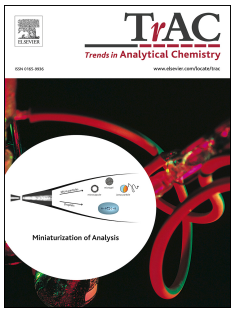
Journal Pre-proof



Practical sample pretreatment techniques coupled with capillary electrophoresis for real samples in complex matrices

Gabor Jarvas, Andras Guttman, Natalia Miękus, Tomasz Bączek, Sunkyung Jeong, Doo Soo Chung, Vladimir Pätoprstý, Marián Masár, Milan Hutta, Vladimira Datinská, Frantisek Foret

PII: S0165-9936(19)30321-8

DOI: https://doi.org/10.1016/j.trac.2019.115702

Reference: TRAC 115702

To appear in: *Trends in Analytical Chemistry*

Received Date: 22 May 2019

Revised Date: 17 October 2019

Accepted Date: 17 October 2019

Please cite this article as: G. Jarvas, A. Guttman, N. Miękus, T. Bączek, S. Jeong, D.S. Chung, V. Pätoprstý, M. Masár, M. Hutta, V. Datinská, F. Foret, Practical sample pretreatment techniques coupled with capillary electrophoresis for real samples in complex matrices, *Trends in Analytical Chemistry*, https://doi.org/10.1016/j.trac.2019.115702.

Practical sample pretreatment techniques coupled with capillary electrophoresis for real samples in complex matrices

Gabor Jarvas[1,2], Andras Guttman[1,2,3], Natalia Miękus[4,5], Tomasz Bączek[4], Sunkyung Jeong[6], Doo Soo Chung[6], Vladimir Pätoprstý[7], Marián Masár[8], Milan Hutta[8], Vladimira Datinská[9,10] and Frantisek Foret[9,10]

[1]Horváth Csaba Memorial Laboratory of Bioseparation Sciences, Research Centre for Molecular Medicine, Faculty of Medicine, University of Debrecen, Debrecen, Hungary
[2]Translational Glycomics Research Group, University of Pannonia, Veszprem, Hungary
[3]SCIEX Separations, Brea, CA 92130
[4]Department of Pharmaceutical Chemistry, Medical University of Gdańsk, Hallera 107, 80-416 Gdańsk, Poland
[5]Department of Animal and Human Physiology, Faculty of Biology, University of Gdańsk, Wita Stwosza 59, 80-308 Gdańsk, Poland
[6]Department of Chemistry, Seoul National University, Seoul 08826, Korea
[7]Institute of Chemistry, Slovak Academy of Sciences, Dúbravská cesta 9, SK-84538 Bratislava, Slovakia
[8]Department of Analytical Chemistry, Faculty of Natural Sciences, Comenius University in Bratislava, Mlynská dolina CH-2, Ilkovičova 6, SK-84215 Bratislava, Slovakia
[9]Institute of Analytical Chemistry of the Czech Academy of Sciences, Brno, Czech Republic
[10]Masaryk University, CEITEC, Brno, Czech Republic

Keywords: analyte preconcentration; capillary electrophoresis; liquid phase separation; microreactors; sample pretreatment

Abbreviations: AFMC - Analyte Focusing by Micelle Collapse; BGE - Background Electrolyte; CMC - Critical Micelle Concentration; DI - Direct Immersion; EF - Enrichment Factor; EKI - Electrokinetic Injection; EOF - Electroosmotic Flow; FASI - Field-Amplified Sample Injection; FASS - Field-Amplified Sample Stacking; FESI - Field-Enhanced Sample Injection; FESS - Field-Enhanced Sample Stacking; GNO - Gold Nanoparticle; GO - Graphene Oxide; HS – Headspace; IME - Immobilized Enzyme Microcolumn; ITME - In-Tube Microextraction; ITP – Isotachophoresis; L - Leading Ion; LE - Leading Electrolyte; LESA - Liquid Extraction Surface Analysis; LIF - Laser-Induced Fluorescence; LOD - Limit of Detection; LPME - Liquid Phase Microextraction; LVSEP - Large Volume Sample Stacking by an Electroosmotic Flow Pump; LVSS - Large Volume Sample Stacking; MCE - Microfluidics Chip Electrophoresis; MS - Mass Spectrometry; MSPE - Micro Solid-Phase Extraction; MSS - Micelle to Solvent Stacking; SDME - Single Drop Microextraction; SPE - Solid Phase Extraction; SPME - Solid Phase Microextraction; T - Terminating Ion; TE - Terminating Electrolyte; tITP - Transient Isotachophoresis; μIMER - Microfluidics-based Immobilized Enzyme Reactor

Abstract

By coupling a sample pretreatment technique of sample clean up and enrichment power with capillary electrophoresis (CE) of high-performance separation, the task of analyzing trace analytes in a complex matrix such as a biological sample can be carried out successfully with ease. This review aims for providing an overview of strategies to couple sample pretreatment techniques with capillary and related microscale (e.g., microchip) electrophoresis, practically adoptable in an automatic manner, without requiring serious modification of existing instruments to install sophisticated interfaces. In-line sample pretreatment techniques based on liquid phase microextraction performed before sample injection and on-line sample preconcentration techniques performed during or after sample injection are discussed with emphasis on the applicability to samples of high conductivity, commonly encountered for biological samples. An overview of the recent developments in microfluidic immobilized enzymatic microreactors which fit excellently to microchip CE is also given.

1. Introduction

It is more than often required to perform sample pretreatment for the analysis of trace analytes in a complex matrix sample such as a biological sample. A recent review paper extensively discussed biological sample preparation methods, mainly off-line and some in-line coupled techniques for chromatography, spectrometry, and electrochemistry as well as a few on-line stacking techniques for capillary electrophoresis (CE) [1].

The most commonly emphasized advantages of CE based separations include high separation efficiency, short analysis time, simple method development, and low sample and solvent consumption. While these claims are true and many, especially bioanalytical, protocols take advantage of some or all of them, CE is still considered as a niche technique in the field of separation sciences. Without a doubt, the major drawback of CE is its limited detection sensitivities associated with the small dimensions of the separation column. Quantitative determination of trace analytes in a complex biological matrix requires the use of extremely sensitive detectors such as laser-induced fluorescence (LIF) or mass spectrometry (MS). However, even the best detection methods may be insufficient without carrying out proper sample cleanup or/and sample preconcentration [2,3].

Herein, we review sample pretreatment techniques in-line and on-line coupled with CE which are easily adoptable without requiring sophisticated apparatuses but with high potential to increase sensitivity or/and improve overall selectivity. All these techniques can be applied for the analysis of complex samples such as biological samples, which is an urgently emerging issue especially for biologically relevant compounds such as biomarkers existing only in a trace amount in a complex biological sample of limited volume. While, some of the recently published reviews also include description of the stacking possibilities suitable mainly for chromatography [1], or CE-mass spectrometry coupling [4], the following text focuses on the sample pretreatment techniques practiced in capillary electrophoresis. For a concise guide to stack samples for CE, please refer to Ref [5].

For the categorization of coupling schemes, a coupling mode is termed as *in-line* (continuous) when the sample pretreatment is coupled to CE via a proper transfer interface before sample injection and *on-line* when sample pretreatment is directly integrated into the CE separation space (in-capillary) during or after sample injection. Note that this categorization is different from that in Brinkman et al. [6] but consistent with that widely used in the literature [1,2].

2. In-line liquid phase microextraction techniques in CE before sample injection

Off-line sample pretreatment techniques such as liquid phase microextraction (LPME) and solid-phase microextraction (SPME) are frequently used in bioanalysis [7,8]. LPME does not require dedicated expensive SPME cartridges, does not suffer from carryover problems, and can be used with small amounts of unconventional, green chemistry solvents including ionic liquids, magnetic ionic liquids, and deep eutectic solvents [9,10,11]. For more information regarding SPME, please refer to excellent recent papers by Pawliszyn et al. [12,13,14]. Similarly, this section does not cover the electroextraction techniques, which were thoroughly reviewed by Oedit and Lindenburg [15] as well as Pedersen-Bjergaard et al. [16]. We will focus on in-line LPME sample pretreatment techniques coupled with CE in an automatic manner with ease.

In-line LPME is a sample preconcentration technique employed prior to injection, also capable of cleaning up sample matrices (including desalting) and sampling gas or solid surface substances. Among various in-line LPME techniques, single drop microextraction (SDME), in-tube microextraction (ITME), and liquid extraction surface analysis (LESA) will be discussed herein. These methods can be easily coupled with CE without modification of existing CE instruments (see Figure 1). Importantly, due to the sample clean up capability, these techniques can be straightforwardly applied to biological samples. Note that in LPME as in most extraction procedures, the extraction efficiency for an analyte strongly depends on its distribution coefficient. It is thus necessary for quantification to take some measures such as using an internal standard [17,18] or standard addition [19].

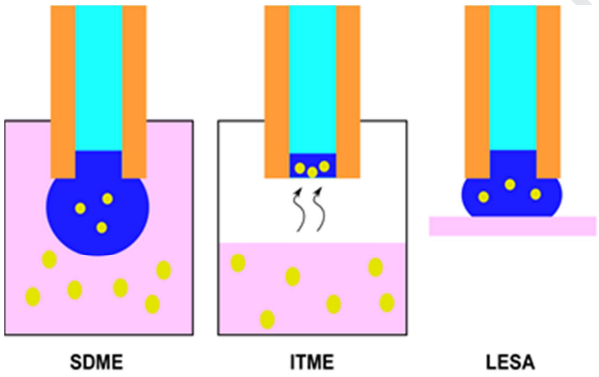


Figure 1. Schematics of three LPME techniques in-line coupled with CE. SDME; single drop microextraction; ITME; in-tube microextraction; LESA; liquid extraction surface analysis.

2.1 Single drop microextraction (SDME)

SDME uses an acceptor drop hanging on a syringe needle tip or capillary inlet end. Since the acceptor drop volume in SDME is less than few microliters, the volume ratio between the sample donor and acceptor phases is very high, resulting in high enrichment factors (EFs). SDME can be performed in a direct immersion (DI) or headspace (HS) extraction mode.

Two-phase DI-SDME is a technique for extracting analytes into an acceptor drop immersed into a sample donor solution. The equilibrium enrichment factor, EF_{eq}, for an analyte obtained via 2-phase extraction from a donor of volume V_d to an acceptor of volume V_a is given by

$$EF_{eq} = \frac{1}{(1/D) + (V_a/V_d)} \qquad \text{(eq 1)}$$

where $D = C_a/C_d$, referring to the distribution coefficient of the analyte, while C_a and C_d are the equilibrium concentrations of the acceptor and donor phases, respectively. Therefore, the maximum EF in a 2-phase SDME when V_a/V_d is negligible is limited to the distribution coefficient *D*, determined by the nature of the solvents and the analyte. Choi et al. overcame this limitation by combining 2-phase SDME with a large pentanol acceptor drop and an on-line sample preconcentration technique, i.e., large volume sample stacking using an electroosmotic flow (EOF) pump (LVSEP), achieving overall EFs of 2000 for pentachlorophenol and benzoic acids [20]. Recently, without filtration or centrifugation, a soil sample prepared in 0.1 M HCl and containing debris was cleaned up and enriched by 2-phase SDME using a pentanol acceptor drop, further enriched by LVSEP after injection, and subsequently analyzed by CE-MS [21].

In 3-phase DI-SDME, analytes are extracted from an aqueous donor phase into an aqueous acceptor drop through a thin organic layer covering the acceptor drop. For ionizable analytes, the pH difference between the donor and acceptor phases serves as the driving force of extraction. Therefore, this technique is not limited by the distribution coefficients as opposed to 2-phase extraction methods. The Chung group demonstrated a 3-phase SDME in-line coupled with CE, wherein a two-layer drop, i.e., a basic aqueous acceptor drop covered with a thin octanol layer, was formed at the inlet end of a separation capillary immersed into an acidic donor solution [22]. The EFs obtained in about 10 min were several hundred to thousands for various analytes [23]. However, it is difficult to preconcentrate amino acids and peptides by 3-phase SDME due to their zwitterionic properties preventing them being partitioned in the middle organic phase. Thus, by blocking the amino groups by fluorescent derivatization, amino acids and peptides having neutral or acidic side chains were preconcentrated several hundred-folds by a 3-phase SDME from an acidic donor solution to a basic acceptor drop and then analyzed by CE-LIF [24]. The 3-phase SDME technique was also successfully in-line coupled to CE-MS for the analysis of illicit drugs in urine [25].

HS-SDME is a 3-phase SDME technique involving a sample donor phase and an acceptor drop in an HS extraction arrangement and is especially advantageous for cleaning up non-volatile matrix components. Although HS-SDME has been widely adopted for various analytical techniques such as GC and LC, mostly it has been limited to off-line modes. Pranaitytė et al. applied off-line HS-SDME to CE for preconcentration and determination of ammonia in human blood, seawater, and milk [26]. Park et al. first applied this technique to CE in an in-line mode. Similar to 2-phase DI-SDME, the extraction from the HS to the acceptor drop was governed by the *D* value described in Eq. 1. Thus, a large single drop was used as an acceptor and on-line LVSEP was performed after large sample injection so that the EFs were several thousand for chlorophenols in wine, with the limit of detections (LODs) of 0.8 ppb obtained using a UV detector built in a commercial CE instrument [27].

2.2 In-tube microextraction (ITME)

Although SDME is a convenient and powerful sample pretreatment technique for CE, it often exhibits instability of the acceptor drop attached to the inlet end of the CE capillary. Careful counterbalancing of the drop shrinkage due to the surface tension should be performed by applying backpressures, especially for long extraction times. To overcome this disadvantage, ITME using a liquid plug inside the separation capillary as an acceptor phase has been recently

developed. For this method, it is not necessary to counterbalance the surface tension since no drop is attached to the capillary. Another advantage of this method is that all the extracted analytes are already inside the separation capillary. To apply ITME for HS extraction, the separation capillary filled with an acceptor plug needs to be held in the HS above the sample, the simplest possible LPME in-line coupling mode with CE. HS-ITME-CE was first used for the analysis of chlorophenols in wine [28], followed by phenol analysis in environmental samples (lake water) [29].

2.3 Liquid extraction surface analysis (LESA)

Analysis of a solid surface sample typically requires homogenization of the bulk sample into a fluid form injectable into an instrument, which may result in considerable dilution into a complicated bulk matrix and is labor and time intensive. In contrast, in LESA, a liquid microjunction is formed on the sample surface, allowing the analytes on the surface to be directly sampled without dilution and complication. LESA was demonstrated for the analysis of dyes in a spot on a thin layer chromatography plate [30]. LESA was then automated using a conductive pipette tip on a commercial robotic arm for spot sampling of various analytes from small molecules to proteins with subsequent MS analysis but without separation [31,32,33]. LESA-CE was applied for detecting pesticides on an apple's skin was demonstrated by using the capillary of a homemade CE setup [34]. A nanoliter-scale liquid microjunction was formed between the capillary tip and the apple skin, and non-infiltrative organophosphorus pesticides on the skin were directly sampled, derivatized using a fluorophore *in-capillary*, and analyzed by CE-LIF. The LODs were ~20 times lower than the tolerance limits set by the US Environmental Protection Agency. However, for a home-made CE setup, LESA-CE requires a skilled operator and long operation times (≥ 30 min) to handle liquids by gravity. Thus, an automated LESA-CE methods using commercial CE equipment with pressure controlled liquid handling was demonstrated [18]. With less manual interventions, LESA-CE was much faster and more reliable. In addition, the sampling spot diameters were reduced from 1~4 mm with the commercial pipette tip to 0.4~0.5 mm with the capillary inlet, allowing higher spatial resolutions. An obvious application of high speed and multiplexed LESA-CE would be mapping of the surface composition of biological or technical samples. Recently, LESA in-line coupled with CE/MS was demonstrated by Duncan et al. [35]. Sampling of endogenous metabolites from various (brain, spinal cord, and kidney) tissue surfaces provided chemical profiling in distinct cellular regions of tissue, critical in diagnosing the disease. Owing to the separation power of CE, isobaric and isomeric species could be differentiated. Metabolites and polypeptides from different locations of various organ surfaces were investigated for regiospecific molecular profiling of biological systems.

3. On-line electrophoretic preconcentration techniques in CE during/after sample injection

On-line approaches for electrophoretic sample preconcentration techniques in CE are based on the on-capillary manipulation of the analyte migration velocity which is the product of the analyte mobility and the electric field. The techniques applicable only to samples in a low conductivity matrix will first be discussed, and then those applicable even to samples in a high conductivity matrix. Since many biological and environmental samples are in complicated matrices of high conductivity, the latter techniques are practically more valuable. A list of representative techniques and their key features are provided in Table 1 [36,5].

Table 1 Selected electrophoretic sample preconcentration techniques used in CE. Symbols: ⊕/⊖ = cations/anions, ○ = neutrals; + = very good, o = limited, – = unsuitable

Type of electrophoretic preconcentration techniques	Analyte charge	Resistance to sample salinity	Prevailing mode of coupling	EF
FASS (field-amplified sample stacking) FESS (field-enhanced sample stacking	⊕⊖	–	on-line on-line	10^1-10^2
LVSS (large volume sample stacking)	⊕⊖	–	on-line	10^2-10^3
FASI (field-amplified sample injection) FESI (field-enhanced sample injection)	⊕⊖	–	on-line on-line	10^2-10^3
pH-driven techniques (dynamic pH junction, pH-mediated stacking)	⊕⊖	+	on-line	10^1-10^3
Sweeping	⊕⊖○	O	on-line	10^1-10^5
AFMC (analyte focusing by micelle collapse)	○	O	on-line	10^1-10^2
MSS (micelle to solvent stacking)	⊕⊖	O	on-line	10^1-10^2
ITP (isotachophoresis) tITP (transient ITP)	⊕⊖	+	off-line, in-line on-line	10^1-10^2

3.1 Electrophoretic preconcentration techniques for samples in low conductivity matrices

Sometimes, analytes are dissolved in a dilute matrix (e.g., analysis of drinking water impurities) and the injected sample constituents are focused after turning on a separation electric potential. In such a case, the increased electric field in the low conductivity sample zone causes the analyte ions to stack (concentrate) into narrow zones at the (front and rear, depending on the charges) boundaries with higher conductivity background electrolyte (BGE) zones. This process is called field amplified (or enhanced) sample stacking (FASS or FESS) or simply stacking. To stack a long plug of sample to achieve high preconcentration without losing the separation quality, the sample matrix must be removed before the separation step. The matrix removal can be carried out either via polarity switching [37] or EOF pumping in an automatic manner [38]. These techniques called as large volume sample stacking (LVSS) can provide EFs of up to several hundreds. The latter automatic mode of LVSS called as LVSEP is obviously more convenient since it does not require current monitoring to switch the polarity of the electric potential but the electroosmotic mobility has to be reduced below the magnitudes of the analyte mobilities. The electroosmotic mobility can be reduced via several ways such as using a coated capillary [39,40,41] or a bare capillary employing EOF modifiers [42], very low BGE pH [43], pH hysteresis [44], or nonaqueous CE with a methanol BGE [45].

In field amplified (or enhanced) sample injection (FASI or FESI), the preconcentration of a low conductivity sample is induced by the sudden migration velocity drop during electrokinetic injection (EKI). Since a large-volume sample solution of low conductivity can be introduced by EKI, FASI is superior over FASS or LVSS in terms of EF values. However, the sampling bias depending on the analyte mobilities must be considered for quantification.

To be more accurate, the requirement of "low conductivity matrix" for the above sample preconcentration techniques should be "lower conductivity sample than that of BGE". The maximum EF of sample preconcentration techniques based on field amplification is given by the conductivity ratio between the BGE and the sample. Then by using a BGE or a short plug of BGE of very high conductivity, a sample in a moderate conductivity matrix can be

preconcentrated [46].

3.2 Electrophoretic preconcentration techniques for samples in high conductivity matrices

The on-line preconcentration techniques described above are applicable only to samples of low conductivity. However, in practice, preconcentration of high conductivity samples is often urgently needed.

3.2.1 Preconcentration by effective mobility changes; dynamic pH junction, sweeping, analyte focusing by micelle collapse (AFMC), and micelle to solvent stacking (MSS)

The sample preconcentration techniques in this section achieve LODs that are at least one or two orders of magnitude lower than those of conventional CE by manipulating the effective mobilities of analytes (Table 1). Dynamic pH junction is a technique wherein a pH gradient between the sample and the BGE zones is generated [47]. Thus, the ionization status of the analytes entering the BGE zone changes, resulting in modified analyte electrophoretic mobilities. It was first reported by Aebersold's group in 1990 [48], and named as dynamic pH junction by Britz-McKibbin and Chen in 2000 [49]. It has been employed for many proteomics and metabolomics researches [50,51,52,53]. This technique could be easily coupled with electrospray ionization MS, and allowed to obtain 200-400 fold higher peak heights while compared to conventional injections for real samples analysis. While applying this preconcentration technique the time-consuming off-line pre-concentration could be omitted, which was recently proved for bacteria analysis by Yan and coworkers [54]. Furthermore, the proteome coverage could be improved together with enhanced robustness and reproducibility while pH junction-CE-MS hyphenated techniques are applied since dynamic pH junction could improve greatly the loading capacity of CE. As an example, the experiments carried out on the complex mouse brain proteome digest analysis by Chen et al could be mentioned [55]. The data presented by Chen et al. suggested that the presented dynamic pH junction based CE-MS system could be successfully applied even for the large-scale bottom-up proteomics. Not only proteomics takes benefits from the dynamic pH junction supported CE-MS-based methods, but also metabolomics. This automated sample enrichment, separation and detection procedure was applied for the low-abundance metabolite analyses and their identification with nanomolar detection limits in biological matrices [53]. In here also the combination of two on-line stacking methods was presented: transient cation isotachophoresis with dynamic pH junction for enhancing the detection of zwitterionic amino acids and other cationic species. Additional techniques include sweeping, analyte focusing by micelle collapse (AFMC), and micelle to solvent stacking (MSS), which are based on analyte interactions with a micellar phase. Sweeping is applied for neutral as well as charged analytes, AFMC for neutral analytes, and MSS for charged analytes (Table 1). Sweeping is based on the accumulation of analytes in a micelle-free matrix at the front of a pseudo-stationary phase composed of micelles, which penetrates the injected sample zone [56]. AFMC occurs when the sample is prepared in a micellar matrix slightly above the critical micelle concentration (CMC) containing a high conductivity electrolyte salt. Micelles in the sample zone transport, release, and accumulate the bound analytes in the micelle dilution zone between the sample and BGE plugs. Analyte release is facilitated by dilution of the surfactant below its CMC, causing micelle collapse [57]. By using a high conductivity solvent containing SDS micelles to form a liquid microjunction, LESA and AFMC were synergistically coupled for the analysis of neutral pesticides on apple skin. The micellar solvent not only improved the surface extraction efficiency but also enabled on-line preconcentration of the injected extract by AFMC [18]. MSS is performed via reversal of the electrophoretic mobility of charged analytes at

the boundary stacking zone between the micelle- and organic solvent-rich zones [58]. All the techniques discussed in this section are suitable for biological samples in high conductivity matrices and thus constitute great utilities for biomedical studies.

3.2.2 Preconcentration with electric field changes; pH-mediated stacking, isotachophoresis (ITP) and transient isotachophoresis (tITP)

pH-mediated stacking leads to the on-line transformation of a high conductivity sample matrix (such as in the case of the biological sample matrices) to a low-conductivity sample matrix which requires only one additional injection step following the sample injection. A plug of high conductivity sample and then an additional plug to neutralize the sample plug are (usually electrokinetically) injected. It has been applied to the analyses of various samples including biological samples [59,60,61].

ITP is typically performed under a suppressed EOF condition using a discontinuous electrolyte system wherein a sample is injected between the leading (LE) and a terminating (TE) electrolytes. While the LE must contain a co-ion (leading ion; L) with higher effective electrophoretic mobility than those of the sample ions, the opposite is true for the TE co-ion (T). When the separation current is applied, after a short time, the sample constituents are arranged into distinct individual zones adjacent each other in decreasing order of their effective mobilities. After completing the zone arrangement, all the zones move at the same migration velocity (steady state ITP). It implies that the electric field of each zone is inversely proportional to the mobility of each constituent. Since the concentration of L (most commonly between 10 and 20 mM) is selected to be much higher than the analyte concentrations, sample band compression always occurs due to the high electric potential drop over the analyte zone according to the Kohlrausch regulating function as described in Eq. 2 (for more details, please refer to [62]):

$$c_a = c_L \frac{\mu_a\left(\mu_L + \mu_C\right)}{\mu_L\left(\mu_a + \mu_C\right)} \quad \text{(eq 2)}$$

where c_a is the steady-state analyte concentration, c_L is the concentration of L, and μ_a, μ_L, and μ_C are the effective mobilities of the analyte, L, and the counter-ion C, respectively. If a trace analyte is present in a concentration too low for the creation of its own regular zone, the analyte is incorporated into the boundary between adjacent fully developed zones, i.e., acts as a pseudo-displacer. In this case (spike ITP mode) the quantitation is based on the peak height or peak area instead of the zone length. Alternatively, the in-line coupling of ITP-CZE in a coupled column arrangement can be used to improve the resolution of trace components. This combination of ITP in the first column to focus the sample into an ideal injection zone (transfer of focused analytes) and CZE in the second column is a common feature in commercial ITP instruments.

A popular form of ITP is transient ITP (tITP), wherein the analytes are first concentrated by ITP and subsequently separated by CE in a single capillary. One scheme of tITP is placing a sample plug dissolved in TE between the two LE zones (BGE in CE). Initially, analytes are focused in the transiently formed LE/sample/TE arrangement at the sample plug front. As the faster L ions overpass the T ions, focusing ceases and the concentrated analytes undergo the CE separation process. Various tITP schemes have been developed [63] and are now widely used since they can be easily implemented with conventional CE instruments using a single capillary [34,64,65,66,67,68]. For real sample analyses, however, the influences of the L or T ions pre-existing in the sample matrix should also be considered [69].

While the early works on ITP used a capillary format, microfluidics chip electrophoresis (MCE)

methods developed in the 1990's [70,71] have been utilized for ITP as well [72,73]. All aspects of MCE meet the demands of green analytical chemistry [74]. However, MCE is often limited due to the high complexity of real matrices, and/or trace concentrations of target analytes in highly saline samples. The short separation channels of MCE devices require injection of simplified samples, while the reduced dimensions of chip channels impose heavy demands on the detection sensitivity. The on-line coupling of various electrophoretic techniques is an effective scheme to integrate sample pretreatment with MCE separations for analyses of complex biological and environmental samples. The first in-line sample pretreatment by combining electrophoretic techniques (ITP and CE) in a chip format was reported by Kaniansky et al. [72]. Small ions, as well as proteins and DNA fragments, can be preconcentrated by ITP [75]. The benefits of ITP-CE in a chip format were reviewed by Smejkal et al. [64]. Jeong et al. [76] described a sensitive tITP-CE method for fluorescein and 2,7-dichlorofluorescein in a highly saline matrix using a polydimethylsiloxane chip with LIF detection. Jung et al. [77] obtained a high EF (2×10^6) from tITP-CE of Alexa Fluor 488 on a borosilicate glass microfluidic chip. Selected proteins were detected in a human urine sample using tITP-CE with UV detection [78]. UV transparent quartz was used to construct microfluidic chips for ITP-CE of quercetin and isorhamnetin [79]. A polymethylmethacrylate chip with coupled separation channels was used for determination of trace nitrite and nitrate, indicators of various neurological diseases, in a cerebrospinal fluid [80]. Complex samples were cleaned up off-line by micro solid-phase extraction (MSPE) prior to ITP-CE analysis. LODs of 3 and 6 nM for nitrate and nitrite, respectively, were achieved using a conductivity detector by large-volume injection (9.9 μL), exceeding the total volume of both separation channels (see Figure 2). Combination of tITP with other sample pre-concentration techniques such as SPME has been shown to even greater improve the sensitivity. Even the problem of band broadening processes observed while SPME is planned to be on-line coupled with CE could be successfully minimalized, which was shown by Wang et al in 2012 [81]. Also, further studies dealing with the in-line combination of commonly used in off-line mode techniques (such as SPE or strong cation exchange (SCX) adsorbents) with CE were carried out. Experiments carried out by Zhang et al. in 2016 proved that the in-line combinations are much less time consuming as well provide the excellent coverage of relevant analytes in the biological samples [82]. Also, the SPE was successfully coupled in-line with CE [83]. Ref. [83] reviewed several CE–ESI–MS methods with in-line SPE microcartridges for the biological analyses such as determination of opioid peptides and neuropeptides in plasma samples [84,85] or in-line SPE-CE with ion-trap (IT) MS detection for analysis of peptides in cerebrospinal fluid [86].

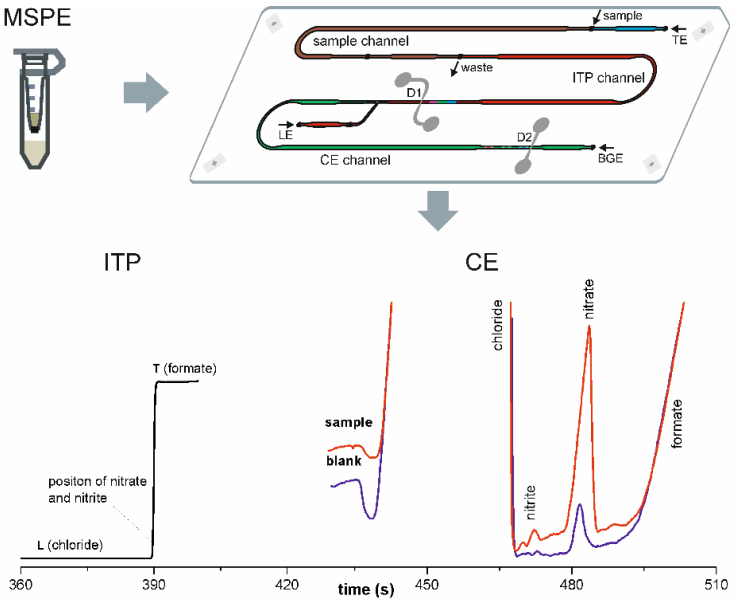


Figure 2. MSPE-ITP-CE analysis of a cerebrospinal fluid sample on a microchip. D1, D2 = conductivity sensors for ITP and CE, respectively. From Ref. [80], with permission.

Recently, the ITP technology has moved towards (micro)preparative purification of nucleic acids [87]. Only small differences exist between the electrophoretic mobilities of short and large nucleic acids. Thus, all DNA fragments, regardless of their sizes, can be focused into a single zone between LE and TE [88]. Kondratova et al. described the ITP method of concentration and isolation of DNA in agarose gel rods [89]. Later developments involve the use of microfluidic devices, as pioneered by Santiago et al. [90]. Thus, ITP is a promising sample pretreatment technique for nucleic acids providing lower losses of trace analytes and EFs of several orders of magnitude [91, 92, 93,94], in contrast to current techniques such as SPE. Applications include DNA purification, extraction, fractionation, and extraction from single cells and/or hybridization. Figure 3 shows a microfabricated device which allowed for ITP focusing of a 50-µL sample volume into a 500-pL zone. The device, composed of Sylgard 184 polydimethylsiloxane resin, achieved highly sensitive detection of both molecular targets (DNA, at 10 fM) and whole bacteria (at 100 cfu/mL).

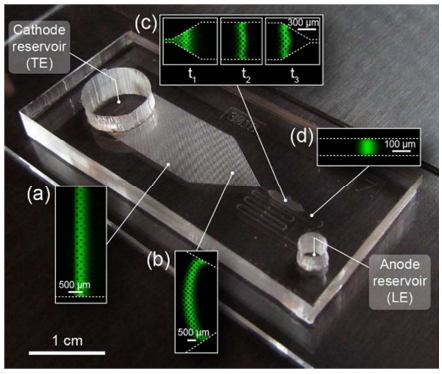


Figure 3. Photograph of a large-volume ITP device. The insets show focusing of a fluorescent

dye. (a) The analyte first focuses uniformly across the entire width of an 8 mm wide region. (b) The wide region tapers down to a width of 100 μm, providing additional geometrical focusing. (c) An intermediate chamber geometry enables smooth refocusing of the interface from the dispersed state. (d) In the final narrow region, the ITP interface rapidly achieves a steady focused state before detection. From Ref. [92], with permission.

4. Sample pretreatment by integrated immobilized enzyme microreactors

Immobilized enzyme reactors are promising tools for bioanalytical research and the applications. These microreactors are complementary to the sample pretreatment techniques described above. Most "-omics" fields utilize immobilized enzymes because of their improved stability and higher resistance against denaturation. Miniaturization of these immobilized enzyme reactors features increased surface-to-volume ratios and concomitantly decreased diffusion limitation, allowing for the development of rapid and high-yield sample pretreatment tools. The fundamentals, advantages, and disadvantages of the technology were well summarized in recent reviews [95, 96], and only recent trends in the field will be discussed.

Enzyme reactors and microcolumns are classified based on the applied immobilization techniques, i.e., entrapment, adsorption, covalent bonding, affinity, or cross-linking and by considering the types of solid support such as micro- and nanoparticles, porous polymer monoliths, membranes, and paramagnetic nanoparticles as well as the inner wall of the reactors/capillaries [97].

Despite the significant progress in microfluidics-based immobilized enzyme reactors (μIMERs), most techniques involve the random immobilization of enzymes on the chromatographic stationary phases via covalent bonds between the functional groups of the stationary phase and the amino groups of the enzyme (including amino groups at active and inactive sites). Therefore, this “general” immobilization approach may result in reduced enzyme activity due to structural deformation induced by covalent bonding or decreasing substrate availability to the active sites caused by steric hindrance (if amino groups in the active sites are involved in the immobilization) [73]. Application of spacers increases the distance between the solid surface and the immobilized enzyme, alleviating difficulties caused by steric hindrance [98]. A more sophisticated approach to limit steric hindrance is orienting immobilization with specially tagged enzymes. His_6 and glutathione-S-transferase are the most commonly used tags with affinities towards nickel and glutathione, respectively. Oriented immobilization reportedly provides higher activity and specificity for the immobilized enzymes [99].

Other trends in sample pretreatment by μIMER include the utilization of graphene oxide (GO), gold nanoparticles (GNPs), and magnetic nanoparticles [100] aiming for the improvement of robustness, repeatability, and hydrophilicity [96]. GO contains numerous oxygen moieties such as epoxy, carboxyl, hydroxyl, and carbonyl groups, and is an ideal supporting material that can be subjected to various chemical modifications. Enzymes can be conjugated to GNPs via thiol groups resulting in very stable immobilized systems. The very high surface-to-volume ratios and narrow size-distributions of GOs and GNPs make them promising packing materials for μIMERs [101,102].

Monoliths are innovative alternatives to conventional particle-based solid support materials and the main advantages of using monoliths in μIMERs are as follows: 1) fast mass transfer due to decreased diffusion resistance; 2) relatively low backpressure owing to the flow-through pores; and 3) simple fabrication using in situ copolymerization (no packing issues). Monolithic type

supports can be categorized into three main classes, such as organic, inorganic, and hybrid. Conventionally used techniques for monolith fabrication include thermal- and photo-initiated polymerization, as well as polycondensation as thoroughly described in a comprehensive review by Svec [103]. Another novel approach for monolith formation is cryogelation which provides large pores ensuring excellent hydrodynamic properties of the monolith. Finally, immobilized enzyme microcolumns (IEM) represent another new trend for microreactors. Szigeti et al. recently reported the oriented immobilization of the endoglycosidase, PNGaseF, on microcolumns for glycomics application [104]. Although IEMs have been used for decades, they have experienced a renaissance since this approach can be readily applied to automated sample pretreatment systems, including liquid handling robots. Since the processivity of the μIMERs is very high compared to the sample amounts separated in CE, they can be coupled in-line. For example, proteins separated by CE can be digested into peptides during their transition from the CE capillary through an extended electrospray needle containing surface immobilized peptidase [105]. Along these lines, integrated and automated workflows are identified as the next promising trends of the field. The Dovichi group is one of the pioneers in integrated and automated techniques hyphenating single or multidimensional CE separations with μIMERs for protein characterization [106,107,108,109].

5. Conclusions

A plethora of electromigration-based approaches have recently been developed allowing for more detailed analytical and biomedical studies, diagnostics, and monitoring of diseases as well as for the evaluation of novel therapeutic compounds. These methods are becoming extremely important for achieving lower LODs of many analytes often present in trace amounts in biological matrices. Combining CE with *in-line* or/and *on-line* sample pretreatment techniques represents a powerful tool for bioanalytical laboratories. Compared to widespread SPE techniques, electromigration techniques do not rely on surface interactions or disposable parts, which may minimize analyte losses in samples containing large biopolymers. In addition, electromigration techniques can be easily automated and combined with on-column detection and fractional collection. Moreover, with a proper selection of electrolyte buffers, a universal instrument can be operated in several modes. While μIMERs are not electrodriven sample pretreatment tools, they can significantly contribute to the improved analytical performance of CE-based separations methods. In-line coupled μIMERs reduce sample loss, increase enzyme stability, and improve productivity, which are key aspects of bioanalytical methods.
We envision that electromigration based sample pretreatment techniques will further emerge, providing a particularly important toolset for researchers in the bioanalytical, biomedical, and clinical diagnostics fields.

Acknowledgments

The authors gratefully acknowledge the support of the MTB Korea V4 joint project from the following sources: National Center for Research and Development in Poland (DZP/V4-Korea-I/20/2018), National Research, Development and Innovation Office of Hungary (K116263, NN127062, 2018-2.1.17-TÉT-KR-2018-00010), Hungarian Government (Bionano_GINOP-2.3.2-15-2016-00017), Hungarian Academy of Sciences (János Bolyai Research Scholarship), ÚNKP-19-4 New National Excellence Program of The Ministry for Innovation and Technology, Project of regional collaboration V4-Korea MTB 8F17003 administered by MSMT with

additional support from European Regional Development Fund-Project „SINGING PLANT“ (No.CZ.02.1.01/0.0/0.0/16_026/0008446), Institute of Analytical Chemistry Czech Academy of Sciences (RVO 68081715), National Research Foundation of Korea (NRF-2018K1A3A1A39088119, NRF-2017K1A3A1A67016126, NRF-2016R1A2B2011878), and Slovak Academy of Sciences (APVV-17-0318, VEGA/1/0787/18, APVV SK-KR-18-0013).

References

[1] Z. Niu, W. Zhang, C. Yu, J. Zhang, Y. Wen, Recent advances in biological sample preparation methods coupled with chromatography, spectrometry and electrochemistry analysis techniques, Trend. Anal. Chem. 102 (2018) 123-146.

[2] F. Kitagawa, K. Otsuka, Recent applications of on-line sample preconcentration techniques in capillary electrophoresis, J. Chromatogr. A 1335 (2014) 43-60.

[3] M.C. Breadmore, A. Wuethrich, F. Li, S.C. Phung, U. Kalsoom, J.M. Cabot, M. Tehranirokh, A.I. Shallan, A.S. Abdul Keyon, H.H. See, M. Dawod, J.P. Quirino, Recent advances in enhancing the sensitivity of electrophoresis and electrochromatography in capillaries and microchips (2014-2016), Electrophoresis 38 (2015) 33-59.

[4] Z.B. Zhang, Y.Y. Qu, N.J. Dovichi, Capillary zone electrophoresis-mass spectrometry for bottom-up proteomics. Trends Anal. Chem. 108 (2018) 23-37

[5] M.C. Breadmore, C.E. Sänger-van de Griend, In-capillary sample concentration in CE - "This is my analyte, how do I stack?", LC GC N. Am. 32 (2014) 174-186.

[6] J.R. Veraart, H. Lingerman, U.A.Th. Brinkman, Coupling of biological sample handling and capillary Electrophoresis, J. Chromatogr. A 856 (1999) 483-514.

[7] L. Arce, L. Nozal, B.M. Simonet, M. Valcárcel, A. Rios, Liquid phase microextraction techniques for simplifying sample treatment in capillary electrophoresis, Trend. Anal. Chem. 28 (2009) 842-853.

[8] R. Ramautar, G.W. Somsen, G.J. de Jong, Developments in coupled solid-phase extraction-capillary electrophoresis 2013-2015, Electrophoresis 37 (2016) 35-44.

[9] J. An, M.J. Trujillo-Rodriguez, V. Pino, J.L. Anderson, Non-conventional solvents in liquid phase microextraction and aqueous biphasic systems, J. Chromatogr. A 1500 (2017) 1-23.

[10] S.K. Kailasa, H.-F. Wu, Single-drop microextraction for bioanalysis: present and future, Bioanalysis 5 (2013) 2593-2596.

[11] N. Miekus, I. Oledzka, A. Plenis, P. Kowalski, E. Bien, A. Miekus, M.A. Krawczyk, E. Adamkiewicz-Drozynska, T. Baczek, Determination of urinary biogenic amines' biomarker profile in neuroblastoma and pheochromocytoma patients by MEKC method with preceding dispersive liquid-liquid microextraction, J. Chromatogr. B 1036-1037 (2016) 114-123.

[12] H. Piri-Moghadam, M.N. Alam, J. Pawliszyn, Review of geometries and coating materials in solid phase microextraction: opportunities, limitations, and future perspectives, Anal. Chim. Acta 984 (2017) 42-65.

[13] N. Reyes-Garces, E. Gionfriddo, G.A. Gomez-Rios, M.N. Alam, E. Boyaci, B. Bojko, V. Singh, J. Grandy, J. Pawliszyn, Advances in solid phase microextraction and perspective on future directions, Anal. Chem. 90 (2018) 302-360.

[14] M. Huq, M. Tascon, E. Nazdrajic, A. Roszkowska, J. Pawliszyn, Measurement of free drug concentration from biological tissue by solid-phase microextraction: In-silico and experimental study, Anal. Chem. 91 (2019) 7719-7728.

[15] A. Oedit, R. Ramautar, T. Hankemeier, P.W. Lindenburg, Electroextraction and electromembrane extraction: Advances in hyphenation to analytical techniques, Electrophoresis 37 (2016) 1170-1186.

[16] D. Fuchs, C.R. Hidalgo, M.R. Payan, N.J. Petersen, H. Jensen, J.P. Kutter, S. Pedersen-Bjergaard, Continuous electromembrane extraction coupled with mass spectrometry–perspectives and challenges, Anal. Chim. Acta 999 (2018) 27-36.

[17] K. Choi, S.J. Kim, Y.G. Jin, Y.O. Jang, J.-S. Kim, D.S. Chung, Single drop microextraction using commercial capillary electrophoresis instruments, Anal. Chem. 81 (2009) 225-230.
[18] S. Jeong, F. Shakerian, D.S. Chung, Analyte focusing by micelle collapse for liquid extraction surface analysis coupled with capillary electrophoresis of neutral analytes on a solid surface, Electrophoresis 40 (2019) 2463-2468.
[19] M.D Víctor-Ortega, F.J. Lara, A.M. García-Campaña, M. Olmo-Iruela, Evaluation of dispersive liquid–liquid microextraction for the determination of patulin in apple juices using micellar electrokinetic capillary chromatography, Food Control 31 (2013) 353-358.
[20] K. Choi, S.J. Kim, Y.G. Jin, Y.O. Jang, J.-S. Kim, D.S. Chung, Single drop microextraction using commercial capillary electrophoresis instruments, Anal. Chem. 81 (2009) 225-230.
[21] J. Kim, K. Choi, D.S. Chung, Synergistic coupling of in-line single drop microextraction and on-line large volume sample stacking for capillary electrophoresis/mass spectrometry, Anal. Bioanal. Chem. 411 (2019) 1067-1073.
[22] K. Choi, Y. Kim, D.S. Chung, Liquid-phase microextraction as an on-line preconcentration method in capillary electrophoresis, Anal. Chem. 76 (2004) 855-858.
[23] Z.A. AlOthman, M. Dawod, J. Kim, D.S. Chung, Single drop microextraction as a powerful pretreatment tool for capillary electrophoresis: A review, Anal. Chim. Acta 739 (2012) 14-24.
[24] Y.K. Park, K. Choi, A.Y.B.H. Ahmed, Z.A. ALOthman, D.S. Chung, Selective preconcentration of amino acids and peptides using single drop microextraction in-line coupled with capillary electrophoresis, J. Chromatogr. A 1217 (2010) 3357-3361.
[25] J. Kim, K. Choi, D.S. Chung, In-line coupling of single-drop microextraction with capillary electrophoresis-mass spectrometry, Anal. Bioanal. Chem. 407 (2015) 8745-8752.
[26] B. Pranaitytė , S. Jermak, E. Naujalis, A. Padarauskas, Capillary electrophoretic determination of ammonia using headspace single-drop microextraction, Microchem. J. 86 (2007) 48-52.
[27] S.T. Park, J. Kim, K. Choi, H.R. Lee, D.S. Chung, Headspace-single drop microextraction with a commercial capillary electrophoresis instrument, Electrophoresis 33 (2012) 2961-2968.
[28] H.R. Lee, S.M. Cho, J. Kim, D.S. Chung, Novel and simple headspace in-tube microextraction coupled with capillary electrophoresis, J. Chromatogr. A 1346 (2014) 117-122.
[29] M.-E. Yue, Q. Lin, J. Xu, T.-F. Jiang, Ionic liquid-based headspace in-tube liquid-phase microextraction coupled with CE for sensitive detection of phenols, Electrophoresis 39 (2018) 1771-1776.
[30] G.J. Van Berkel, A.D. Sanchez, J.M.E. Quirke, Thin-layer chromatography and electrospray mass spectrometry coupled using a surface sampling probe, Anal. Chem. 74 (2002) 6216-6223.
[31] V. Kertesz, G.J. Van Berkel, Fully automated liquid extraction-based surface sampling and ionization using a chip-based robotic nanoelectrospray platform, J. Mass Spectrom. 45 (2010) 252-260.
[32] D. Eikel, M. Vavrek, S. Smith, C. Bason, S. Yeh, W.A. Korfmacher, J.D. Henion, Liquid extraction surface analysis mass spectrometry (LESA□MS) as a novel profiling tool for drug distribution and metabolism analysis: the terfenadine example, Rapid Commun. Mass Spectrom. 25 (2011) 3587-3596.
[33] E.C. Randall, J. Bunch, H.J. Cooper, Direct analysis of intact proteins from Escherichia coli colonies by liquid extraction surface analysis mass spectrometry, Anal. Chem. 86 (2014) 10504-10510.
[34] I.H. Sung, Y.W. Lee, D.S. Chung, Liquid extraction surface analysis in-line coupled with capillary electrophoresis for direct analysis of a solid surface sample, Anal. Chim. Acta 838 (2014) 45-50.
[35] K.D. Duncan, I. Lanekoff, Spatially defined surface sampling capillary electrophoresis mass spectrometry, Anal. Chem. 91 (2019) 7819-7827.
[36] S. Hamidi, A. Jouyban, Pre-concentration approaches combined with capillary electrophoresis in bioanalysis of chiral cardiovascular drugs, J. Pharm. Sci. 21 (2015) 229-243.
[37] J.F. Flores-Aguilar, L.C. Medrano, E. Perez-Escalante, J.A. Rodriguez, R.L. Camacho-Mendoza, I.S. Ibarra, Large-volume sample stacking with polarity switching for analysis of azo dyes in water samples by capillary electrophoresis, Int. J. Environ. An. Ch. (2019) DOI: 10.1080/03067319.2019.1618461.

[38] F. Kitagawa, O. Osanai, I. Nukatsuka, LVSEP analysis of cationic analytes in non-aqueous capillary electrophoresis, Chromatography (2019) DOI: 10.15583/jpchrom.2019.008.
[39] J.H. Lee, O.-K. Choi, H.S. Jung, K.-R. Kim, D.S Chung, Capillary electrophoresis of nonprotein and protein amino acids without derivatization, Electrophoresis 21 (2000) 930-934.
[40] M.-S. Chun, D.S. Chung, Large volume sample stacking in capillary electrophoresis of weakly acidic compounds using coated capillaries at high pH, Anal. Chim. Acta 491 (2003) 173-179.
[41] M.-S. Chun, D. Kang, Y. Kim, D.S. Chung, Protein analysis with large volume sample stacking with an electroosmotic flow pump: a potential approach for proteomics, Michrochem. J. 70 (2001) 247-253.
[42] M. Albert, L. Debusschere, C. Demesmay, J.L. Rocca, Large-volume stacking for quantitative analysis of anions in capillary electrophoresis II. Large-volume stacking without polarity switching, J. Chromatogr. A 757 (1997) 291-296.
[43] Y. He, H.K. Lee, Large-volume sample stacking in acidic buffer for analysis of small organic and inorganic anions by capillary electrophoresis, Anal. Chem. 71 (1999) 995-1001.
[44] J.H. Han, M.-S. Chun, A. Riaz, D.S. Chung, Large-volume stacking in capillary electrophoresis using pH hysteresis of the electroosmotic flow in a bare fused-silica capillary, Electrophoresis 26 (2005) 480-486.
[45] B. Kim, D.S. Chung, Large-volume stacking in capillary electrophoresis using a methanol run buffer, Electrophoresis 23 (2002) 49-55.
[46] T. Kawai, N. Ota, A. Imasato, Y. Shirasaki, K. Otsuka, Y. Tanaka, Profiling of *N*-linked glycans from 100 cells by capillary electrophoresis with large-volume dual preconcentration by isotachophoresis and stacking, J. Chromatogr. A 1565 (2018) 138-144.
[47] L. Wang, D. MacDonald, D.D. Chen, Capture efficiency of dynamic pH junction focusing in capillary electrophoresis, Electrophoresis 37 (2016) 1143-50.
[48] R. Aebersold, H.D. Morrison, Analysis of dilute peptide samples by capillary zone electrophoresis, J. Chromatogr. 516 (1990) 79-88.
[49] P.Britz-McKibbin, D.D.Y. Chen, Selective focusing of catecholamines and weakly acidic compounds by capillary electrophoresis using a dynamic pH junction, Anal. Chem. 72 (2000) 1242-1252.
[50] P. Yan, K. Zhang, L. Wang, W. Tong, D.D. Chen, Quantitative analysis of microcystin variants by capillary electrophoresis mass spectrometry with dynamic pH barrage junction focusing, Electrophoresis (2019) doi:10.1002/elps.201900042
[51] D.D. Chen, X. Shen, L. Sun, Capillary zone electrophoresis-mass spectrometry with microliter-scale loading capacity, 140 min separation window and high peak capacity for bottom-up proteomics, Analyst 142 (2017) 2118-2127.
[52] G. Zhu, L. Sun, N.J. Dovichi, Dynamic pH junction preconcentration in capillary electrophoresis-electrospray ionization-mass spectrometry for proteomics analysis, Analyst 141 (2016) 5216-5220.
[53] R. Lee, A.S. Ptolemy, L. Niewczas, P. Britz-McKibbin, Integrative Metabolomics for Characterizing Unknown Low-Abundance Metabolites by Capillary Electrophoresis-Mass Spectrometry with Computer Simulations, Anal. Chem. 79 (2007) 403-415.
[54] P. Yan, K. Zhang, L. Wang, W. Tong, D.D. Chen, Quantitative analysis of microcystin variants by capillary electrophoresis mass spectrometry with dynamic pH barrage junction focusing, Electrophoresis (2019) doi:10.1002/elps.201900042
[55] D.D. Chen, X. Shen, L. Sun, Capillary zone electrophoresis-mass spectrometry with microliter-scale loading capacity, 140 min separation window and high peak capacity for bottom-up proteomics, Analyst 142 (2017) 2118-2127.
[56] J.P. Quirino, S. Terabe, Exceeding 5000-fold concentration of dilute analytes in micellar electrokinetic chromatography, Science 282 (1998) 465-468.
[57] J.P. Quirino, P.R. Haddad, Online sample preconcentration in capillary electrophoresis using analyte focusing by micelle collapse, Anal. Chem. 80 (2008) 6824-6829.

[58] J.P. Quirino, Micelle to solvent stacking of organic cations in capillary zone electrophoresis with electrospray ionization mass spectrometry, J. Chromatogr. A 1216 (2009) 294-299.
[59] S.D. Arnett, C.E. Lunte, Investigation of the mechanism of pH-mediated stacking of anions for the analysis of physiological samples by capillary electrophoresis, Electrophoresis 24 (2003) 1745-1752.
[60] X. Zhong, L. Hao, J. Lu, H. Ye, S.-C. Zhang, L. Li, Quantitative analysis of serotonin secreted by human embryonic stem cells-derived serotonergic neurons via pH-mediated online stacking-CE-ESI-MRM, Electrophoresis 37 (2016) 1027-1030.
[61] J.A. Gillogly, C.E. Lunte, pH-mediated acid stacking with reverse pressure for the analysis of cationic pharmaceuticals in capillary electrophoresis, Electrophoresis 26 (2005) 633-639.
[62] F.M. Everaerts, J.L. Beckers, T.P.E.M. Verheggen (Eds.), Isotachophoresis: theory, instrumentation and applications (Journal of Chromatography Library, Vol. 6), Elsevier, Amsterdam, 1976.
[63] T. Hirokawa, H. Okamoto N. Ikuta, B. Gas, Optimization of operational modes for transient isotachophoresis preconcentration-CZE, Anal. Sci. 17 (2001) i185-i188.
[64] P. Smejkal, D. Bottenus, M.C. Breadmore, R.M. Guijt, C.F. Ivory, F. Foret, M. Macka, Microfluidic isotachophoresis: A review, Electrophoresis 34 (2013) 1493-1509.
[65] F. Foret, E. Szoko, B.L. Karger, Trace analysis of proteins by capillary zone electrophoresis with on-column transient isotachophoretic preconcentration, Electrophoresis 14 (1993) 417-428.
[66] I. Valaskova, E. Havranek, Isotachophoretic analysis of inorganic ions, J. Chromatogr. A 836 (1999) 201-208.
[67] F. Kvasnicka, Application of isotachophoresis in food analysis, Electrophoresis 21 (2000) 2780-2787.
[68] Q. Zhu, G.K.E. Scriba, Analysis of small molecule drugs, excipients and counter ions in pharmaceuticals by capillary electromigration methods–recent developments, J. Pharm. Biomed. Anal. 147 (2018) 425-438.
[69] A. Riaz, D.S. Chung, Calibration of migration times of variable salinity samples with internal standards in capillary electrophoresis, Electrophoresis 27 (2006) 553-562.
[70] A. Manz, D.J. Harrison, E.M.J. Verpoorte, J. Fettinger, A. Paulus, H. Ludi, H.M. Widmer, Planar chips technology for miniaturization and integration of separation techniques into monitoring systems: capillary electrophoresis on a chip, J. Chromatogr. A 593 (1992) 253-258.
[71] L. Lin, J.-M. Lin, Design and preparation of microfluidics device, in: J.-M. Lin (Ed.), Cell analysis on microfluidics, Springer Nature, Singapore, 2018.
[72] D. Kaniansky, M. Masár, J. Bielčíková, F. Iványi, F. Eisenbeiss, B. Stanislawski, B. Grass, A. Neyer, M. Jöhnck, Capillary electrophoresis separations on a planar chip with the column-coupling configuration of the separation channels, Anal. Chem. 72 (2000) 3596-3604.
[73] D. Kaniansky, M. Masár, R. Bodor, M. Žúborová, E. Ölvecká, M. Jöhnck, B. Stanislawski, Electrophoretic separations on chips with hydrodynamically closed separation systems, Electrophoresis 24 (2003) 2208-2227.
[74] N. Szczepańska, M. Rutkowska, K. Owczarek, J. Płotka-Wasylka, J. Namieśnik, Main complications connected with detection, identification, and determination of trace organic constituents in complex matrix samples, Trends Anal. Chem. 105 (2018) 173-184.
[75] T. Hirokawa, Y. Takayama, A. Arai, Z. Xu, Study of a novel sample injection method (floating electrokinetic supercharging) for high-performance microchip electrophoresis of DNA fragments, Electrophoresis 29 (2008) 1829-1835.
[76] Y. Jeong, K. Choi, M.K. Kang, K. Chun, D.S. Chung, Transient isotachophoresis of highly saline samples using a microchip, Sensor Actuat. B-Chem. 104 (2005) 269-275.
[77] B. Jung, R. Bharadwaj, J.G. Santiago, On-chip millionfold sample stacking using transient isotachophoresis, Anal. Chem. 78 (2006) 2319-2327.
[78] R. Wu, W.S.B. Yeung, Y.-S. Fung, 2-D t-ITP/CZE determination of clinical urinary proteins using a microfluidic-chip capillary electrophoresis device, Electrophoresis 32 (2011) 3406-3414.

[79] B. Ma, X. Zhou, G. Wang, H. Huang, Z. Dai, J. Qin, B. Lin, Integrated isotachophoretic preconcentration with zone electrophoresis separation on a quartz microchip for UV detection of flavonoids, Electrophoresis 27 (2006) 4904-4909.
[80] M. Masár, R. Bodor, P. Troška, Microchip capillary electrophoresis of nitrite and nitrate in cerebrospinal fluid, in: A.V. Schepdael (Ed.), Microchip capillary electrophoresis protocols, methods in molecular biology, Springer Science, New York, 2015.
[81] Y. Wang, B.R. Fonslow, C.C.L. Wong, A. Nakorchevsky, J.R. Yates III, Improving the comprehensiveness and sensitivity of sheathless CE-MS/MS for proteomic analysis, Anal. Chem. 84 (2012) 8505-8513.
[82] Z. Zhang, L. Sun, G. Zhu, O.F. Cox, P.W. Huber, N.J. Dovichi, Nearly 1000 Protein Identifications from 50 ng of Xenopus laevis Zygote Homogenate Using Online Sample Preparation on a Strong Cation Exchange Monolith Based Microreactor Coupled with Capillary Zone Electrophoresis, Anal. Chem. 88 (2016) 877-882.
[83] I. Kohler, J. Schappler, S. Rudaz, Microextraction techniques combined with capillary electrophoresis in bioanalysis, Anal. Bioanal. Chem. 405 (2013) 125–141.
[84] F. Benavente, S. Medina-Casanellas, J. Barbosa, V. Sanz-Nebot, Investigation of commercial sorbents for the analysis of opioid peptides in human plasma by on-line SPE-CE, J. Sep. Sci. 33 (2010) 1294–1304.
[85] E. Hernandez, F. Benavente, V. Sanz-Nebot, J. Barbosa, Evaluation of on-line solid phase extraction-capillary electrophoresis-electrospray-mass spectrometry for the analysis of neuropeptides in human plasma, Electrophoresis 29 (2008) 3366–3376.
[86] F.W. Tempels, W.J. Underberg, G.W: Somsen, G.J. de Jong, On-line coupling of SPE and CE-MS for peptide analysis, Electrophoresis 28 (2007) 1319–1326.
[87] V. Datinska, I. Voracova, U. Schlecht, J. Berka, F. Foret, Recent progress in nucleic acids isotachophoresis, J. Sep. Sci. 41 (2018) 236-247.
[88] M. Fraňo, K. Džuganová, P. Koiš, M. Masár, DNA fragment separations by on-line combination of capillary isotachophoresis–capillary zone electrophoresis with UV detection, Electrophoresis 37 (2016) 3084-3088.
[89] V.N. Kondratova, O.I. Serd'uk, V.P. Shelepov, A.V. Lichtenstein, Concentration and isolation of DNA from biological fluids by agarose gel isotachophoresis, Biotechniques 39 (2005) 695-699.
[90] C. Eid, J.G. Santiago, Isotachophoresis applied to biomolecular reactions, Lab Chip 18 (2018) 11-26.
[91] A. Rogacs, L.A. Marshall, J.G. Santiago, Purification of nucleic acids using isotachophoresis, J. Chromatogr. A 1335 (2014) 105-120.
[92] X.F. van Kooten, M. Truman-Rosentsvit, G.V. Kaigala, M. Bercovici, Focusing analytes from 50 µL into 500 pL: On-chip focusing from large sample volumes using isotachophoresis, Sci. Rep. 7 (2017) 10467, DOI:10.1038/s41598-017-10579-5.
[93] V. Datinská, I. Voráčová, J. Berka, F. Foret, Preparative concentration of nucleic acids fragments by capillary isotachophoretic analyzer, J. Chromatogr. A 1548 (2018) 100-103.
[94] F. Foret, V. Datinská, I. Voráčová, J. Novotný, P. Gheibi, J. Berka, Y. Astier, Macrofluidic Device for Preparative Concentration Based on Epitachophoresis, Anal.Chem. (2019), DOI: 10.1021/acs.analchem.8b05860
[95] K. Meller, M. Szumski, B. Buszewski, Microfluidic reactors with immobilized enzymes-characterization, dividing, perspectives, Sensor Actuat. B-Chem. 244 (2017) 84-106.
[96] J. Ma, L. Zhang, Z. Liang, Y. Shan, Y, Zhang, Immobilized enzyme reactors in proteomics, Trends Anal. Chem. 30 (2011) 691-702.
[97] L. Hajba, A. Guttman, Continuous-flow biochemical reactors: Biocatalysis, bioconversion, and bioanalytical applications utilizing Immobilized microfluidic enzyme reactors, J. Flow Chem. 6 (2016) 8-12.

[98] S. Datta, L.R. Christena, Y.R.S. Rajaram, Enzyme immobilization: an overview on techniques and support materials, Biotech 3 (2013) 1-9.
[99] M. Naldi, A. Tramarin, M. Bartolini, Immobilized enzyme-based analytical tools in the -omics era: Recent advances, J. Pharmaceut. Biomed. 160 (2018) 222-237.
[100] D.M. Liu, J. Chen, Y.P. Shi, Advances on methods and easily separated support materials for enzymes immobilization, Trend. Anal. Chem. 102 (2018) 332-342.
[101] M. Safdar, J. Sproß, J. Jänis, Microscale immobilized enzyme reactors in proteomics: Latest developments, J. Chromatogr. A 1324 (2014) 1-10.
[102] J. Iqbal, S. Iqbal, C.E. Müller, Advances in immobilized enzyme microbioreactors in capillary electrophoresis, Analyst 138 (2013) 3104-3116.
[103] F. Svec, Porous polymer monoliths: Amazingly wide variety of techniques enabling their preparation, J. Chromatogr. A 1217 (2010) 902-924.
[104] M. Szigeti, J. Bodnar, D. Gjerde, Z. Keresztessy, A. Szekrenyes, A. Guttman, Rapid N-glycan release from glycoproteins using immobilized PNGaseF microcolumns, J. Chromatogr. B 1032 (2016) 139-143.
[105] J. Krenkova, K. Kleparnik, F. Foret, Capillary electrophoresis mass spectrometry coupling with immobilized enzyme electrospray capillaries. J. Chromatogr. A 1159 (2007) 110-118.
[106] R. Wojcik, M. Vannatta, N.J. Dovichi, Automated enzyme-based diagonal capillary electrophoresis: application to phosphopeptide characterization, Anal. Chem. 82 (2010) 1564–1567.
[107] Y. Li, R. Wojcik, N.J. Dovichi, A replaceable microreactor for on-line protein digestion in a two-dimensional capillary electrophoresis system with tandem mass spectrometry detection, J Chromatogr A. 1218 (2011) 2007–2011.
[108] R.M. Schoenherr, M. Ye, M. Vannatta, N.J. Dovichi, CE-microreactor-CE-MS/MS for protein analysis, Anal Chem. 79 (2007) 2230–2238.
[109] S. Mou, L. Sun, N.J. Dovichi, Accurate determination of Peptide Phosphorylation Stoichiometry Via Automated Diagonal Capillary Electrophoresis Coupled with Mass Spectrometry – Proof of Principle, Anal Chem. 85. (2013) 10692-10696.

Highlights

- Recent advances and major trends in sample pretreatment for capillary electrophoresis are summarized.
- In-line and on-line sample pretreatment techniques are discussed with emphasis on biological samples.
- This review aims at providing an overview of strategies to couple sample pretreatment techniques with capillary and microchip electrophoresis.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: