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Interfaces between Microfluidics and Mass Spectrometry

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Synonyms

Coupling microfluidic devices to mass spectrometers; Interfacing Lab-on-a-Chip platforms with mass spectrometers using MALDI and ESI

Definition

The ►mass spectrometer (MS) is an analytical tool that provides information about sample composition based on the mass-to-charge ratio (m/z). In order to analyze biological samples by MS, the relevant analytes must be driven into the gas phase, and ionized (charged). Two techniques are currently the most popular for the ionization of biological analytes are ESI (►Electrospray Ionization) and MALDI (Matrix-Assisted Laser Desorption/Ionization). In ESI, an electric field is applied to a solution of analyte to form a spray of charged droplets. Subsequent solvent evaporation and ion release enables analysis by MS. In MALDI, the analyte is co-crystallized with a solid matrix to form a dry spot on a surface. Under vacuum, the sample is then irradiated with a laser, which desorbs the sample from the surface and ionizes it. The developers of these so-called *soft* ionization techniques, which enabled, for the first time, the capacity to analyze large biomolecules such as proteins, were awarded the 2002 Nobel Prize in Chemistry.

Overview

In the last decade, mass spectrometry has emerged as one of the most powerful tools in bioanalysis. MS is commonly integrated with high performance liquid chromatography (HPLC) and other separation tools; as separations has been the most popular application for microfluidics, it is perhaps natural that interest has grown in interfacing mass spectrometry with microfluidics. While the focus of this chapter is on the microfluidics-MS interface, we note that one of the most popular applications for mass spectrometry is in the field of proteomics (the study of the complement of proteins expressed in a system), which has emerged as the next great scientific challenge in the post-genome era. Thus, many of the descriptions in this essay are viewed through the lens of proteomics; for more comprehensive information on this topic we refer the reader to other chapters of the Encyclopedia (►proteomics in microfluidic devices, ►integrated micro devices for biological applications).

Basic Methodology

Mass Spectrometers

The principle of operation of mass spectrometers is that electric and/or magnetic fields can be used to manipulate ions in space, and that their trajectory is a function of mass and charge. Ions of interest can be manipulated as a beam or trapped by confining electrodes, leading to different mass resolution and accuracy depending on the type of MS used. Although mass spectrometers have been used for approximately a century, the analysis of high molecular weight compounds has only become possible with advances in electronic instrumentation and the development of soft ionization techniques. Currently, it is possible to characterize analytes with molecular weight over 500 kDa [1].

Of special importance is the technique of tandem mass spectrometry (MS/MS), which integrates two stages of mass analysis. A particular ion of interest (called the parent or precursor ion) is selected in the first MS stage, allowed to fragment, and then analyzed further by the second MS stage. This technique has become important in proteomics, as protein sequences can be elucidated from the pattern of fragmentation. The ultimate identification of proteins requires complex database searching algorithms (e. g., SEQUEST [2]), and presents big challenges for data analysis (► mass spectrometry).

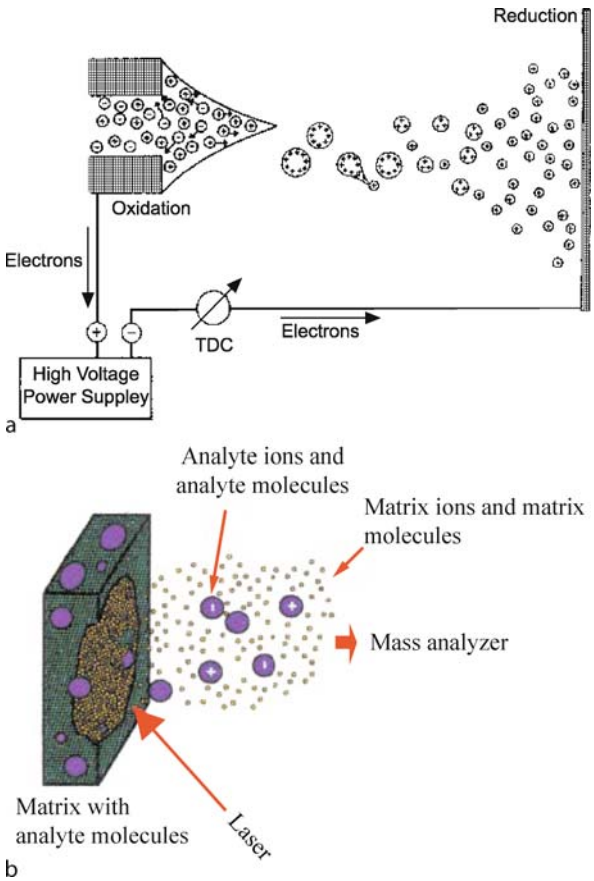
Ionization Methods

Several methods can be used to ionize samples, enabling the analysis by mass spectrometry. Two *soft* ionization methods are currently the most popular, ESI and MALDI. In ESI [3], an electric field is used to generate an aerosol of charged droplets from the end, or *tip*, of a fluid-filled tube. A schematic depicting the ESI process [4] is shown in Fig. 1a. The applied field leads to accumulation of charge close to the edge of the ESI tip. When the forces generated by charge repulsion overcome those associated with surface tension, the meniscus of the liquid assumes a conical shape, called a Taylor cone. When working properly, the shape of the cone is well defined, and results in a spray of droplets leading to the spectrometer.

After spraying, each charged droplet shrinks due to solvent evaporation. As the forces driven by coulombic repulsion and surface tension become similar, the surface-charged liquid droplet becomes unstable and disintegrates. This phenomenon is called coulombic fission, and can be predicted by the Rayleigh stability limit [3] As the droplets reduce to nanoliter volumes after successive bursts of fission, charged analytes are released into the gas phase. Two mechanisms have been proposed to explain this process: solvent evaporation from droplets containing just one ion; and direct release of ionized samples from droplets. As microfluidic devices handle volumes of liquids in the order of nanoliters, integrating microchannels with ESI requires dedicated nozzles (called nanoelectrospray, or nanospray, tips) capable of spraying minute volumes. The principles that apply for the stability of nanospray tips are the same as for conventional ESI.

A second popular soft ionization method is MALDI. In this technique, analytes are co-crystallized with a matrix (typically a small, acidic, organic molecule), with an absorption maximum close to the wavelength of a laser used to irradiate the substrate. This process is typically performed in vacuum (although it has been shown to be feasible at atmospheric pressure); after the analytes have been desorbed and ionized (in MALDI, fast heating caused by the laser pulse desorbs analytes into gas phase; however, the process by which proteins become charged is still a topic of study), they are guided to the MS for analysis [5] (Fig. 1b).

ESI and MALDI have distinct advantages and disadvantages, such that they are complementary for many applications. Of the two techniques, ESI is the softer one, allowing for ionization of intact multi-molecule complexes; however, in the presence of high concentrations of salts or other unwanted constituents, the formation of ions can be suppressed (making analysis impossible). This effect is less pronounced in MALDI, which can produce ionized products even in the presence of salts; but MALDI spectra



Interfaces between Microfluidics and Mass Spectrometry, Figure 1 Schematic depictions of (**a**) ESI and (**b**) MALDI mass spectrometry. In (**a**), the electric field causes an accumulation of positive charges at the liquid meniscus. When coulombic repulsion forces overcome the forces associated with surface tension, a spray is formed. Additional mechanisms (see text) lead to the release of analytes from the droplets. Reprinted with permission from Kebarle et al. [4]. In (**b**), analytes are co-crystallized with a matrix which has an absorption maximum near the wavelength of the laser used to desorb the analytes for analysis by MS. Reproduced by permission of Annual Reviews [5]

tend to be very noisy in the mass range below 500 daltons (Da), due to the presence of matrix ions. MALDI usually generates singly charged analytes, making data analysis trivial; ESI generates many differently charged species for each analyte, which makes interpretation of spectra more challenging, as complex mixtures lead to spectra with hundreds of peaks. Finally, in MALDI, the intensity of the signal associated with a particular analyte is not easily related to the amount of sample; this contrasts with ESI, where the intensity of the signal increases linearly with the analyte concentration over a wide range.

ESI and MALDI are considered to be the most appropriate candidates for coupling microfluidic devices to mass spectrometry. In what follows, we present a survey of the different kinds of microfluidic-MS interfaces reported in the literature and discuss the most promising geometries.

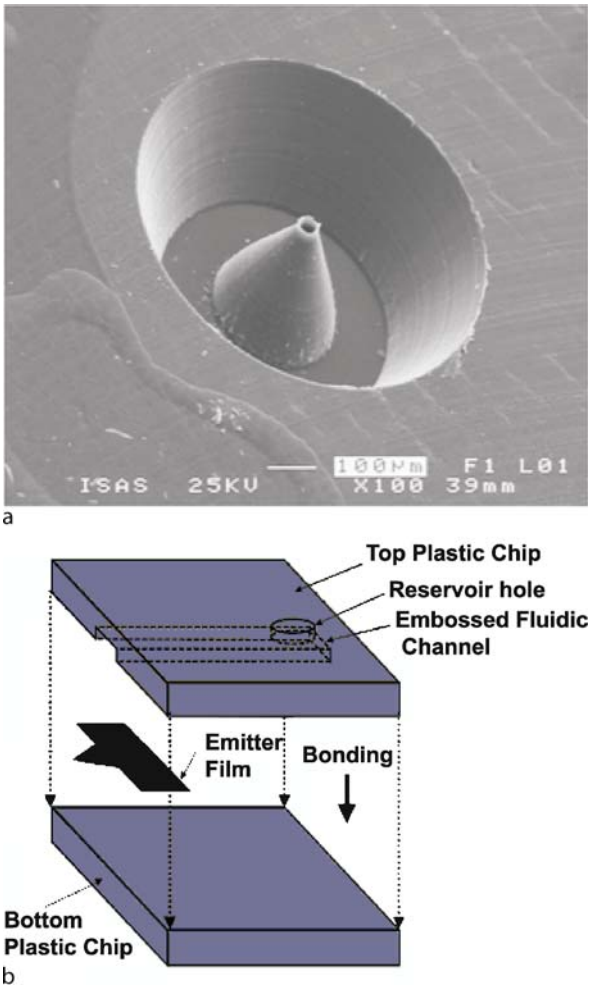
Key Research Findings

Electrospray Ionization

A variety of strategies for integrating microfluidic devices with nanoelectrospray ionization have been reported. Proteomic sample solutions are pumped through microchannels pneumatically or by electroosmotic flow (EOF) at $\sim 100 - 300\,\mathrm{nL/min}$; samples are typically dissolved in low-pH buffers modified with organic solvents suitable for positive mode mass spectrometry, with detection limits in the fmol-amol range. These methods can be broadly classified by how the electrospray is generated, including: direct spray from channels [6]; spray from mated, conventional tips [7]; and (3) spray from microfabricated tips [8–12].

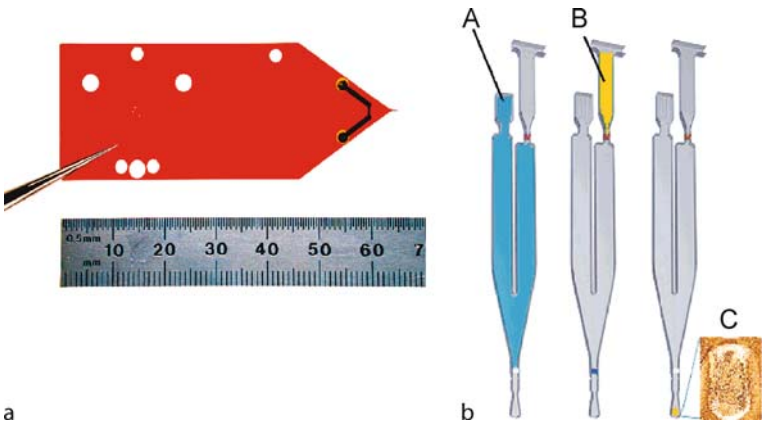
The simplest approach for interfacing microchannels with mass spectrometry is to electrospray directly from a channel (i. e., the unmodified edge of a device). As reported by Xue et al. [6] the first microchannel-ESI interface exhibited limited performance due to eluent spreading at the edge of the chip resulting from the non-tapered geometry and the hydrophilicity of the substrate. Others improved the method by mechanically tapering the edge of substrates and by integrating hydrophobic coatings on the edges of the devices. However, these methods do not match the performance of conventional nanospray tips, and the strategy of spraying directly from channels has been largely abandoned.

A second strategy for interfacing microchannels with mass spectrometry is the coupling of microfluidic devices to conventional pulled glass capillary tips. These devices are capable of efficiently sampling analytes into the spectrometer, generating mass spectra with sensitivities similar to those of conventional techniques. For example, Lazar et al. [7] reported sub-attomole detection of peptides using a glass microfluidic device mated to a conventional electrospray tip. A major drawback for this strategy, however, is in separation resolution: bands of analytes mix as they pass through dead volumes in the interface between chip and capillary. As a result, this device geometry is not likely to be useful for most applications.



Interfaces between Microfluidics and Mass Spectrometry, Figure 2 Two nanospray sources: (**a**) nozzle fabricated by micro milling of PMMA, reproduced by permission of [8]; (**b**) nozzle formed by sandwiching a 2D parylene tip between cover plates. Reprinted with permission from Kameoka et al. [10]

A third strategy for microfluidic-nanospray interfaces, microfabricated, tapered electrospray tips [8–12] is the most promising that has been reported. In fact, several devices with this configuration are now available commercially (for example, from Advion Biosciences and Agilent Laboratories). Several authors have fabricated devices capable of sustaining a stable spray with no dead volume between the channel and tip. For example, Fig. 2a shows a micro-milled electrospray nozzle in poly(methyl



Interfaces between Microfluidics and Mass Spectrometry, Figure 3 Two commercial microfluidics-MS interfaces: (**a**) a polyimide microchannel device with integrated ESI tip formed by laser ablation. Reprinted with permission from Yin et al. [11] and (**b**) schematic of MALDI CD device (see text for details). Reprinted with permission from Gustafsson et al. [15]

methacrylate) (PMMA) fabricated by Schilling et al. [8] The performance of the spray is dependent on the nozzle diameter and apex angle. Large diameter nozzles (100 μm) have to be coated with fluorinated polymer to prevent liquid spreading, and performance depends on the state of the coating. The best performing tip geometry had a 30 μm wide nozzle with a 60° angle, with no coating; this device was capable of generating a stable spray for several hours. Xie et al. [9] used vapor-deposited parylene-C to fabricate ESI tips on silicon microfluidic devices, enabling integrated liquid chromatography with mass spectrometry detection with comparable performance to conventional techniques. The drawback for these devices is the complexity involved in their fabrication, requiring many sequential photolithography steps in a cleanroom. However, parylene is a material with high chemical resistance, and may be a useful choice for the construction of nanospray tips in future work. For example, Kameoka et al. [10] constructed a nanospray tip comprising a parylene film sandwiched between two plastic plates (Fig. 2b). This device is relatively easy to fabricate, and demonstrates that a 3D hydrophobic nozzle is not required; a stable spray of bioanalytes can be obtained by using a planar, triangular parylene tip.

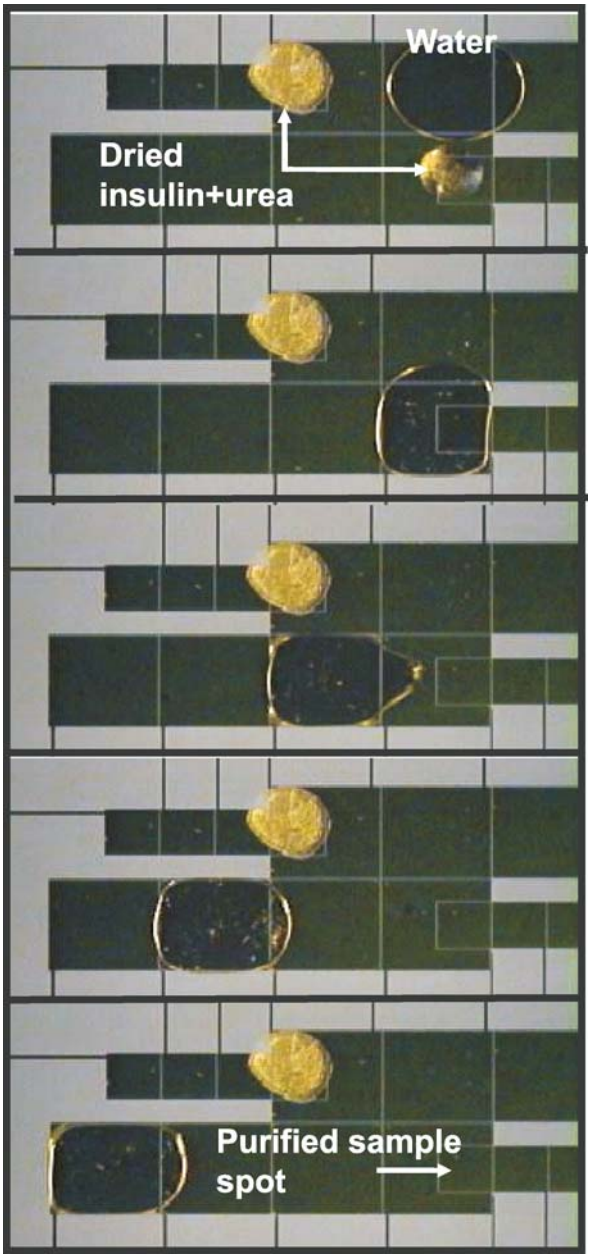
The most promising microfabricated ESI interface may be the one developed by Yin et al. [11], which features a nanospray tip formed by laser ablation (355 nm) of a polyimide substrate (Fig. 3a). The fabrication required to form these tips is relatively simple, and the devices integrate separation and sample enrichment modules which lead to mass spectrometry analysis with peak resolution, limits of detection and signal-to-noise ratio (S/N) similar to those obtained by conventional macro-scale methods. Complex protein mixtures from blood plasma were characterized using this platform [12], with a detection limit in the low femtomole range; this device is now commercially available (Agilent Laboratories).

MALDI

MALDI is an alternative to ESI for an interface between microfluidic platforms and MS. The geometry of MALDI detection targets features arrays of crystallized sample spots on an open surface, and the process is (in general) performed under vacuum. Thus, MALDI is not an obvious match for interfacing with microfluidics; despite this, several interfaces with microfluidics have recently been developed.

Enclosed microchannels are by definition not accessible to laser desorption/ionization, which requires an open surface from which analytes can be sampled into the spectrometer. Several strategies have been adopted to circumvent this challenge, including the elution of bands of analytes from microfluidic devices onto an open substrate, where they are dried and analyzed. Alternatively, Brivio et al. [13] developed means to desorb analytes directly from enclosed channels through sub-micron pores in the device cover. Musyimi et al. [14] employed a rotating ball to transfer analytes from polymer microchannels to a MALDI-MS system without compromising the vacuum required for mass spectrometry.

In one of the most complete microfluidic systems developed for mass spectrometry applications to-date, Gustafsson et al. [15] developed a MALDI interface for compact disk (CD)-based microfluidics, a technology in which reactions and separations are powered by centrifugal forces on a spinning device (CD Lab-on-a-Chip). Fig. 3b demonstrates the operation of an individual analysis region (each CD contains 96 such regions). The sample



Interfaces between Microfluidics and Mass Spectrometry, Figure 4
Video sequence (top-to-bottom) depicting digital microfluidics-based analysis of a sample containing insulin and urea. The large electrodes are used to move a water droplet to the dried spot, where it selectively dissolves the urea. Because the rinsing droplet primarily touches clean surfaces on the surrounding electrodes, it is easily moved away, leaving behind an (invisible) insulin film, ready for analysis by MALDI-MS. Reproduced by permission of [20]

is loaded (A), and then washed and eluted from a reversed-phase column (B). Finally, the sample is co-crystallized with a MALDI matrix (C) for analysis by mass spectrometry. In proof-of-principle experiments, the CD technology enabled the identification of tryptic digests with higher certainty than in identical analyses using conventional techniques (i. e., steel MALDI target), with better sequence coverage, and the same resolution and mass accuracy. This platform is now commercially available.

Future Directions for Research

Two factors favor the use of nanoelectrospray ionization for coupling microfluidic devices to mass spectrometers. The first is the similarity between the conventional pulled-glass capillary tips and the nanospray nozzles developed for microdevices discussed in the previous section; the second springs from the linear geometry of microfluidic channels. Thus, we believe that nanospray ionization techniques are the most likely to be used for the construction of robust interfaces between microfluidics and mass spectrometry for most applications in the future [16].

Despite the assessment above, we can't help but imagine that MALDI will continue to be an important tool used with Lab-on-a-Chip devices, given how amenable it is to high-throughput analysis (i. e., probing densely packed arrays of sample spots). The solutions that have been developed for interfacing MALDI-MS with enclosed microchannels (described above) are ingenious; however, such solutions are probably not practical for widespread use. Several alternative solutions have recently emerged that are not *microfluidics* per se, but may be a better match for MALDI-MS. For example, some authors have demonstrated the construction of arrays of patterned hydrophobic regions [17] or the use of piezoelectric pipets to dispense nanoliter amounts of liquid in silicon etched wells [18] Others have used electrospray to deposit homogeneous protein spots directly on MALDI targets; using this approach, Wang et al. [19] characterized 3.5 fmol of Angiotensin by mass spectrometry.

Digital microfluidics (DMF) is a related technique in which droplets are moved on a substrate by means of electrowetting and/or dielectrophoretic forces (►digital microfluidics, ►applications based on electrowetting). DMF been used to process proteomic samples and form arrays of spots for analysis by MALDI-MS [20]. As shown in Fig. 4, the technique has been used to perform in situ sample cleanup on an open substrate, after which, samples were interrogated by MALDI-MS, with similar detection efficiencies, resolution, and S/N as conventional techniques. DMF may enable the processing of several analytes at the same time, and is thus a promising tool for implementing high-throughput profiling proteomics using MALDI-MS.

The trend towards laboratory miniaturization is leading to the necessity of robust and reliable interfaces between MS and microfluidics. Although general tools for wide-

spread use are not yet available, it is clear that technologies are becoming more effective and reproducible. (see Freire and Wheeler [16] for a review). Of paramount importance in future work will be functional characterization of the devices when in contact with complex proteomic samples, evaluating, among other things, undesirable non-specific adhesion, long term operation, and the capacity to deliver the analytes of interest to the MS for analysis. In particular, the latter requires an efficient release of sample from MALDI targets or ionization when sprayed from a liquid containing salts, impurities and large abundance proteins that may conceal the desired signal.

Cross References

- ► Proteomics in Microfluidic Devices
- ► Integrated Micro Devices for Biological Applications
- ► Mass Spectrometry
- ► Digital Microfluidics
- ► Lab-on-a-Chip (General Philosophy)
- ► Applications Based on Electrowetting

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Interface Tracking

- ► Interface Capturing Schemes for Free-Surface Flows

Interfacial Electrohydrodynamics

- ► Interfacial Electrokinetic Flow

Interfacial Electrokinetic Flow

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