1

Capillary Electrophoresis–Mass Spectrometry Interfacing: Principles and Recent Developments

Lukas Naumann, Jasmin Schairer, Alisa Höchsmann, Elahe Naghdi, and Christian Neusüß

Aalen University, Faculty of Chemistry, Beethovenstraße 1, 73430 Aalen, Germany

1.1 Introduction

Capillary electrophoresis (CE) is an attractive separation technique in many fields of analytical chemistry. This is primarily due to its selectivity based on charge and size. Mass spectrometry (MS) is an excellent characterization tool. Thus, the coupling of CE with MS is highly desirable, though by far not so widespread as liquid chromatography (LC) with MS. Separation techniques can be coupled with MS either offline or online. CE is a microfluidic technique with an inner diameter (i.d.) of the separation capillary in the 20–100 µm range. The overall volume of CE capillaries (length is typically 20–100 cm) is in the order of 1 µl, with injection volumes in the low nl range. Thus, fraction collection for subsequent offline MS characterization is difficult and rarely performed for subsequent MS characterization. Only when CE is combined with matrix-assisted laser desorption/ionization (MALDI) fractions can these be collected for offline MALDI–MS characterization (see Section 1.7). Mostly electrospray ionization (ESI) is used in online coupling approaches due to its ability to ionize efficiently most of the molecules being important in bioanalysis. The coupling of CE with ESI–MS is obvious since ionic compounds are separated in most electromigrative techniques and the low flow rates in CE perfectly fit to ESI. This is especially the case when nanoESI is considered: the ionization in flow rates below 1 µl/min without additional nebulizing gas is known for sensitivity and reduced matrix effects. However, CE–ESI–MS interfacing is complicated by two major aspects: (i) the variable flow rates in CE (electroosmotic flow [EOF]) and (ii) the required voltages both for CE and for ESI. Both electrical fields need to be contacted at the end of the separation capillary without hampering the separation efficiency. Thus, special designs are needed for CE–MS, which are more complicated than those applied for LC–ESI–MS.

Capillary Electrophoresis–Mass Spectrometry for Proteomics and Metabolomics: Principles and Applications, First Edition. Edited by Rawi Ramautar and David D. Y. Chen.

In this chapter, we summarize the actual status of CE–MS interfaces and discuss the most relevant techniques. Homebuilt systems are also included, although commercialization of efficient ionization techniques has made significant progress in the last decade. Due to the importance of ESI in bioanalysis, we focus on CE–ESI–MS and start with general considerations of CE–ESI–MS interfaces. This is followed by a section about the widespread sheath liquid (SL) interface setups, including both the traditional coaxial design and modern nanoflow sheath liquid (nanoSL) approaches. Afterward, sheathless approaches are presented and discussed with a focus on the commercially available porous-tip interface. Then, miscellaneous CE–ESI–MS interfaces are briefly summarized. Next, coupling microchip electrophoresis (MCE) with MS is discussed in a short section. Thereafter, alternative ionization techniques are discussed including MALDI and inductively coupled plasma (ICP). The chapter ends with a short concluding discussion and outlook on future trends.

1.2 General Considerations of CE–ESI–MS

ESI is by far the most widely applied ionization technique for CE–MS. Many interface designs have been developed addressing the special needs regarding the handling of variable flow rate in CE and the provision of electrical contact for both CE and ESI. Most relevant CE–ESI–MS interface designs are presented in Figure 1.1.

All indicated designs will be discussed in the following sections. However, we start with a short overview about ESI properties (Section 1.2.1) and general aspects of CE–MS (Section 1.2.2 and 1.2.3).

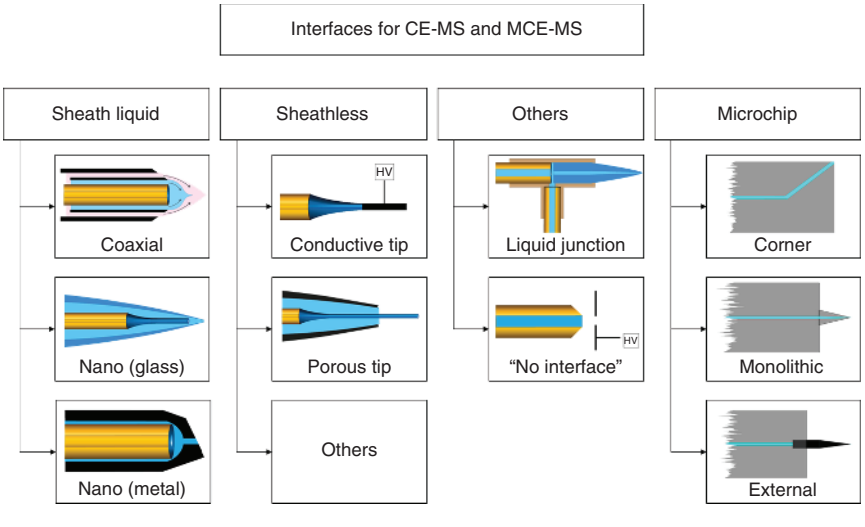

Figure 1.1 Most important interface designs for CE–ESI–MS and MCE–ESI–MS.

1.2.1 Electrospray Ionization

In principle, electrospray (ES) ionization creates ions in gas phase from a solution. To perform ESI, a conductive liquid along an electrical field is required to apply electrospray voltage. ESI takes place in three steps: (i) creation of the fine droplets in strong electrical field, (ii) droplet desolvation and shrinkage (Coulomb repulsions), and (iii) formation of the gas-phase ions from the charged offspring droplets.

The applied electrical gradient penetrates the liquid surface at the tip of the sprayer and forms a meniscus. Further, meniscus deforms and a cone, is called a Taylor cone, is created. A fine jet of charged droplets emerges from the cone apex with sufficient electrical field. The charge at the droplet surface corresponds to the electrical field's polarity and voltage. The subsequent solvent evaporation leads to a cascade of Coulomb repulsions, where the surface charge at the droplet overcomes the surface tension. It leads to smaller charged droplets. Two different models describe the subsequent formation of the gas-phase ions: the charge residue model (CRM) [1] and the ion evaporation model (IEM) [2]. The CRM suggests Coulomb repulsions continue until an ultimate droplet is formed which contains only a single analyte molecule. This molecule retains a part of the droplet's excess charge and becomes a free gas-phase ion. Proteins are expected to follow the CRM due to their relatively large size and the presence of their polar side chains, which are located at the protein surface to stabilize their solvation within a single droplet [3, 4]. Small molecules are expected to leave the shrinking droplet according to the IEM.

In general, the nanoESI provides better ionization efficiency than classical ESI because of the smaller initial droplet size, which is related to the initially formed Taylor cones. As a result, the sensitivity is higher in nanoESI. In Figure 1.2 the Taylor cone size is illustrated for the three mostly used CE–MS interface designs.

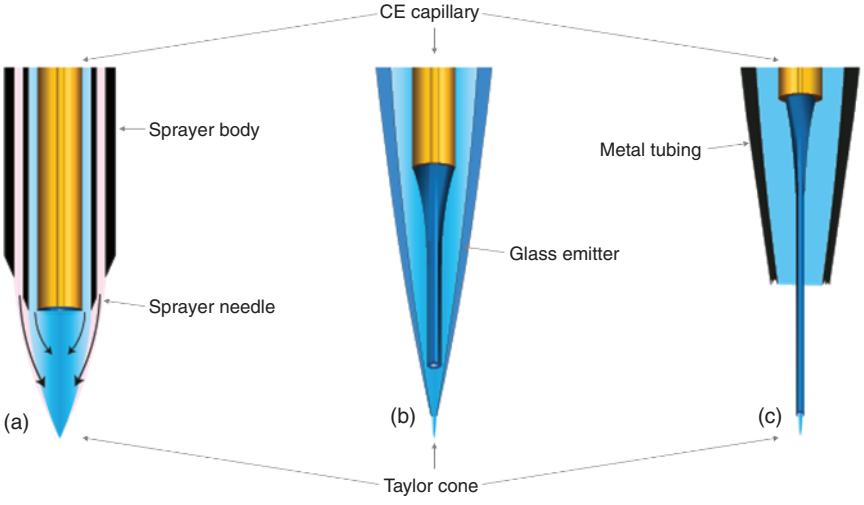

Figure 1.2 Comparison of the Taylor cone size of different spraying approaches in CE–ESI–MS at the same scale. (a) TTS, (b) nanoflow sheath liquid interface, and (c) porous-tip interface.

It obviously shows the benefit of the nanoSL (Figure 1.2b) and sheathless interface (Figure 1.2c) compared with the coaxial sheath liquid interface (Figure 1.2a) regarding initial droplet size.

However, the kind of solvent also strongly influences the ESI efficiency. A mixture of mostly water-based background electrolyte (BGE) with a high content of a medium polar solvent (methanol, acetonitrile, isopropanol) is aspired, potentially (partly) compensating or even outperforming the effect of dilution in SL interfaces.

ESI is prone to analyte signal suppression by high buffer concentration, nonvolatile components, and surfactants inherent to its liquid-phase mechanism. Besides, nonvolatile components may cause source contamination in ESI, producing high background signals. Thus, similar to LC–MS, volatile BGEs and solvents are generally used in CE–MS to increase ionization efficiency of the analytes and to avoid contamination of the MS. Nevertheless, the possibility of MS source contamination is slightly lower because of the lower CE capillary effluent than the LC column effluent [5].

1.2.2 Electrical Circuit in CE–ESI–MS

After the voltage-driven separation in the CE capillary, ESI requires a second electric circuit between the capillary exit and the MS endplate. In order to avoid any peak broadening, both electrical circuits share the same electrode, placed close to the end of the separation capillary. Ideally the potentials of both the CE and ESI can be chosen independently, in order to be flexible in the CE separation and achieve a stable and reproducible electrospray. Depending on the mass spectrometer, two approaches are possible: the shared electrode of CE and ES is grounded (mass spectrometers from, e.g. Agilent Technologies and Bruker Daltonics) or carries a potential (mass spectrometers from, e.g. Thermo Fisher Scientific, Waters, Sciex). The grounded electrode at the CE outlet/ES simplifies the independent choice of CE parameter and ES conditions. Also, the maximum CE voltage (typically 30 kV) can be used for separation, when the same polarity is applied to CE and ES. Nevertheless, it is also possible to perform efficient separation and stable ES when the shared CE outlet/ES electrode is put on (ES) voltage. In this case the remaining voltage from CE needs to be considered, as well as the involved currents. Typically, the currents of CE and ESI are very different (CE: 5–50 μA, ESI: <1 μA). Therefore, an additional electrode at the end of the CE capillary is needed! The difference between these currents needs to be handled by the ESI source of the mass spectrometer. Additional resistors guiding the high currents out of the system might be required, in case the ES source is not able to handle these high currents remaining from the CE. If “special” CE conditions are used in CE, so that the resistance of the CE and the applied voltage at the CE inlet result in an appropriate ES voltage at the end of the CE capillary, no additional electrode is required. Such a CE–MS system has been described as an “interface-free” CE–MS system [6, 7] (see Section 1.5.2).

The electrical circuits of the CE and the ES can be closed with a conductive coating on the capillary tip or an auxiliary (sheath or makeup) liquid, respectively, as

previously mentioned. These various approaches lead to different interfaces (see Figure 1.1), which are discussed in this paragraph, regarding flexibility, sensitivity, robustness, ease of use, etc.

1.2.3 CE Modes and Conditions in CE–MS

Capillary zone electrophoresis (CZE) is the mostly used CE–MS mode. Beside the technical aspects of CE–MS coupling, as they are discussed in this chapter, several practical parameters such as BGE, a suitable coating, the polarity, and the EOF should be considered and optimized [8]. Although pH is the most effective parameter in CZE, the BGE type strongly influences the selectivity and separation efficiency as well. This is a major drawback of CE–MS – in contrast to LC–MS, where the restriction to volatile additives is of minor importance. Several other CE modes mostly are not MS compatible. This includes micellar electrokinetic chromatography (MEKC), capillary sieving electrophoresis using sodium dodecyl sulfate, and capillary isoelectric focusing (CIEF). Nevertheless, direct CIEF–MS is possible when incompatible additives other than ampholytes are omitted. To overcome the MS incompatibility of several CE methods, CE–CE–MS workflows have been developed recently for the coupling of any CE method to the ESI–MS through a CZE as a second dimension to remove interfering compounds from the first dimension [9]. The prevention of nonvolatile additives also restricts application of dynamic coating; therefore, static coatings (adsorbed or covalently bound) are necessary [10].

The EOF is an important parameter in CE and CE–MS. A low, high, or reversed EOF can be beneficial depending on the mobility of the target analyte. Generally, the EOF cannot be directed backward from the interface to the CE inlet in CE–MS, as either SL (SL interfaces) or air (sheathless interfaces) would penetrate into the CE capillary and disrupt the separation. Depending on the interface, an EOF toward the MS can be desired. This is especially important for sheathless interfaces. At least a small EOF typically stabilizes both the separation and the ES; however, a small EOF bears the risk of reduced method robustness.

Independent of the interface, as it is discussed below, offline and online preconcentration methods increase the sensitivity of the overall method. Online approaches in CE–MS include stacking, dynamic pH-junction, transient ITP, and SPE methods. These methods can be limited in CE–MS due to the absence of an outlet vial. However, exchange of SL or recently developed valve approaches (2-capillary approach – see Section 1.3.2) will provide flexibility regarding online preconcentration techniques.

1.3 Sheath Liquid Interfaces

The earliest, most straightforward, and commonly used interface type is the sheath liquid interface. A conductive sheath liquid closes the electrical circuits of the CE separation and the ESI, which takes place at the terminus of the CE capillary by mixing the CE effluent with the SL. The constant delivery of an additional SL to the

CE effluent stabilizes the flow rate, predominantly independent of the EOF. This allows a wide range of CE BGEs regarding composition and ionic strength.

Since the SL flow rate is mostly much higher than the CE effluent, the SL dominates the chemistry of the electrospray. Therefore, the composition of the SL can be chosen in order to achieve the best ionization efficiency. This reduces the negative impact of the dilution. Usually, sheath liquids consist of aqueous organic solvent mixtures with a low percentage of a volatile acid or base. The organic solvent supports the ionization by reducing the surface tension, which affects, namely, the drop size formed within the electrospray. Volatile acid or base can promote (de-)protonation of the analyte molecules. Choosing the right sheath liquid composition can be a challenging process regarding the various parameters to consider. A sheath liquid needs to connect two different electric circuits with different magnitudes of voltage and current. This works for both polarities in CE and the ionization process, respectively. In this way, both cations and anions can be separated and detected by positive or negative ESI–MS. Even a separation at one polarity and the ionization at another polarity in ESI–MS is possible. Therefore, the routinely used sheath liquids decouple the separation and ionization conditions, as such providing an optimum for both. Sheath-flow chemistry can be optimized regarding, e.g. sensitivity, by the addition of additives to the sheath liquid [11]. These additives can improve the ionization efficiency and overcome the negative effects of MS-incompatible buffers by exchanging the counter ions. Also, H/D exchange experiments are possible. In addition, reactions between separation and detection can be performed without post-capillary peak broadening, because of the absence of a reaction coil required in LC–MS. When low EOF systems are used for the CE separation, ions from the SL or the complete SL can penetrate into the separation capillary. This strongly interferes with the separation and needs to be avoided. To prevent SL migration into the CE capillary, the height level of the CE inlet vial and capillary terminus needs to be balanced.

1.3.1 Coaxial Sheath-Flow ESI–MS Interface

Fundamentally, the standard CE–MS interface is based on the work of Smith and coworkers (Figure 1.2a). They reported the usage of an auxiliary solvent composition around the terminus of the CE separation capillary. This auxiliary solvent provides a sheath flow around the capillary outlet, which establishes an electric contact for the grounding of the CE separation and enables the ESI voltage. This coaxial sheath-flow interface approach, commercialized by Agilent Technologies (at that time Hewlett-Packard), became known as the so-called triple-tube sprayer (TTS). The TTS consists of several parts, as shown in Figure 1.2a. The main parts are the spray needle, the connections for sheath liquid and nebulizing gas, and the sprayer body. The TTS is constructed as follows: The CE capillary is fixed within the sprayer and forms the spray tip (first tube). The sheath liquid is delivered through the sprayer body and the sprayer needle (second tube), which encircles the CE capillary. The SL provides the liquid contact with the CE buffer and thereby closes the electric circuit. The TTS approach typically requires a SL flow of several µl/min,

making it necessary to have a third tube providing a nebulizing gas for assistance in droplet formation. The nebulizing gas is delivered through the sprayer body, which forms the outermost, third tube around the spray needle. The sheath liquid added to the capillary effluent with an isocratic flow rate acts as the outlet buffer for the CE and maintains a spray that is independent of the buffer composition and the magnitude of the EOF.

Regarding the construction of the TTS, there are a few parameters to consider, which affect the CE separation and the electrospray such as CE capillary (protrusion and shape), sprayer needle (material and positioning), and nebulizing gas flow. If the capillary protrudes too far out, it is possible for the liquid connection between the CE buffer and the sheath liquid to be interrupted. This results in a current drop and arching which can damage the capillary. The recommended protrusion of the CE capillary is around 0.1 mm. The quality, namely, the shape of the cut of the capillary outlet, affects the electrospray. Using a capillary with a crushed terminal tip may result in the detection of more baseline noises in the electropherogram. The spray needle can be made of either stainless steel or platinum. In general, the chosen material does not affect the CE separation, but the lifetime of the spray needle material can differ. The positioning and shape of the spray needle have a big influence on the separation and the electrospray. The wrong positioning of the spray needle, for example, too far out, can negatively affect the separation, leading to more background signals. The reason for this effect is a more consistently nebulizing gas flow along the spray needle. This can result in higher solvent flow, followed by earlier migration times (suction effect) and poor peak separation. The nebulizing gas flow (pressure) must be balanced between a proper and stable spray and interference with the separation due to the suction effect (pressure difference between both ends of the CE capillary). The resulting hydrodynamic flow causes peak broadening, decreased separation efficiency, and shorter migration times. To compensate for this suction effect and to increase the resolution of the separation, a vacuum can be applied on the inlet vial at the CE instrument. On the other hand, a pressure of 10–50 mbar at the inlet of the CE can be used to prevent SL entering into the CE capillary, when low EOF systems are used.

The TTS is commercially available for Agilent and Bruker LC–MS ESI sources (same electrical circuit design). The orthogonal mounting of the TTS (off-axis, Figure 1.3a) minimizes the contamination of the MS inlet by large droplets and neutral mobile-phase additives. It also reduces the number of neutral molecules, which enter the MS inlet because of the absence of the acceleration in the electrical field of the ESI source. This can reduce the contamination of the MS. The sheath liquid flow of 1–20 µl/min is delivered either with a syringe pump or with an LC pump in split-flow mode. The flow rate range of the nebulizing gas (nitrogen) is between 3 and 10 l/min. Typical limits of detection for CE–MS without any further preconcentration are often in the order of 1 µM [5]. The substantial dilution of the CE effluent by the sheath liquid limits the sensitivity.

Robustness, the easy handling and its automatization made the TTS the standard sheath liquid interface for CE–MS. However, the sensitivity of the TTS is an important issue because of the high dilution of the CE effluent (analyte, nl/min)

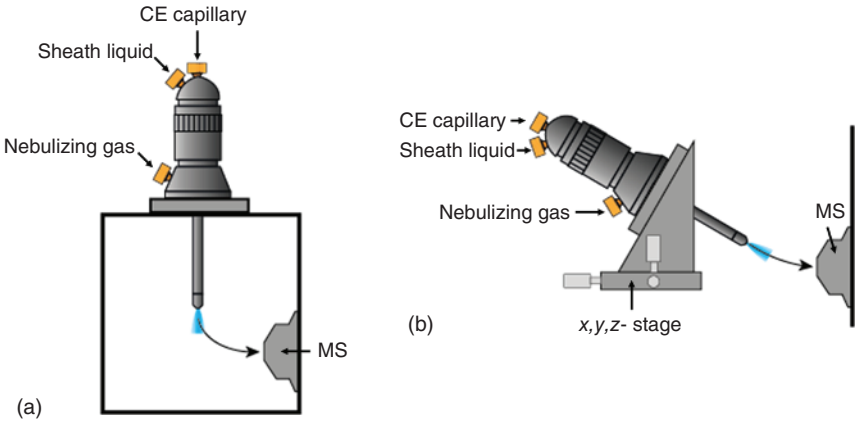

Figure 1.3 Schematic overview of possible positioning of the coaxial sheath liquid interface. (a) Off-axis positioning and (b) improved on-axis positioning. Source: Modified from Ferré et al. [12].

with the SL (µl/min). Typical starting points for the optimization of the sensitivity of the TTS interface are the sheath liquid (flow rate, additives), nebulizing gas (flow rate), ESI source (parameters), and CE capillary (positioning, shape of the capillary tip). By adjusting the sheath liquid flow rate, the dilution of the CE effluent can be reduced, and thereby the sensitivity rises. The standard sheath liquid flow rate is about 4 µl/min, but it is possible to get a stable spray with flow rates of 2 µl/min. Additives such as DMSO which has a high gas-phase proton affinity can improve the sensitivity of protein identification for bottom-up proteomics [13].

Further improvements of the TTS have been developed: (i) optimized operation mode without nebulizing gas flow [14], (ii) establishing a nanospray by using the TTS [15], (iii) horizontal TTS mounting [12], and (iv) different geometries of the sprayer parts (see TTS revisions by Agilent). The idea of the optimized operation mode without nebulizing gas flow is to overcome the disadvantages of the nebulizing gas such as suction effect. Therefore, an optimization of the following operation parameters takes place to achieve a stable electrospray: drying gas temperature, capillary voltage, sheath liquid flow rate, and capillary protrusion. This parameter optimization can improve, for example, the sensitivity of antibody separations by a factor of two and increase the S/N ratio of basic endogenous compounds by 0.5–4.5 times compared with the standard operation mode of the TTS [14]. Ferré et al. [12] changed the mounting and the direction of the sprayer to improve the sensitivity of the TTS (compare Figure 1.3). Therefore, the standard off-axis positioning of the sprayer (Figure 1.3a) is changed to an on-axis positioning (Figure 1.3b). Furthermore, the nebulizing gas flow is disabled, and sheath liquid flow rate is reduced. This combination allows a better ionization efficiency regarding the lower analyte dilution, and a higher number of ions can enter the inlet of the MS. These optimizations resulted in an improved sensitivity of 4.5 up to 12.7 times for an amino acid standard.

1.3.2 Nanoflow Sheath Liquid ESI–MS Interface

The dilution by the relatively large SL flow rates is the major disadvantage of the TTS. The developments of the nanoSL interface have the goal to improve sensitivity by reducing the SL flow rate. At the same time, the advantages of the SL concept, i.e. the flexibility, robustness, and ease of use, are supposed to be maintained. Sometimes, the nanoSL interface is also called junction-at-the-tip interface.

Most nanoSL interfaces are laboratory-specific setups. Different materials and geometries have been used. The following testimonials are common for all setups: The separation capillary from the CE is introduced into an emitter. The CE capillary is positioned close behind the emitter tip and is mostly not protruding from the emitter. Sheath liquid is supplied to the setup to stabilize the spray and to close the electric circuits. The emitter is placed close to the transfer capillary of the MS (1–10 mm) to enhance the transmission efficiency. Bonvin et al. calculated an increased efficiency of 12% for a nanoSL interface compared with only 1% for the TTS setup [4]. The nanoSL interface is often placed on-axis, while the TTS is mostly placed off-axis in front of the transfer capillary of the MS. A nebulizing gas, mandatory in the TTS approach, is not needed for spray formation because the small emitter opening and the low flow rates allow spontaneous Taylor cone formation under these conditions.

Several nanoSL interface designs have been developed over the last two decades since their introduction by Hsieh et al. [16]. Figure 1.4 gives an overview of the main developments.

The first attempt on a nanoSL CE–MS system was done by F. Hsieh et al. [16]. This setup is illustrated in Figure 1.4a. An uncoated borosilicate glass micropipette was used as the emitter, and the CZE capillary was positioned at the end of the emitter. A second capillary was inserted into the emitter to deliver a sheath liquid from a reservoir. A stainless steel wire was inserted into the emitter tip to close the two electric circuits. The electrophoretic separation was accomplished by applying voltage to the separation capillary inlet and a second voltage directly on the liquid flow within the emitter via the stainless steel wire.

An approach based on an external SL reservoir containing the electrode (Figure 1.4b) was introduced by the group of J. Dovichi in 2010 with several optimizations over the following years [18]. Later, this system was commercialized as the EMASS-II by CMP Scientific. The nanoESI takes the liquid needed from the reservoir which is at ambient pressure and connected to the emitter via polymer tubing. Since the electrode is inserted in the sheath liquid reservoir, a potential drop between the electrode and the emitter occurs. That is why this system is called “electro-kinetically pumped sheath flow nanospray interface” [18]. However, because the emitter is on higher potential than the reservoir (positive ESI), the voltage drop would cause an EOF away from the emitter [20, 21]. Additionally, this interface works similarly in positive and negative ESI modes without changing the setup [20]. This implies that the consumption of the liquid by the ESI is the driving force of liquid flow in the glass emitter. However, the resistance of the SL needs to be considered for these interfaces, i.e. requiring a rather conductive SL. A proper SL

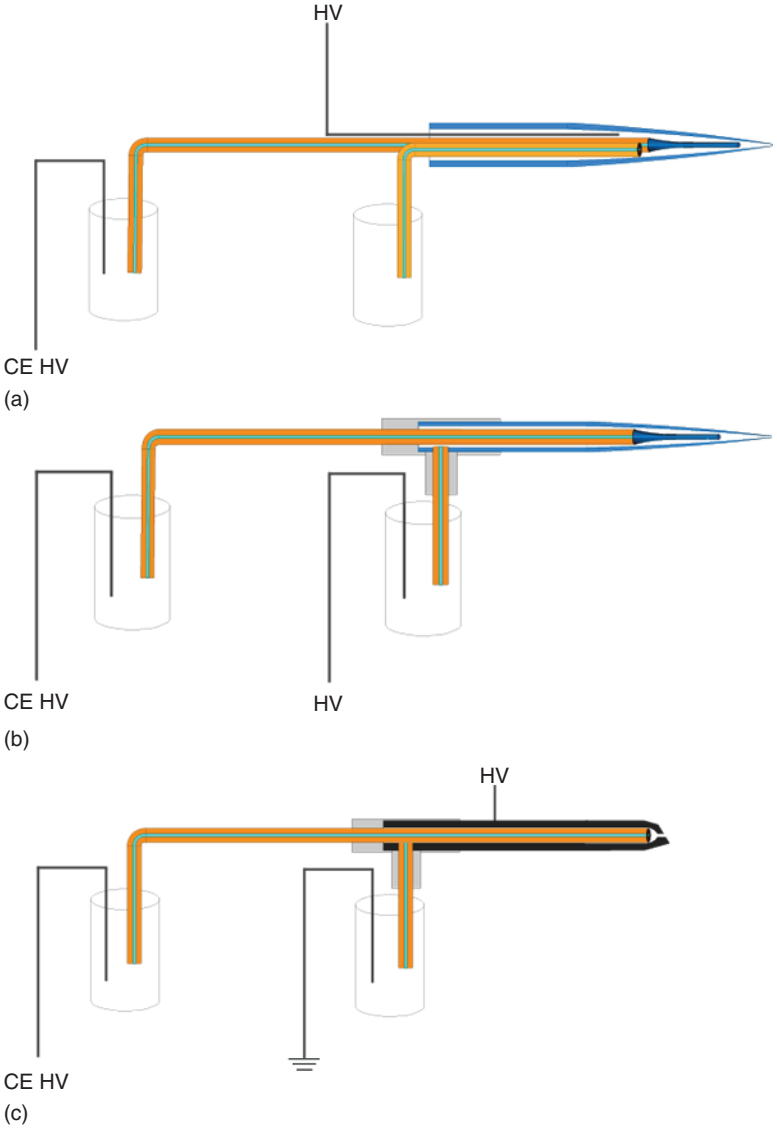

Figure 1.4 Setup of various nanoflow sheath liquid interfaces. (a) First nanoflow sheath liquid interface setup with electrode wire and SL capillary introduced in the glass emitter. Source: Modified from Hsieh et al. [16]. (b) Nanoflow sheath liquid interface setup with sheath liquid reservoir containing the electrode [17, 18]. Source: Modified from Maxwell et al. [19]. (c) Nanoflow sheath liquid interface setup with sheath liquid reservoir and with a metal emitter. Source: Modified from Maxwell et al. [19].

conductivity reduces the risk that the remaining CE voltage at the emitter is higher than the intended ES voltage.

The approaches mentioned so far used glass emitters. The emitters can be produced by pulling glass micropipettes using a commercial micropipette puller at low costs and with good reproducibility [18]. Besides their chemical inertness, glass emitters are transparent and therefore allow the observer an insight into the system. Glass emitters are available in different geometries like tapered and beveled tips. However, beveled tips from fused silica capillaries are quite difficult to produce and maintain [19].

The opening diameter of the emitter is an important parameter in nanoSL interfaces. In general, emitters with a tip opening between 10 and 40 µm are used in nanoSL setups. A point of discussion remains about the correlation between emitter tip opening, flow rate, and signal intensity. In one study, an increased intensity was reported with a larger emitter diameter for the detection of angiotensin II [18]. The correlation between flow rates and tip opening was studied by Reschke and Timperman [22]. In some experiments, they observed increased flow rates when the tip opening was larger. However, since no clear tendency could be observed in all experiments, it was assumed that slight experimental changes might create differences in the interface performance. Our recent studies demonstrate that an increased diameter results in a slight increase in the flow rate. However, the applied voltage has a major effect on the flow rate. The resulting peak intensity is directly related to the flow rate; thus, smaller emitter i.d. can result in increased sensitivity. However, clogging of the emitter is an important issue. The smaller the emitter, the more prone it is to clog. Since the CE capillaries have larger i. d. than the emitter tip, particles from the injected sample can clog the emitter. This can be reduced by using larger emitter openings.

Another important parameter to be considered is the positioning of the separation capillary within the nanoSL emitter. Depending on the emitter geometry and the separation capillary used, different dead volumes in the emitter tip remain. A large dead volume leads to broad signals, thus reducing separation efficiency. If a standard capillary with an outer diameter (o.d.) of 360 µm is placed in the emitter, a large volume in the emitter tip remains as shown in Figure 1.5a.

The dead volume can be significantly reduced when the o.d. of the capillary is decreased by grinding or etching (using hydrofluoric acid [HF]). Such capillaries can

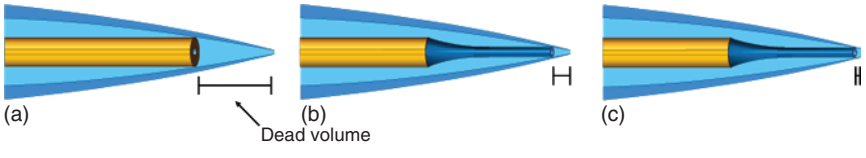

Figure 1.5 Comparison of dead volume in the emitters' tip for the nanoflow sheath liquid interface. (a) Unetched capillary within an emitter with small tip opening. (b) HF-etched capillary within the same emitter as in (a). (c) HF-etched capillary within an emitter with larger tip opening. Source: Modified from Sun et al. [18].

be placed closer to the emitter tip (Figure 1.5b). If the tip opening is increased, the volume in the tip can be further reduced (Figure 1.5c). Nevertheless, no peak broadening was observed, when the etched capillary was pulled slightly out of the emitter, showing the robustness of this parameter [20]. There are even approaches where the separation capillary is protruding from the emitter tip when the tip opening is larger than the capillaries o.d. [23, 24].

Another important parameter is the positioning of the emitter in front of the MS. The distance between the emitter tip and the MS inlet can strongly influence the signal intensity. Sun et al. studied the influence of this distance using angiotensin II [18]. The distance from the emitter tip to the MS inlet was changed starting at 0.6 mm and ending with the largest distance of 2.7 mm. The results showed an optimum of 1.7 mm distance between the emitter's orifice and the MS entrance. However, the optimum distance depends on the applied voltage and the geometry of the MS inlet. The emitter–MS distance is observed to be robust for a certain range of variations (±10%).

In 2010, the group of David D. Y. Chen introduced a system using a tapered outer tube of stainless steel as an emitter (Figure 1.4c) [17, 19]. The separation capillary is placed at the end of the hollow metal emitter. The emitter is designed in a way that the i.d. at the emitter tip is smaller than the capillaries o.d. The volume contained between the capillary terminus and the inner walls of the needle tip constitutes a flow-through microvial that replaces both outlet vial and terminal electrode of a typical CE instrument, providing electric contact while allowing the analytes to pass through to the needle tip [19]. A tee union is used to supply the sheath liquid.

Instead of the previously used glass emitter, this interface uses a metal emitter. These emitters are manufactured, for example, using stainless steel tubing which is cut to small pieces whose tip is then tapered [25]. The production of beveled tips requires more equipment and time. The metal is heated to its melting point and then quickly withdrawn. The ends are cut using a ceramic cutter. Afterward, the tip is wrapped with cellophane and ground with a rasp to the desired dimensions. Then, the tape is removed and the emitter can be used [26]. The advantage of a beveled tip is that it is rugged and durable and therefore can be used for longer measurements. A tapered tip instead of a flat tip provides a better and more stable ion current, which is an important parameter for robust nanoESI measurements and reproducible results [27]. Apart from these, the same influences as for the glass emitter are important: the emitter must be placed close to the MS. To get the best sensitivity, the optimal flow rate of the emitter must be evaluated. Similar to the glass emitter, the amount of dilution of the sample is important. When the dilution can be decreased, higher sensitivities can be achieved. However, the smaller the emitters, the higher the risk of emitter clogging or breaking [28]. A challenge arising in metal emitters is the formation of gas due to electrochemical reactions on the metal surface. These reactions lead to mechanical and oxidative stress that reduces the emitter lifetime. Therefore, noble metals should be used because they are more resistant to oxidative stress [25]. The gas formation is problematic because gas bubbles in the emitter tip potentially lead to unstable spray conditions.

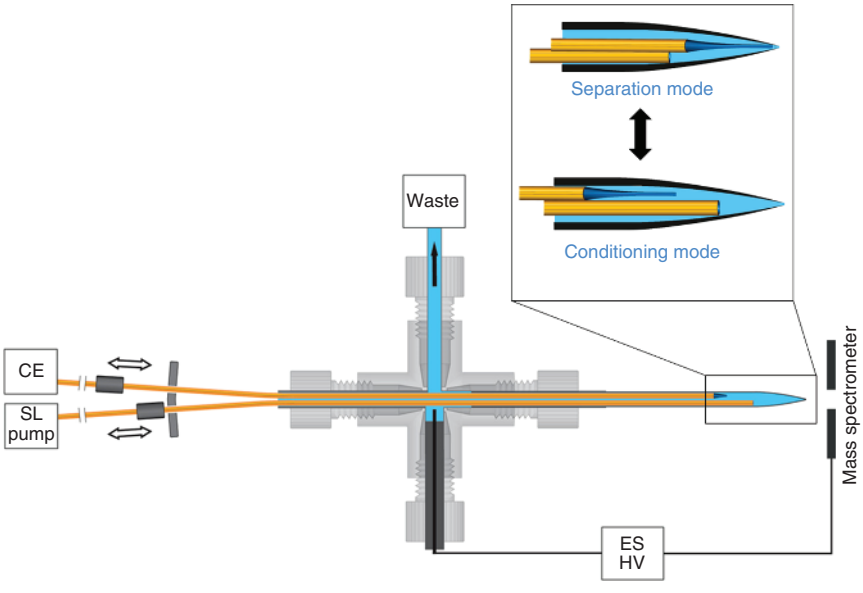

Figure 1.6 Two-capillary approach of a nanoflow interface by [21]. Capillary positioning in separation and conditioning modes. Source: Modified from Höcker et al. [21].

Based on the spread of the nanoSL after its commercialization, several improvements to the nanoSL interface have been published. Recently, we published a two-capillary nanoSL approach based on an idea from Mike Knierman [21]. The concept is illustrated in Figure 1.6.

This novel type of nanoSL interface consists of a glass emitter, in which both the separation capillary and the sheath liquid capillary are placed [21]. The two capillaries are inserted into the borosilicate emitter via a PEEK cross. The separation capillary is etched so that it can be placed closer to the emitter tip (Figure 1.5). Both CE and ES voltages are connected via an electrode inserted into that cross, and its last part is used as a drain for excess sheath liquid (open system).

The key aspect of this interface is a kind of valve function provided by the movable SL and CE capillaries enabling the interface to be switched between two modes: In the so-called conditioning mode, the separation capillary is positioned behind the sheath liquid capillary. Anything leaving the CE capillary is flushed out of the system without entering the ESI–MS. During separation, the CE capillary is placed in front of the SL capillary into the emitter tip. In this so-called separation mode, the interface works similar to the previously described setup (EMASS-II). The option to flush out anything leaving the CE capillary in conditioning mode enables unique functions not capable by any other CE–MS interface: (i) Coating and recoating of the capillary are possible without removing any part of the interface. (ii) Not the whole separation window needs to be sprayed into the MS, similar to the use of a classical rotary valve in LC–MS. Therefore, salts and any other matrix not intended to be sprayed into the MS can be removed. (iii) Particles from the sample (or formed after

sample injection in the BGE) do not reach the emitter tip, leading to reduced clogging and, thus, strongly improving the ruggedness of the nanoSL interface. (iv) Interfering components from the BGE (e.g. ampholytes) are sprayed only in separation mode where analytes are supposed to be detected. (v) The flushing of the CE capillary does not lead to enrichment of BGE in the emitter. Thus, ES conditions are kept more constant and analyses are more reproducible. In the other nanoSL setups (e.g. the EMASS-II), the enrichment of BGE in the emitter and the penetration of SL into the CE capillary are major issues as the emitter opening is smaller than the SL and CE capillaries, and mostly open systems are used facilitating the nanoESI. Overall, this 2-capillary approach leads to reduced contamination of the MS, improved ruggedness (less clogging), and simplified handling in the case of condition steps. An additional detector would even allow signal-dependent workflows.

In summary, nanoSL interfaces provide a higher sensitivity than the TTS. This is primarily due to the flow rates of low nl/min to low µl/min range. The improvement factor depends on the actual settings but is typically at least one order of magnitude [20]. However, several parameters, such as emitter opening, capillary and emitter positioning, SL composition (SL conductivity), and ES voltage, need to be optimized. Thus, a transfer of a method from the TTS to the nanoSL interface requires some experience. Also, emitter clogging and more complicated handling limit its further spread. Nevertheless, new approaches, such as the use of movable CE and SL capillaries in the emitter, strongly improve the applicability of nanoSL interfaces.

1.4 Sheathless Interfaces

Compared with other interface designs, the main characteristic of sheathless interfaces is that the electric circuit of the CE is closed, and a stable spray is created without the support of a sheath liquid. Sheathless interface approaches try to increase the signal intensity of CE–MS measurements by bypassing dilution. However, since the BGE is directly sprayed, the composition of the BGE determines not only the separation but also the ES properties. Thus, separation and ionization efficiency need to be compromised in many cases. To achieve a stable electrospray, the o.d. of the capillary terminus is typically decreased to support the formation of stable Taylor cones. Therefore, three different methods can be used: etching the tip with concentrated HF, mechanical sharpening, or flame pulling. Flame pulling decreases the i.d. alongside the o.d.

The first versions of the sheathless interface used either a capillary tip coated with conductive material (Figure 1.8a) or a metal wire (Figure 1.8b,c) to close the electric circuits of CE and MS. Further developments provided a conductive liquid to establish the electric contact outside of the separation capillary. Those approaches include porous-tip interfaces, where the electric contact by the conductive liquid is established by ions diffusing through the thin-walled separation capillary tip or other approaches such as using a crack or hole in the separation capillary. The commercialized porous-tip interface is described in Section 1.4.1, while a selection of other sheathless interfaces are described in Section 1.4.2.

1.4.1 Porous-Tip Interface

The most widely used sheathless CE–MS interface is based on the work of Moini [30] and consists of an etched capillary with a porous tip on the interface side. For the creation of the porous tip, the capillary tip is etched with HF until the wall is thin enough (5–10 μm) so that ions can diffuse through the glass wall. While etching the capillary, it is important to flush it with either N_2 or water, in order to prevent the widening of the capillary i.d. and thus interfering with the ES. An electric contact is established without a liquid junction, and, therefore, without diluting the analytes, no nebulizing gas is required. This approach was first introduced as the high-sensitivity porous sprayer (HSPS) design (Beckman Coulter) and later sold as a combination of a CE instrument with the porous-tip interface as the CESI 8000 (Sciex, shown in Figure 1.7).

The commercial porous-tip interface (CESI) includes a capillary (i.d. 30 μm, o.d. 150 μm, length 90 cm) with a porous tip (i.d. 30 μm, o.d. 40 μm) that is inserted in a stainless steel ESI sprayer and positioned close to the MS. A conductive liquid establishes the electric contact for both the separation and the ES. While the conductive liquid is static, it should be replaced regularly to remove electrochemical degradation products and to replenish vaporized conductive liquid [30]. A small pressure can be applied on the conductive liquid reservoir in order to exchange continuously the conductive liquid. As conductive liquid, either acidic solutions or the BGE is used. The porous capillary tip protrudes from the ESI sprayer by around 5 mm to allow dilution-free spraying of the analytes. To support the ionization of the

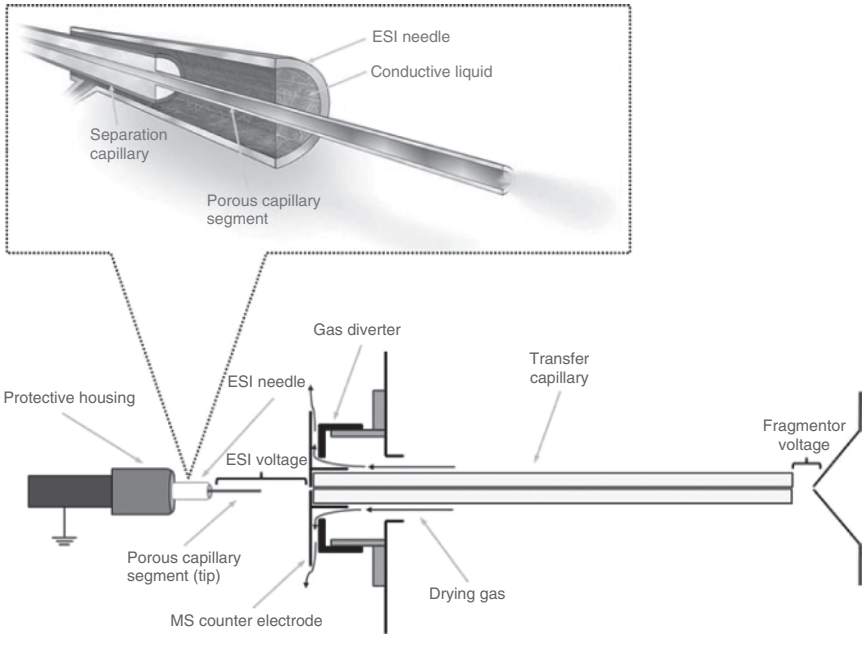

Figure 1.7 Principle of the commercialized porous-tip interface (CESI, Sciex). Source: Bonvin et al. [31] / with permission from John Wiley & Sons.

analytes in the BGE without ionizing the conductive liquid, the ES voltage needs to be adjusted for each application independently. The applied ES voltage depends on the BGE composition and the distance between the capillary tip and the MS. The voltage range for the formation of an optimized spray is narrow. For the separation of low-molecular weight analytes at pH 3 using the prototype of the HSPS, the voltage range for optimal ionization was between 1150 and 1250 V [31]. Drying gas flow rate and drying gas temperature are crucial parameters that need to be adjusted to create a stable spray. A high drying gas flow rate can lead to a distorted spray, while in the case of pure aqueous BGEs high drying gas temperatures were required [31]. The ionization can be further improved by adding organic solvents such as isopropanol to the BGE. In contrast to sheath liquid interfaces, where the SL mainly affects the formation of the electrospray and the BGE mainly the separation, sheathless interfaces are more restricted. Since the BGE needs to provide optimal conditions for the CE separation and the ESI, the possible applications are limited. In the case of protein separation, it was shown [32] that it is possible to add 5% IPA to the selected BGE without significantly decreasing the separation.

One critical parameter in sheathless interfaces is the necessity of a small flow directed to the MS side of the CE capillary. Generally, a small flow rate results in sensitive measurements for all sheathless approaches, including the porous-tip interface. In an experiment where angiotensin I was pressure-driven injected through the porous-tip capillary, a spray could be achieved for flow rates between 4.2 and 336 nl/min. This translates to electroosmotic mobilities between 2.9×10^{-5} and 233×10^{-5} $cm^2/V\,s$ [33]. For low EOF systems (e.g. when using neutral coatings), typically a small pressure is applied to create a stable flow that is directed toward the MS and supports the ionization. To generate a stable and reproducible spray, a continuous flow rate is required. This is often problematic in the case of protein containing samples where proteins absorb at the capillary wall and change the EOF over time [32]. When applying pressure, the separation can decline because of the parabolic flow profile in a pressure-driven system. The suction caused by the electrospray causes a small flow rate in sheathless interfaces as well.

Apart from the commercial CESI interface (Sciex), which is compatible with mass spectrometers from various vendors, other lab-built alternatives and modifications were published. Instead of using an axial approach it is possible to use an orthogonal approach by spraying the analytes from a grounded TTS. The nebulizer is sealed shut while the conductive liquid is filled through the sheath liquid tube before the first measurement. Compared with the traditional usage of the TTS, it is necessary to increase the distance between the capillary tip and the sprayer tip, and lower voltages need to be applied to prevent the conductive liquid from being ionized. Compared with the on-axis approach, this version has proven to be less sensitive [32]. Other approaches [20] use an additional glass emitter with a wide opening (100 μm) where the porous capillary extends the emitter. The electric circuit is closed by a separate electrode. The same setup was used as a nanoSL interface with the CE capillary not protruding through the narrow-opened glass emitter tip (30 μm).

For porous-tip interfaces, typically 30 μm i.d. capillaries are used. The porous-tip interface works best for small inner tip diameters because of the stable electrospray

and higher MS sensitivities. However, a small i.d. limits the possible sample loading volume. There are several ideas trying to overcome this disadvantage, e.g. connecting a capillary with a larger i.d. (100 μm) to a short porous-tip capillary with a smaller i.d. (20 μm) [34]. The two capillaries are connected by inserting the smaller capillary in the larger capillary and gluing them together. This allows for separate replacement of the tip capillary and the separation capillary. A sensitivity increase of 11-fold can be detected when comparing the new approach with a conventional porous-tip interface (i.d. 30 μm). In both cases, the same percentage of total capillary volume was injected. On the contrary, the sudden decrease of the i.d. increases the risk of capillary clogging. Another similar idea is decreasing the bore size of the emitter tip [35]. After etching the emitter down to a wall thickness of 20–30 μm, the etched capillary tip is narrowed with a CO_2 laser puller to create an i.d. of 1.3 μm at the capillary opening. After creating a tapered tip, the etching is continued until a porous tip with a 5–10 μm i.d. tip and 10 μm wall thickness is achieved. Compared with the conventional CESI, the nanoCESI had higher (3.6-fold) and more stable signals, and no additional pressure to support the formation of a stable spray was required. The interface is restricted due to its increased risk of capillary clogging, e.g. the precondition of the capillary with 1 M NaOH turned out to be problematic. When combining the porous-tip approach with an adjustable metallic needle [36], it is possible to pull back the porous tip into the conductive liquid, using the BGE-filled needle as the outlet. This allows for more stacking and preconcentration possibilities. For example, it is possible to use an EOF directed at the inlet during preconcentration. For the commercialized CESI, it would be only possible to apply this technique until the BGE passes the porous section and the connection is lost.

Generally, the porous-tip interface has several advantages. Due to the distance between the capillary and the electrode, bubble formation does not impact the electrospray stability. Additionally, bubble accumulation at the electrode can be prevented by replenishing the conductive liquid regularly. Another advantage is the lower risk of clogging due to a steady inner capillary diameter over the whole capillary. Similar to other sheathless interfaces, the analytes are not further diluted because no liquid junction occurs which allows overall for better sensitivity. The sensitivity improvement compared with the TTS is typically between one and two orders of magnitude depending on application [20, 32]. Further to decreasing the effects of dilution, no additional contaminations can be introduced by a sheath liquid. However, the BGE is solely responsible for the separation and the ionization. Therefore, nanoSL interfaces can have a similar sensitivity. The effect of dilution in the nanoSL interface is counteracted by an increased ionization efficiency due to organic solvent in the SL. Another restriction for the standard porous-tip interface is the fact that only capillaries with an i.d. of 30 μm are available.

1.4.2 Other Sheathless Interfaces

This chapter introduces a selection of additional sheathless interfaces that are commonly used. Figure 1.8 shows an overview of these other approaches which establish an electric contact without diluting the analytes. Since operation parameters and

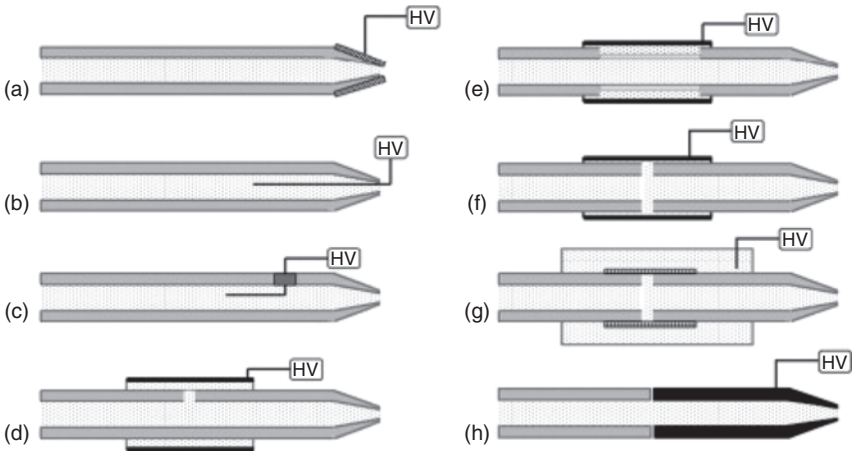

Figure 1.8 Different approaches of sheathless interfaces. (a) Conductive coating applied to the emitter tip; (b) wire inserted at tip; (c) wire inserted through hole; (d) split-flow interface with a metal sheath; (e) porous, etched capillary walls in metal sleeve; (f) junction with metal sleeve; (g) microdialysis junction; and (h) junction with conductive emitter tip. Source: Maxwell and Chen [29] / with permission from Elsevier.

limitations of these interfaces are heavily dependent on the specific interface design, we focus on the most important concepts only. A more detailed discussion can be found in the review of Maxwell et al. [19].

One of the first sheathless interfacing types is the conductive-tip approach (Figure 1.8a) which to this day is further evolving. Its main characteristic is that the nonconductive capillary tip is coated with a conductive material to close the electric circuits. The conductive tip needs to be long lasting in order to result in reproducible data. This can be achieved through varying the emitter-coating material and the emitter-coating procedure. For coating, several different conductive materials such as metals, graphite, and polymer coatings can be used. Depending on the application, emitter lifetimes can range between less than an hour and over 100 hours. A sign that an emitter coating is destroyed is the decrease of the signal intensity over time, but the definition that outlines the lifetime of an emitter coating varies. Each of the materials has several advantages and different restrictions, and, in this chapter, only a small overview is presented. While coating emitters with silver is a simple procedure, silver has a poor electrochemical stability at high voltages. On the other hand, gold coatings have a higher electrochemical stability, but gold is less physically stable on glass. A proposed solution to increase the lifetime is the use of gold-coated emitters where the gold was chemically deposited on underlying silver. Compared with a pure silver coating, the lifetime of the gold/silver coating is significantly increased from around 80 to 600 hours [37].

There are several different methods to produce an emitter coating, and the method should be chosen according to the analytical requirements and the selected coating material. Coating methods can range from vacuum deposition over electrochemical deposition [37] to gluing of particles or whole foils [38] on the emitter tip. A recent

application [38] that coated the emitter by gluing gold foil to the emitter tip reached lifetimes of 180 hours. An important aspect for the choice of the appropriated coating is its dependence on the application because some coatings are not compatible with the addition of organic solvent to the BGE, while other coatings are not compatible with a basic BGE.

The short lifetimes of the capillaries due to the degradation of the coating or tip clogging can be counteracted by disconnecting the tip from the separation capillary. This allows for the use of rather short-living tips while prioritizing a simple and reproducible coating procedure [39]. In general, the introduction of a second capillary as a tip might decrease the separation capability of the CE setup due to misalignments and the creation of dead volumes.

To avoid the complicated coating of the emitter, other approaches were developed including a discontinuity near the end of the capillary which can be a crack through the capillary, a small hole (Figure 1.8d), or a short gap between two capillaries (Figure 1.8f). These possibilities all generate the electric contact with the help of an external liquid. When the gap between the two capillary parts is larger and an auxiliary liquid is mixed with the CE effluent, the approach is called liquid junction and described in Section 1.5.1. Interface designs, where the conductive liquid is not actively mixed with the BGE, are sheathless interfaces and will be further described in the next paragraph. In all cases, a small part between the electric contact and the spray tip is created where the CE conditions no longer apply.

One of these approaches is an interface with a small crack in the capillary where the electrical contact is established outside of the capillary through a platinum electrode surrounded by conductive liquid. It was possible to achieve peak heights 18.2-fold higher than for the TTS [40]. A similar approach is the creation of a small opening in the capillary wall without completely cutting the capillary making the interface easier to handle. In the past, this idea was restricted because etching or other available processes were unable to create reproducible capillary openings. A recent approach to cut reproducible openings into the capillary wall was achieved by using a CO_2 laser [41, 42]. To minimize analyte loss, it is possible to use a microdialysis membrane [43] to cover the crack [40], allowing only small molecules to transfer between the inside and outside of the capillary.

However, all these alternative sheathless interfaces are not as widely distributed as the porous-tip interface. This is due to the commercialization of the CESI interface, which makes porous-tip interfaces widely accessible and easy to handle. In order to apply the other (not commercialized) sheathless interfaces, the operator needs to reproduce often rather complicated production protocols using special equipment.

1.5 Other CE–ESI–MS Interfaces

1.5.1 Liquid Junction

The liquid junction interface is a CE–ESI–MS interface, which uses an auxiliary liquid (makeup liquid – others also call it “sheath liquid”) at the capillary terminus

to close the electric circuits of the CE separation and the ESI. The idea of this kind of CE–MS interfacing is the combination of the sensitivity of sheathless interfaces (Section 1.4) with the advantages of the sheath liquid interfaces (Section 1.3).

The general setup of the liquid junction interface consists of the CE separation capillary, the makeup liquid capillary, connector (e.g. tee union), and the spray capillary. The makeup liquid is usually added and mixed to the CE effluent within a gap of 20–200 µm between the CE separation capillary and the spray capillary/emitter [44]. The reproducible adjustment of the gap between separation capillary and spray capillary is a crucial step in liquid junction interfaces. Compared with the nanoSL interfaces, which are based on the "junction-at-the-tip" principle (separation capillary surrounded by a sheath liquid-filled emitter), the distance without an electrical field for the CE separation is longer, which can have an adverse effect on the zone dispersion.

Due to the limitations of the first interface approach such as contamination of the makeup liquid, band broadening [45], and reproducible capillary, new approaches have been developed, based on a tee union connector or on a microchip (most recent). Wachs et al. designed and characterized a self-aligning liquid junction electrospray interface [45]. The main part of the interface is the PEEK tee for the connection of separation capillary, makeup liquid, and spray capillary. The spray capillary can be made of stainless steel or fused silica. The width of the gap between the CE capillary and the sprayer capillary is not determined. The improvement of the signal-to-noise ratios compared with the TTS is about three times [45]. Krenkova et al. [46] designed and characterized a hybrid liquid junction-based interface used with an automated CE instrument (Figure 1.9).

This liquid junction interface is based on a polyimide chip that contains a star structure for the reproducible positioning of the separation capillary with an unobstructed flow of the makeup liquid/spray liquid around its exit and the ESI spray channel. The chip is mounted within a plastic cylinder. This cylinder has three connections for the CE separation, the auxiliary liquid, and the waste capillaries. The cylinder assists the mounting of the planar terminus of the CE capillary within the star structure on the chip. The dimensions of the channels on the chip have been designed to keep the adverse effect on the zone dispersion negligible. The lifetime of the chip was specified with more than 100 analyses. The chip-to-chip repeatability of the microchips was tested out as well without any significant influence on the CE–MS analysis. The improvement of the signal-to-noise ratios according to the coaxial sheath liquid interface was about 9–31 times [46].

The liquid junction approach provides makeup liquid flow rates mostly well below 100 nl/min, depending on the ESI voltage. That results in an analyte dilution comparable with the sheathless interface with the ability of using standard CE capillary dimensions for the separation (i.d. of 50 or 75 µm). In contrast to the sheathless interface, the makeup liquid allows the improvement of the electrospray conditions by using compositions of aqueous organic solvent mixtures with a low percentage of a volatile acid or base. This decouples the CE separation (EOF) and the electrospray formation. The independence of the EOF within the capillary for the electrospray

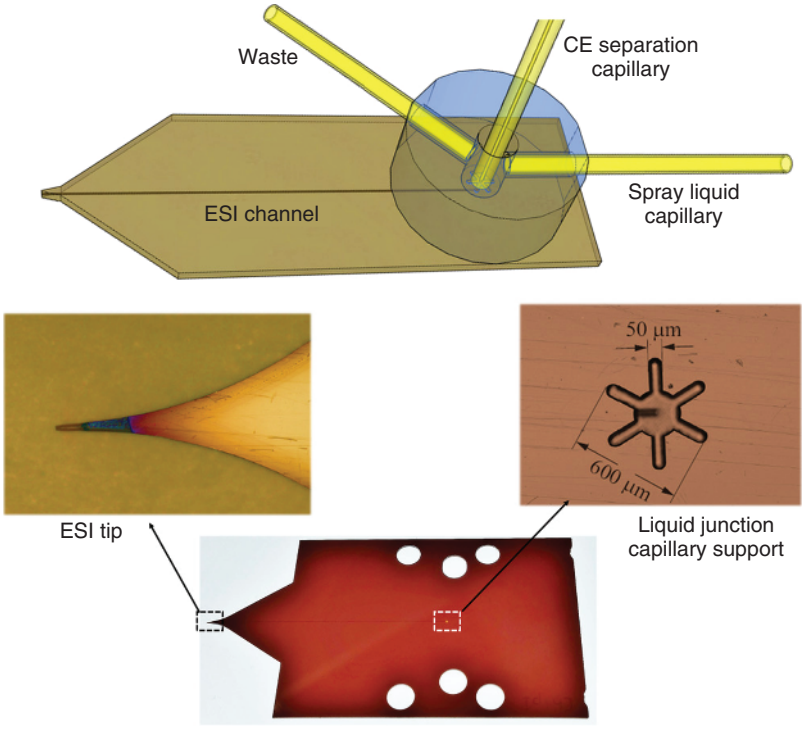

Figure 1.9 Scheme of a hybrid liquid junction interface. Source: Krenkova et al. [46] / with permission from John Wiley & Sons.

allows a wider range of CE applications, e.g. neutral capillary coatings. Furthermore, the low flow rate allows the usage of narrow electrospray tips that lead to high electric field strength even at lower voltages for nanoESI. The emerging nanospray has a better ionization efficiency and sensitivity, and is less affected by ion suppression effects.

However, the liquid junction approach is not as widely distributed as the nanoflow approaches of nanoSL and porous-tip interface. This is due to the absence of a commercialized interface and the challenge of a reproducible alignment of CE capillary and spray capillary to overcome, for example band broadening and to achieve homogeneous mixing of CE effluent with makeup liquid.

1.5.2 Interface-Free CE–MS

Generally, the current in CE is much higher than the ES current. Therefore, an electric contact is needed at the end of the CE capillary. When the CE current is brought to the ES value (<1 µA), the applied voltage at the CE inlet can be used for direct ES without an extra electrode. This concept has been published as "interface-free" approach by Frantisek Foret's group a few years ago [6, 7]. A schematic design of "interface-free" CE–MS arrangement is shown in Figure 1.10.

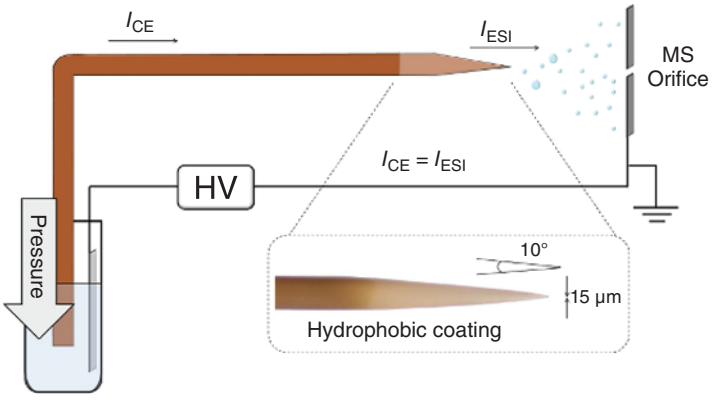

Figure 1.10 Schematic design of "interface-free" CE–MS. Source: Tycova et al. [7] / with permission from Elsevier.

The simplicity of this approach can be recognized in the figure. The applied voltage at the inlet separation serves for the separation and subsequent ESI. A single electrical circuit ranges from the CE inlet to the MS. The electrospray tip was formed at the outlet of the separation capillary by grinding on fine sandpaper and final polishing with fiber-optic lapping film. A hydrophobic external coating was applied on the outer surface of the electrospray tip. A significant improvement in the nanoES stability was achieved compared with the bare fused silica surface used before [7]. In order to keep a sufficient high potential for the ES, the resistance of the CE capillary needs to be high. This can be achieved by a low-conductivity BGE and/or narrow capillaries (here an i.d. of 20 µm was used). The narrow capillaries provide benefits regarding the separation efficiency and ES properties but limit the injected volume and bear the risk of clogging.

In summary, integration of the separation capillary with the nanospray without using junctions or fittings makes this "interface-free" approach simple without scarifying the sensitivity. However, the flexibility is limited, and CE performance can be compromised due to size of the capillary and choice of BGE.

1.6 Microchip Electrophoresis–MS Interfaces

To perform a rapid, comprehensive, and cost-effective analysis, the micro-total analysis systems (µTAS) concept was introduced as a major research field. The analytical µTAS are attractive for the analysis of biological samples since they offer the prospect of integrating several functional elements on a single platform and require only a small amount of sample.

MCE, as a valuable separation technique, attracts special attention owing to its excellent characteristics of rapid analysis even in seconds, low sample and reagent consumption, and high throughput. On the other hand, MS provides the structural information for high-quality qualitative identification and good sensitivity for the

quantitative determination of target molecules. As a result, the application scope of MCE–MS covers various analytical studies such as gene analysis, proteomics, and medicine development and provides sensitive and universal detection along with the potential addition of MS/MS information.

Although MCE–MS is commercially available and its applicability has been practically proven, MCE–MS remains an active research area. MCE–MS was founded on glass microchips, because glass is a chemically inert material, has well-defined surface properties, and can be used to generate an EOF. Later on, the fabrication process has been expanded to include the use of various materials including polydimethylsiloxane (PDMS), polymethyl methacrylate (PMMA), and cyclic olefin copolymer (COP). In terms of fabrication time, cost, labor intensiveness, and simplicity, polymer materials come up with significant improvements over the glass. Application of these polymeric materials to the fabrication of MCE–MS devices allows the disposable use. Since clogging occurs frequently in the microchannel, disposability is one of the important factors for selecting the chip material in MCE–MS.

In general, the coupling of MCE and MS is based on the same principle as CE–MS, whereas more sophisticated interfacing hardware is required due to the smaller scale and different geometry (planar system versus capillary format). Among the several available interfaces, two soft ionization methods, namely, ESI and MALDI, have been applied to MCE–MS, which ionize analytes from solutions and dried samples, respectively. Flow rates typically achieved in microfluidic channels are on the order of several tens of nl/min, which is compatible with miniaturized ESI. Hence, ESI is the predominant approach for online coupling. In contrast, MALDI is generally restricted to offline coupling (see Section 1.7.1).

Two different critical issues should be considered to hyphenate MCE and ESI–MS. First, the electrical connection to define the electrophoretic field strength along the separation channel and the generation of the electrospray for MS detection. Similar to CE–MS, it can be applied using makeup flow (MUF) channels (analogous to sheath-flow approach for CE–ESI–MS), liquid junctions, and conductive emitter coatings or ion-permeable membranes (analogous to the sheathless method for CE–ESI–MS). Second, a miniaturized electrospray is needed after separation in the planar chip. The MCE–ESI–MS concepts can be divided into five approaches including blunt end of microchip, external capillary, external emitter, corner of microchip, and monolithic emitter as illustrated in Figure 1.11.

Ramsey et al. attempted coupling the MCE with ESI–MS using a *blunt glass microchip* (Figure 1.11a) [48]. In order to obtain an electrical contact, a side channel was fabricated and connected to the separation channel before the exit of the separation channel. The makeup liquid provides both electrical contact and additional liquid for ES. Since the nanospray is established directly from the planar edge of the microchip, the fluid tends to spread over the glass surface and form large droplets, resulting in excessive band broadening. Moreover, higher electrospray voltages are required to form the spray, and consequently, the separation efficiency and sensitivity were reduced.

To overcome the drawback of the blunt end, the connection to an *external capillary* (Figure 1.11c) or an *emitter* (Figure 1.11d) was introduced. This is known as the

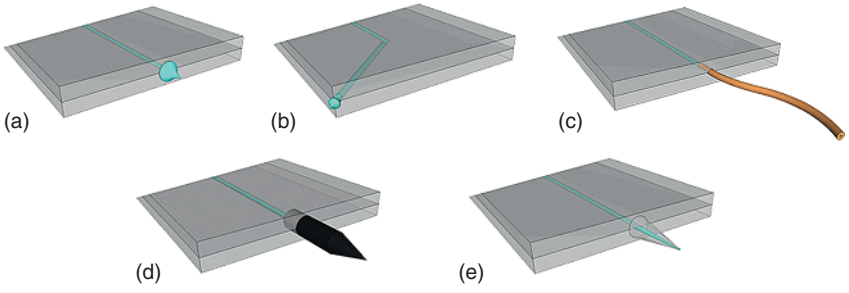

Figure 1.11 Different approaches to generate the nanospray in MCE–ESI–MS: (a) blunt end, (b) corner, (c) external capillary, (d) external emitter, and (e) monolithic emitter. Source: Ohla and Belder [47] / with permission from Elsevier.

off-chip technique. In this approach, a commercially available tapered capillary or emitter is manually attached to the microchannel outlet using a PEEK screw/sleeve or adhesive. In principle, these devices can be coupled to MS using all CE–MS interfaces described above; however, often sheathless devices are used. Due to some difficulties in high-precision drilling of the exit hole of the separation channel and placing the external emitter/capillary, a liquid junction MCE–MS was introduced [49, 50]. The junction channel is created by adding a cross-channel on the chip, which left a small gap between the end of the separation channel and the inlet of the emitter tip. Then the cross-channel is flushed with a conductive liquid. This liquid provides the electrical contact and additional liquid for ES. Although an external capillary/emitter provides a stable ES, it requires a manual postprocessing in addition to microfabrication and also easily results in large dead volumes at the intersection and decreased separation efficiency.

In response to the requirement for a fully dead volume-free interface, the sharp *corner of the microchip* and the monolithically fabricated emitter were produced as a tip (Figure 1.11b). Ramsey et al. modified their MCE, so that the separation channel terminates at the corner of a thin glass microchip, providing a sharp two-dimensional feature for an electrospray tip [51]. A side channel is connected to the separation channel just 200 µm before its exit. The electrode was immersed in the side channel reservoir. A schematic of the device and the generated electrospray are shown in Figure 1.12.

This MCE–ESI–MS device has been commercialized as "ZipChip" (908 devices). Using a similar interface, IntaBio (now Sciex) introduced microchip-imaged CIEF coupled with MS.

In these devices, a makeup liquid is used to provide both the electrical contact and an additional liquid for ESI. In addition to the independent adjustment of the separation and ionization potentials, an advantage of the makeup liquid approach is the possibility to promote the spray process by adding reagents without affecting the electrophoretic separation. It is accompanied by analyte dilution; as a consequence, the risk of sacrificing the detection sensitivity and band broadening increases. However, this dilution often can be made up with better ionization due to organic

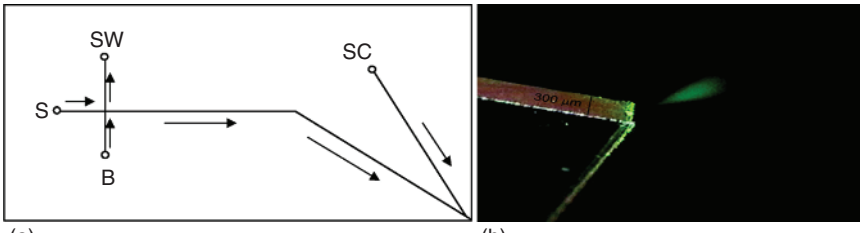

Figure 1.12 (a) Schematic diagram of the MCE with the sharp corner to produce the nano-electrospray. The reservoirs are labeled S (sample), B (buffer), SW (sample waste), and SC (side channel). The arrows show the EOD direction through applied voltage. (b) Image of the electrospray plume generated from the corner of an MCE–ESI–MS acquired with a CCD camera. Source: Mellors et al. [51] / with permission from American Chemical Society.

solvent in the ESI process (see Section 1.2.1). Earlier, chips using zero, one, and three MUF channels were compared. The authors concluded that, while LODs are improved for drugs without MUF, ESI is more robust and easier to optimize with MUF [50].

A *monolithically fabricated glass emitter* for MCE–ESI–MS was reported by Belder et al. [52]. They enclosed a cone at the end of a Borofloat glass microchip and then drew the protruding cone out to form a sharp tip with a diameter of few micrometers. The tip was next cut and treated with HF to remove any debris. A schematic diagram of a microfluidic glass chip with a monolithically integrated nanospray emitter is shown in Figure 1.11e.

To eliminate the sample dilution, several sheathless MCE–MS systems were developed using on-chip and off-chip emitter tips. In the most commonly used sheathless electrospray designs, the voltage is applied onto a conducting layer at the emitter tip. Therefore, a durable conducting coating is needed. The shape, inner size, and surface properties of the emitter must be considered. Conductive polymeric, graphite, and metallic coatings have been used in various applications. Similar to sheathless CE–MS interfaces (see Section 1.4.2), the common drawback to this technique is the short lifetime and limited robustness of coated tips. In a recently developed alternative design [53], an ion-conductive hydrogel membrane is placed in a Y-shaped microfluidic supporting channel at the end of the separation channel and contacted via platinum electrodes to ensure a reliable electrical connection without sample loss or dilution. Although a dead volume-free MCE–MS device is very attractive, clogging of the fine ES tip and/or narrow separation channel will be troublesome during replicate measurements. Therefore, the introduction of disposable chip substrates such as PDMS, PMMA, and COP is desired in such highly integrated microchips. In comparison with glass processing, patterning of polymer substrates is relatively rapid and inexpensive, and sharp ES emitter structures can be easily patterned as integral parts of separation microdevices. For a more detailed view on MCE–MS, we refer to [47, 54].

1.7 Alternative Ionization Techniques for CE–MS

By far, the most widely used technique for ionization of nonvolatile analytes in liquid phase is ESI. However, CE has also been combined with MALDI–MS or with inductively coupled plasma mass spectrometry (ICP–MS). These two techniques are discussed briefly below. Rarely, other ionization techniques like atmospheric pressure chemical ionization (APCI), atmospheric pressure photoionization [55], or dielectric barrier discharge ionization (DBDI) [56] are used. The main idea of performing CE–APPI–MS and CE–APCI–MS is the option to use nonvolatile BGEs, e.g. in the context of MEKC [55] or microemulsion electrokinetic chromatography (MEEKC). However, on the one hand, alternative techniques exist, and on the other hand, the application in the field of metabolomics and proteomics is scarce.

1.7.1 CE and MCE Combined with MALDI–MS

CE–MALDI–MS is mostly performed offline: using the coaxial triple tube sheath liquid interface as in ESI, the electrical circuit of the CE is closed. The sheath liquid supplied in µl/min leads to reasonable amounts of liquid to be placed on each spot. The dilution caused by the SL is not critical since the solvent is evaporated before the analysis. However, the SL influences the crystallization process essential for subsequent MALDI. This offline combination of CE and MALDI–MS has been applied to proteomics workflows. The decoupling of separation and MS allows for extensive measuring time (MS and MS/MS experiments) and later remeasurements of interesting fractions. However, CE–MALDI lacks sensitivity since the absolute amount of sample delivered is rather small, compared with microLC with roughly two to three orders of magnitude higher sample loading capabilities. In CE–MS, several buffer systems such as phosphate and borate are incompatible due to low volatility of electrolytes. Especially in ESI–MS, the nonvolatile additives cause low ionization efficiency and lower analyte detectability. To overcome this limitation, the combination of CE with MALDI has been introduced. However, salt adducts also disturb the analysis in MALDI.

When coupling MCE to MALDI, it is possible to use a simple approach, i.e. the MCE separation is performed in the open microchannels. After solvent evaporation, the chip is transferred to the MALDI source. This method was first described for the rapid separation of biological samples within a few seconds [57].

Unlike offline CE–MALDI–MS, the online coupling is challenging because the MALDI target is under vacuum while the CE operates at atmospheric pressure. As a unique approach for online coupling of CE and MCE to MALDI–MS, a rotating ball interface was developed [58, 59]. In this interface, the output of the separation channel is machined to allow the direct contact deposition of effluent onto the rotating ball interface. Afterward, a matrix is added to the surface of the ball, and UV laser is irradiated for MALDI–MS detection. The interface has the advantage of decoupling the ionization process from the separation step.

ESI is predominant for online CE–MS, while MALDI is used as an offline coupling approach. MALDI–MS offers some advantages including attomole detection

sensitivity and a tolerance to nonvolatile constituents of the BGE. For a more detailed discussion on interfacing techniques as well as applications on CE–MALDI–MS, we refer to the reviews by Huck et al. [60] and Zhong et al. [44].

1.7.2 CE–ICP–MS

CE can be coupled to ICP–MS providing information on the (heavier) atoms present in the sample, and is therefore useful for elemental and species analyses. The coupling is performed using a miniaturized approach similar to LC–ICP–MS coupling. Similar to CE–ESI–MS a SL is typically applied for closing the electrical circuit of the CE. Dilution is not an issue; however, the small injection volume of CE restricts sensitivity. In bioanalysis, PTMs of proteins or peptides with rare and heavy atoms are the target for CE–ICP–MS. To obtain more insight into the methodology of CE–ICP–MS, see Michalke [61].

1.8 Concluding Remarks and Outlook

The coaxial TTS is still the reference for CE–MS interfacing. This is due to its ease of use, flexibility, and robustness. However, the development of nanoESI interfaces in the last decade has significantly improved the sensitivity of CE–MS. The signal intensity is improved by at least one order of magnitude, partially up to two orders of magnitude using sheathless or nanoSL designs ([20] and references therein). Since both designs have been commercialized, these nanoESI interfaces are increasingly used, especially in bioanalysis where the sensitivity is a major factor. However, both the porous tip (CESI) and the nanoSL (EMASS-II) still have limitations with regard to ease of use, flexibility, and robustness. Further developments can be expected to increase ease of use and robustness, leading to a further spread of CE–MS.

Compared with LC–ESI–MS, CE–ESI–MS is considered to be less concentration sensitive. However, nanoESI interfacing technology overcomes this limitation of CE–ESI–MS: a simple model calculation leads to identical peak concentration in both techniques (CE: 50 µm i.d. × 70 cm capillary, 30 nl injection, 6s peak width, ESI flow rate: 300 nl/min ↔ LC: 20 µl injection, 6s peak width, ESI flow rate: 200 µl/min). Several effective parameters such as the preconcentration possibilities, the solvent type, and the ESI geometry, however, need to be considered for a more detailed comparison. The proven sensitivity down to the mid ng/l (pptv) level [61] demonstrates that the limitation of CE–MS regarding sensitivity has been overcome. These model calculations and recent experimental results show that CE–MS can be performed with similar sensitivity compared with LC–MS despite the injection of lower volumes. Therefore, sensitive interfacing will enable CE–MS to be applied in many fields of bioanalysis where its selectivity is beneficial compared with LC–MS. CE–MS will be increasingly applied to the analysis of metabolites, intact proteins, and PTMs as subject of this book. These applications will continuously force future development in interfacing at the same time. Similarly, MCE–MS can play a major role in future bioanalytical µTAS developments. In conclusion, the

improved CE–MS interface technology will further promote CE–MS in many fields of (bio)analytical applications.

List of Abbreviations

APCI	atmospheric pressure chemical ionization
BGE	background electrolyte
CE	capillary electrophoresis
CIEF	capillary isoelectric focusing
COP	cyclic olefin copolymer
CRM	charge residue model
CZE	capillary zone electrophoresis
EOF	electroosmotic flow
ES	electrospray
ESI	electrospray ionization
HF	hydrofluoric acid
HSPS	high-sensitivity porous sprayer
ICP	inductively coupled plasma
ICP-MS	inductively coupled plasma – mass spectrometry
i.d.	inner diameter
IEM	ion evaporation model
LC	liquid chromatography
MALDI	matrix-assisted laser desorption/ionization
MCE	microchip electrophoresis
MEEKC	microemulsion electrokinetic chromatography
MEKC	micellar electrokinetic chromatography
MS	mass spectrometry
MUF	makeup flow
nanoSL	nanoflow sheath liquid
o.d.	outer diameter
PDMS	polydimethylsiloxane
PMMA	polymethyl methacrylate
SL	sheath liquid
TTS	triple-tube sprayer
μTAS	micro-total analysis systems

References

1 Dole, M., Mack, L.L., Hines, R.L. et al. (1968). Molecular beams of macroions. *J. Chem. Phys.* 49 (5): 2240–2249.

2 Iribarne, J.V. and Thomson, B.A. (1976). On the evaporation of small ions from charged droplets. *J. Chem. Phys.* 64 (6): 2287.

3 Hommerson, P., Khan, A.M., de Jong, G.J., and Somsen, G.W. (2011). Ionization techniques in capillary electrophoresis-mass spectrometry: principles, design, and application. *Mass Spectrom. Rev.* 30 (6): 1096–1120.

4 Bonvin, G., Schappler, J., and Rudaz, S. (2012). Capillary electrophoresis-electrospray ionization-mass spectrometry interfaces: fundamental concepts and technical developments. *J. Chromatogr. A* 1267: 17–31.

5 Lindenburg, P.W., Haselberg, R., Rozing, G., and Ramautar, R. (2015). Developments in interfacing designs for CE–MS: towards enabling tools for proteomics and metabolomics. *Chromatographia* 78 (5–6): 367–377.

6 Tycova, A. and Foret, F. (2015). Capillary electrophoresis in an extended nanospray tip-electrospray as an electrophoretic column. *J. Chromatogr. A* 1388: 274–279.

7 Tycova, A., Vido, M., Kovarikova, P., and Foret, F. (2016). Interface-free capillary electrophoresis-mass spectrometry system with nanospray ionization-analysis of dexrazoxane in blood plasma. *J. Chromatogr. A* 1466: 173–179.

8 Neusüß, C., Römer, J., Höcker, O., and Jooß, K. (2018). Coupling of capillary electromigration techniques to mass spectrometry. In: *Capillary Electromigration Separation Methods*, Handbooks in Separation Science (ed. C.F. Poole), 293–311. Amsterdam, Netherlands: Elsevier.

9 Schlecht, J., Jooß, K., and Neusüß, C. (2018). Two-dimensional capillary electrophoresis-mass spectrometry (CE–CE–MS): coupling MS-interfering capillary electromigration methods with mass spectrometry. *Anal. Bioanal. Chem.* 410 (25): 6353–6359.

10 Huhn, C., Ramautar, R., Wuhrer, M., and Somsen, G.W. (2010). Relevance and use of capillary coatings in capillary electrophoresis-mass spectrometry. *Anal. Bioanal. Chem.* 396 (1): 297–314.

11 Causon, T.J., Maringer, L., Buchberger, W., and Klampfl, C.W. (2014). Addition of reagents to the sheath liquid: a novel concept in capillary electrophoresis-mass spectrometry. *J. Chromatogr. A* 1343: 182–187.

12 Ferré, S., Drouin, N., González-Ruiz, V., and Rudaz, S. (1641). Evaluation of a nanoflow interface based on the triple-tube coaxial sheath-flow sprayer for capillary electrophoresis-mass spectrometry coupling in metabolomics. *J. Chromatogr. A* 2021: 461982.

13 Hahne, H., Pachl, F., Ruprecht, B. et al. (2013). DMSO enhances electrospray response, boosting sensitivity of proteomic experiments. *Nat. Methods* 10 (10): 989–991.

14 Drouin, N., Schappler, J., Wenz, C., and Greiner, M. (2018). Improved CE/MS Sensitivity by Operating the Triple-Tube Coaxial Sheath-Flow Sprayer Without Appling Nebulizing Gas. *Technical Overview 5991-9073EN-D3*. USA: Agilent Technologies.

15 Liu, C.C., Zhang, J., and Dovichi, N.J. (2005). A sheath-flow nanospray interface for capillary electrophoresis/mass spectrometry. *Rapid Commun. Mass Spectrom.* 19 (2): 187–192.

16 Hsieh, F., Baronas, E., Muir, C., and Martin, S.A. (1999). A novel nanospray capillary zone electrophoresis/mass spectrometry interface. *Rapid Commun. Mass Spectrom.* 13 (1): 67–72.

17 Zhou, W., Zhang, B., Liu, Y. et al. (2019). Advances in capillary electrophoresis-mass spectrometry for cell analysis. *TrAC, Trends Anal. Chem.* 117: 316–330.

18 Sun, L., Zhu, G., Zhang, Z. et al. (2015). Third-generation electrokinetically pumped sheath-flow nanospray interface with improved stability and sensitivity for automated capillary zone electrophoresis-mass spectrometry analysis of complex proteome digests. *J. Proteome Res.* 14 (5): 2312–2321.

19 Maxwell, E.J., Zhong, X., Zhang, H. et al. (2010). Decoupling CE and ESI for a more robust interface with MS. *Electrophoresis* 31 (7): 1130–1137.

20 Höcker, O., Montealegre, C., and Neusüß, C. (2018). Characterization of a nanoflow sheath liquid interface and comparison to a sheath liquid and a sheathless porous-tip interface for CE–ESI–MS in positive and negative ionization. *Anal. Bioanal. Chem.* 410 (21): 5265–5275.

21 Höcker, O., Knierman, M., Meixner, J., and Neusüß, C. (2021). Two capillary approach for a multifunctional nanoflow sheath liquid interface for capillary electrophoresis-mass spectrometry. *Electrophoresis* 42 (4): 369–373.

22 Reschke, B.R. and Timperman, A.T. (2011). A study of electrospray ionization emitters with differing geometries with respect to flow rate and electrospray voltage. *J. Am. Soc. Mass Spectrom.* 22 (12): 2115–2124.

23 González-Ruiz, V., Codesido, S., Far, J. et al. (2016). Evaluation of a new low sheath-flow interface for CE–MS. *Electrophoresis* 37 (7–8): 936–946.

24 Konášová, R., Koval, D., Hošek, J., and Kašička, V. (2021). Investigating the position of the separation capillary and emitter tube tips in a nanoflow sheath-liquid CE–ESI–MS interface to decouple the ESI potential. *Talanta* 228 (1): 122212.

25 Sauer, F., Sydow, C., and Trapp, O. (2020). A robust sheath-flow CE–MS interface for hyphenation with Orbitrap MS. *Electrophoresis* 41 (15): 1280–1286. https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/full/10.1002/elps.202000044.

26 Tseng, M.-C., Chen, Y.-R., and Her, G.-R. (2004). A beveled tip sheath liquid interface for capillary electrophoresis-electrospray ionization-mass spectrometry. *Electrophoresis* 25 (13): 2084–2089.

27 Choi, S.B., Zamarbide, M., Manzini, M.C., and Nemes, P. (2017). Tapered-tip capillary electrophoresis nano-electrospray ionization mass spectrometry for ultrasensitive proteomics: the mouse cortex. *J. Am. Soc. Mass Spectrom.* 28 (4): 597–607.

28 Tseng, M.-C., Chen, Y.-R., and Her, G.-R. (2004). A low-makeup beveled tip capillary electrophoresis/electrospray ionization mass spectrometry interface for micellar electrokinetic chromatography and nonvolatile buffer capillary electrophoresis. *Anal. Chem.* 76 (21): 6306–6312.

29 Maxwell, E.J. and Chen, D.D.Y. (2008). Twenty years of interface development for capillary electrophoresis-electrospray ionization-mass spectrometry. *Anal. Chim. Acta* 627 (1): 25–33.

30 Moini, M. (2007). Simplifying CE–MS operation. 2. Interfacing low-flow separation techniques to mass spectrometry using a porous tip. *Anal. Chem.* 79 (11): 4241–4246.

31 Bonvin, G., Veuthey, J.-L., Rudaz, S., and Schappler, J. (2012). Evaluation of a sheathless nanospray interface based on a porous tip sprayer for CE–ESI–MS coupling. *Electrophoresis* 33 (4): 552–562.

32 Haselberg, R., Ratnayake, C.K., de Jong, G.J., and Somsen, G.W. (2010). Performance of a sheathless porous tip sprayer for capillary electrophoresis-electrospray ionization-mass spectrometry of intact proteins. *J. Chromatogr. A* 1217 (48): 7605–7611.

33 Busnel, J.-M., Schoenmaker, B., Ramautar, R. et al. (2010). High capacity capillary electrophoresis-electrospray ionization mass spectrometry: coupling a porous sheathless interface with transient-isotachophoresis. *Anal. Chem.* 82 (22): 9476–9483.

34 Wang, C., Lee, C.S., Smith, R.D., and Tang, K. (2013). Capillary isotachophoresis-nanoelectrospray ionization-selected reaction monitoring MS via a novel sheathless interface for high sensitivity sample quantification. *Anal. Chem.* 85 (15): 7308–7315.

35 Kawai, T., Ota, N., Okada, K. et al. (2019). Ultrasensitive single cell metabolomics by capillary electrophoresis-mass spectrometry with a thin-walled tapered emitter and large-volume dual sample preconcentration. *Anal. Chem.* 91 (16): 10564–10572.

36 Moini, M. and Martinez, B. (2014). Ultrafast capillary electrophoresis/mass spectrometry with adjustable porous tip for a rapid analysis of protein digest in about a minute. *Rapid Commun. Mass Spectrom.* 28 (3): 305–310.

37 Trapp, O., Pearce, E.W., Kimmel, J.R. et al. (2005). A soft on-column metal coating procedure for robust sheathless electrospray emitters used in capillary electrophoresis-mass spectrometry. *Electrophoresis* 26 (7–8): 1358–1365.

38 Zhang, H., Lou, C., Li, J., and Kang, J. (1624). A gold foil covered fused silica capillary tip as a sheathless interface for coupling capillary electrophoresis-mass spectrometry. *J. Chromatogr. A* 2020: 461215.

39 Chen, Y.-R. and Her, G.-R. (2003). A simple method for fabrication of silver-coated sheathless electrospray emitters. *Rapid Commun. Mass Spectrom.* 17 (5): 437–441.

40 Hirayama, A., Abe, H., Yamaguchi, N. et al. (2018). Development of a sheathless CE–ESI–MS interface. *Electrophoresis* 39 (11): 1382–1389.

41 Vermeire, P.-J., van Schepdael, A., and Petersen, N.J. (2020). Development of a novel sheathless CE–ESI–MS interface via a CO_2 laser ablated opening. *Talanta* 214: 120853.

42 Johnson, R.T., To, N.H., Stobaugh, J.F., and Lunte, C.E. (2017). The development of a sheathless interface for capillary electrophoresis electrospray ionization mass spectrometry using a cellulose acetate cast capillary. *Chromatographia* 80 (7): 1061–1067. https://link.springer.com/article/10.1007/s10337-017-3326-y.

43 Severs, J.C., Harms, A.C., and Smith, R.D. (1996). A new high-performance interface for capillary electrophoresis/electrospray ionization mass spectrometry. *Rapid Commun. Mass Spectrom.* 10 (10): 1175–1178.

44 Zhong, X., Zhang, Z., Jiang, S., and Li, L. (2014). Recent advances in coupling capillary electrophoresis-based separation techniques to ESI and MALDI–MS. *Electrophoresis* 35 (9): 1214–1225.

45 Wachs, T., Sheppard, R.L., and Henion, J. (1996). Design and applications of a self-aligning liquid junction-electrospray interface for capillary electrophoresis–mass spectrometry. *J. Chromatogr. B* 685 (2): 335–342.

46 Krenkova, J., Kleparnik, K., Luksch, J., and Foret, F. (2019). Microfabricated liquid junction hybrid capillary electrophoresis–mass spectrometry interface for fully automated operation. *Electrophoresis* 40 (18–19): 2263–2270.

47 Ohla, S. and Belder, D. (2012). Chip-based separation devices coupled to mass spectrometry. *Curr. Opin. Chem. Biol.* 16 (3-4): 453–459.

48 Ramsey, R.S. and Ramsey, J.M. (1997). Generating electrospray from microchip devices using electroosmotic pumping. *Anal. Chem.* 69 (6): 1174–1178.

49 Zhang, B., Liu, H., Karger, B.L., and Foret, F. (1999). Microfabricated devices for capillary electrophoresis–electrospray mass spectrometry. *Anal. Chem.* 71 (15): 3258–3264.

50 Schwarzkopf, F., Scholl, T., Ohla, S., and Belder, D. (2014). Improving sensitivity in microchip electrophoresis coupled to ESI–MS/MS on the example of a cardiac drug mixture. *Electrophoresis* 35 (12–13): 1880–1886.

51 Mellors, J.S., Gorbounov, V., Ramsey, R.S., and Ramsey, J.M. (2008). Fully integrated glass microfluidic device for performing high-efficiency capillary electrophoresis and electrospray ionization mass spectrometry. *Anal. Chem.* 80 (18): 6881–6887.

52 Hoffmann, P., Häusig, U., Schulze, P., and Belder, D. (2007). Microfluidic glass chips with an integrated nanospray emitter for coupling to a mass spectrometer. *Angew. Chem. Int. Ed. Engl.* 46 (26): 4913–4916.

53 Scholl, T., Dietze, C., Schmidt, M. et al. (2018). Sheathless coupling of microchip electrophoresis to ESI–MS utilising an integrated photo polymerised membrane for electric contacting. *Anal. Bioanal.Chem.* 410 (23): 5741–5750.

54 Wang, X., Yi, L., Mukhitov, N. et al. (2015). Microfluidics-to-mass spectrometry: a review of coupling methods and applications. *J. Chromatogr. A* 1382: 98–116.

55 Hommerson, P., Khan, A.M., de Jong, G.J., and Somsen, G.W. (2008). Comparison of electrospray ionization and atmospheric pressure photoionization for coupling of micellar electrokinetic chromatography with ion trap mass spectrometry. *J. Chromatogr. A* 1204 (2): 197–203.

56 Zhang, Y., Ai, W., Bai, Y. et al. (2016). An interface for online coupling capillary electrophoresis to dielectric barrier discharge ionization mass spectrometry. *Anal. Bioanal. Chem.* 408 (30): 8655–8661.

57 Liu, J., Tseng, K., Garcia, B. et al. (2001). Electrophoresis separation in open microchannels. A method for coupling electrophoresis with MALDI–MS. *Anal. Chem.* 73 (9): 2147–2151.

58 Musyimi, H.K., Guy, J., Narcisse, D.A. et al. (2005). Direct coupling of polymer-based microchip electrophoresis to online MALDI–MS using a rotating ball inlet. *Electrophoresis* 26 (24): 4703–4710.

59 Musyimi, H.K., Narcisse, D.A., Zhang, X. et al. (2004). Online CE–MALDI–TOF MS using a rotating ball interface. *Anal. Chem.* 76 (19): 5968–5973.

60 Huck, C.W., Bakry, R., Huber, L.A., and Bonn, G.K. (2006). Progress in capillary electrophoresis coupled to matrix-assisted laser desorption/ionization – time of flight mass spectrometry. *Electrophoresis* 27 (11): 2063–2074.

61 Michalke, B. (2016). Capillary electrophoresis-inductively coupled plasma mass spectrometry. *Methods Mol. Biol.* 1483: 167–180.