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Chip-MS: Coupling the large with the small

Andrew J. de Mello reviews developments in on-chip mass spectrometry

Over the past decade the miniaturization of analytical techniques and methods has become a highly visible and dominant trend in the physical and biological sciences.[1] Development in this area has primarily been driven by a need for rapid, on-line measurements at low concentrations within fields such as DNA analysis, drug discovery, pharmaceutical screening, medical diagnostics, environmental analysis and chemical production. The advantages associated with shrinking analytical systems are well known and include improved efficiency with respect to sample size, application, response times, cost, analytical performance, integration, throughput and automation.[2]

The evolution of Lab-on-a-Chip technology is in some ways analogous to the development of the integrated circuit during the latter part of the twentieth century. Improvements in fabrication methods facilitated the miniaturization of most electronic components to a level where currently over 10 million transistors can be routinely compressed onto a standard microprocessor chip. It is this scale of integration (through miniaturization) that has primarily defined the huge gains in processor performance over the past thirty years. Nevertheless, although miniaturization of chemical analysis systems affords distinct advantages in terms of analytical performance, it is quite obvious that the very nature of chemical and biological systems poses constraints on the degree of miniaturization needed or even desired. For example, when performing capillary electrophoresis on a microfabricated chip device, injection volumes may typically range between 10^{-14}–10^{-10} dm³. This means that for a diagnostically relevant sample concentration of 1 nM, only 10–10 000 molecules are available for analysis. If the injection volume is reduced much further a point is soon reached at which no analyte molecules at all will be introduced into the analysis system. Moreover, even for analyte volumes on a pL scale, it is evident that detection becomes a key issue in determining the

practicality of microfluidic systems. Indeed, the adaptation of conventional detection protocols for measurement in small volumes has closely accompanied the development of lab-on-a-chip technology, and it has long been realized that ultimate size limits for chip-based systems are primarily set by the system detector. Consequently, it is clear that high-sensitivity detection is essential when performing any kind of analysis on a small scale.

Small volume detection within planar chip devices has conventionally been based around optical measurements. This is primarily due to the optical properties of the materials used in chip fabrication. The most common substrates include glass, quartz and polymeric materials, all of which possess good transparency in the visible regions of the electromagnetic spectrum. A cursory survey of the literature demonstrates that common methods for on-chip detection include laser-induced fluorescence (LIF), absorption, indirect fluorescence, chemiluminescence, electrochemistry, refractive index variation, Raman spectroscopy and electrochemiluminescence methods.[1] Of these, LIF approaches have proved by far the most the popular due to their exceptional sensitivity and low mass detection limits. However, LIF techniques suffer from significant drawbacks that prohibit their use universally. These include relatively high instrumental costs and the fact that the majority of molecular species do not fluoresce (or are not easily converted to fluorescent species). The development of new detection protocols is therefore of considerable importance.

Most microfluidic chip devices have been developed for specific applications. These include DNA/RNA separations,[3] small-molecule organic synthesis,[4] DNA amplification,[5,6] immunoassays[7] and cell manipulations.[8] In many of the above applications a single analyte is targeted for analysis. However, more usually structural identification and quantitation of individual sample components is highly desirable or even necessary. On-chip

spectroscopic detection cannot easily provide this information and consequently alternative analytical techniques must be considered. This mini-review describes how one such conventional technique, mass spectrometry, has been used in conjunction with chip-based analytical systems to provide for high-throughput sample identification and structure elucidation.

Seeking structure: mass spectrometry

Over the past two decades mass spectrometry (MS) has developed into one of the most powerful detection techniques used in liquid phase analyses.[9] The mass and structural information afforded by MS provide an insight into the fundamental properties of the analyte under investigation. Furthermore, the capability of variable selectivity allows trade-offs to be made between general detection and targeted sample analysis at low detection levels.

The success of MS as an analytical detector interfaced to standard separation techniques (such as CE and HPLC) has primarily been due to the development of methods for sampling ions directly from solution. This has occurred in a number of forms. Early studies utilized both electron ionization (EI) and chemical ionization (CI) sources as interfaces with LC.[10] The higher pressures associated with CI afforded efficient heat transfer and allowed larger amounts of solvent to be vaporized in the ion source. Consequently, a number of successful interfaces with CI-MS were demonstrated. These included direct liquid introduction (DLI)[11] and the thermospray technique.[12] The thermospray interface introduced by Vestal enabled the direct coupling of condensed phase separation methods and MS. This development allowed for easy sampling from solutions but posed constraints on the required volatility and polarity of the solvent medium. In addition, modification of fast atom bombardment (FAB) methods to allow desorption from a liquid surface facilitated

direct MS analysis of flowing solutions.[13] Unfortunately, these 'continuous-flow' techniques proved restrictive in their application due to experimental issues.

Electrospray ionization

Undoubtedly the single most important advance in liquid sampling methods for MS analysis has been the development of the electrospray technique. Electrospray ionization (ESI), originally proposed by Dole[14] and later pioneered by Fenn[15] in the 1980s, provides both a route to facile coupling of condensed phase separation methods and MS and also the ionization of involatile and polar analytes. The general mechanism of ESI can be summarized as follows: a flowing analyte stream (*e.g.*, the eluent from a CE separation) is forced through a needle or a capillary held at high potential. The high electric field causes the fluid to form a Taylor cone, which is enriched with positive ions at the tip. Positively charged droplets are then expelled from the tip of the Taylor cone by the electric field to form a mist of small droplets (pneumatic nebulization). The droplets subsequently move under the influence of a potential and pressure gradient towards the MS analyzer. During this migration period 'Coulomb explosion' and evaporation act to shrink the droplet size, ultimately resulting in fully de-solvated ions (Fig. 1). Controlled heating of the interface and the application of a nebulizing gas flow are commonly used to facilitate droplet evaporation.

Over the past decade, ESI-MS has become a standard technique for the analysis of many analytes, most notably peptides and proteins.[16] This, in large part, has been due to its simple interfacing with high-performance separation techniques (such as HPLC[17] and CE[18])

and a need for only minimal sample preparation. As noted previously, planar chip devices have been hugely successful in improving analytical performance and reducing analysis times when applied to electrophoretic and chromatographic separations. Not surprisingly then, the combination of ESI-MS and microchip-based analytical systems offers the possibility of high-speed, high-efficiency structural molecular analysis.

Chip-MS

The ability to interface microfabricated structures with MS has been directly facilitated by the development of the nano-electrospray (nano-ESI) ionization source.[19] By fabricating extremely fine spraying capillaries (a few microns in diameter), low liquid flow rates (nL min^{-1}) can be used to sustain a stable electrospray, thus enabling high-sensitivity MS analysis on extremely small sample volumes. It is clear that both the capillary dimensions and flow rates used in nano-ESI match those encountered in many microfluidics devices. Consequently, interest in microchip-ESI-MS has escalated over the past 5 years.

On a fundamental level microfluidic devices are ideal for sample delivery to ESI-MS. They provide small volumes of sample at low flow rates. Reduced channel dimensions afford minimal dead volumes and little sample wastage. Furthermore, through integration of sample handling and reaction steps, complex processing can be achieved in an integrated and automated fashion. Finally, fabrication of multiplexed units *via* micromachining methods is effortless and facilitates high-throughput parallel

analysis. Examples of advances in all these areas are now presented.

The first examples of interfacing microfluidic chips with ESI-MS were reported in 1997. Barry Karger and co-workers at Northeastern University described multichannel glass chips that were used to perform enzymatic digestion and also to deliver different samples of standard peptides and proteins into an ESI-MS at flow rates between 100 and 200 nL min^{-1}.[20,21] At the same time, scientists at Oak Ridge National Laboratories reported a broadly similar approach to microchip-ESI-MS, except that fluid delivery was effected using electro-osmotic pumping with no external pressure source.[22] In addition, Aebersold and co-workers at the University of Washington constructed a hybrid capillary/microfabricated device for automated, sequential infusion of peptide samples into an ESI-MS. Using this approach detection limits as low as 1 fmol µL^{-1} were achieved.[23]

A primary concern in all three studies is that the interface should allow for the creation of a stable electrospray. In the aforementioned studies solutions exiting from the channel terminus are electrosprayed directly, without the use of a more conventional capillary or needle. This is clearly ideal in terms of system complexity but means that the formation of the Taylor cone (Fig. 2) and subsequent electrospray may be ill-defined, since the finer the electrospray nozzle the more efficient and stable the electrospray process. Direct solutions to this problem have included coating the surface of the outlet edge with a hydrophobic reagent.[21] This acts to prevent liquid from spreading and ensures efficient droplet and electrospray formation. Other approaches have utilized short lengths of capillary (acting as electroosmotic pumps) between the chip and microsprayer.[23]

Further development of the general approach was reported by Figeys and co-workers in 1998 through the demonstration of a nine-position sample handling microdevice interfaced with a tandem MS system.[24] Studies demonstrated low fmol µL^{-1} sensitivity and compatibility with the automated analysis of proteins separated by 2-D gel electrophoresis. In a separate publication the authors also presented a microfluidic module for the generation of solvent gradients which sequentially eluted peptides adsorbed on a SPE cartridge. This system was successfully used for frontal analysis of complex peptide mixtures.[25]
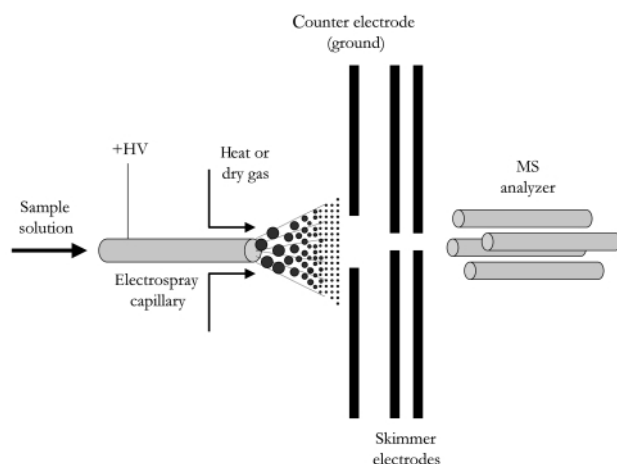

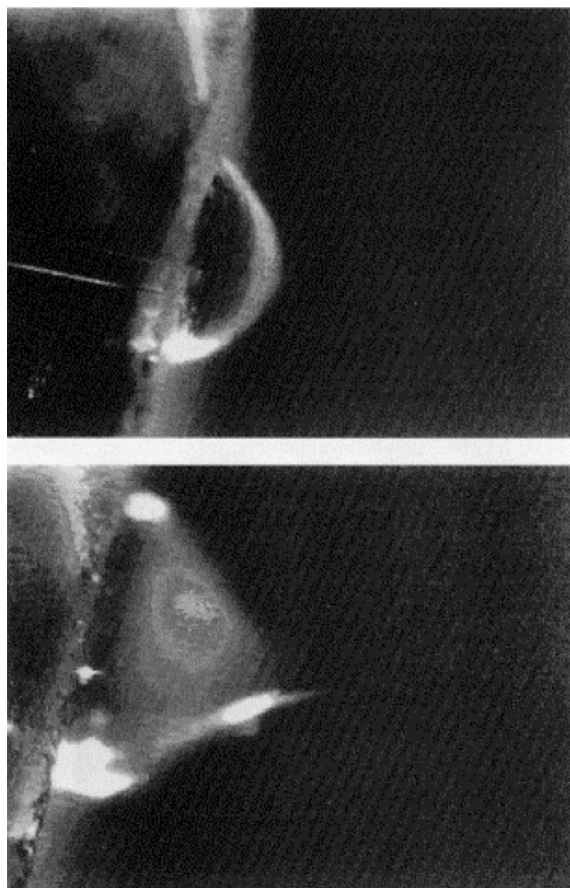**Fig. 1** Basic features of an electrospray interface.

Fig. 2 Images of (top) a 12 nL water droplet forced through a channel opening by positive pressure and (bottom) the Taylor cone and electrospray generated at a channel opening by applying a 3 kV potential between a microchip and target electrode. Reproduced from ref. 22 with permission.

electrospray device reported by Gary Schulz.[29] Using deep reactive ion etching, electrospray nozzles with diameters as small as 15 μm were successfully formed and used in the generation of nano-electrospray of liquid samples.

A common issue associated with the use and mass application of microfluidic structures is cost. Micromachining of traditional materials, such as glass, silicon and quartz, although well established, is often expensive, time-consuming and laborious. These drawbacks are significant since the successful adoption of lab-on-a-chip technologies as routine laboratory tools relies heavily on their low cost, ease of fabrication and functionality. The use as plastics or polymers as substrate materials offers a robust, rapid and economical route to the mass fabrication of microfluidic devices. Polydimethylsiloxane (PDMS) has shown great promise as a substrate material for microfabricated devices.[30] It is elastomeric, optically transparent, durable, non-toxic, chemically inert, cheap and can be structured using a variety of simple methods. Prototype devices microfabricated in PDMS have been successfully used for the delivery of peptide mixtures to ESI-MS systems.[31] Importantly, the polymer has been shown to be compatible with electrospray ionization, electroosmotic pumping and mass spectrometry. More recently, Jack Henion at Cornell University reported the fabrication of Zeonor 1020 CE microchips and their coupling with ESI-MS for the analysis of small organic molecules.[32] The use of Zeonor as an appropriate substrate material for microfluidic devices is noteworthy because of its wide use in the manufacture of compact discs and digital video disks. Lee and co-workers have also used polymers to create electrospray sources using MEMS technology.[33] The

As shown previously, in infusion analysis dedicated electrospray tips are not always necessary since an electrospray can be generated at the channel exit due to the high electric fields at the exposed liquid surface. Unfortunately, large dead volumes associated with droplet formation make this approach unsuitable for performing chip CE-ESI-MS. Accordingly, alternative interfacing techniques have been developed to allow for post-column detection. These have included the use of a short transfer capillary attached to the channel exit and, more interestingly, the integration of a microfabricated pneumatic nebulizer at the electrospray exit port (Fig. 3).[26] The implementation of an on-chip nebulizer dramatically reduces dead volumes, improves electrospray stability, and obviates the need for any external electrospray tip.

Nevertheless, the use of an external electrospray tip can be used to good effect, as demonstrated by Ramsey and colleagues.[27] Using a replaceable electrospray tip attached to the end of a microchannel, stable electrospray generation can be achieved through application of voltages at fluid reservoirs.

Without any pressure assistance, high speed CE-MS analysis of peptide mixtures could be performed in a repetitive, automated fashion. Furthermore, using time-of flight (TOF) MS, sub-attomole quantities of sample could be detected within millisecond time frames. Other examples of miniaturized electrospray nozzles include a microfabricated silicon nozzle (with a 1–3 μm orifice) reported by Terry Lee[28] and a monolithic silicon
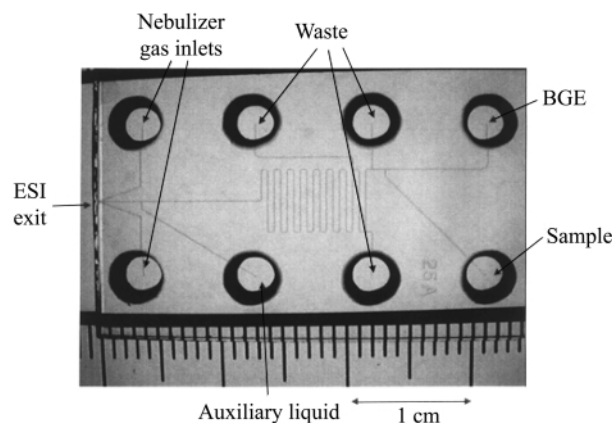

Fig. 3 Photograph of a microdevice with an integrated pneumatic nebulizer for CE-electrospray mass spectrometry. Reproduced from ref. 26 with permission.
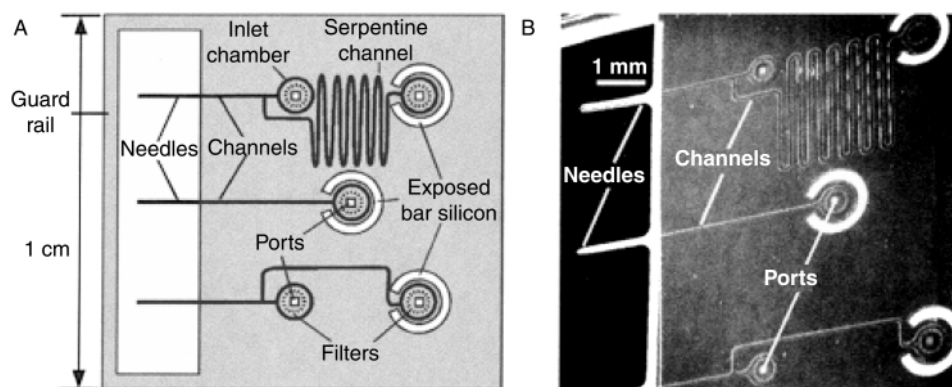
Fig. 4 (A) Diagram of the layout of an electrospray-chip incorporating needle sources (electrospray emitters). (B) Scanning electron micrograph of an actual chip. Reproduced from ref. 33 with permission.

use of needle-like parylene electrospray sources provides performance characteristics similar to conventional pulled fused silica capillaries, and obviates the need for a nebulizing gas flow. A typical electrospray chip incorporating hollow needle structures, channels, chambers and filters is shown in Fig. 4. Frantisek Foret and co-workers used the advantages of plastic processing techniques to machine disposable devices for high-throughput ESI-MS analyses.[34] Sample wells within the microdevice were arranged in the format of a standard 96-well plate, with individual wells being connected to independent electrospray ports *via* microchannels. Using this novel approach 96 peptide samples were analyzed in 480 s, corresponding to a theoretical throughput of approximately 720 samples h[−1]. Further simplifications of the fabrication process have recently been reported by Jentaie Shiea at the National Sun Yat-Sen University in Taiwan. Open channels (375 µm in width, 300 µm in depth) were structured on planar PMMA substrates by cutting into the surface with a sharp knife. A stable electrospray was generated from sharp channel termini and a multiplexed design allowed the sequential analysis of up to eight individual samples.[35]

The ionization conditions encountered in ESI-MS are mild and thus allow for the efficient analysis of many biomolecules. However, sample matrix interferences often pose severe limitations on its use in the analysis of large biopolymers. Consequently, the ability to integrate sample cleanup stages within microfluidic sample delivery systems will be highly desirable in the analysis of 'real' samples. Richard Smith and colleagues at Pacific Northwest National Laboratory have constructed a microfabricated device for microdialysis cleanup of biological samples prior to analysis by ESI-MS.[36] The microchip was used in-line to de-salt

both DNA and protein samples prior to ESI-MS analysis. This simple but effective approach to desalting greatly enhances signal-to-noise ratios when compared to direct infusion of non-dialysed samples (Fig. 5).

MS-on-a-Chip?

As described the integration of microfabricated chip devices with conventional mass spectrometry is proving an important addition to the analytical toolkit. However, until recently little attention has focussed on the benefits of downsizing the mass spectrometer itself. The development of miniature mass spectrometers has been motivated by a need for portable instruments in applications such as environmental

monitoring, process analysis, chemical and biological weapon detection and medical diagnostics. In theory, almost all mass analyzers can be miniaturized. However, the need to maintain (or even improve) performance means that some are better suited to downscaling than others. For example, many mass analyzers suffer from reduced mass resolution and usable mass range when their dimensions are reduced. Mass resolution in TOF analyzers is dependent on time resolution, and thus the length of the flight path. Consequently, miniaturization poses experimental constraints.

Although, the complexity and capabilities of modern tandem mass spectrometers will be difficult to transfer to planar chip formats, exciting progress is being made into the downsizing of ion
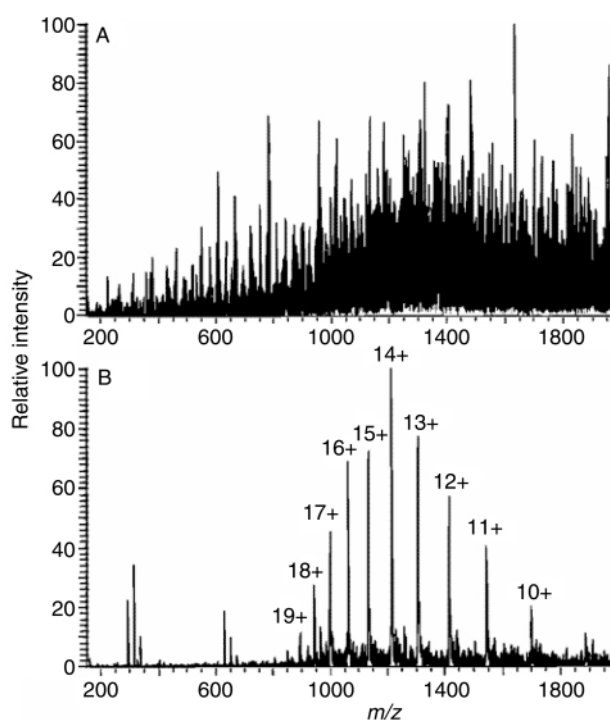

Fig. 5 (A) ESI-mass spectrum of 5 µM horse heart myoglobin in 500 mM NaCl, 100 mM Tris and 10mM EDTA by direct infusion; (B) ESI-mass specrum of previous myoglobin sample after on-line microdialysis. Reproduced from ref. 36 with permission.
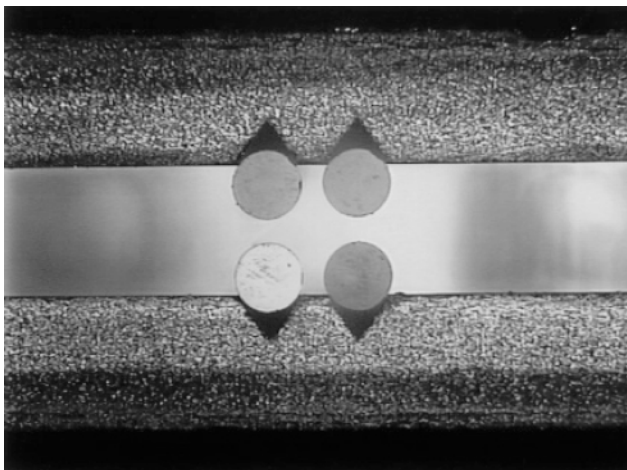
Fig. 6 End-on view of microengineered quadrupole electrostatic lens, with 500 μm diameter electrodes. Courtesy of R.R.A. Syms, ICSTM, 2001.

College, London, have recently demonstrated continuous-flow, solution-phase compound library generation using silicon microchips and TOF-MS.[4,43] As the pharmaceutical industry moves towards developing drugs 'tailored' to specific population genotypes (pharmacogenomics) the synthesis and screening of large numbers of structurally-related molecules gains ever-greater importance. Chip-MS may provide the ideal route towards the automation of these processes.

trap mass analyzers,[37,38] quadrupole mass analyzers[39,40] and time-of-flight mass spectrometers.[41] For example, Stephen Taylor at Liverpool University and Richard Syms of Imperial College of Science, Technology and Medicine have micromachined miniaturized quadrupole lenses. Their approach is based on cylindrical glass electrodes mounted in two anisotropically etched silicon substrates that are separated by precision spacers (Fig. 6). Micromachining the quadrupole lens enables the system to self align, thus optimizing constructional precision. Using a field emitter array as the ion source, the micromachined quadrupole as the mass filter and a CMOS electrometer as the ion detector, preliminary demonstrations of mass selection (resolution of 2.7 u at mass 40) have been performed. Recently, more refined studies of the MEMS mass filter indicate that the resolution (at 10% peak height) varies approximately as $1/S$ (where S = sensitivity) with a best case resolution of 31 (at mass 40).[40] If the size and weight of quadrupole mass analyzers can be further reduced by MEMS fabrication then a number of advantages will almost certainly follow. These include low costs associated with fabrication, operation at higher pressures (due to reductions in the ion mean free path length), lower power operation (since electrode voltages can be reduced) and the possibility of fully integrated quadrupole mass analyzers driven by associated on-chip electronics.

Bob Cotter and colleagues at The Johns Hopkins University and the University of Maryland have recently developed a microfabricated coaxial time-of-flight mass spectrometer.[41] The TOF analyzer employs a reflectron energy correction scheme and is less than 9 cm in length. The mass spectrometer uses low acceleration voltages (<500 V), and mass spectra with mass resolutions as high as 2500 can be measured. Current studies are focussing on reducing the dimensions of the analyzer and electronics to yield a portable, hand-held mass spectrometer.

Further details of other developments in the area of miniaturized mass spectrometers can be found in an excellent review by Graham Cooks and colleagues.[42]

Outlook

The need to miniaturize any analytical technology is driven primarily by the potential gains that downscaling yields. Although by definition lab-on-a-chip devices should be physically small with respect to their conventional counterparts, this is generally not the driving force in development. The notions of improved analytical performance, component integration, increased throughput and automation are far more important. Accordingly, the marriage between chip-based analysis systems and the conventional mass spectrometer, although perhaps not obvious in size terms, is quite natural in terms of integration and added value. In particular, the sample volumes and flow rates encountered within microfluidic systems are very close to those required for efficient electrospray formation. This makes interfacing facile. Moreover, the ease with which multichannel systems can be fabricated within chip-based formats provides an excellent opportunity for maximizing throughput in mass spectrometric analyses. This, perhaps, will have its greatest implications within the fields of drug discovery and combinatorial library generation, where the ability to handle and structurally assign large numbers of compounds is still one of the primary bottlenecks in high-throughput screening strategies. Indeed, researchers at Imperial

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