLME-back extraction-CZE method for the determination of mercury(II), using 1-(2-pyridylazo)-2-naphthol and L-cysteine as chelating reagents for DLLME and back extraction, respectively [100]. Lately, we have reviewed recent advances in DLLME for organic compounds analysis in environmental water [101]. Also, DLLME applications prior to CE have been increasingly carried out. Zhang et al. [102] developed a DLLME-MEKC method for trace analysis of six carbamate pesticides in apples. The LODs at a signal-to-noise ratio of 3 ranged from 2.0–3.0 ng/g. The recoveries of the six carbamates for apple samples at spiking levels of 20.0 and 100.0 ng/g ranged 85.5–108.1% and 85.4–113.3%, respectively. Deng et al. [103] also used a DLLME-MEKC method for the determination of 2,4-dihydroxy-benzophenone (HBP) and 2-hydroxy-4-methoxy-benzophenone (HMBP) in environmental water samples after topical skin application. Under the optimum conditions, enrichment factors (EFs) of 208.9-fold for HBP and 194.6-fold for HMBP were obtained, as well as the LODs of 0.52 μmol/L for HBP and 0.29 μmol/L for HMBP. The coupling of DLLME-CE has been widely applied to different matrices, such as forensic [104], water [105, 106], and juice [107] samples.

2.1.4.3 CPE

CPE, based on the clouding phenomena of nonionic surfactants, has become an alternative to conventional solvent extraction due to a number of possible advantages such as low cost, environmental safety, high capacity to concentrate a wide variety of analytes of widely varying nature with high recoveries, and high EFs [108]. Similar to solvent extraction, CPE is based primarily on the hydrophobic interaction between solutes and surfactants. So, any hydrophobic species can be extracted into the surfactant-rich phase and thereafter interfere possibly with the analysis of the targeted analytes [109]. For CPE procedure, samples are adjusted to a certain pH value. Surfactant micelles (usually Triton X-114 is used) are added into sample solution. The mixture is then immersed in a thermostatic bath. In this step, a cloudy solution is formed and the analytes in the water samples are extracted into the surfactant-rich phase. After the mixture is centrifuged and cooled in an ice bath to increase the viscosity of the surfactant-rich phase, the supernatant aqueous phase is removed carefully with a pipette and the organic phase is remained for instrumental analysis. However, the injection of high-concentration surfactant into the electrophoretic capillary may decrease both the separation efficiency and the reproducibility. In order to solve this problem, two effective methods are usually employed.

The first one is that some organic solvents such as methanol, ethanol, and ACN are used as the diluting solvent for the back extraction after the extraction [109, 110]. Wu et al. [110] developed a CPE-MEKC method for the extraction and determination of triptonide in traditional Chinese herb *Tripterygium wilfordii* Hook F. After the extraction by Triton X-114, a 250 μL aliquot from the extraction surfactant-rich phase was diluted to 400 μL with ethanol to reduce its viscosity before separation by MEKC. Under optimum conditions, an EF of 25 was obtained and the LOD of triptonide was found to be 3.156×10^{-7} mol/L. The developed method had been successfully applied to the determination of triptonide in *T. wilfordii* tablet and spiked

urine matrix, indicating its feasibility and reliability. Zhong et al. [111] utilized ACN as the diluting solvent to reduce the surfactant-rich phase viscosity.

The other one is that dual-CPE often mediated by pH values is used to avoid the above problem. After the first CPE procedure, basic solution was added into the surfactant-rich phase for the second CPE. The analytes entered into the aqueous phase for CE analysis. Yin et al. used pH-mediated dual-CPE as a preconcentration and cleanup procedure for CE determination of phenol compounds [112] and auxins [109]. The results had demonstrated the feasibility of CPE to enrich target analytes and to eliminate potential matrix interference.

2.2 Solid and semisolid samples

Extraction techniques for solid and semisolid samples are generally exhaustive to guarantee efficient recoveries in different types of samples since low levels analytes are generally present in complex matrix of solid and semisolid samples. In general, Soxhlet extraction, SFE, microwave-assisted extraction (MAE), sonication-assisted extraction (SAE), PLE, and MSPD are commonly used for enhancing extraction efficiency. Although Soxhlet extraction as a classic method is still often used, SFE, MAE, SAE, PLE, and MSPD have allowed more efficient extraction of the analytes from matrix, considering the reduced extraction time and organic solvent consumption.

2.2.1 SFE

SFE, performing an automatic sample preparation, relies on supercritical fluids for extraction in which a wide variety of compounds existing in complex matrices can be selectively extracted. Various solvents are available as supercritical fluids, including CO_2, nitrous oxide, ethane, propane, *n*-pentane, ammonia, fluoroform, sulfur hexafluoride, and water [113]. It is an attractive technique because of its high diffusivity combining with high, easily tunable solvent strength and the ease of collection and concentration of extracts. CO_2 is currently the solvent of choice as its low toxicity, nonflammability, high purity, and low cost. However, the use of CO_2 is restricted by its inadequate solvating power for highly polar analytes, which can, to some extent, be boosted by using an appropriate modifier [114]. There are several parameters that affect extraction recovery, such as temperature and pressure, extraction time, flow rate, choice of modifier, and collection mode (e.g., solvent, SPE trap, empty vessel) [115]. So far, such commercial instruments are also available. Hurtado-Fernández et al. [115] reviewed some SFE applications to the determination of antioxidant phenolic compounds prior to CE. Other interesting applications of SFE include the determination of nitrosamines in sausages [115], heterocyclic amines in meat samples [116], and riboflavin vitamins in food samples [117]. In the above researches, commercial instruments were used and the extraction processes were optimized. In addition, on-line SFE-CE was used for the enrichment and determination of riboflavin vitamins in food samples [118]. The results showed the SFE-CE method could effectively reduce the sample manipulation processes, total extraction time, and cost. The key role of SFE is evident: simplification and automation of the sample treatment with the possibility to integrate two sequential steps (cleanup of the sample and selective extraction of the analytes from complex matrix).

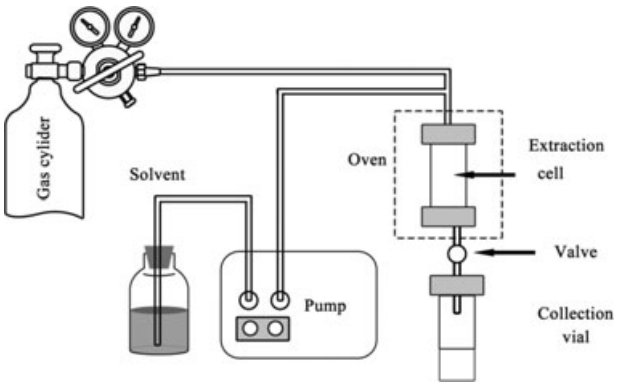

Figure 7. Schematic diagram of a PLE system. Adapted with permission from [119]. Copyright (2000), Elsevier.

2.2.2 PLE

PLE, also known as pressurized fluid extraction (PFE), pressurized solvent extraction, accelerated solvent extraction, and enhanced solvent extraction [114], provides quantitative extractions with reduced consumption of solvents and shortened extraction time. The basic set-up for PLE has been described in detail [114, 119, 120] and the schematic diagram is shown in Fig. 7 [119]. The system consists of a stainless steel extraction cell where the programmed parameters (temperature and pressure) are kept at their specified values by electronically controlled heaters and pumps. By pressurizing the sample cell, it is possible to keep the organic solvent in a liquid phase at the relatively high extraction temperatures sometimes used (even up to 200°C) [119]. Commercial, automated systems are also available and miniaturized versions of the PLE system have also been developed. The most important variables affecting the efficiency of the PLE process are the extraction solvent, the extraction temperature, and time. Typically, some organic solvents, water, and mixtures of low- and high-polar solvents generally provide efficient extractions of analytes. Leon et al. [121] investigated methanol, water, and hexane at two different temperatures, 100°C and 175°C, for the extraction of transgenic maize metabolomics. The results showed that water was the solvent that extracted the highest number of compounds at 175°C and hexane was a more selective solvent in this particular case. There are also some other extraction solvents for PLE prior to CE, for example, 70% ethanol [122], water [123], and dichloromethane [124]. Recently, surfactant-assisted PLE has become very popular

[125]. As water is too polarized to dissolve most bioactive compounds from plants, it is difficult to use this "green" environmentally benign solvent for extraction at room temperature [125]. So, surfactant added in water becomes an alternative. In this research [125], experimental parameters such as the type and concentration of surfactant and extraction time were evaluated systematically. Under the optimum conditions, the extraction efficiencies were compared with Soxhlet extraction using organic solvent. The results showed the surfactant (SDS)-assisted PLE coupled to MEKC method was a green, rapid, and effective approach for the extraction and analysis of flavonoids in *Costus speciosus* flowers [125].

2.2.3 SAE

SAE utilizes acoustic vibrations to cause cavitation in the liquid [114], and cavitation enhances the removal of analytes from the matrix to the extraction solvent. Variables affecting the extraction efficiency of SAE mainly include extraction solvent, extraction temperature, and time. The separation technique of CE coupled with SAE has been extensively developed and applied. Fakhari et al. [126] optimized ultrasound-assisted extraction procedure for the analysis of opium alkaloids in papaver plants. The optimized extraction conditions for SAE were an extraction time of 1 h, an ultrasonic frequency of 60 kHz with water–methanol (80:20, v/v) at 40°C as the extraction solvent. Moreover, filtration and rinsing steps after extraction are usually needed. Rodríguez-Gonzalo et al. [127] used SAE for extracting the herbicide residue in potatoes. And then a cleanup step of the organic extracts was carried out with SPE, using an Oasis MCX sorbent.

2.2.4 MAE

MAE is based on heating organic solvent or solvent mixtures by applying microwaves to the sample and extraction solvent. Like SAE, the extraction efficiency of MAE is mainly affected by extraction solvent, extraction temperature, and time. The most typically used solvents include methanol [128], 4% H_3BO_3 aqueous solution [129], acetone and water mixed solvent (3:7, v/v) [130], deionized water [131], and methanol [132] for ultratrace triorganotin compounds, quaternary ammonium herbicides, chlorogenic acid, catechin and epicatechin, and antioxidant constituents.

2.2.5 MSPD

MSPD has found particular applications as an analytical process for the simultaneous disruption, cleanup, and extraction of solid, semisolid, and highly viscous biological samples [133]. This technology involves mechanically blending a small amount of sample matrix with an appropriate sorbent followed by washing and elution of compounds with a small volume of solvent. Many materials such as octadecylsiloxane (C_{18}, C_8, etc.), underivatized silicates (silica gel, sand, etc.), and other organic (graphitic fibers) or inorganic (Florisil, alumina, etc.) solids are available as dispersants/sorbents of MSPD [134]. Factors needed to be optimized for extraction efficiency mainly include sorbents, ratio of sample to sorbents, elution solvents and volume, and use of chemical modifiers (e.g., acids, bases, or chelating agents). Our group has employed atrazine-MIPs as MSPD sorbents (MI-MSPD) for concurrent cleanup of samples and extraction of triazines at trace levels in soil, fruit, and vegetable samples followed by MEKC, and the whole procedure is shown in Fig. 8 [134]. Compared with other researchers that used Florisil [135] and MFE®-pack amino sorbent [136] as sorbents, our method proved more selective for the analytes extraction.

3 CE on-line stacking

Despite the attempts to enhance detection sensitivity by off-line enrichment procedures (sample pretreatment and preparation), the main limitation of CE-based analytical techniques lies with its extremely small injection volume, typically in the nanoliter range, three orders of magnitude smaller than the usual injection amount of HPLC [137]. Fortunately, a number of strategies have been developed to improve the sensitivity in CE through the use of on-line enrichment techniques, which is collectively known as stacking [4]. Generally, CE on-line stacking includes four basic modes, that is, field amplication (FA), sweeping, pH regulation, and isotachophoresis. Their modified/derivatized and hyphenated techniques together with dual/multiple stacking modes have also attained rapid development in recent years.

3.1 FA

FA stacking, one of the most popular and simplest stacking techniques, was first introduced by Mikkers et al. [138]. It is based on the conductivity difference between sample and CE buffer. The sample ions are prepared in a matrix of lower conductivity than that of the buffer. The lower the conductivity is, the higher the electric field is, and then the higher the ion velocity is. Therefore, the electric field in the sample zone is much higher than that in the separation buffer. Once the voltage is switched in, the sample ions migrate faster than they do in the separation buffer. When the ions pass the boundary between the sample matrix and the buffer, the ions will experience significant decrease in velocity because of the dramatic decrease of local electric field [139], and thus the stacking will happen at the boundary.

FA can simply be realized by diluting sample with a pure solvent or by placing a section of pure solvent in front of the sample in capillary [4]. The simplest FA stacking modes consist of the hydrodynamic injection (normal stacking mode, NSM) and electrokinetic injection (field-enhanced sample injection, FESI) [140]. Large-volume sample stacking (LVSS) is known as one of the NSM. Chien and Burgi [141] used the

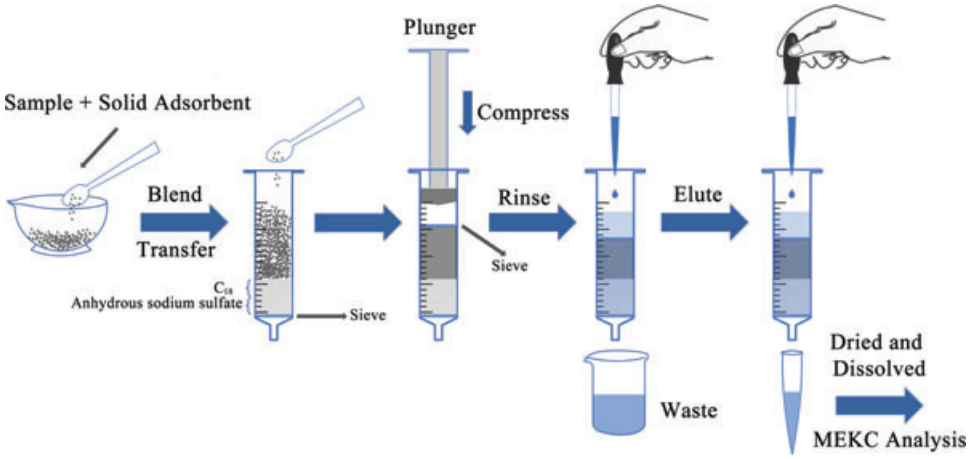

Figure 8. Schematic illustration of the MSPD procedure. Reprinted with permission from [134] Wiley-Blackwell.

buffer pumped into the capillary with polarity reversing for separation. This technique removed the sample matrix immediately after stacking by temporarily reversing the polarity in order to assure EOF to push the matrix plug out of the capillary [141]. But a single injection of large volume of sample does not always result in improved sensitivity. In fact, an increased sample plug boosts dispersive effects and results in band broadening [142]. This can be improved by FESI. Pressure-assisted electrokinetic injection (PAEKI) is an effective mode. The technique is based on the concept that the movement of running buffer in the capillary column due to EOF can be balanced during sample injection by an externally applied pressure in the opposite direction under a given electric field, to achieve a stationary state of the running buffer inside the column [137]. At balance, the injection time can extend up to 1200 s in CZE-MS and 3600 s in CZE-UV [143].

Recently, Zhang et al. [144] developed a facile way of extremely large volume electrokinetic stacking of cationic molecules in MEKC by EOF modulation with strong acids in sample solution, and obtained more than 1000-fold increase in sensitivity in CE under optimized conditions, as compared with the normal hydrodynamic injection without sample stacking. Figure 9 shows the schematic illustration of sample injection and stacking [144]. Here, electrokinetic stacking injection was performed by inserting one end of the capillary, which was already conditioned and filled with separation buffer, into a sample solution with low pH sample matrix. As seen from Fig. 9, a voltage of 10 kV (anode at the inlet) was then applied across the capillary (Fig. 9A) and the sample solution was sucked into the capillary by EOF. Cationic analytes continuously migrated toward the cathode until they encountered the micelles, where they lost most of their positive charges, stacked in the low conductivity band. A steady state as shown in Fig. 9B was established and could be sustained for a quite long time (with gradual expanding of the low conductivity stacking zone) so that a large amount of sample could be injected through the capillary. After the sample injection, the sample vial was exchanged with the separation buffer vial (Fig. 9C) and a voltage (27.5 kV, anode at the inlet) was applied. The pH of the sample zone increased

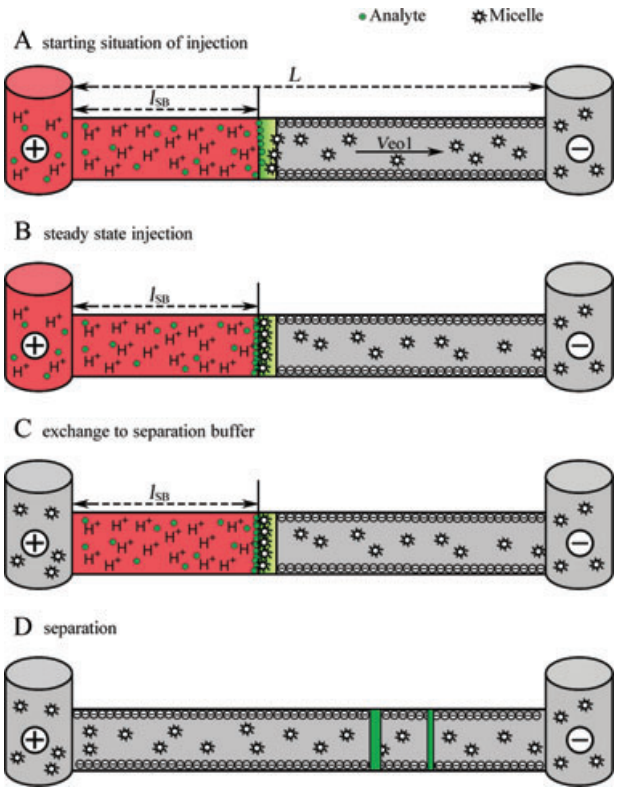

Figure 9. Schematic illustration of sample injection and stacking. The red area with labels of H represents sample solution in phosphate buffer. The green circles represent the analytes. The light green-yellow area represents the low conductivity band. The gray area with micelle represents the separation buffer. L is the total capillary length, l_{SB} is the sample buffer length, V_{eo1} is the local EOF velocity. Reprinted with permission from [144]. Copyright (2009) American Chemical Society.

and EOF was resumed gradually because of the dissociation of silanol. The steady state was disturbed and the separation of analytes was fulfilled (Fig. 9D).

Some highlighted applications of FA in CE are summarized in Table 3 [139, 140, 142–154], that is, using such FA

Table 3. Overview of FA stacking applications

Analytes	Samples	FA modes	Detection	Limit of detection	Remarks	Ref.
Halogenated phenols	Water	PAEKI	MS/MS	7.4–37.1 ng/L	–	[143]
Oxymatrine and matrine	Water	EKSI[a)]	UV	0.81 and 0.18 ng/L	MEKC	[144]
Nonsteroidal drugs	Water	LVSS	UV	0.03–0.3 μg/L	MEEKC	[145]
Sulfonamides	Porcine liver and Water	LVSS	UV	0.9–4.2 μg/L	MEEKC	[146]
C_1-C_9 aliphatic amines	Water	LVSS	LIF	1.2–3.8 pmol/L	–	[147]
Antidepressants	Human plasma and urine	FESI	UV	3.7–51.5 μg/L	SPME combined with FESI	[140]
Monophthalates	Urine	PAEKI	MS	0.53–1.3 ng/mL	–	[148]
Phenazine-1-carboxylic acid	Soil	NSM	UV	0.02 mg/kg	SPE combined with NSM	[149]
Plasma arginine and dimethylated arginines	Blood	FESI	UV	0.01–0.02 μmol/L	–	[150]
p-hydroxybenzoic acid and parabens	Water	LVSS	DAD	0.7–2.1 ng/mL	NACE	[151]
5-sulfosalicylic acid, matrine and oxymatrine	Herb medicine	FESI	UV	0.06–0.2 ng/mL	–	[139]
Sulfonylurea herbicides	Water and grape	LVSS	DAD	0.04–0.12 μg/L and 0.97–8.30 μg/kg	SPE combined with LVSS	[152]
β-lactam antibiotics	Water	LVSS	DAD	0.08–0.80 μg/L	SPE combined with LVSS	[153]
Nonsteroidal drugs	Saliva	LVSS	DAD	0.1 μg/L	SPE combined with LVSS	[142]
Glufosinate and aminomethylphosphonic acid	Grape	PAEKI	LIF	6.34–16.32 pg/mL	–	[154]

a) Electrokinetic stacking injection.

modes as LVSS [142,145–147,151–153], PAEKI [143,148,154], FESI [139, 140, 150], electrokinetic stacking injection [144], and NSM [149]. Various compounds such as halogenated phenols, SAs, sulfonylurea herbicides, plasma arginine, and dimethylated arginines have been determined after FA enrichment from environmental samples (e.g., water and soil samples), biological samples (e.g., urine, blood, plasma, and liver), food samples (e.g., grape), and pharmaceutical samples (e.g., herb medicine). Although FA stacking is an efficient and popular on-line enrichment procedure, it only works with low-conductivity matrices. Sweeping, another on-line enrichment procedure used recently, can overcome this drawback.

3.2 Sweeping

Sweeping, first introduced by Quirino and Terabe [155] has attracted wide attentions and applications so far. The theory is based on that a zone of sample having the same conductivity as that of the background but without micelle (usually using SDS) is injected into a capillary filled with a micelle-containing buffer. Once the high voltage is applied, the two zones will be forced to move against each other and analytes will be "swept" toward and extracted into the micellar phase, forming a narrow front at the micellar zone [4]. So, the concentration ability is highly based on the affinity between analytes and pseudo-stationary phase (micelles) in MEKC. Although the enrichment efficiency of sweeping is independent of EOF, the swept zone would be driven by EOF and the analytes may arrive at the detector when the sweeping has not finished. Therefore, the buffer with very low pH value is used to decrease EOF [156]. And SDS is the most popular alternative micelles for on-line sweeping. Sweeping techniques have been demonstrated for the analysis of hydrophobic compounds [156], multiple abused drugs [157], aromatic amines [158], morphine and its four metabolites [159], tobacco-specific *N*-nitrosamines [160], voriconazole [161], and so on. Furthermore, Maijó et al. [162] developed anion selective exhaustive injection-sweeping in MEKC for the analysis of nonsteroidal anti-inflammatory drugs (NSAIDs).

Another procedure combining FESI with sweeping is called selective exhaustive injection-sweeping. The optimization of different parameters such as SDS concentration, sample matrix composition, water plug, high-conductivity

buffer (HCB) plug, and sample injection time was performed [162]. So far, the innovation of sweeping technique mainly lies in the exploration of new pseudo-stationary phases. Su et al. [163] used the ionic liquid-type cationic surfactants 1-cetyl-3-methylimidazolium bromide and *N*-cetyl-*N*-methylpyrrolidinium bromide as the pseudo-stationary phases for sweeping of seven benzodiazepines. Results revealed that *N*-cetyl-*N*-methylpyrrolidinium bromide exhibited superior sweeping power relative to those of 1-cetyl-3-methylimidazolium bromide. So, it can be seen that sweeping is a very strong on-line enrichment method and more applications are expected in CE. In addition, the focusing mechanism of sweeping under different experimental conditions has been detailed, discussed, and schematically illustrated by Aranas et al. [10].

3.3 pH regulation

Although FA and sweeping are the commonly used on-line enrichment methods to concentrate analytes, in analysis of real biological samples that are not strong electrolytes, pH regulation is especially preferred. The pH regulation creates a discontinuous acidic–basic boundary to make weak analytes lose their speed suddenly during stacking [4]. Dynamic pH junction, first mentioned by Britz-Mckibbin and Chen [164], in which two electrolytes are required at different pH values to form a sharp pH junction boundary, and thereby making the sample be dissolved in a different type electrolyte for the focusing of weakly acidic, basic, or zwitterionic analytes based on their pK_a values [165]. The principal mechanism takes advantage of velocity-difference induced focusing, in which an analyte migrates differentially within two distinct segments of background electrolyte, resulting in the compression of the analyte into a narrow zone prior to its reaching the detector [166]. Zhang et al. [167] demonstrated the scheme of dynamic pH junction and applied the technique for the preconcentration of benzoic acid and sorbic acid.

Another mode of pH regulation is pH-mediated stacking introduced by Lunte et al. [168–170] and it has been used to preconcentrate samples in highly conductive solutions. In this mode, a solution of analytes is sandwiched between plugs of highly acidic and highly basic solutions. When voltage is applied, the H^+ and OH^- ions will migrate toward each other, converting the original sample into a low conductivity zone where the analytes migrate quickly until they reach the boundary with the buffer and the stacking happens. Wu et al. [171] used a pH-mediated acid stacking method for preconcentration of IgG in human serum. The sample was prepared with 50 mmol/L phosphate buffer (pH = 7.4), and running buffer was 50 mmol/L borax (pH = 9.3) containing 10 mmol/L SDS. Under the optimum conditions, the detection sensitivity for IgG was improved 40.3-fold using a 100 s electrokinetic injection as compared with a 6 s hydrodynamic injection and a very clean electropherogram only containing IgG peak was obtained, indicating the complex serum matrix was eliminated well by the removal of HSA and dilution. The LOD of IgG was found to be 0.1 mg/L. This suggested that the developed method was successfully applied for the determination of IgG in human serum with satisfactory results.

3.4 ITP

As for the determination of analytes in real samples, it is almost evitable of the high ionic strength in matrices, for instance, urine, serum, and seawater. Because of little conductivity differences between sample zone and buffer, the high ionic matrix constituents do not allow an efficient sample stacking. Fortunately, tITP mode is especially suitable for high-salt sample. The tITP is based on the use of at least two small volumes of electrolytes of different mobilities, such as a leading electrolyte (LE) and a terminating electrolyte (TE), which contains ions with, respectively, faster and slower electrophoretic mobility than that of analytes, to focus the analytes that possess intermediate mobility. The sample solution is introduced between the LE and TE solution. When the voltage is applied, a potential gradient is established between the electrolyte and sample zones, and the field strength is inversely proportional to the mobility of the ions in every capillary zone. At equilibrium, every analyte moves as a discontinuous band according to its respective mobility, with high mobility ions migrating prior to low mobility ions. During the moving process, such stacking of sample zones always occurs at the identical velocity of leading ion. In this way, high concentration analytes in narrow sample zone can be obtained [172]. The stacking is achieved by introducing a plug of LE followed by a section of sample solution with TE, or reversely, by introducing a plug of sample solution with LE followed by a section of TE [4]. The tITP method has been successfully applied to the on-line enrichment of nonsteroidal anti-inflammatory drugs [173], ionic liquid entities [174], doping substances [172], and peptides [49].

3.5 Combined-stacking techniques and other modality of stacking

All of the above on-line preconcentration techniques can be used for the analysis with enhanced sensitivity in CE. Moreover, the coupling of multiple modes of on-line stacking is becoming popular. Also, other modality of stacking has been increasingly developed and applied.

Zhu et al. [175] overcame the deficiencies of LVSS in separating low-mobility and neutral analytes through combining LVSS with sweeping in CE, and successfully employed the approach to enrich and separate neutral and anionic analytes simultaneously. Careful optimization of the enrichment and separation conditions allowed the EFs of peak height and peak area of the analytes to be in the range of 9–33 and 21–35 comparing with the conventional injection mode, respectively. The five analytes were baseline separated in 15 min and the LODs ranged from 26.5 to 55.8 ng/mL. The method proved to be a simple, fast, and high-sensitivity strategy to

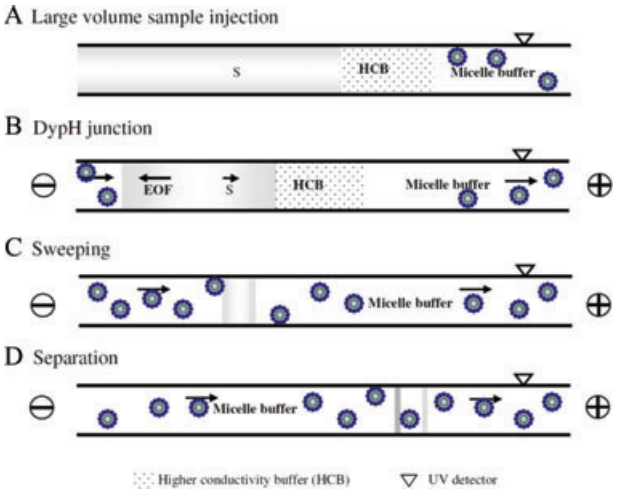

Figure 10. Mechanism of LVSS-DypH-sweeping method. Reprinted with kind permission from [177] Springer Science and Business Media.

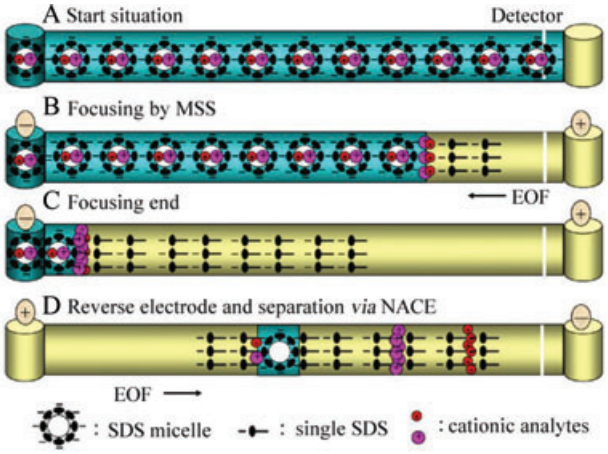

Figure 11. Schematic evolution of analyte focusing in MSS-NACE: (A) filling the whole capillary with the micellar sample from the inlet vial and the outlet vial was filled with the running buffer, (B) removal of the sample matrix with the running buffer during sample focusing under a reverse potential, (C) the focusing process finished after a certain focusing time, (D) separation of the focused analytes by NACE. Reprinted with permission from [181]. Copyright (2011), Elsevier.

simultaneously determine differently chargeable targets existing in complex matrices. Cheng et al. [176] combined three stacking techniques, including LVSS, dynamic pH junction, and sweeping for analysis of methotrexate (MTX) and its eight metabolites in whole blood by MEKC, and about 40-fold sensitivity increase was attained compared with that the single CZE mode. Cheng et al. [177] also adopted such triple-stacking CE mode to monitor MTX and its eight metabolites in cerebrospinal fluid (CSF). The LODs (hydrodynamic injection 2 psi, 99.9 s) were observed to be 0.1 μM for MTX, 7-OHMTX, MTX-$(Glu)_n$, $_{n=2–5}$ and 0.2 μM MTX-$(Glu)_6$, and 0.3 μM for DAMPA and MTX-$(Glu)_7$. Compared with 1 μM MTX proposed as a minimum effective antileukemic concentration [178], the LODs provided sufficiently levels for detecting MTX and its metabolites in CSF samples. Schematic diagrams of the mechanism of LVSS-DypH-sweeping are shown in Fig. 10 [177]. The capillary was rinsed with buffer containing micelles. After that, HCB with lower pH was loaded for collecting large volume samples (Fig. 10A). The analytes would have negative charges while in the sample matrix (higher pH), and migrate toward the detector. By applying the suitable voltage, the EOF turned toward the inlet direction (Fig. 10B). The pH junction was produced between sample zone and HCB zone for stacking. The analytes were dissociated in the basic sample zone and protonated in acidic HCB zone. When analytes reached the pH junction boundary, their mobility was close to zero, causing the stacking effect. Then, the SDS buffer swept through and stacked into a compact zone (Fig. 10C). The separation was conducted in MEKC mode (Fig. 10D).

Interestingly, multiple stacking is further applied from CE to microchip electrophoresis. Wang et al. [179] established an on-chip multiple-concentration method combining chitosan sweeping, reversed-field stacking, and field-amplified sample stacking for ultrasensitive detection of bacteria. A concentration EF of approximately 6000 was obtained as compared to using no concentration step, and the LOD of *Escherichia coli* was 145 CFU/mL. The multiple-concentration methodology was also applied for the quantification of bacteria in surface water, and satisfactory results were achieved.

Quirino and Guidote [180] described two-step stacking of organic anions by sweeping and other stacking modality of MSS using cationic cetyltrimethylammonium micelles in co-EOF CZE. The swept analytes were brought by the micelles to the MSS boundary where the second stacking step was induced by the presence of organic solvent in the background solution. A 20–29, 17–33, and 18–21 times increase in peak height sensitivity was obtained for the test hypolipidaemic drugs, nonsteroidal anti-inflammatory drugs, and herbicides, respectively. The LODs were from 0.05 to 0.55 μg/mL.

The development of MSS was for the first time extended to NACE by Zhu et al. [181]. Under optimal conditions, this method afforded LODs of 0.002 μg/mL and 0.003 μg/mL for berberine and jatrorrhizine, respectively. In contrast to conventional NACE, the concentration sensitivity was improved 128–153-fold. Figure 11 depicts the simple model of MSS-NACE [181]. Initially (Fig. 11A), the whole capillary column is filled with the micellar sample solution (dark green blank parts) by hydrodynamic injection (20.0 psi, 1 min) from the inlet vial to a waste vial, and the outlet vial is filled with the running buffer. Then, under a reverse potential, the running buffer is brought into the capillary from the outlet vial by the EOF and a part of sample matrix is removed (Fig. 11B). Simultaneously, because the SDS micelles have negative charges, the micelles will continuously migrate and collapse into the running buffer where the concentration of the SDS drops below its CMC, thereby releasing and focusing the transported organic cations at the MSS boundary. After a certain

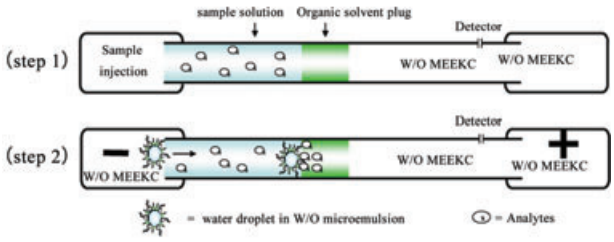

Figure 12. Illustrations of a modified NSM step coupled to water-in-oil (W/O) MEEKC model. (Step 1) A capillary was conditioned with a W/O microemulsion solution of pH 8.0, and then an organic solvent plug (1-propanol; 50 mbar for 10 s) was introduced into the capillary. The penicillin sample prepared in deionized water was pressure-injected into the capillary (50 mbar for 60 s). (Step 2) W/O microemulsion solution was placed at the inlet end of the capillary followed by the application of a 29 kV. Reprinted with permission from [183]. Copyright (2011), Elsevier.

focusing time, a majority of the sample matrix is replaced by the running buffer (Fig. 11C). Finally, the inlet vial of micellar sample solution is changed to a vial of running buffer, and then a positive voltage is applied (Fig. 11D), consequently, the focused analytes are separated via NACE.

Sun et al. [182] developed a novel stacking system of normal moving reaction boundary, which was formed by an alkaline buffer and double acidic buffers (*viz.*, acidic sample and blank buffers). With the system, phenazine-1-carboxylic acid (PCA) with low pK_a leaking into the blank buffer from the sample buffer could be efficiently stacked by the prolonged moving reaction boundary formed between the alkaline buffer and blank buffer. The stacking system, coupled with sample pretreatment, could achieve a 214-fold increase of PCA sensitivity under the optimal conditions and the LOD of PCA in soil was decreased to 17 ng/g.

Huang et al. [183] described for the first time, the ability of a NSM on-line concentration step coupled with water-in-oil (W/O) MEEKC, using six common penicillin antibiotics. In addition to the sensitivity enhancement, the resolution of several test compounds was improved as compared to that of conventional W/O MEEKC. This study demonstrated that the NSM W/O MEEKC method was successfully used to detect trace levels of penicillin compounds in porcine organs samples after a simple SPE sample treatment. Figure 12 illustrates the model of modified NSM step coupled to W/O MEEKC [183]. First, the capillary was filled with a pH 8 microemulsion solution, and then an organic solvent plug (1-propanol, 50 mbar for 10 s, 1.04 cm) was introduced into the capillary. The penicillin sample prepared in deionized water was introduced hydrodynamically into the capillary (50 mbar for 60 s) (step 1). After the sample was injected, a separation voltage of −29 kV was applied with the microemulsion solution in the inlet vial (step 2). This step allowed the analytes to be stacked in the organic solvent zone because of the difference in solution viscosity; meanwhile, the negatively charged water droplets also entered the stacked sample zone to sweep the analytes, then the separation proceeded by W/O MEEKC.

4 Concluding remarks

In this review, we have summarized the development of sample enrichment techniques prior to CE analysis, and attempted to provide a comprehensive overview of recent advances of relevant extraction techniques as well as several on-line stacking modes and their coupling concentration techniques in the last 5 years. Various available enrichment techniques continue to be used and improved, and new ones are continually being explored and developed, for trance and ultra-trace analysis of diverse targeted analytes in a variety of matrices with enhanced detection sensitivity in CE. Among the techniques for sample preparation, on-line SPE and SPME are fast gaining ground due to its integration of several sample preparation steps into one, showing rapid, simple, solvent saving, and sensitive. Some not widely used techniques such as SBSE, MSPD, and CPE also have many opportunities in CE analysis. DLLME is still a young member in the sample preparation prior to CE, and it can be expected to better develop in the future. However, some extractants from solid and semisolid samples especially require further purification, and extractant cleanup is usually still carried out by tedious conventional methods and high sensitivity is still need to be explored. So some combined preparation methods such as SPE-DLLME, SPME-DLLME, and so on can be expected to find a place. The on-line SDME-LLLME-CE [90, 91] facilitated commercial CE instrument to directly handle complex matrices, and relatively high sensitivity was attained. Also, combined stacking methods are developing increasingly such as LVSS-dynamic pH junction sweeping [176, 177]. It is worthwhile to note that the combined stacking methods can provide greater EFs than that single used. Many applications have also appeared focusing on the combined techniques of off-line and on-line enrichment to remarkably improve the sensitivity in CE, such as SPME-FESI [140], SPE combined with NSM [149], and SPE combined with LVSS [142, 152, 153]. For the enrichment techniques in CE, various coupling modes and their automation and instrumentation indicate great prospects, which are urgently to be further motivated in order to achieve super CE-based analytical performances.

Generally, that maximizing the detection sensitivity achieved while minimizing the analysis time required is the first objective and challenge in CE analysis. Increasing the analysis speed is essential when large numbers of samples need to be analyzed, or when profiling of the samples requires several techniques and methods for the analysis of different groups of compounds. The second is purity after preparation of the complicated sample matrices. Highly selective extraction materials and methods are increasingly developed for cleanup of matrices and selective recognition and enrichment of analytes. The third is environmental friendly—reducing or eliminating the use of organic solvents. More new solvents of extraction and dispersion are investigated to meet the requirement of eco-benignity, and solvent-free enrichment techniques are developed for CE. In addition, the use of chemometrics, in particular, experimental design methodology, can

enable to achieve the optimal extraction conditions quickly in a relatively small number of experiments, leading to rapid and solvent saving and thereby environmentally friendly separation and determination by CE. Developing more and more advanced enrichment methods is expected to overcome the main challenges. We strongly encourage further works studying possible solutions to various problems besides such mentioned above, and therefore expanding applications to numerous complicated matrices for a wider variety of analytes, to fully demonstrate the advantages and potentials of CE.

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