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REVIEW

Developments in Interfacing Designs for CE–MS: Towards Enabling Tools for Proteomics and Metabolomics

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Abstract Capillary electrophoresis–mass spectrometry (CE–MS) can be considered a useful analytical technique for the analysis of charged compounds in the fields of proteomics and metabolomics. Currently, the commercially available co-axial sheath–liquid interface is generally employed for coupling CE to MS in most application areas. Although it has proven to be rather robust for various proteomics, glycomics and metabolomics studies, the intrinsically low-flow separation property of CE is not effectively utilized in this set-up. In this type of interfacing the sheath–liquid (typical flow-rate between 1 and 10 µL/min) dilutes the CE effluent (flow-rate between 20 and 100 nL/min), thereby reducing the detection sensitivity. Over the past few years some significant developments that aim to overcome this limitation have been made in interfacing techniques for CE–MS, which resulted in an increased interest of CE–MS for proteomics and metabolomics. This paper provides an overview of these developments and the utility of CE–MS employing the new interfacing techniques is demonstrated by representative examples in the fields of proteomics, glycomics and metabolomics. Finally, general conclusions and perspectives are provided.

Keywords Capillary electrophoresis · Mass spectrometry · Interfaces · Proteomics · Metabolomics

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Introduction

Capillary electrophoresis–electrospray ionization-mass spectrometry (CE-ESI-MS) has emerged as a powerful tool for the analysis of charged compounds in biological samples [1–3]. CE–MS can be used for the characterization of a wide variety of analytes, e.g., metabolites, peptides, glycans and intact proteins [4–6]. So far, the co-axial sheath–liquid interface has been primarily used for the coupling of CE to MS [7, 8]. In this design, the sheath–liquid is provided co-axially to the end of the CE capillary as a terminal electrolyte reservoir, thereby providing a closed electrical contact (see "Advancements in CE–MS Interface Designs" for details). For many applications, this interfacing technique provided an acceptable performance and robustness [9–12]. However, a limitation of the use of the sheath liquid is that it dilutes the CE effluent [13], thereby compromising the achievable concentration sensitivity. This may have been one of the reasons why CE–MS lagged behind LC–MS in the field of proteomics (apart from intact protein analysis), glycomics and metabolomics. The low loadability (nL injection volumes) of CE is also mentioned as an obstacle to obtain improved concentration sensitivities and/or low concentration detection limits. In order to overcome this issue, various chromatographic- and electrophoretic-based preconcentration techniques have been developed to enhance the concentration sensitivity of CE–MS. The reader is referred

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to more dedicated literature for an overview concerning these techniques [14–16].

Recently, the field of CE–MS has benefitted from improvements in interfacing designs and, as a result, CE–MS gained significant interest as a tool for proteomics, glycomics, metabolomics and bio-analytical research [2, 17–19]. These fields can profit from enhanced sensitivity, as biological samples often contain many low-abundant species and may also be volume-restricted. In this context, the porous tip sheathless interface of Moini [20], the flow-through microvial interface of Maxwell et al. [21], the electro-osmotic flow (EOF) driven sheath–liquid interface of Wojcik et al. [22], and the porous emitter sheathless CE–MS interface of Wang et al. [23] emerged as important recent developments. In this paper, a critical and in-depth overview of these new interfacing techniques is given, with a primary focus related to the level of sensitivity improvement that can be achieved. Attention is devoted to those interfaces that gained an increased interest for proteomics and metabolomics over the past few years. Furthermore, two interfacing techniques are commercially available now, i.e. the porous tip sheathless interface and the flow-through microvial interface and, therefore, they may have the potential of becoming broadly used without being an expert user. Representative examples illustrate the applicability of the new CE–MS approaches in the fields of proteomics (both bottom-up and top-down), glycomics and metabolomics. Finally, conclusions and perspectives are provided.

Advancements in CE–MS Interface Designs

Soon after the appearance of CE as a liquid-phase ultra-high resolution micro-separation technique in 1981 [24], efforts to couple CE with MS as an information-rich detection method started. Even more so since the short path length of spectrophotometric detection in CE provided a limited sensitivity. These efforts culminated in the development of the co-axial sheath–liquid ESI interface for CE–MS (sometimes also called the triple tube CE–MS interface), which was brought to market by Hewlett-Packard (now Agilent Technologies) in 1995. This interface has been the commercial, standard solution for CE–MS interfacing until today. The interface couples CE seamlessly to Agilent Technologies MS instruments and has been co-used by Bruker to interface CE systems to its family of mass spectrometers. Essential pre-requisites of this interface were met by both manufacturers viz. to share a common ground for both the CE- and the electrospray current circuits and to provide the voltage for ESI from the MS. Coupling mass spectrometers from other manufacturers with CE in which the MS inlet is at ground and the ESI-voltage is delivered from the interface spray needle is possible but more arduous [25, 26].

Currently, the field of CE–MS interfacing is changing. New methods that abolish or minimize the usage of a sheath liquid have been introduced in the market or are on the verge of becoming commercialized.

Requirements for CE-ESI-MS Interfacing

Atmospheric pressure (AP) ESI was first reported by Yamashita and Fenn [27] and also by Aleksandrov et al. [1, 28]. The method allowed the formation of a spray of (sub)micrometer-sized charged droplets under the influence of an electrical field from which eventually charged molecules (ions) enter the MS via the inlet orifice. Initially, AP ESI was confined to flow-rates of maximally 10 µL/min. In order to establish a stable spray at higher flow-rates common in HPLC, Bruins et al. [29], Huang et al. [30] and Henion [31] came up with an interface concept which they called “ion spray” to emphasize the difference of this approach with ESI. This approach, where spray formation is assisted pneumatically by a co-axially delivered flow of nitrogen gas, has become the standard ESI method for a wide range of HPLC methods. In HPLC the solvent is delivered to the interface by the pump and drives the solutes towards the point of detection. The voltage to establish the electrospray field is applied at the outlet spray needle in LC–MS. In CE, charged analytes migrate electrophoretically from the inlet side towards the point of detection and into the outlet reservoir under the influence of an electrical field. Simultaneously, the background electrolyte (BGE) in the capillary may be driven towards the outlet reservoir by the EOF. Electrodes in the inlet and outlet reservoir close the electrical circuit. When coupling CE to MS, the outlet reservoir must be replaced in order to close the electrical circuit of the CE system and provide a contact to establish electrospray. Therefore, in CE–MS interfacing two electric circuits of different magnitude and (sometimes) different signs are required to drive the BGE and analytes towards the interface and to form the electrospray. The electrical circuitry has to manage currents in µA and nA range, direct the fields properly and incorporate ground in a safe manner. Moreover, like in HPLC–MS, volatile BGEs and solvents are generally used in CE–MS to avoid contamination of the MS.

Co-Axial Sheath–Liquid CE–MS Interface

Smith et al. [32] were the first to propose the co-axial delivery of a solvent to the end of the CE separation capillary as a terminal electrolyte reservoir. Based on this work, the triple tube sprayer was developed by engineers at Hewlett-Packard. The current design of the co-axial sheath–liquid interface is given in Fig. 1. The co-axial sheath–liquid CE–MS interface has a number of advantages. The sheath

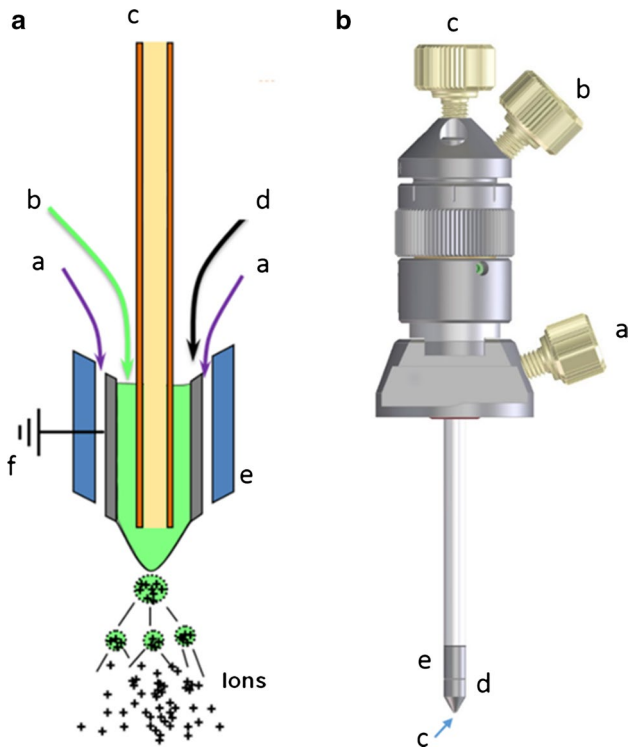

Fig. 1 **a** Pictorial representation of Agilent co-axial sheath–liquid CE–MS interface: *a* nebulizing gas, *b* Sheath liquid, *c* CE-capillary with BGE, *d* Stainless steel spray needle 0.4 mm i.d., 0.5 mm o.d., *e* outer tube, *f* ground connection. **b** Engineering sketch of the co-axial sheath–liquid CE–MS interface (graphics courtesy from Agilent Technologies)

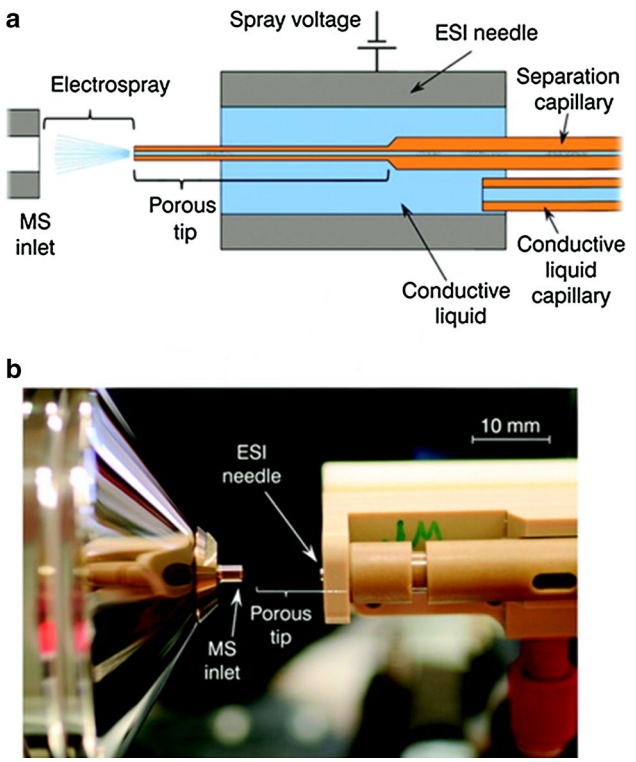

Fig. 2 The porous tip sheathless interface **a** schematic and **b** photograph of the prototype interface. Taken with permission from Ref. [41]

liquid which is added to the CE effluent at a flow-rate of typically 1–10 µL/min and the pneumatic assistance render spray formation independent of BGE composition and the presence of the EOF. The sheath–liquid composition (typically consisting of aqueous alcohol mixtures with a low percentage of a volatile acid like formic or acetic acid) dominates ESI chemistry. Thus, one can, for example, separate the analytes as anions in CE but detect them in their cationic form by MS. The sheath liquid is also a crucial part of the separation system as it should enable closure of the electrical circuit. Consequently, either co- or counter-ions from the sheath liquid will enter the capillary during the separation. If no common ions exist in BGE and sheath liquid, depletion phenomena may occur and OH^- or H^+ ions will enter. Overall, this can lead to changes in the CE performance (peak widths, migration time order, etc.) [33]. The spray needle (Fig. 1) is grounded and as such there is a common return path for the CE- and ESI-current. In Agilent and Bruker CE–MS systems the ESI voltage is applied on the MS inlet. This interface is compliant with different ionization modes besides ESI, such as AP chemical ionization and AP photo-ionization. Specific detail of these interfaces is the orthogonal orientation of the spray needle to the MS inlet orifice which allow neutrals and big droplets pass. Bubbles formed by electrolysis in the spray needle are easily transported out. Over the past decade, the usefulness, versatility and robustness of this interface has been demonstrated in various application areas [2, 4, 6, 12, 22, 34]. Typical limits of detection (LODs), without usage of preconcentration methods, are often in the order of 1 µM in sample concentration (depending on the MS method used) [9, 11, 35–37].

The co-axial sheath–liquid interface design, however, compromises sensitivity since the combined EOF and sheath–liquid flow is high compared with flow-rates used, e.g., in nanoflow HPLC and thus lack the sensitivity enhancement occurring in nano-ESI. Based on common sheath–liquid flow rate/EOF ratios, volumetric dilutions in the order of a factor 100 or more are frequently obtained. However, by optimization of the sheath–liquid composition the sensitivity decrease can be minimized to about one order of magnitude as compared to sheathless CE–MS in a similar set-up [38]. Moreover, recently the addition of reagents to the sheath liquid has been studied for their potential to improve the detection of analytes [39]. Important to note is that a hydrodynamic flow may occur through suction at the capillary end in the interface, especially when wider i.d. capillaries (75 and 100 µm) are used. As a

counter measure, the inlet vial must be under-pressurized. Therefore, since the introduction of the co-axial sheath–liquid interface in 1995 efforts have continued to interface CE with MS without delivering a Sheath–liquid, now commonly called sheathless CE–MS interfaces. However, only in the recent past this work has resulted in practical approaches.

Porous Tip Sheathless CE–MS Interface

Moini [20] proposed to etch the end of the CE separation capillary with hydrofluoric acid (HF) which renders the end of the fused-silica capillary porous. Via this porous section small ions and electrons can enter or leave the capillary [40]. The capillary was placed in a stainless steel ESI needle. Co-axially a conductive liquid is delivered to the outside of the porous end, which makes a hydraulic electrical contact between the ESI needle and the CE separation BGE. This essential concept has been implemented by Beckman Coulter in a prototype system in 2010 (Fig. 2) [41], and brought to market very recently (now Sciex Separations, CESI 8000). In this set-up, the voltage for electrospray can be applied to the spray needle or at the MS inlet (depending on the MS). Since the CE capillary is grounded through the conductive liquid capillary in the outlet vial of the CE, the electrospray voltage is delivered and controlled by the current monitor. To allow the formation of the porous tip by HF etching, it is required that the CE capillary has a thin wall. Therefore, the use of 150 µm o.d. and 30 µm i.d. fused-silica capillaries is practical. As the spray is formed solely from the BGE, the usage of positively or negatively coated capillaries that generate an EOF has been reported or a slight gas pressure on the inlet vial is applied when neutrally coated capillaries are used [42].

Though in principle one could use this interface with those MS systems that provide the ESI voltage from the MS inlet side, this CE–MS interface is at this stage a proprietary solution requiring the usage of Sciex CESI 8000 system, CE separation capillaries with porous tips and mass spectrometers with proper adapters (AB Sciex, Thermo and Bruker). Several groups have reported their results with the prototype CESI 8000 system [38, 41–44]. Using optimized sheath–liquid and sheathless approaches in CE–MS, the latter typically provides 10- to 100-fold improved concentration sensitivity [38, 42, 43]. The loss in sensitivity in sheath–liquid CE–MS can be partly compensated by using capillaries with larger i.d. as they allow injection of larger sample amounts. Interestingly, different LODs ratios for analytes have been reported when comparing the porous tip with the sheath–liquid interface [38]. Longevity of the porous tip capillaries of about 200 injections has been reported [44]. Since the usage of standard 365 × 50 or × 75 µm i.d. capillaries is prohibited by design of the interface, replacement capillaries have to be acquired from the manufacturer. This might be considered as a potential limitation of this approach since a major benefit of CE over other separation techniques, namely the cheap capillary, is discarded in this way. Another limitation is that this interface can only operate under relatively low CE currents (preferably below 10 µA) and therefore the composition and concentration of the BGE should be carefully considered.

Flow-Through Microvial CE–MS Interface

In a similar way as in the original co-axial sheath–liquid CE–MS interface work by the group of Smith et al. [32], the group of Maxwell et al. [21] reported a CE–MS interface with a metal spray needle and a co-axially delivered modifier [45]. In contrast to Smith, these authors sprayed from a 22' gauge steel needle with optimized exit geometry. The CE separation capillary is moved forward in this needle until it stops after which it is pulled back minimally to allow a hydraulic liquid connection with the BGE in the separation capillary. In the set-up in Fig. 3, the ESI voltage is established at the spray needle while the MS entrance is at ground. Careful and safe control of the magnitude and direction of the electrical fields and of the CE and ESI currents is required. The modifier solvent used typically consists of aqueous alcohols with 0.1 % acetic acid and is delivered at sub µL/min flow-rates. Apart from providing a wet electrical connection, the modifier solvent flow helps align the flow of BGE and sample constituents coming out of the separation capillary towards the needle exit [46]. This design allows the usage of standard CE capillaries (365 µm o.d. × 50 µm i.d.) providing stability and ease of use of the interface. This interface is now being commercialized by CMP scientific (EMASS-I, http://cmpscientific.com/index.html). Recently, Lindenburg et al. [47] compared the flow-through microvial interface with the conventional co-axial sheath–liquid interface for the profiling of cationic metabolites. This study revealed slightly better sensitivity of the flow-through microvial CE–MS interface. Sensitivity was dependent on the flow-rate of the modifier solvent. The authors also reported that this interface does not tolerate high CE currents as formation of electrolysis gases may accumulate in the spray needle and will hamper the electrical contact. This implies that the choice of a BGE is a critical aspect when working with this interface.

EOF-Driven Sheath–Liquid CE–MS Interface

In a number of publications, Wojcik et al. [22] propagate the use of a co-axially, EOF-driven sheath liquid with a borosilicate spray tube emitter for CE–MS [48–52]. The essential details are given in Figs. 4 and 5. A

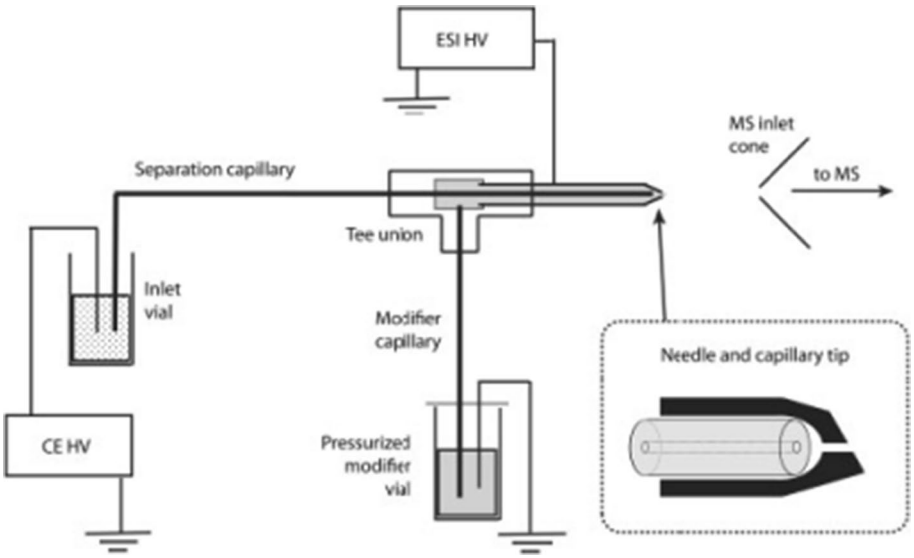

Fig. 3 Schematic overview of the flow-through microvial interface. Taken with permission from Ref. [21]

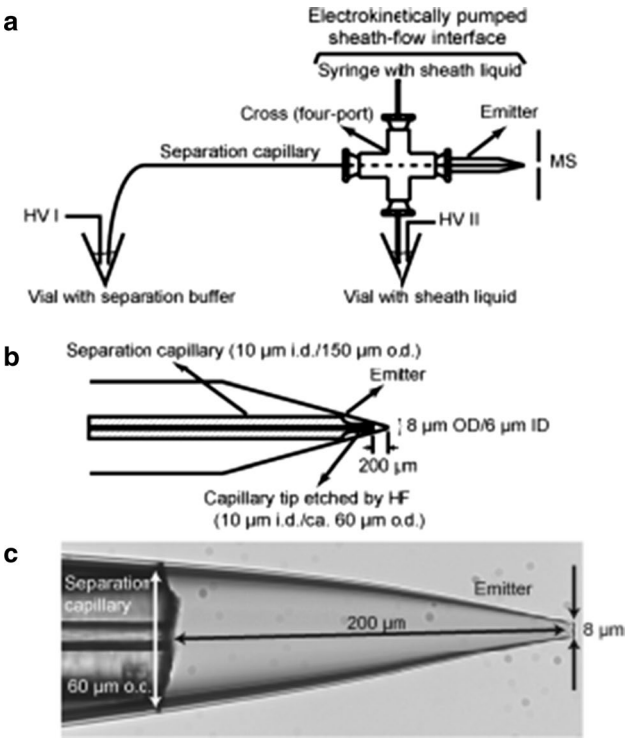

Fig. 4 **a** Schematic of CE-ESI-MS system; **b** schematic representation of the etched capillary in the electrospray emitter; **c** micrograph of the etched capillary in the emitter. Taken with permission from Ref. [52]

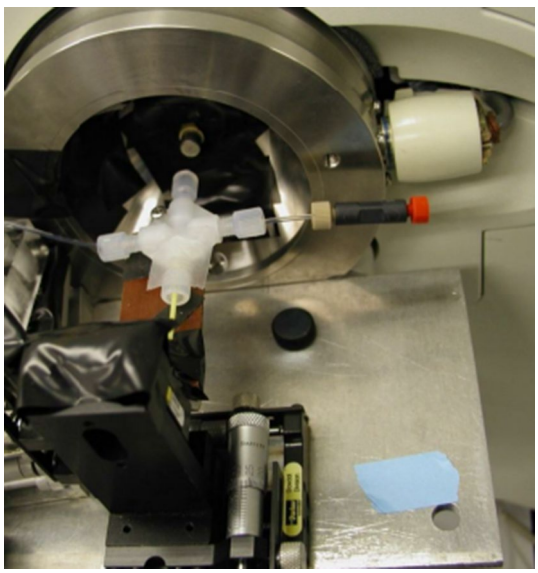

Fig. 5 CE–MS interface setup with thermo LCQ ion trap mass spectrometer. The interface was positioned on a home-made assembly used for nano-LC–MS applications

fused-silica capillary 150 µm o.d. × 10 µm i.d. is used for the CE separation. Its end is etched down to 60 µm o.d. allowing positioning close to the emitter orifice. The borosilicate emitter is home-made from a 1 × 0.75 mm glass tube. The emitter orifice is about 8 µm. As capillaries from borosilicate glass are known to be less robust than fused-silica, modest reliability has been reported (personal communication). On the other hand, by using 10 µm i.d. capillaries in the CE separation the exit spray acts as a true nanospray. Exceptional sensitivity is reported down to nM in solute concentration in the sample. Overall, since this design is still not commercialized, its use will be limited to experts in the field. So far, this interface has only been used by the Dovichi lab and, therefore, for a potential widespread use the performance of this approach should be evaluated by other research labs as well.

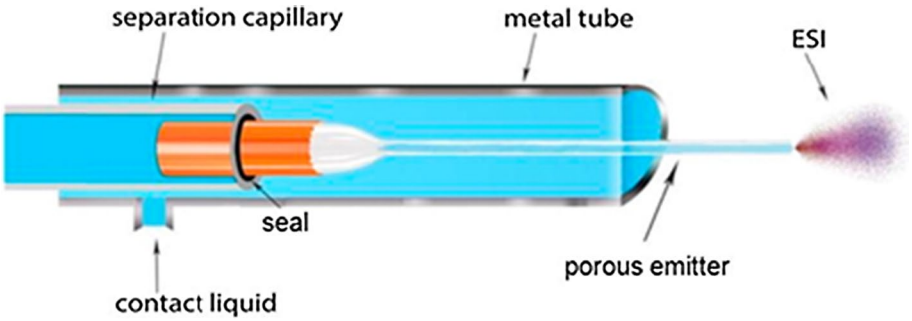

Fig. 6 Schematic view of the sheathless porous tip emitter design. Taken with permission from Ref. [23]

Porous Emitter CE–MS Interface

Recently, Smith et al. [32] reported an approach to a CE–MS interface. In the final implementation, they describe a fused-silica emitter with a porous tip (90 µm o.d.) which is placed inside the separation capillary (365 × 100 µm i.d.) and glued to fix its position [23]. The assembly is contained in a T-piece where the assembly of separation and emitter capillaries is shifted through a steel tube (1.0 mm i.d.). The third leg of the T-piece is connected to a contact liquid (2 % acetic acid), which is delivered by a syringe. Prior to each run the contact liquid is refreshed. The ESI voltage is applied to the steel tube (Fig. 6). Essential in this work though is the combination of the narrow i.d. emitter with 100 µm i.d. CE separation capillary. In this way, the interface eliminates the necessity of working with very narrow i.d. separation capillary as in the approaches by Moini and Dovichi. As higher currents are in principle present in larger bore capillaries, assessing the performance of this interface at high CE current conditions should be evaluated. The EOF in the separation capillary is suppressed by coating the inner surface with hydroxyl propyl cellulose. The CE separation used is a capillary isotachophoretic (CITP) method allowing sample injection up to 2.5 µL or 33 % of the volume of the separation capillary. Since the CITP provides analyte focusing, peptide concentrations down to sub nM could be obtained.

Applications

Proteomics

Over the past few years, CE–MS has been extensively used for the characterization of proteins and peptides. This has been done both on the intact protein level (i.e. top-down proteomics), as well as on the peptide level (i.e. bottom-up proteomics) [3, 53, 54]. In this section, the potential of CE–MS using novel interfacing techniques is demonstrated for some illustrative proteomics studies. For a comprehensive overview of CE–MS-based proteomics studies, we refer to the recent reviews of Haselberg et al. [3], Heemskerk et al. [53] and Zhao et al. [2].

Top-Down Proteomics

For intact proteins, CE–MS has been most commonly used to characterize and identify potential naturally-occurring isoforms [55–58] or modifications [59–61] of the protein. Since the CE separation is a function of charge, size and shape of a compound, small differences in the size or charge of proteins may be sufficient for separation. For example, protein deamidation replaces an amide by an acidic carboxylic acid moiety leading to a change in pI. Consequently, the protein's electrophoretic mobility changes significantly [55]. Moreover, since the species are separated, their molecular weight can be determined accurately using MS detection. As discussed in "Advancements in CE–MS Interface Designs", especially when using the conventional coaxial sheath–liquid CE–MS interface, the required sensitivity might not be achieved due to dilution of the CE effluent. In most studies described above, aqueous solutions with a high protein concentration are analyzed and the sensitivity of the CE–MS set-up is not a real issue. However, when protein concentrations are low or the heterogeneity leads to very disperse products the sheath–liquid CE–MS approach might not be sensitive enough for detailed characterization. It was demonstrated that the porous tip sheathless interface can provide LODs for intact proteins that are about two order of magnitude lower when compared to the same analysis performed with a sheath–liquid interface [38]. This was attributed to enhanced analyte ionization efficiencies and decrease in background noise obtained with the porous tip interface. Overall, this enabled the detection of intact proteins in the (sub-)nM-range.

In bottom-up proteomics, fragmentation is commonly used to sequence separated peptides and, subsequently, to identify proteins. For intact proteins, however, that has long been a challenge. With the advent of top-down proteomics using techniques like electron-transfer dissociation and higher energy collisional dissociation, efficient intact protein fragmentation has become achievable. Over the last

two years, a few studies have demonstrated that efficient CE separations can be combined with a top-down proteomics approach [62–64]. In all cases, coupling was achieved using the interface designed by Dovichi. High concentrations of standard proteins (μM-range) were injected to demonstrate the feasibility of top-down proteomics in CE–MS. Moreover, this approach has also been applied to characterize unknown proteins in both cell lysates [62] and culture filtrates [63]. No indications about minimum protein amounts or concentrations required for good sequence coverage is given.

Bottom-Up Proteomics

In the field of peptide analysis by CE–MS, bottom-up proteomics applications have been dominant. Since the latest update on this topic [53], a few new studies have appeared [65–68]. All these studies are from the group of Dovichi and, therefore, make use of the EOF driven sheath–liquid interface ("Advancements in CE–MS Interface Designs"). With this interface good sensitivity can be obtained, allowing peptide identification from *Escherichia coli* digest injections as low as 400 fg [65]. Up to 600 peptides, corresponding to 160 proteins, could be identified in this study (using 84 ng digest injections). This number could be improved by making use of a neutrally-coated capillary as it provides higher separation efficiencies and more time to obtain tandem mass spectra, resulting in the identification of over 1,250 peptides from an injection of 100 ng of *E. coli* digest in a single-shot approach [66]. This was just slightly lower than using UPLC–MS/MS and, interestingly, complementary peptide identifications were obtained with both techniques. Additional sensitivity improvement in the CE–MS method was achieved by introducing a sample preconcentration approach [67]. By dissolving the sample in a buffer of medium pH and using a low-pH BGE, a dynamic pH junction was formed where analytes are concentrated. With this approach a 10- to 20-fold sensitivity improvement was obtained. Until this point, their set-up was not automated, i.e. requiring manual intervention during CE–MS experiments. Recently, the authors introduced an autosampler to allow automation of their analysis [68]. Over eight injections, this automated CE–MS system provided average RSDs for migration time, peak intensity, and peak area of 3, 24 and 19 %, respectively, for 340 peptides with high intensity. These relatively high numbers were attributed to temperature changes in laboratory and evaporation of solvent during the 8 h analysis time. Their system showed to be capable of reproducibly identifying over 1,000 peptides from an *E. coli* tryptic digest in a 1-h analysis time.

Tryptic peptide mapping is also routinely used in the biotechnology industry to confirm primary sequence and to analyze posttranslational modifications of recombinantly-produced therapeutics (i.e. biopharmaceuticals). A therapeutic monoclonal antibody was studied by tryptic peptide mapping using LC–MS and CE–MS, where in the latter case both the sheath liquid and porous tip interface were used [69]. With LC–MS 97 % sequence coverage was achieved. The 3 % not covered consisted of 11 peptides containing 3 or fewer amino acids. Both CE–MS systems allowed separation and detection of the 11 small peptides. However, with the sheath–liquid CE–MS system, one large peptide was not detected due to poor ionization, dropping the sequence coverage to 94 %. Employing the CE–MS system with the porous tip interface resulted in 100 % sequence coverage. The porous tip sheathless CE–MS method using a bare fused-silica capillary provided the most optimal resolution, compared to a co-axial sheath–liquid CE–MS system using both a bare fused-silica and a neutrally-coated capillary. This improvement was explained by the nebulizing gas used in the sheath liquid interface, inducing a laminar flow in the capillary resulting in peak broadening. The porous tip sheathless CE–MS system also provided significantly less noise as compared to the sheath–liquid CE–MS system. On the other hand, the sheath–liquid CE–MS system proved to have better repeatability in terms of migration time and peak height. The porous tip sheathless interface was also used for CE–MS impurity profiling of the therapeutic peptide aviptadil [70]. A comparison with LC–MS was made; more impurities were detected by CE–MS than by LC–MS (15 vs. 2, respectively). Interestingly, none of the impurities found with CE–MS could be observed with LC–MS and vice versa. Moreover, LODs were about 2,500 times lower with CE–MS as compared to LC–MS. Besides characterizing biopharmaceuticals, the quantitation of residual host-cell proteins in the formulation is very important. CE–MS using the EOF-driven sheath–liquid interface was recently used for this purpose [71]. By using isotopically-labeled peptide standards, three known host-cell proteins could be confidently quantified at the picomole level.

Glycomics

CE–MS is widely used for the analysis of carbohydrates, glycoproteins and glycopeptides [72]. Both the porous tip interface and the flow-through microvial interface have been used for glycomic studies. CE–MS using a porous tip sheathless interface in combination with a neutrally-coated capillary was used to study glycoform profiles of intact pharmaceutical proteins [73]. Analysis of human erythropoietin revealed more than 250 different isoforms (of which 74 glycoforms) with estimated glycoform concentrations ranging between 0.53 and 950 nM. The isoforms were separated over a 20-min time window (Fig. 7a). The separation was mainly due to differences

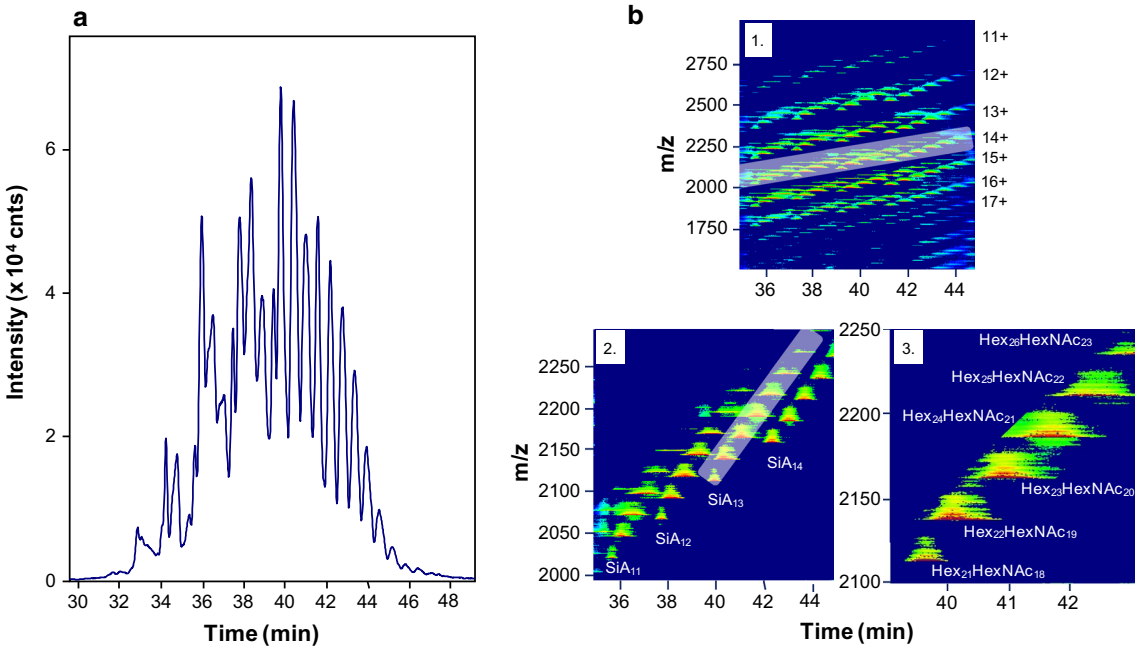

Fig. 7 CE–MS of rhEPO employing the porous tip interface in combination with a neutrally coated capillary. **a** BPE; **b1** contour plot with zooms of **b2** the 14+ charge state of the glycoforms and **b3** the SiA_{13} sialoforms of the 14+ glycoforms. Taken with permission from Ref. [73]

in the number of sialic acid residues, leading to almost a 2-min migration time shift with the addition of a sialic acid unit (Fig. 7b). Differences in the hexose-N-acetylhexoseamine content also led to small differences in electrophoretic mobility, and thus partial separation. Similarly, the analysis of recombinant human interferon-β-1a lead to the assignment of up to 80 isoforms (18 glycoforms). The authors compared their results with the literature and concluded that in comparison with sheath–liquid CE–MS similar or improved glycoform coverage of both proteins was obtained, while injecting at least ten times less sample amount.

Maxwell et al. [74] evaluated the potential of CE–MS using a flow-through microvial interface for carbohydrate analysis. The authors showed that replacement of the CE outlet vial by the flow-through microvial interface for coupling to MS did not affect separation by using LIF detection measurements, implying that the suction effect often reported for sheath–liquid CE–MS is not present in this set-up. For the separation of negatively charged 8-aminopyrene 1,3,6-trisulfonate (APTS)-labeled carbohydrates, reverse polarity separations were carried out using bare fused-silica capillaries under acidic conditions. This approach was also used for the analysis of native and APTS-labeled *O*-acetylated *N*-glycans in fish serum [75]. The method enabled to investigate structural variations of *O*-acetylated sialic acid isomers and was able to discern between salmons that received a stress treatment and controls.

Metabolomics

As CE–MS is an electrodriven-based separation technique that specifically targets charged analytes, the field of metabolomics can benefit greatly from it, since a major part of the metabolome is comprised of charged (or chargeable) compounds [34]. Until now, CE–MS employing the co-axial sheath–liquid interface has been primarily used for metabolomics studies [18, 76]. Recently, the porous tip sheathless interface and the flow-through microvial interface have shown good potential to improve the concentration sensitivity of CE–MS and as a result the metabolic coverage [43, 47]. For example, Ramautar et al. [43] and Hirayama et al. [44] used the porous tip sheathless interface for metabolic profiling of human urine. Both studies employed fused-silica capillaries and 10 % acetic acid as BGE and reported a significant increase in the number of molecular features detected in human urine in comparison with the conventional co-axial sheath–liquid CE–MS method. Ramautar et al. [43] reported relative standard deviations (RSDs) for migration times and peak areas of test compounds below 2–12 %, respectively, which were comparable with values obtained by sheath–liquid CE–MS. Furthermore, LODs between 11 and 120 nM were obtained and the number of molecular features was increased threefold as compared to sheath–liquid CE–MS (i.e., 900 vs. 300). Hirayama et al. [44] demonstrated that, in comparison with conventional sheath–liquid CE–MS, a fivefold improvement in LODs was obtained

with the porous tip sheathless interface for 21 cationic metabolite standards, ranging between 4 and 800 nM. Application of the method to human urine showed a tenfold increase in the number of detected molecular features in comparison with conventional CE–MS. The same porous tip sheathless CE–MS approach has been used for metabolic profiling of mouse cerebrospinal fluid (CSF), urine and plasma, allowing for the detection of circa 350 molecular features in CSF, circa 400 in mouse plasma and circa 3,500 features in urine [77]. This demonstrates the suitability of the porous tip sheathless CE–MS method for highly sensitive metabolic profiling of volume-limited samples. CE–MS employing an EOF driven sheath–liquid interface or the recent sheathless interfacing design of the group of Smith have not been evaluated for metabolomics yet, however, given the results that were obtained in the field of proteomics (see above), these designs should also be of interest for metabolic profiling studies.

Lindenburg et al. [47] evaluated the performance of CE–MS employing a flow-through microvial interface for 35 cationic metabolites. Figure 8 shows the comparison between the flow-through microvial interface and a conventional sheath–liquid CE–MS interface. Using TOF–MS for detection, 10 % acetic acid as BGE, and a sheath–liquid (75:25 % (v/v) isopropanol/water containing 0.1 % acetic acid) flow-rate of 300 nL/min, the authors found that, on average, the sensitivity was improved three times and the LOD five times (ranging between 10 nM and circa 3 μM). Moreover, RSDs for migration times were below 2 %, while with sheath–liquid CE–MS RSDs were below 5 %. The improvement in migration-time repeatability was explained by the lack of a nebulizer gas in the case of the microvial interface and, therefore, the vacuum effect induced by the nebulizer was not present. The analysis of a single zebrafish embryo resulted in the detection of 110 molecular features, which was a twofold improvement as compared to sheath–liquid CE–MS. Soliman et al. [78] developed a CE–MS method employing a flow-through microvial interface for the analysis of potential prostate cancer biomarkers (sarcosine and its isomers, L-proline, L-cysteine, L-leucine, L-glutamate, and L-kynurenine) in human urine. CE separation was carried out on a positively charged, PEI-coated capillary using 2 % formic acid in 50 % methanol as BGE. The sheath–liquid was the same as the BGE and was delivered at a flow rate of 200 nL/min. An excellent separation resolution between sarcosine and its isomers was obtained. Furthermore, acceptable precision and accuracy of standard addition calibration of all potential biomarkers were obtained, both intra-day and inter-day. Overall, the results demonstrated that the flow-through microvial interface can be used in repeatable way for metabolite analysis in human urine.

The majority of reported CE–MS-based metabolomics applications, regardless the used interfacing technology, deals with the cationic metabolome. CE–MS with negative electrospray ionization often results in rather low analyte responses [79, 80]. The performance of the recently introduced CE–MS interfacing techniques with respect to improving the analysis of anionic metabolites has not been reported yet. It would be highly interesting to evaluate the performance of the interfaces for anionic metabolic profiling studies in order to assess their potential for overcoming

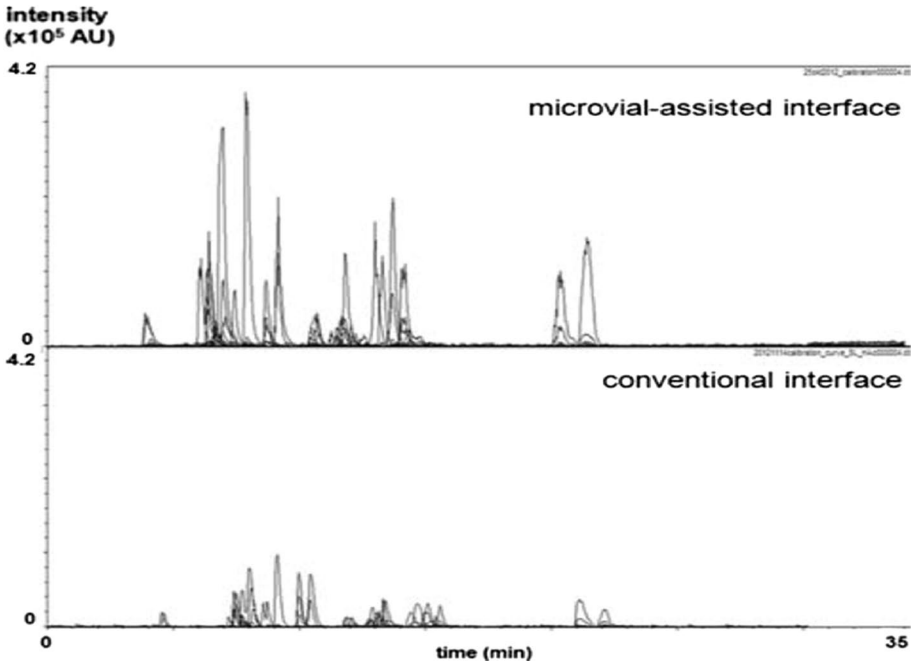

Fig. 8 Multiple extracted ion electropherograms of 35 cationic metabolites (25 μM) obtained by CE–MS using a flow-through microvial interface (*upper electropherogram*) and a sheath–liquid interface (*lower electropherogram*). Taken with permission from Ref. [47]

a major limitation in CE–MS based metabolomics, i.e., being the analysis of anionic metabolites. Moreover, the newly developed interfaces should all be carefully studied with respect to long-term performance and robustness in order to evaluate their suitability for clinical metabolomics studies that often involve the analysis of hundreds or even thousands of biological samples.

Conclusions

In this review, we have described the recently introduced CE–MS interfaces that are in our opinion promising for enhancing the coverage of the proteome and metabolome by CE–MS. Therefore, we have mainly focused on sensitivity. Compared with conventional sheath–liquid CE–MS, significant progress has been booked over the past few years. Especially the sheathless porous tip interface and the flow-through microvial interface emerged as very promising interfacing techniques for highly sensitive proteomics and metabolomics studies at different laboratories. The feasibility of the EOF-driven sheath–liquid interface and the recent porous tip emitter interface of the Smith group has only been shown in the field of proteomics so far.

In general, the potential of CE–MS in the clinical lab is strongly dependent on the reliability, robustness and sensitivity of the interfacing method. As such, the sheath–liquid interface approach can be considered a relatively robust and routine method for practical usage though with compromised sensitivity. All recently introduced alternatives that are described in this review offer enhanced sensitivity compared to the sheath–liquid interface, but should be further evaluated with regards to reliability and robustness to assess how suitable they will become for routine clinical omics research. The sheathless porous tip interface holds the potential of most optimal sensitivity but the long-term stability, robustness and ease of use in practice need to be demonstrated. The flow-through microvial interface has potential to become a viable alternative, however, at present it is still in a relatively preliminary stage. For this interface, it was demonstrated that it remedies the suction effect that takes place in sheath–liquid CE–MS. As this effect is known to affect separation, we recommend that the other interfaces should be studied as well with respect to this. Though, in principle, suction from the nebulizer gas cannot occur in the porous tip interface, as there is no nebulizer gas. The recent CE–MS interfaces reported by Dovichi and Smith, are at this stage expert approaches lacking commercial support.

An important aspect is the versatility of the interfaces. The sheath–liquid interface can be used with a relatively wide variety of BGEs; this remains to be seen with the other interfaces. For example, 1 M formic acid is often used as BGE in sheath–liquid CE–MS for the profiling of cationic metabolites in biological samples [4]. However, this BGE cannot be used at this concentration level with the porous tip sheathless and the flow-through microvial interface due to the high background currents. In principle, lower concentrations of this BGE can be employed with these interfacing techniques, however, at the expense of separation efficiency, which is highly needed for the analysis of complex samples. Therefore, 10 % acetic acid is used instead of 1 M formic acid, yielding significantly lower background currents but comparable separation efficiencies for metabolomics studies [43, 47]. Moreover, an interface that can be operated with standard capillaries, i.e., $\pm$360 μm outer diameter and 50 μm inner diameter, has an advantage both in user-friendliness, and in analytical performance as well costs. In order to further expand the role of CE–MS in clinical omics studies, the feasibility of the new approaches should also be demonstrated for the analysis of anionic compounds using alkaline BGE conditions in combination with ESI–MS detection in negative ion mode.

In principle, all interfacing techniques discussed in this review can be coupled to an MS of choice, but this requires highly skilled users and, often, modification of the MS source. In practice, this means that for many end-users the available MS equipment will determine the practical application of CE–MS. From there, the user will be directed to a (commercial) solution. The application or type of biological question determines the type of MS to be used, which then dictates the type of interfacing to be employed for CE–MS studies.

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