Electrospray ionization source geometry for mass spectrometry: past, present, and future

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The geometry of an electrospray ion source plays important roles in the processes of analyte desolvation, ionization, transportation, and detection in a mass spectrometer. We provide a brief account of the scientific principles involved in developing an electrospray ion source, and in the various geometries used to improve the sensitivity of mass spectrometry. We also present some popular configurations currently available and outline future trends in this research area.

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1. Brief introduction to electrospray

Electrospray ionization (ESI) is a soft ionization method, allowing the formation of gas phase ions through a gentle process that makes possible the sensitive analysis of non-volatile and thermolabile compounds. Consequently, the use of the ESI source in the field of mass spectrometry (MS) has greatly facilitated the study of large biomolecules, as well as pharmaceutical drugs and their metabolites. Thus, the ESI source has evolved with the growth of proteomics and drug discovery research [1]. The analysis of carbohydrates, nucleotides, and small polar molecules comprises a few of the many other applications in which ESI-MS is currently used. Together with matrix assisted laser desorption ionization (MALDI), another soft ionization technique, ESI has revolutionized biomedical analysis. In 2002, half the Nobel Prize for Chemistry was awarded to ESI developer John Fenn, who shared it with Koichi Tanaka, who made an outstanding contribution to the development of MALDI.

The concept of the ESI source is deceivably simple, as some aspects of its functioning are still not well understood. The technique involves a number of steps, including the formation of charged droplets, desolvation, ion generation, declustering, and ion sampling. As the name suggests, the basis of this technique lies in using a strong electric field to create an excess of charge at the tip of a capillary containing the analyte solution. Charged droplets exit the capillary as a spray and travel at atmospheric pressure down an electrical gradient to the gas conductance-limiting orifice or tube. Gas phase ions are then transported through different vacuum stages to the mass analyzer and ultimately the detector. ESI has successfully been coupled with a variety of mass analyzers. Each analyzer has different advantages and the choice of which to use is based on the requirements of the application. Comprehensive information about the coupling of ESI with various mass analyzers can be found in Cole’s 1997 compilation of review articles on the topic [2].

2. Milestones in the evolution of electrospray

Since 1968, when Dole used ESI and a nozzle-skimmer system to produce charged gas-phase polystyrene [3], the ESI source has continued to undergo various changes in its size, material, and geometry. These transformations were made to optimize both the ionization efficiency and the efficiency of transferring gas phase ions into the mass analyzer.

The evolution of the electrospray-source design has also reflected the coupling of the ion source to separation systems, such
as high performance liquid chromatography (HPLC) and capillary electrophoresis (CE). This has brought new challenges in generating ions at different flow rates, minimizing contamination of the interface, and optimizing overall efficiency. Leading instrument manufacturers are directly involved in providing innovative solutions to the industry’s demand for more sensitive, reliable mass spectrometers.

The purpose of this review is to present some milestones in the evolution of the ESI source to achieve the designs and the capabilities currently offered by commercial manufacturers. We discuss some important developments achieved by research groups with respect to ESI design. We also present a basic theoretical background on droplet formation and current theories of gas phase ion creation in order to provide the reader with a better understanding of the reasoning behind the modifications in source design. While other atmospheric pressure ion sources use similar geometries, this review will focus on only electrospray ionization.

3. Theory

A number of publications have presented details of the electrochemical nature of the electrospray process [4–6]. When a large potential difference is applied between an electrode shaped as a wire and a counter electrode, a strong electric field is created at the tip. In the case of ESI, a high voltage is applied to a capillary containing the analyte solution. For simplicity, we consider the application of positive potential only. Due to the electric field gradient at the tip, charge separation occurs in the solution as anions migrate towards the capillary walls, and cations travel towards the meniscus of the droplet formed at the tip (Fig. 1) [7].

In order to direct charged species into the mass spectrometer, a series of counter electrodes is used in order of decreasing potential. Typically, the principal counter electrode can be either the curtain plate of the mass spectrometer or a transport capillary, which will be discussed later. The optimal potential difference between the sprayer and the principal counter electrode depends on experimental parameters, such as the charge state of the analyte, the solution flow rate, the solvent composition, and the distance between the tip and the counter electrode. While different mass spectrometers may require different applied voltages on the sprayer and the counter electrode, the potential difference between them is similar (typically 2–5 kV) [8]. In the presence of an electric field, liquid emerges from the tip of the capillary in the shape of a cone, also known as the “Taylor cone” (Fig. 1) [9]. When the electrostatic repulsion between charged molecules at the surface of the Taylor cone approaches the surface tension of the solution – known as reaching the Rayleigh limit – charged droplets are expelled from the tip. The droplets containing excess positive charge generally follow the electric field lines at atmospheric pressure toward the counter electrode. However, trajectories will also be affected by space charge and gas flow.

The mechanism of forming the Taylor cone is not entirely understood, but it is known that, under certain conditions, the morphology of the spray emitted from the capillary tip can change [10]. The various spray modes strongly depend on the capillary voltage and are related to pulsation phenomena observed in the capillary current [10]. Juraschek and Röllgen showed that liquid flow rate, capillary diameter, and electrolyte concentration can all impact the spray mode. Controlling the spray mode is thus crucial in achieving a stable spray and an optimal signal. However, this becomes particularly difficult when the mobile phase composition changes during gradient elution conditions necessary in many LC-MS applications. To address this problem, Valaskovic and Murphy have developed an orthogonal optoelectronic system capable of identifying many spray modes under different conditions [11]. The system automatically optimizes the ESI potential in response to changes in flow rate and solvent composition.

The size of the spray droplets released from the Taylor cone, highly dependent on flow rate and capillary diameter, is critical to the efficient ionization of the analyte.

Since a small droplet contains less solvent, desolvation and ionization can be more efficient. Because less fission is required to produce ions, the salt concentration in the final offspring droplets may be lower compared to a droplet that has undergone more evaporation-fission cycles. As a result, the background noise in the mass spectrum may be reduced [12]. In addition, with smaller droplets, analytes that are not surface active will have a greater chance of being transferred to the gas phase rather than being lost in the bulk of the parent droplet residue [12].
The solvent is typically a combination of acidified water and an organic modifier. The role of the organic solvent is to lower the surface tension of the liquid, facilitating the formation of gas phase ions. As the solvent evaporates, the droplet shrinks and the electrostatic repulsion between charges within the droplet increases. When the Rayleigh limit is approached, offspring droplets begin to break away unevenly in a process also known as “coulombic fission” [7]. Evaporation of the offspring droplets leads to a new fission series and the process repeats itself to produce smaller and smaller droplets. The eventual creation of gas phase ions from these droplets is thought to be a combination of two mechanisms known as the “ion evaporation mechanism” (IEM), initially proposed by Iribarne and Thomson [13], and the “charged residue model” (CRM), put forward by Dole et al. [3,14] and supported by Rollgen et al. [13]. The fundamental difference between the two lies in the mechanism of forming gas phase ions.

IEM suggests that, when the electric field on a charged droplet is high enough, single, solvated, analyte molecules carrying some of the droplet charge are ejected into the gas phase. This happens because the potential energy of the ions near the surface becomes high enough to allow evaporation to occur [16].

By contrast, the CRM maintains that gas phase ions are formed when successive fissions lead to a charged droplet containing a single analyte.

Although it is difficult to determine which of the two mechanisms is more accurate, extensive studies [17–19] seem to suggest that CRM is the preferred mechanism in the case of forming charged globular proteins in the gas phase.

Kearbale also concluded that charging a single protein in the evaporating droplet is due to small ions found at the surface of the droplet [18]. The mechanism of forming small analyte ions is still not clear. Charging analyte molecules can occur through more than one process [20]. Charge separation (in the ESI source), adduct formation, gas phase molecular reactions, and electrochemical reactions may also contribute to ionization during the electrospray process.

Efficient transport of ions and charged droplets from the sprayer into the mass spectrometer is challenging and depends on parameters such as interface arrangement and gas throughput into the instrument [8]. As gas and ions are transported from atmospheric pressure into vacuum, strong cooling of the mixture occurs during expansion. Under these conditions, polar neutral molecules may cluster with analyte ions and it is therefore very important to achieve efficient desolvation within the atmospheric pressure region. One instrument manufacturer (MDS SCIEX) introduced a method of desolvation (described in more detail in Section 5.1.2.), whereby heated auxiliary nitrogen gas is directed at an angle from the direction of the spray (TurbolonSpray™) [21,22]. In addition, counter current nitrogen gas flow (Curtain Gas™, further illustrated in Fig. 5) emanating from in front of the gas conductance limiting orifice provided additional desolvation [8]. The advantage of the Curtain Gas is that neutral molecules, such as solvent, are carried away from the sampling orifice by the nitrogen flow, while charged molecules are directed through the gas flow under the influence of the electric field. Counter current gas flows can also aid in declustering as a result of collisions between ions and gas molecules. Since nitrogen is inert, it cannot form covalent bonds with the ions and clustering during transfer to vacuum is prevented. While its name varies depending on the manufacturer, counter current gas flow is an important feature of the ESI ion source. In addition to using inert gas flow, heating the ion source or gas conductance limiting orifice or capillary is also a common way to aid declustering and desolvation [23].

4. Historical highlights

4.1. Development of the traditional ESI source

Although the work of Fenn and colleagues demonstrated the applicability of ESI to generation of biomolecular ions, it was Dole and co-workers who led the way in the late 1960s by building the first electrospray based mass analysis system [3], as shown in Fig. 2.

A sharp hypodermic needle was used to spray polystyrene solution into an evaporation chamber. A Teflon plate supported the needle as well as a gas inlet introducing nitrogen as a bath gas. A nozzle-skimmer system and two stage differential pumping were used to reduce pressure in the analyzer region and permit sampling of the supersonic free jet without disturbing the molecular flow [24]. The voltage applied on the needle was maintained at approximately −10 kV (negative polarity used), while the voltages on the first and second collimating plates were −3 kV, and −1.4 kV, respectively. Dole measured mass-to-charge ratios (m/z) by determining the retarding potential of ions through a grid preceding a Faraday cup detector.

Intrigued by Dole’s ESI work, and having himself been a pioneer of supersonic free jets as molecular beam sources, Fenn reproduced Dole’s experiments with the same observations but discontinued research in this area due to technical limitations [25]. Years later, Fenn reprised his experiments on molecular beams and used ESI coupled to a quadrupole mass analyzer for ion detection. Recognizing flaws in Dole’s ion source design as well as in the interpretation of results, Fenn modified the electrospray ion source by reducing the distance between the hypodermic needle and end plate containing the nozzle [26] (Fig. 3). As a result, the applied sprayer voltage could be significantly lower.
In contrast with Dole’s ion source, Fenn’s ESI chamber was built with metal walls to prevent charge build up, and had significantly smaller dimensions. Fenn’s ESI source later evolved into what is known as the Fenn-Whitehouse design [27]. The additional characteristic is a glass capillary that allows ions from atmospheric pressure to enter the first vacuum stage of the instrument. The capillary dimensions can be chosen to allow the same flux of drying gas and ions as a regular thin-plate orifice [24]. Increasing the capillary diameter may be desirable in order to increase the acceptance and to decrease the chance of blockage. However, for a fixed gas throughput, this must be associated with a corresponding increase in capillary length, potentially lowering performance.

An alternative to using the counter current gas for ion desolvation is the heated metal capillary, introduced by Chowdhury et al. [23]. With this configuration, gas throughput depends on both capillary dimensions and temperature. The heated metal capillary inlet (ion pipe), as shown in Fig. 4, is currently used on Thermo Electron instruments. However, a number of other manufacturers have developed modified versions of this device, including heated resistive tubes [28] and heated metal capillaries with different inlet/outlet dimensions [29]. The combination of a heated metal capillary and the counter current gas makes possible even more efficient ion desolvation.

ESI-MS interfaces comprising multiple capillaries have been developed for various applications, such as ion ion reaction monitoring [30,31], separate introduction of analyte and calibrant using a multiple sprayer source [32], and improved ion transmission when used in combination with an electrodynamic ion funnel [33].

Other modifications to the heated capillary inlet include offsetting the exit of the capillary from downstream ion optics devices (such as a skimmer) [34,35]. The advantage of this design is that some large solvent clusters exiting the transfer capillary are prevented from entering the next vacuum stage, while the lighter analyte ions follow the gas flow through the ion optics.

Figure 2. Configuration of Dole’s first electrospray based mass analysis system. (Reprinted with permission from [3], ©1968, American Institute of Physics).

Figure 3. Configuration of the electrospray ion source coupled with a quadrupole mass analyzer designed by Fenn and co-workers in 1984. (Reprinted with permission from [22], ©1984, American Chemical Society).
Recently, Varian also adopted this modification by positioning a heated capillary outlet off axis from a subsequent RF multipole ion guide [36].

In 1987, Bruins et al. introduced the pneumatically assisted nebulizer for the electrospray interface, also known as ion spray. Fig. 5 shows the details of the ion spray interface [37].

The nebulizer gas was beneficial for coupling LC with ESI-MS, because it helped to stabilize electrospray for flow rates up to approximately 0.2 mL/min. It also tolerated larger distances between the sprayer and the counter electrode, reducing the occurrence of corona discharge [37]. Bruins observed that the spraying process was less dependent on the sprayer position relative to the orifice than without nebulizer assistance, and that better sensitivity was obtained if the sprayer was pointed off axis instead of directly(215,442),(301,558) at the orifice. The reasoning behind the off axis geometry was that, by sampling the periphery of the spray, finer droplets entered the mass spectrometer while the larger droplets struck the curtain plate. This led to improved performance because finer droplets are easier to desolvate. Currently, the off axis sprayer geometry has evolved to an orthogonal sampling position. More detail is given in Section 5 below.

Figure 4. Schematic of the Ion Max source from Thermo Electron using a heated metal capillary for ion desolvation and transfer.

Figure 5. Schematic of the ion spray configuration developed by Bruins in 1987.
4.2. The CE-MS interface

While ion spray became standard for LC-MS at higher flows, CE-MS coupling was more complicated due to the large variety of solvent compositions required for separation and problems associated with electrospray ionization. In addition, differences in flow rate requirements also presented problems. Three common ways of interfacing CE-MS are shown in Fig. 6.

The most common method of CE-MS interfacing is the coaxial sheath flow interface, first described by Smith et al. in 1988 [38]. In 2003, approximately 77% of CE-MS users employed this type of interface, due to its reproducibility and robustness [39]. By allowing a conducting liquid to provide electrical contact between the stainless steel capillary maintained at high voltage and the fused silica capillary carrying the analyte solution, the interfacing of capillary zone electrophoresis (CZE) with ESI-MS could be achieved with many buffer systems that were previously impractical. This is significant because aqueous buffers of high ionic strength are normally not tolerated by ESI-MS. Buffer ions can lead to charge neutralization and corona discharge as well as formation of undesirable adducts with the analyte resulting in loss of signal and high sensitivity and high background. By using organic solvents, such as acetonitrile or methanol, as the sheath liquid, buffers of ionic strength up to 0.2 M can be used. The sheath liquid prevents direct contact between the high voltage electrode and the analyte solution, thus avoiding electrochemical modification of analytes. An additional advantage of using the sheath flow interface is minimization of corona discharge as a result of reducing the ionic strength of the sprayed solvent [38].

Olivares et al. developed the sheathless interface in 1987 by using a stainless steel capillary to create electrical contact with the analyte solution [39]. Accounting for about 11% of CE-MS interfaces in 2003 [40], this method evolved with a focus on low flow rates for improved sensitivity.

Other sheathless methods were subsequently developed, such as sharpening the CE-capillary tip in order to produce a stable spray, while the ESI electrode was made by installing a piece of gold wire [41] or coating the capillary end with a metal or alloy [42]. The advantages of the sheathless interface are that analyte dilution and ion suppression due to the sheath flow can be eliminated. Sensitivity can also be improved by up to an order of magnitude over sheath-flow designs [43].

A third method of interfacing CE-MS was the liquid-junction interface developed by Henion and colleagues [44,45]. A liquid junction between the CE capillary and the ESI emitter was built using a stainless steel tee equipped with a buffer reservoir. This interface reduced the complication brought about by the different flow rate requirements of CE and ESI [46], but peak broadening and mechanical difficulties limited the general applicability of this technique [42].

4.3. Miniaturization of electrospray

In an attempt to reduce flow rates and thus produce smaller droplets to improve the ionization efficiency, various miniaturized ESI sources were developed [47]. Caprioli et al. [48,49] developed an on line ESI source equipped with a sprayer tip of 10–20 μm internal diameter and capable of operating at flow rates of 300–800 nL/min. The emitter was created by burning off the polyimide coating of a fused silica capillary tip and tapering it by etching with hydrofluoric acid to give droplets in the micron-diameter range. Caprioli named his technique microelectrospray, a term that currently describes a flow rate range of 0.2–4 μL/min [50]. Operation in this flow-rate regime may involve various means for pulling silica capillaries and applying the electrospray voltage [51].

In 1995, Caprioli introduced the idea of using the LC-column tip combined with microelectrospray technology [52] to spray directly towards the mass-spectrometer inlet. This was significant because it triggered the evolution of analytical LC columns towards miniaturized versions of microbore and nanobore capillary columns necessary for low flow rate electrospray [50].

Nanoelectrospray, introduced by Wilm and Mann more than a decade ago [53], was a particularly successful technique due to its ability to form droplets

![Figure 6. Schematic of the three most common ways to interface CE-MS: a) coaxial sheath flow; b) sheathless; and, c) liquid junction.](http://www.elsevier.com/locate/trac)
100–1000 times smaller in diameter than droplets formed by conventional ESI. The technique was named to reflect the nanometer (nm) sized droplets as well as the flow rate of nanoliters per minute (nL/min) [54]. Essentially, the solution infusion system was eliminated, as a few microliters of solution were transferred to the tip of a gold coated glass capillary. The emitter tip was located within a few mm of the inlet aperture, and a stereomicroscope was used to accurately position the sprayer in front of the inlet. By applying a low potential onto the conductive capillary surface, a fine spray was generated and the charged droplets were directed towards the counter electrode under the effect of the electric field gradient. Despite an estimated efficiency of 500 times greater than traditional ESI [54], nanoelectrospray has some limitations, including plugging of the emitter tip and lower reproducibility associated with differences in tip morphology.

While the term “nanoelectrospray” initially referred to Wilm and Mann’s off line technique, evolving technology has made possible the use of on line flow rates down to tens of nL/min. The term nanoelectrospray has thus expanded to encompass both on line and off line techniques.

For nanoelectrospray, the effects of tip geometry, flow rate and solvent composition have been investigated by a number of groups [55–57].

Motivated by the fact that, at certain solvent compositions, the sensitivity decreased dramatically, Vanhoutte et al. compared the effects of various mobile phase compositions using several types of electrospray capillaries. These included combinations of uncoated or gold coated, tapered or non-tapered fused silica capillary tips, as well as stainless steel emitters. They found that the gold coated, tapered tips were the most effective, because they covered the most extensive range of solvent composition for the tested conditions without loss of sensitivity [55].

Schmidt and Karas used combinations of model compounds to examine the effect of flow rate on ion signal [56]. They determined that, at flow rates of a few nL/min, signal suppression caused by differences in surface activity of the analytes was insignificant compared to that at higher flow rates (> 50 nL/min).

Li and Cole studied the often observed shift in charge state as a result of changing experimental parameters for nanoelectrospray [57]. Parameters such as tip diameter, flow rate, analyte concentration and solvent composition can all affect the observed ions and the charge states.

5. Currently available commercial sources

Since recognition of ESI as an invaluable bioanalytical tool in the late 1980s, research groups have attempted to exploit the capabilities of ESI by modifying the source geometry in order to allow a wider range of flow rates and in source fragmentation, as well as improved sensitivity, efficiency and practicality. Niessen’s 1998 and 2003 review articles [58,59] are a good starting point for those interested in the trends that have stimulated ESI source evolution. Despite numerous improvements, commercial ESI sources still retain many features of the original configuration.

5.1. Source configuration and its relationship to flow rate

A parameter of critical importance in ESI-MS is the flow rate of the solution. Depending on the application for which the mass spectrometer is used, the ESI source needs to be adapted to handle the incoming flow of solution, whether it is direct infusion, or LC or CE eluent.

5.1.1. Low flow rate. While flow rates from approximately 1 nL/min up to several mL/min are typically used with the assistance of a nebulizer, traditional nanoelectrospray relied simply on the electrostatic attraction between the mobile phase and the counter electrode to generate and disperse the charged droplets. The nanoflow regime can generally be defined as using flow rates of less than 1 nL/min and can extend to levels of less than 1 nL/min [55]. As mentioned previously, nanoflow ESI can be used independently (off line) or coupled to a separation system (on line), such as CE or HPLC. Depending on experimental requirements, the commercially available nanoelectrospray emitters vary in material, tip shape, diameter, and the configuration used for electrical contact. On line analyses typically use higher flow rates (0.1–1 nL/min), and the tips are usually fabricated from fused silica or metal. Off line analyses typically use flow rates in the low nL/min range, with coated or uncoated glass or quartz emitters. A number of groups have also described modified nanoflow sprayers with various coatings [60–62] or inserted fibers [63].

There are many different configurations for nanoflow LC-MS coupling [64], although they all include an LC column and a narrow diameter emitter tip. The simplest combination involves coupling an upstream LC column to the emitter tip using some type of low dead volume union [65]. The union can also serve as the electrical contact for the electrospray process, although it is also possible to use conductive tips or other electrode configurations [64,66]. The advantage of decoupling the LC column from the sprayer is simple, inexpensive replacement of sprayer tips, should they become damaged or plugged. However, the coupling must be done carefully to maintain chromatographic performance.

An alternative combination involves packing the LC column material directly into the sprayer tip to eliminate any dead volume after the column [67–70]. Tapered sprayers containing LC column packing are available commercially from companies such as New Objective Inc. The column preparation typically involves passing a
slurry containing column packing material through a
tapered sprayer \[71,72\]. Sometimes, a small frit is in-
cluded inside the tip of the sprayer to help confine the
packing material. The elimination of post column dead
volume helps to ensure optimal chromatographic per-
formance. In addition, it has been suggested that the
presence of packing material in the tapered sprayer acts
similar to a filter, extending tip lifetimes by preventing
plugging. The drawback with this configuration is that
tip replacement due to blockage or damage requires the
entire column to be replaced. Electrical contact can be
established at the sprayer tip or distal to the tip end.

Amirkhani and co-workers recently compared the
efficiency of four different sheathless electrospray emitter
configurations for a nanoflow LC system \[73\]. Two of the
configurations used on-column emitters with the applied
voltage either at the outlet or the inlet end of the
column, and the other two had emitters coupled to
columns, with the electrical connection at either the
sprayer tip or the low dead volume union. It was
demonstrated that all the configurations worked equally
well, provided the connections were made with minimal
dead volume \[73\].

Currently, most major MS manufacturers (e.g., Thermo
Electron, MDS SCIEX, Agilent Technologies, and Waters/
Micromass) make the nanoelectrospray source available.
The number of applications using electrospray at low flow
rates is very large, and outside the scope of this paper.
Wood et al.’s 2003 review article is a good starting point
and a source of references \[47\].

5.1.2. High flow rate. If the flow rate is in the range
0.05–3 mL/min, sensitivity can be an issue, due to the
decrease in ionization efficiency resulting from large
droplet size. ESI-MS is widely interfaced with LC, so high
flow rates are often necessary. One solution to this
problem is to employ a splitter that allows only a limited
volumetric flow rate to reach the MS interface. Under
these conditions, part of the eluent is wasted and con-
nection dead volume is a common problem. A more
practical approach adopted by manufacturers is to
sample the spray from a region, peripheral to the main
droplet trajectory, where the mist is much finer. This is
done by simply re-orienting the sprayer relative to the
interface so that the fine droplets from the exterior of
the spray plume can enter the sampling inlet, while the
majority of large droplets are directed away from the
entrance \[74\]. To minimize contamination, many major
MS manufacturers now sample orthogonally from the
spray plume for many applications (e.g., Agilent Tech-
nologies, Waters/Micromass, and MDS SCIEX). An
example of this is shown in Fig. 7 for an Agilent Tech-
nologies source incorporating an additional asymmetrical
lens.

The asymmetrical lens is essentially a half-full, partial
cylinder electrode operated at high voltage during the
electrospray process \[75\]. The purpose of the electrode is
to help initiate and sustain electrospray. Conversely,
other groups have attempted to use various types of
auxiliary electrodes \[76–78\] to focus ions at atmospheric
pressure.

Also exploiting the advantages of orthogonal sampling
is the Waters/Micromass Zspray™ \[79,80\] interface,
shown schematically in Fig. 8. The spray is first sampled
orthogonally through the sampling cone into a low
pressure chamber. An extraction cone (skimmer) is ori-
ented at a right angle relative to the axis of the spray to
sample for a second time into the next differentially
pumped vacuum stage. The double orthogonal sampling
system prevents solvent and neutral molecules from
entering the analyzer, resulting in reduced chemical
background \[81\]. Larger cone apertures are used to

![Figure 7. Schematic of an ESI source configuration developed by Agilent Technologies.](http://www.elsevier.com/locate/trac)

![Figure 8. Schematic of the Zspray™ source configuration used by Waters/Micromass.](http://www.elsevier.com/locate/trac)
compensate for transmission losses. The ability to disassemble and to clean the sampling cone without breaking vacuum is an additional advantage contributing to the ruggedness of the Zspray source.

Although some MS manufacturers (e.g., MDS SCIEX, Waters/Micromass, and Agilent Technologies) have opted for orthogonal sampling, Thermo Electron uses a sampling angle at $60^\circ$ from the ion optics axis. In its Ion Max source, the function of the angled position of the sprayer is similar to the orthogonal one.

Sprayer orientation is not the only important parameter when it comes to optimizing sensitivity while using high flow rates. For conventional high flow rate ESI sources, the nebulizer gas is an essential component. Air or nitrogen is typically used to disperse the emerging solution into small droplets and to direct the droplets on the trajectory chosen for optimal sampling.

To vaporize the large amounts of solvent emerging from the sprayer as efficiently as possible, manufacturers have introduced additional features to assist the nebulizer gas. MDS SCIEX developed the TurboIonSpray source [21,22], in which heated nitrogen gas that is released from a unit external to the sprayer is used to assist evaporation of the spray droplets at atmospheric pressure. More recently, MDS SCIEX took this design a step further, creating the TurboVTM source, shown in Fig. 9. In this case, two heated auxiliary nitrogen sources are oriented to achieve very efficient desolvation. The improved desolvation permits stable, sensitive operation for flow rates of greater than 1 mL/min [82].

Thermo Electron also integrated auxiliary nitrogen gas for desolvation purposes, except that it flows directly from the nozzle of the Ion Max source [83]. Similar to the TurboV, the Ion Max uses a high temperature source heater incorporated into the housing of the ion source. This probe configuration is referred to as Heated Electrospray Ionization (H-ESI) and it tolerates the use of higher flow rates than previously possible on Thermo Electron instruments.

5.2. Interface

Various ion sampling interface configurations have been developed [8]. The sampling orifice can be built in a disc, in the top or the bottom of a cone, or in glass or metal tubes. Some MS manufacturers now equip their instruments with conical interfaces. Intuitively, the conical shape is meant to improve the electric field density at the sampling orifice in an attempt to improve ion transmission. To avoid potential contamination, the sampling cone can be heated to evaporate residuals from its surface. Since high temperatures are required for this purpose, ceramic materials have often been used for the interface. Ceramic produces no outgassing, and is thought to have low sample adsorptivity, thus reducing memory effects on the interface surface [84].

6. Multiple sprayers

Commercial multiple sprayer systems for ESI have evolved mainly as a result of industry’s increasing requirements for high throughput sample analysis, particularly for pharmaceutical samples. With a multiple sprayer source and suitable equipment, LC-MS analyses can be done quickly and efficiently. However, the first attempts at building multiple ESI sprayers were motivated by other reasons. Smith et al. [30,85] were the first to describe the coupling of two ESI sprayers in order to generate ions of opposite polarity and study ion-ion chemistry. McLuckey et al. also interfaced up to three ion sources (two ESI sources and one atmospheric sampling glow discharge ionization (ASGDI) source) onto a quadrupole ion trap mass spectrometer in order to control the charge states of reactant and product ions [86,87]. Other multiple source combinations have also been described, incorporating ESI with sources such as atmospheric pressure chemical ionization (APCI) [88,89].

Using a linear array of capillary electrodes, Rulison et al. demonstrated the feasibility of increasing sample throughput by using parallel capillaries [90]. They also observed that the Taylor cones at the ends of the array were being deflected due to end effects caused by the electric field.

In 1994, Kostiainen and Bruins proposed that multiple sprayers could be used to improve ion current stability under high flow rate conditions (i.e., above 200 $\mu$L/min) [91]. Four years later, Kassel and Zeng developed a dual ESI source for purification and simultaneous analysis of combinatorial libraries using two separate LC-MS eluent streams [92]. Although this development paved the way
for further efforts in high throughput parallel analysis, Kassel’s system required knowledge of the particular analyte that each sprayer was generating. Dual ESI sources have also been used to introduce an internal calibration solution separately from the analyte solution [93–96]. This configuration provides benefits compared with a single solution containing both analyte and calibrant because it avoids preferential ionization.

In 1999, Kassel et al. [97] and De Biasi et al. [98] described indexed multiple sprayer systems that allow sequential sampling of each spray by a mass spectrometer, thus making it possible to identify ions from their respective eluent stream. Currently, publications in which fast LC-MS analyses of samples are discussed indicate that the Waters/Micromass multiplexed ESI source (MUX) is the most commonly used for the analysis of multiple LC eluent streams [99–101]. Tiller’s 2003 review paper provides useful references regarding biological applications using the MUX system as well as other types of parallel analysis [102]. The MUX system attaches to the Zspray source of the mass spectrometer and is fitted with either four or eight channels. As a rotating aperture allows only one spray at a time to reach the sampling cone, spectra of compounds eluting from individual separation columns can be quickly acquired.

However, a significant problem with MUX-type systems is a decrease in sensitivity of approximately three-fold at high flows [103]. Yang et al. attributed the drop in sensitivity to several reasons, such as difficulty in optimizing sprayer position and lower desolvation efficiency due to large amounts of solvent introduced into the source in combination with a desolvation gas that was counter-current relative to the spray instead of the traditional coaxial flow. The sensitivity decrease is expected to be larger for nanoflow operation, where sprayer position can be more critical.

In 2002, Schneider et al. [104] developed an alternative multiple sprayer system that appeared to solve the sensitivity problem. By installing an atmospheric pressure ion lens [76,105] on each sprayer of a dual sprayer and a four sprayer ESI source, they were able to maintain high sensitivity by optimizing the applied lens potential as well as sprayer position. An additional advantage of the system was that sprayers could be enabled or disabled by changing the lens potential, potentially a faster method than using mechanical devices.

7. Recent developments in ESI sources

The quest for a superior source design capable of effectively focusing more of the electrospray generated ion cloud through the sampling aperture of the instrument is still on going. Due to gas phase collisions occurring under atmospheric pressure conditions and space charge effects, only a small percentage of the spray plume is actually sampled. There is still significant room for improvement in ion sampling efficiency.

7.1. High velocity nebulizers

One approach developed by Zhou et al. [106] involved installing an industrial air amplifier between the electrospray probe and the sampling orifice. The concentric, high velocity gas flow was meant to control the expansion of the spray plume as well as assist in the desolvation of ions. In addition, focusing of ions could be achieved by applying an optimized voltage of up to 3 kV onto the amplifier. Thus, when both the air amplifier and the high voltage were used, Zhou’s ion source configuration seemed to provide some improvement in ion-sampling efficiency. Suppression of background chemical noise was also observed.

7.2. Improved nanoflow configurations

Schneider et al. developed a novel interface for nanoflow ESI (Fig. 10) that provides two separate atmospheric pressure regions for droplet desolvation as well as two regions for removing unwanted particles [107].

The first desolvation stage makes use of the traditional counter-current gas to eliminate neutral particles and solvent. Next, charged particles, ions and gas travel through the heated laminar flow chamber, where additional desolvation takes place at optimal temperature. Once they reach the particle discriminator area, ions are carried by the gas streamlines through the discriminator space and into the sampling orifice, while large charged particles with high momentum escape from the gas streamline trajectory under the influence of the strong electric field. This source design provided improved signal and ion current stability for flow rates from a few nL/min up to 1 μL/min as well as improved performance for gradient elution nanoLC-MS in both positive-ion and negative ion mode [108].

7.3. Desorption electrospray ionization

Cooks and co-workers recently described a new method of ionization in which analytes are desorbed from surfaces by nebulizing the charged droplets of an electrospray plume at a surface [109]. This technique is referred to as desorption electrospray ionization (DESI). The exact mechanism of ion formation in DESI is not well understood, although the technique has been successfully used for analyzing a number of samples, including pills and TLC plates [110]. Efficient sampling of desorbed ions requires careful optimization of the angles and the spacings between the surface, the electrospray emitter, and the mass spectrometer inlet.

7.4. Coupling ESI to micro-analytical systems

In the rapidly expanding area of microchip chemical analysis, currently known as ‘Micro-Total Analysis
Systems” (µ-TASs) and the related “Lab-on-a-chip”, ESI has become the preferred interface method for MS detection [111–113]. More than a decade after being introduced [114], µ-TASs have been used in a wide range of biological applications, drug discovery, and clinical diagnostics. References regarding applications can be found in review papers [115–120].

The interest in miniaturized analytical systems rose due to the potential for integrating time consuming steps, such as sample preparation, chemical reaction, separation, and detection, into a fast, highly automated technique, while at the same time using minute sample quantities. Constantly evolving microfabrication techniques adapted from the semiconductor industry are used to produce the microfluidic devices [117,121,122].

Although on-chip optical detection is often used in microfluidic applications, MS detection is desirable due to its suitability for analyzing biological molecules. In addition, the low flow rates (nL–µL/min) used with microfluidic devices are optimal for the ESI source.

However, integrating ESI-MS with a microchip is not easy. Initial work involved using traditional ESI emitters, such as a section of fused silica capillary or a nanoelectrospray needle attached to a specific microchannel exit, but these types of sources were prone to contamination problems from bonding agents, poor reproducibility, and dead volume problems [121,123,124]. Work has therefore focused on building the ESI emitter as part of the microdevice and a range of materials, from silicon based to polymers, has been tested for fabrication of the ESI emitter [117,121].

Agilent Technologies has introduced a polymer based HPLC-MS microfluidic chip [125]. The chip contains enrichment and analytical columns packed with reverse-phase material, a face-seal rotary valve for switching between loading and separation positions, and a conical ESI emitter created directly on the chip structure by laser ablation. Gradient reverse phase separation of peptides has been achieved at flow rates of 100–400 nL/min. The system was reported to be stable with various solvents, sensitive, and robust. Since the various connections necessary for traditional nanoelectrospray were eliminated, dead volume problems as well as analyte dispersion were minimized [125].

While the combination of microfluidic devices with MS may have great potential, many barriers still need to be overcome. Currently, CE methods appear to be most common for sample separation on microfluidic chips, because they are less complex to miniaturize than HPLC [126]. Difficulties, such as integrating pumping systems for controlling flow and creating gradients, still limit widespread commercialization of microfluidic-LC systems [127].

For high throughput analyses, Advion BioSciences successfully introduced a fully automated nanoelectrospray system, called the NanoMate™. This microchip device contains an array of nozzles to which the sample is automatically delivered for MS analysis. Short analysis times are possible due to a simplified spray optimization procedure [128,129]. Although the NanoMate was initially intended for use in combination with microfluidic separation devices [128], it has been integrated with sources from other manufacturers (e.g., Waters/Micromass, MDS SCIEX, and Thermo Electron). The key advantages of the NanoMate include elimination of sample carry over and simplification of the tuning procedure [130].

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**Figure 10.** Schematic of the particle discriminator interface developed by MDS SCIEX.
8. Conclusion and future outlook

Because ESI-MS is used in many areas of chemistry, there is an immense number of publications covering modifications to the ion source for various applications. This article has attempted to sketch a basic picture of how ESI works and how it has evolved, and continues to do so, towards new designs. Undoubtedly, commercial ESI sources will continue to evolve to meet growing demands for improved sensitivity and robustness. Further improvements are likely to include increasing the gas throughput to sample a larger number of ions, even though these types of approaches are typically associated with higher costs due to the increased pumping requirements. Research is also likely to continue to increase the ion sampling efficiency by electrostatic and aerodynamic focusing, without the need to increase system pumping. With MS more popular than ever, it is fascinating to see that the capabilities of ESI-MS are still expanding.

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