MARKUS HAAPALA
Heated Nebulizer Microchips for Mass Spectrometry

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Division of Pharmaceutical Chemistry
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University of Helsinki
Heated Nebulizer Microchips for Mass Spectrometry

by

Markus Haapala

ACADEMIC DISSERTATION

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This study was carried out at the University of Helsinki, in the Center for Drug Discovery and the Division of Pharmaceutical Chemistry, Faculty of Pharmacy, during the years 2005-2010. Funding was provided by the Graduate School of Chemical Sensors and Microanalytical Systems and the Finnish Funding Agency for Technology and Innovation (projects *Biofunctional Microchips* nos. 40177/05 and 40380/06 and *Microchip based ionization methods for fast mass spectrometric analysis* no. 40399/08).

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ABSTRACT

Miniaturized analytical techniques are of increasing interest owing to the faster operation, better performance, ability to analyze much smaller sample volumes, reduced waste production, and lower cost relative to conventional systems. Heated nebulizer (HN) microchips are microfabricated devices that vaporize liquid and mix it with gas. They are used with low liquid flow rates, typically 0.5–10 μL/min, and have previously been utilized for atmospheric pressure chemical ionization (APCI) and atmospheric pressure photoionization (APPI). Conventional APCI and APPI sources are seldom feasible at such low flow rates. In this work HN chips were developed further and new applications were introduced.

The requisite for enhancing the performance of HN chips is to understand their principal properties. A new method for thermal and fluidic characterization of miniature gas jets was developed and used to study the heated vapor jet produced by the HN chips. Thermal behavior of the chips was also studied by temperature measurements and infrared imaging. Different types of HN chips were compared and the origins of the differences clarified.

The high temperature of the HN microchips allows efficient vaporization of high boiling analytes. An HN chip was applied to the analysis of crude oil – an extremely complex sample – by microchip APPI Fourier transform ion cyclotron resonance mass spectrometry. With the chip, the sample flow rate could be reduced significantly without loss of performance and with greatly reduced contamination of the ion source and ion inlet of the MS. Microchip APPI provided efficient vaporization of high molecular weight components in crude oil.

With the same chip as for APCI and APPI, the first microchip version of sonic spray ionization (SSI) was presented. Ionization was achieved simply by applying high (sonic) speed nebulizer gas without heat, corona discharge, or high voltage. SSI significantly broadens the range of applicability of the HN chips, from small stable molecules to labile biomolecules. The performance of the microchip SSI source in terms of flow rate dependence, linearity, limits of detection, and repeatability was confirmed to be acceptable.

The HN microchips were also used to connect gas chromatography (GC) and capillary liquid chromatography (LC) to MS, using APPI for ionization. Microchip APPI allows efficient ionization of both polar and nonpolar compounds with capillary LC and other low flow rate separation methods, whereas with electrospray ionization (ESI) only polar and ionic molecules are ionized efficiently. The combination of GC with MS with atmospheric pressure ionization showed that, with HN microchips, GCs can easily be used with MS instruments designed for LC-MS. The presented GC-μAPPI-MS method for polycyclic aromatic hydrocarbons (PAH) and capillary LC-μAPPI-MS method for steroids showed good quantitative performance.

The same high performance microfabrication methods used for the HN microchips were successfully utilized in the fabrication of the first integrated LC–HN microchip. In a single microdevice, there were structures for a packed LC column channel, micropillar frit, channel for optional optical detection, and heated vaporizer. Nonpolar and polar
analytes were efficiently ionized by APPI, as demonstrated with PAHs and selective androgen receptor modulators. Ionization of nonpolar and polar analytes had not been possible with previously presented chips for LC–MS since they relied on ESI. With use of APPI-MS, preliminary quantitative performance of the new chip was evaluated in terms of limit of detection, linearity, and repeatability of signal response and retention time. Determination of fluorescent compounds was demonstrated with use of laser induced fluorescence for optical detection.

A new ambient ionization technique for mass spectrometry, desorption atmospheric pressure photoionization (DAPPI), was presented, and its application to the rapid analysis of compounds of various polarities on surfaces was demonstrated. The DAPPI technique is based on an HN microchip delivering a heated jet of vaporized solvent, e.g., toluene, and a photoionization lamp. The solvent jet is directed toward sample spots on a surface, causing desorption of analytes from the surface. Photons ionize the analytes via reactions similar to those in APPI, and the ions are directed into an MS. The direct analysis of pharmaceuticals from tablets was successfully demonstrated as an application.

All in all, the HN microchips were demonstrated to be universal ion sources, which can be used with any API MS, connected to GC or LC, and used with one of several ionization techniques. New integrated LC–HN chip showed good potential for miniaturized LC-MS. The new DAPPI technique was shown to be suitable for rapid analysis of various surfaces and analytes.
LIST OF ORIGINAL PUBLICATIONS

This doctoral thesis is based on the following six original publications hereafter referred to by their Roman numerals (I-VI):


Author’s contribution to the publications included in this thesis:

I The experimental work was carried out and the publication written equally by the author and Ville Saarela.

II The experimental work, excluding the microfabrication, was carried out by the author and Dr. Jeremiah Purcell. The article was written by the author with contributions from Dr. Jeremiah Purcell and others.

III The experimental work, excluding the microfabrication, was carried out by the author, Dr. Jaroslav Pôl, and Dr. Tiina Kaupila. The article was written by Dr. Jaroslav Pôl with contributions from the author and Dr. Tiina Kaupila.

IV The experimental work, excluding the microfabrication, was carried out by the author. The publication was written by the author with contributions from Ville Saarela (microfabrication) and others.

V The experimental work was carried out equally by the author and Ville Saarela. The publication was written by the author and Ville Saarela with contributions from others.

VI The experimental work, excluding the microfabrication, was carried out by the author and Dr. Jaroslav Pôl. The publication was written by the author with contributions from others.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>SSI</td>
<td>sonic spray ionization</td>
</tr>
<tr>
<td>TAGA</td>
<td>trace atmospheric gas analysis</td>
</tr>
<tr>
<td>μAPPI</td>
<td>microchip atmospheric pressure photoionization</td>
</tr>
<tr>
<td>μTAS</td>
<td>micro total analysis system</td>
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<tr>
<td>VUV</td>
<td>vacuum ultraviolet</td>
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1 INTRODUCTION

Analytical chemistry studies the composition of materials on the basis of their chemical and physical properties. Besides chemistry itself, analytical methods are important in the biosciences, medicine, environmental research, and many branches of industry. Modern analytical methods commonly involve a separation step, in which the compounds in a sample are separated, and a detection step, in which the separated compounds are detected. One example of this is liquid chromatography-mass spectrometry (LC-MS), in which LC is used for separation and MS for detection.

Mass spectrometry is not only a detection method but a powerful analytical technique used widely in many areas of chemistry and other sciences. The first step in measuring the mass spectrum of any sample is ionization. All mass spectrometers work in vacuum, which ranges from $10^{-3}$ to $10^{-10}$ mbar depending on the type of mass analyzer. Thus the sample has to be either transferred into vacuum before ionization or ionized at atmospheric pressure and the ions then transferred into vacuum. In all mass spectrometers up to the 1980s and in many instruments still today, ionization takes place in vacuum. This creates a limit on the amount of sample that can be transferred from LC to MS because when a liquid vaporizes, its volume expands by a factor of several hundred and a large gas load is created in the vacuum system. Since the LC eluent cannot be pumped directly into the MS, it was for many years impossible to connect LC with MS.

The first devices used to couple LC to MS relied on ionization techniques in which ionization took place in vacuum or at significantly reduced pressure. The amount of eluent was reduced, for example, by splitting the liquid flow before ionization to decrease the gas load to the mass spectrometer. The first techniques to become commercial were the moving-belt interface, direct liquid introduction, thermospray, and fast atom bombardment. Of these techniques, thermospray was the most popular and allowed the broad breakthrough of LC-MS as an analytical technique. Today, these techniques are all but obsolete, and commercial LC-MS instruments rely instead on atmospheric pressure ionization methods. All experiments in this work have been performed with use of atmospheric pressure ionization techniques. Vacuum ionization techniques are still popular for other than LC-MS instruments; electron ionization is widely used in gas chromatography–mass spectrometry (GC-MS), for example. Another common vacuum ionization technique, used extensively in the biosciences, is matrix assisted laser desorption ionization (MALDI).

1.1 Miniaturization of analytical devices

Miniaturization of analytical devices has attracted much interest during the last three decades. The general aims of miniaturization are to gain speed; reduce sample, reagent (solvent), and energy consumption; and to enable cost-effective manufacturing of analytical devices. The ultimate goal is a micro total analysis system ($\mu$TAS) including, for example, sample preparation, separation, and detection within a single microchip.
μTAS is commonly used today to refer to the field of research on miniaturized analytical systems. The materials, or substrates, for microfabrication of microfluidic components and components of microelectromechanical systems (MEMS) are usually in the form of round wafers of 100 or 150 mm size. This is because the tools and methods for microfabrication are largely derived from the semiconductor industry, which also uses wafers. New materials and methods for fabricating specifically microfluidic and MEMS components have also been developed along with the growing interest in the field. The materials and methods for microfabrication relevant in the context of this work are shortly reviewed in the following subsections. A more comprehensive review of microchip-based devices related to mass spectrometry has recently been presented by Sikanen et al. 7

1.1.1 Materials for microfabrication

The most common materials for fabrication of microfluidic and MEMS components are silicon, glasses, and polymers. Silicon has excellent properties for MEMS devices including mechanical strength, high thermal conductivity, and adjustable electrical conductivity. In addition, it is compatible with the fabrication of microelectronics, which makes it possible to integrate electronics with mechanical and fluidic components. Thanks to the years of experience with silicon fabrication in microelectronics, a wide variety of fabrication methods are available. Perhaps the most important feature of silicon processing relevant to microfluidics is the ability to fabricate structures with very high aspect ratios (height/width), narrow and deep channels or thin and high micropillars, for example. Some properties may be advantageous or disadvantageous depending on the proposed use. For example, the high thermal conductivity is advantageous if a uniform temperature distribution is required but not if the temperature distribution needs to be localized. The high mechanical strength of silicon allows it to be used as a material for masters in replication methods, but the good electric conductivity makes it impossible to fabricate devices requiring high potential differences, such as electrophoretic separation devices. 8,9

Glass encompasses a wide class of materials with essentially limitless composition and properties. However, most glass wafers used in microfabrication are borosilicate glass, quartz, or fused silica. Glass materials are electric insulators and thus good for electrophoretic and other high voltage applications. They are also transparent to visible light, which is ideal for optical detection or visual observation with a microscope. The microfabrication methods for glasses are more limited than those for silicon, and high aspect ratios are difficult to achieve. Glass–glass bonding is easier than silicon–silicon bonding, however, and silicon–glass anodic bonding is a widely applied method for bonding cover wafers to microfluidic and MEMS devices. 8

Polymers offer a wide choice of materials and material properties. The fabrication methods for polymers also range widely. In general, polymers are considered to be a simpler and cheaper alternative to traditional silicon and glass. Properties affecting the fabrication and applicability of polymers in microfluidics include glass transition temperature, melting temperature, coefficient of thermal expansion, solvent compatibility, water contact angle, transparency, and porosity. 8,10
1.1.2 Methods for microfabrication

The methods and tools for microfabrication of microfluidic and MEMS come largely from the semiconductor industry, although some processes have been specifically developed. The fabrication is performed in dedicated cleanrooms where temperature, humidity, and especially particle contamination are tightly controlled. In principle, the minimum feature size of the components restricts the fabrication conditions in that possible contaminating particles are ones smaller than the minimum feature size. Thus, many microfluidic devices with feature sizes of roughly 100 μm could be fabricated outside cleanrooms. Nevertheless, this is seldom done since other environmental conditions besides particle contamination are controlled clearly better in cleanrooms.

Microstructures are typically created by lithography and etching. In photolithography, a polymeric photoresist is first applied on the substrate and then exposed with UV light through a transparent photomask with opaque patterns. When the resist is developed, a patterned layer of resist is left on the substrate, defining the structures to be created by further processing, typically etching. The photoresist itself can act as a mask for etching or it can be used to pattern another mask, for example, a silicon dioxide thin film hard mask on silicon. Photoresist thickness is usually from hundreds of nanometers to a few micrometers. Some photoresists, such as SU-8, can also be used to create final structures directly by lithography. Advanced lithographic methods can provide pattern sizes well below 100 nm, the costs of equipment and photomasks increasing with decreasing feature size.9

In direct imprinting/replication methods, structures are created by patterning with a 3D shaped mold piece. The patterns on the mold are mirrored to the workpiece (wafer), i.e., an extrusion on the mold makes a channel on the workpiece and vice versa. Imprinting methods include (hot) embossing and casting. In embossing, a mold is pressed with high force against a workpiece, which may be heated. The mold is made of a hard material, typically silicon or metal, and the patterns are transferred onto the softer workpiece. Polymers are common materials amenable to embossing. In casting, liquid monomer is applied on a mold and polymerized, and the mold is detached. A typical material suitable for casting is polydimethylsiloxane (PDMS) elastomer, which thanks to its high elasticity allows even retrograde molds to be used.8,9

Etching methods are classified into wet and dry methods, or into isotropic and anisotropic methods. Wet etching is done in solution and dry etching uses reactive gases in plasma conditions. In isotropic methods etching takes place in every direction at the same rate, while anisotropic methods have directional dependence. Important factors in etching include selectivity and etching rate. Selectivity is the ratio of the etching rate of the mask to that of the material being etched. Some selectivity is always required, and ratios as high as 60,000:1 can be achieved. Etching rate varies widely with the method and material and may be as much as micrometers per minute. Both selectivity and etching rate become critical when high aspect ratios with deep structures are required. Typical examples of isotropic wet etching are glass etching in HF and metal etching with various acids. Anisotropic wet etching can be achieved only with crystalline materials, a common example being silicon etching in KOH. Isotropy in dry etching is controlled by process...
parameters such as gas composition, flow rate, and pressure, and plasma power. A widely used anisotropic dry etching method is deep reactive ion etching (DRIE) of silicon, which is the method of choice to achieve high aspect ratio silicon structures.\textsuperscript{8,9}

Bonding of wafers is commonly done to achieve closed microstructures. Bonding of dissimilar materials is more challenging than bonding of a material with itself since differences in surface chemistry and thermal expansion need to be taken into account. Bonding may be direct or with an intermediate layer (adhesive) in between. Usually bonding requires that elevated temperature and force are applied. Examples of direct bonding are silicon–silicon fusion bonding at about 1000 °C and glass–glass fusion bonding at about 600 °C (with Pyrex glass). A widely used bonding method with MEMS and microfluidic devices is silicon–glass anodic bonding (field-assisted thermal bonding) at 300–400 °C. A special bonding method is PDMS bonding, which may be either nonpermanent or permanent depending on the surface treatment. In adhesive bonding, an intermediate layer of adhesive material is used to join the wafers. Typical adhesives are photoresist materials or other polymers. The intermediate layer could cause problems in the final device due to differences in surface chemistry and other properties. A nice application is SU-8 adhesive bonding, in which SU-8 wafers are bonded with an SU-8 intermediate layer, resulting in uniform properties of the final structures.\textsuperscript{8,9}

1.2. Atmospheric pressure ionization sources and their miniaturized versions

Atmospheric pressure ionization (API) sources for mass spectrometry were presented in 1958 by Knewstub and Sudgen.\textsuperscript{11,12} The first commercial API source for LC-MS was based on work by the group of Horning and Carroll.\textsuperscript{13-16} A heated vaporizer was used for LC eluent vaporization and corona discharge for ionization. Beta radiation from a $^{63}$Ni foil was also used in the early versions. Other API devices for gas analysis\textsuperscript{17} and LC-MS\textsuperscript{18,19} and methods for them were subsequently described, while the most successful commercial instrument was the Sciex TAGA (trace atmospheric gas analysis). The real breakthrough in API methods, however, came in the early 1990s as the consequence of developments in electrospray ionization (ESI) and several commercial API instruments presented in the late 1980s.

Modern API mass spectrometers are designed so that multiple ionization methods can be applied with the same instrument merely by changing the ion source. To be precise, an ion source is the device that generates the ions, for example an ESI or atmospheric pressure chemical ionization (APCI) source, but in general also the first stages of an API mass spectrometer are considered to be parts of the ion source. Since the pressure difference between the atmosphere and a mass analyzer may be up to 12 orders of magnitude, ions have to be transferred into the mass analyzer by differential pumping through two or more vacuum sections with decreasing pressure. The first transition from atmospheric pressure may be through a small orifice in a plate or a capillary of several centimeters length. The pressure in the first vacuum stage is typically in the order of 0.1–10 mbar. Various techniques can then be used to transfer the ions further, to the next stage,
which may be another pumping stage or the first part of the mass analyzer. An example of an API mass spectrometer including electrospray and differential pumping stages is presented in Figure 1. In this design, a curtain gas is flowing against the spray and the ions to prevent the orifice becoming dirty or clogged. In the following subsections, the most common API methods in use today are shortly reviewed and their principles presented. All API methods can be used for both positive and negative ionization simply by reversing all voltages. With one exception, positive ionization was used in this work and the discussion here is limited to positive ionization.

Figure 1. Schematic view of a typical API mass spectrometer with three pumping stages.

1.2.1 Electrospray ionization

When a high electric potential is applied to a solvent exiting a narrow capillary or a sharp tip, the solvent breaks into thin threads, which fragment further into small droplets. This phenomenon, later called electrospray, was first described by Zeleny in 1914. Electrospray ionization as a method for producing large macroions was presented by Dole in 1968. At the time, however, API-MS had not been developed. ESI as an ionization method for MS was introduced by Fenn et al. in 1984, and Whitehouse et al. described an LC-MS interface based on electrospray in 1985. The early ESI interfaces worked only for low μL/min flow rates and thus the pneumatically assisted electrospray (ionspray) of Bruins et al., which allowed flow rates up to 200 μL/min, was an important advance. The final breakthrough of ESI took place after the discovery of its ability to ionize large intact biomolecules (proteins), which made it possible to determine their molecular weight (MW) accurately. This resulted in a rapid increase in LC-MS applications particularly in the life sciences.

The principle of liquid spraying in ESI is illustrated in the upper part of Figure 2. When a high potential is connected to a needle carrying a liquid, the liquid forms a sharp cone, called a Taylor cone, at the end of the needle. In the case of positive potential, the cone is positively charged and the negative charges in the solution migrate to the needle surface. At the tip of the Taylor cone the liquid forms a thin jet, which disintegrates into
charged droplets due to Rayleigh instability. These droplets fly away from the needle due to electrostatic forces and undergo processes that finally lead to single gas-phase ions. First, neutral solvent molecules evaporate from the droplets, decreasing the size of the droplets but preserving the charge. This continues until the Rayleigh limit is reached, i.e., the electrostatic repulsion of the charges exceeds the surface tension that holds the droplet together. As a result, the droplet undergoes Rayleigh fission, where it loses a small percentage of its mass but a relatively large percentage of its charge, and several small offspring droplets are produced.

![Diagram of ESI process](image)

**Figure 2.** The principle of ESI with ion evaporation and charge residue models presented.

Models called ion evaporation and charge residue have been presented for the final gas-phase ion generation process. The ion evaporation model (Figure 2 top) of Iribarne and Thomson\(^{28}\) assumes that, when a droplet loses solvent by evaporation, the electric field strength on the droplet surface becomes large enough for the droplet to emit ions or solvated ions into gas phase. When an ion is emitted, the charge of the droplet decreases but the mass is practically unchanged. Subsequent evaporation–ion emission cycles follow. The charge residue model (Figure 2 bottom) of Dole et al.,\(^{21}\) on the other hand, assumes that the droplets proceed further through the cycle of solvent evaporation and fission until finally there is just one analyte ion and some solvent in a droplet. The gas-phase ions are formed when the solvent molecules evaporate. It is believed that ion evaporation is the mechanism of ionization for small ions, while the charge residue model applies to large molecules.\(^{29,30}\) The boundary between these mechanisms has been
suggested to be from a few to several thousand Daltons depending on the type of molecule.31

Practically all commercial ESI sources are designed for joining LC to MS and can handle flow rates up to at least several hundred microliters per minute. As compared with the typical 1–10 μL/min for traditional electrospray, such high flow rates are achieved by assisting the spraying with a high velocity gas flow that promotes nebulization and evaporation of the solvent. The nebulizer can also be heated or an additional heated gas flow may be used to assist the ionization process allowing eluent flow rates as high as 2 mL/min with some instruments.

The ionization mechanism of electrospray makes it a concentration-sensitive mechanism. Decreasing the flow rate while keeping the sample amount the same (i.e., increasing the concentration) increases sensitivity and decreasing the flow rate while keeping the sample concentration the same does not affect sensitivity. Electrospray is thus inherently suitable for miniaturization.32,33 In protein characterization, for example, the sample amount may well be very small and a low flow rate ionization method is needed. Electrospray is easily miniaturized by drawing a glass capillary to a very narrow tip, with an i.d. of just a few micrometers. Such narrow tips can be used for analyzing samples of just 1 μL volume, and the flow rate is on the order of tens of nanoliters per minute. The nanospray method, as it is called, was introduced in 1994 by Wilm and Mann.32,33 It is commercially available34,35 and widely used in bioanalysis, especially for proteins.

Almost all of the research on miniaturization of API methods for mass spectrometry has concentrated on miniaturization of ESI. This is due to the straightforward fabrication of ESI devices by various microfabrication methods, the advantages of nanospray (reduced flow rate, increased sensitivity), and the similarity of the flow rates of ESI and microfluidic separation methods. In contrast to ESI, APCI and APPI have been developed for connecting traditional LC to MS, and optimal sample flow rates of APCI and APPI are typically in the order of hundreds of microliters per minute.

Tens of different microfabricated ESI devices have been presented since the first microchip ESI devices described by Ramsey and Ramsey36 and Xue et al.37 in 1997. ESI chips have been fabricated out of a variety of materials including silicon, glass, and polymers such as PDMS and SU-8. Silicon has been a popular material thanks to its advantageous properties and well-established microfabrication methods. Polymers offer a wide variety of properties and fabrication methods and have also been used extensively. Although glass has good properties, possibilities for microfabrication are somewhat limited and it has been used less than silicon and polymers. The field of microfabricated ESI devices has been reviewed extensively several times. Lazar et al.38 and Koster and Verpoorte39 have reviewed microfluidic devices connected to MS via ESI, with also devices for separation and sample pretreatment integrated with ESI. The microfabrication technologies and microfabricated devices for mass spectrometry, including microfabricated ion sources, were recently reviewed by Sikanen et al.7 from a more general perspective. Microchip-based ESI devices are also available commercially.40,41
1.2.2 Sonic spray ionization

Sonic spray ionization (SSI), first presented by Hirabayashi et al. in 1994, is an API method closely related to ESI. During the past ten years, sonic spray has been used in a variety of applications mainly as an alternative ionization method for LC-MS. In SSI, nebulization of solvent is achieved with high pressure and high speed gas flow in a pneumatic nebulizer. The speed of the gas flow in the nebulizer is approximately the speed of sound, hence the name sonic spray. Since neither voltage nor heat is used in SSI, the internal energy of the produced ions is very small, and SSI is an even softer ionization method than ESI.44 In most applications of SSI, some (high) voltage has nevertheless been used, which means that these methods are, strictly speaking, not SSI but closer to ESI. Hirabayashi et al. originally suggested that charged droplets are produced in SSI as a result of non-uniform ion concentrations of positive and negative charges in small droplets where the non-uniformity is due to a surface double layer. Later, Takats et al. presented results suggesting that charge formation follows Dodd’s statistical charging model (which Hirabayashi et al. originally ruled out) and that the final ion production takes place through the charge residue model. The statistical charging model as it applies to SSI means that the statistical unbalance in natural charge distribution in solution and droplets leads to a net charge in some of the initial droplets. A low flow rate version of SSI, with flow rate down to 100 nL/min, has been reported.

1.2.3 Atmospheric pressure chemical ionization

Atmospheric pressure chemical ionization for LC-MS was originally reported by Horning et al. in 1974, and several applications were demonstrated with their APCI system. Research on APCI was continued at several commercial laboratories, one of the results being the Sciex TAGA system. LC-APCI-MS with a Sciex TAGA was presented by Henion et al. in 1982, and high-speed LC-APCI-MS was demonstrated in 1986. APCI was widely commercialized in the late 1980s in combination with the API-MS instruments that had been introduced for LC-ESI-MS. Since then, APCI has been widely used for LC-MS, though ESI is still the most popular ionization method.

The main working principles of commercial APCI sources are closely similar. The same kind of vaporizers are also used in atmospheric pressure photoionization (APPI) discussed in section 1.2.4 below. Historically, alternative methods for liquid nebulization and vaporization for APCI have been presented, but they are not of interest here. A typical APCI source with heated nebulizer (HN) is presented in Figure 3. The eluent coming into the source is nebulized pneumatically by a high velocity gas flow in a co-axial nebulizer. The eluent capillary is inserted into a larger i.d. capillary, which in some devices is inside a third tubing used for auxiliary gas. After nebulization, the solvent spray is heated causing complete vaporization of droplets. The temperature of the vaporizer is adjustable and may be up to 500 °C. In contrast to ESI, high voltage plays no role in the nebulization process and the produced vapor is neutral. Ionization of the analytes is achieved by means of a corona discharge that initiates gas-phase ionization processes.
Corona discharge is the electric discharge that may occur in the non-uniform electric field around a high-voltage electrode with small radius of curvature — a thin wire or a sharp needle, for example. The counter electrode of the discharge is considered to be large and far away and it plays no role in the discharge process. Corona discharge consists of a corona plasma region close to the curved electrode and a unipolar region further away. The formation of a corona discharge is initiated by an exogenous ionization event (e.g. background radiation) of a neutral atom or molecule in the region of very high electric field strength close to the discharge electrode. A free electron and a positive ion are produced in the ionization. In the case of a positive corona, the discharge electrode is at a positive potential, and the electron is accelerated towards it. The electron undergoes inelastic collisions with neutral species and in the course of these collisions more free electrons and positive ions are produced. The produced electrons undergo the same process, precipitating an electron avalanche towards the electrode. Since all the electrons migrate toward the electrode and ions cannot achieve enough kinetic energy to initiate ionization, the avalanche will collapse after the initial ionization unless more secondary electrons are produced for further avalanches. These are produced in the region outside the corona plasma region by photoionization by photons emitted in de-excitation processes occurring in the plasma and are then accelerated into the plasma region to initiate further avalanches.

The positive ions produced by corona discharge are the initial source of charge in APCI. In air, corona discharge produces mainly \( N_2^+ \) and \( O_2^+ \) ions. Because of atmospheric moisture and solvent vapor, these ions transfer their charge to water and solvent molecules, and finally protonated solvent molecules or clusters are formed. The most common mechanism for analyte ionization is then proton transfer from protonated solvent to analyte (reaction (1)), which can take place if the proton affinity (PA) of the analyte (M) is larger than that of the solvent (S). This mechanism is highly simplified, it should be noted, since the cluster size of solvent and analyte affects their proton affinities and thereby the whole process. Impurities in the atmosphere and buffers and additives in the LC eluent may also influence the process, complicating it significantly.

\[
S_mH^+ + M \rightarrow MH^+(S)_1 + S_{m-1}
\]

The main differences between ESI and APCI are temperature and the ionization process. Ionization in APCI is a chemical gas-phase ion–molecule reaction process, whereas in ESI ionization takes place in the liquid phase. Because ESI is a very soft...
ionization method and causes minimal fragmentation, even very large labile molecules can be ionized. In APCI, in turn, the high temperature required for vaporization limits its use to relatively stable molecules of MW up to approximately 1000. Although ESI is by far the most widely used ionization technique in API-MS, it nevertheless has some clear limitations. Because the ionization takes place in liquid phase, ESI ionizes effectively only ionic and polar compounds and is ineffective for nonpolar compounds. APCI provides efficient ionization of less polar compounds, in addition to polar and ionic compounds. Moreover, since solvent polarity is a critical factor in ESI, only polar and medium polar solvents can be used. APCI allows the use of nonpolar solvents as well, and can thus be used with normal phase LC, which widens the range of LC applications compared with ESI. Another major issue in ESI is ion suppression due to interfering ions from the sample or eluent, because in the ionization process total ion concentration in the droplets is a key factor. APCI is a gas-phase process and inherently less susceptible to ion suppression.48

1.2.4 Atmospheric pressure photoionization

Atmospheric pressure photoionization was presented in 2000, simultaneously by two independent research groups, as a new ionization method for LC-MS.49,50 The research was motivated by the need to widen the range of compounds analyzable by LC-MS to nonpolar compounds, which are not ionizable with ESI or APCI. Since its introduction, and commercialization in 2000 by Syagen Technologies, APPI has gained interest and has been used in a wide variety of applications. The principles and applications of APPI have lately been reviewed.51,52

In APPI, a co-axial pneumatic nebulizer and heated vaporizer similar to those in APCI are used to vaporize the solvent. Instead of a corona discharge, ionization is achieved by vacuum ultraviolet (VUV) radiation from a krypton discharge lamp. Ionization of analytes (M) takes place by direct photoionization (reaction ((2))) or, more commonly, through gas-phase reactions enhanced with a dopant compound. The latter method is sometimes called dopant-assisted APPI, although the normal term is APPI whether a dopant is used or not.

\[
\text{(2) } M + h\nu \rightarrow M^{+} + e^- 
\]

The original two APPI instruments relied on different VUV lamps, and the difference has continued until now. The PhotoMate APPI source from Syagen uses a radio frequency (rf) excited lamp, which has higher photon output than the direct current (dc) powered lamp used in the PhotoSpray source from Applied Biosystems. In certain applications, the rf excited lamp may enable direct photoionization, but in most cases use of a dopant is advantageous.52 The ionization mechanisms in dopant-assisted APPI have been discussed by Kauppila et al.53 When a dopant (D) is used, the first step in the ionization process is direct photoionization of the dopant (3).

\[
\text{(3) } D + h\nu \rightarrow D^{+} + e^- 
\]
After this, there are two common ways to analyze ionization. If the ionization energy (IE) of the analyte is smaller than that of the dopant, charge exchange may take place, producing a radical cation of the analyte (4).

\[ D^{**} + M \rightarrow D + M^{**} \]  

The other path is proton transfer through solvent (S) molecules or clusters. First the dopant donates a proton to the solvent (5), and then the protonated solvent donates a proton to the analyte (6). In this case the PA of the analyte has to be higher than that of the solvent molecule or cluster.

\[ D^{**} + nS \rightarrow S_nH^+ + (D-H)^* \]

\[ S_nH^+ + M \rightarrow MH^+(S)_m + S_{n-m} \]

The main advantage of APPI is that neutral and nonpolar compounds, which are poorly ionized by ESI or even APCI, can be efficiently ionized. Since polar compounds are ionized as well, it is a universal ionization method in regard to analyte polarity. Moreover, APPI, like APCI, is compatible with the nonpolar solvents used in normal-phase liquid chromatography.\(^{54}\) In regard to ion suppression, APPI is more tolerant than either ESI or APCI.\(^{55,56,52}\) Because of the high temperature vaporization, APPI, like APCI, can only be used for relatively small thermally stable molecules.

### 1.2.5 Heated nebulizer microchips

The optimal flow rate with commercial APCI and APPI sources is typically a few hundred microliters per minute. With low flow rates of tens of microliters per minute or less, sensitivity decreases significantly, and the use of commercial APCI and APPI with low flow rates is usually not feasible. Since miniaturized APCI or APPI sources are not yet available, APCI and APPI methods have mostly been limited to traditional LC. Low flow rates have nevertheless been used in some studies. APCI has been combined with supercritical fluid chromatography,\(^{57}\) open tubular liquid chromatography,\(^{58}\) and capillary electrophoresis (CE).\(^{59}\) In addition, APPI has been used to combine CE\(^{60-62}\) and capillary electrochromatography (CEC)\(^{63,64}\) with MS. Commercial APPI at a flow rate of 2 μL/min has also been used successfully in the analysis of crude oil asphaltenes\(^{65}\) suggesting that APPI sources differ in terms of low flow rate performance. These sources have been manufactured conventionally or modified from commercial sources, however, and lack the advantages of modern microfabrication.

The first microfabricated heated nebulizer for APCI was presented in 2004 by Östman et al.\(^{66}\) Figure 4 (a) shows the microchip from both sides. The chip consists of silicon and glass chips bonded together. The silicon part has wet etched structures for fluidic inlets, an eluent–gas mixer, a vaporizer channel, and a nozzle. The HN chip creates a heated jet of
eluent vapor, and a corona needle is used to initiate APCI outside the chip in front of the nozzle. Three microfluidic connectors (Nanoports) provide the gas and eluent connection. An improved version of the HN chip and an initial thermal characterization of it were presented by Franssila et al.\textsuperscript{67} This chip, illustrated in Figure 4 (b), relies on a fused silica capillary for eluent introduction and a single Nanoport connector for gas. The chip dimensions are reduced significantly. Both wet and DRIE etched versions of the chip have been fabricated. An all-glass version of the HN microchip (Figure 4 (c)) was presented by Saarela et al.\textsuperscript{68} Using glass alone provides improved thermal control of the chip since thermal conductivity of glass is only a fraction of that of silicon. The thermal and fluidic behavior of the improved silicon–glass and all-glass chips were characterized in detail in this work by a new high resolution temperature profiling method.\textsuperscript{1}

![Figure 4](image.png)

**Figure 4.** (a) The first version of the heated nebulizer microchip with three Nanoport connectors. On the bottom side (left) are the etched channel structures and metallic heater and on the top side (right) the fluidic connectors. (b) Improved silicon–glass and (c) all-glass versions of the heated nebulizer microchip with Nanoport connectors.

The newest version of the heated nebulizer microchip is presented in Figure 5 (a). This chip is a reduced-size all-glass version, which is used with custom-made chip holders. Two versions of the holder are presented in Figure 5 (b) and (c). The gas connection is made without a Nanoport connector, directly to the gas inlet hole on the chip. Either a flat bottom connector (b) or an o-ring seal inside the holder (c) is used for gas-tight sealing.
Miniaturized APPI based on the first version of the HN microchip was presented by Kauppila et al.\textsuperscript{69} For APPI, dopant (toluene) was mixed with eluent and a VUV lamp was used for ionization. The improved version of the HN microchip (Figure 4 (b)) was used by Östman et al.\textsuperscript{70} to connect a GC to a triple quadrupole API-MS with APCI providing ionization. Normally, GC-MS is performed with specially designed GC-MS instruments with vacuum ionization, but the HN microchips provide easy combination of GCs with API-MS instruments originally intended for LC-MS. For example, connection of a GC to an Orbitrap mass spectrometer has recently been demonstrated with the chip.\textsuperscript{71} The improved silicon–glass chip has also been used to connect a capillary LC (capLC) to MS using APCI,\textsuperscript{72} and the capability for quantitative analysis was demonstrated with a set of neurosteroids. Normally, capillary LC with flow rates lower than 20 μL/min is only feasible with ESI. Luosujärvi et al. used the silicon–glass HN chip as an API source for an ion trap MS and performed GC-MS analysis of PCBs with ionization by APCI and APPI.\textsuperscript{73}

In this work, a silicon–glass heated nebulizer chip (Figure 4 (b)) was used as a microchip APPI (μAPPI) source for connecting GC to MS for the analysis of polycyclic aromatic hydrocarbons (PAH), and an all-glass chip similar to that in Figure 4 (c) was used for combining capLC to MS for the analysis of steroids.\textsuperscript{IV} Analysis of crude oil by μAPPI combined with Fourier transform ion cyclotron resonance mass spectrometry\textsuperscript{II} (μAPPI FT-ICR MS) is also presented. In addition to the several applications with APCI and APPI, the HN microchip was employed for SSI.\textsuperscript{III} Use of the chip for SSI opens the

\textbf{Figure 5.} (a) Current version of the all-glass HN chip from top and bottom. (b) Chip in a holder made of aluminum and Macor. (c) Chip in a holder made of Sintimid polymer.
way to the analysis of labile biomolecules, which is impossible with APCI and APPI. Moreover, initial results have been published describing use of the chip for ionspray.\textsuperscript{74} Recently Keski-Rahkonen et al.\textsuperscript{75} used the HN microchip as an atmospheric pressure thermospray ionization (APTSI) source by employing only heating and low gas flow to the chip. No external ionization was applied. Further, Ahonen et al.\textsuperscript{76} used the newest version of the HN microchip in the first μAPPI-based method for LC-MS analysis of biological samples. Anabolic steroids in urine samples were analyzed by a capLC-μAPPI-MS/MS method.

1.3 Miniaturization of chromatography

Efforts toward miniaturized chromatography historically began with the development of miniaturized gas chromatographic systems.\textsuperscript{77} The focus of the present work is on liquid chromatography, nowadays the most versatile of all separation techniques and extensively used in numerous branches of chemical research. LC is a diverse technique allowing the analysis of compounds ranging from small volatile molecules to the largest proteins, and methods based on LC cover a vast area of chemical analysis from ion chromatography to polymer analysis. Commercial LC instruments are large and solvent consumption is typically in the range of 0.2–2 mL/min. Capillary and nano LC are widely available for flow rates down to tens of nanoliters per minute. However, low flow rates involve considerable challenges with solvent mixing and gradient delays, connections, and dead volumes, and the instruments are often as large as normal LC devices. The goal of miniaturized LC systems is, ultimately, to integrate the total analytical system on a microchip. The practical advantages would include faster analysis, lower solvent and power consumption, ability to analyze minimal sample volumes, and the avoidance of problematic connections.

Miniaturization of integrated devices for liquid chromatographic and electrophoretic separation and detection has been studied extensively during recent years. Most research on microfluidic separation devices has focused on CE, CEC, and related techniques, while miniaturization of LC has received considerably less attention.\textsuperscript{39,78,79} Nevertheless, LC is widely accepted as a more robust and reliable technique than CE and is nowadays the most widely used separation technique in analytical chemistry. The main reason for this neglect is that miniaturization of LC is more complicated. Development of miniaturized LC requires challenging new approaches to integrate pumps, injector, sample transfer channels, column, and detector on a single microchip. Furthermore, LC microchips should be able to withstand high pressure – a challenging requirement for chip materials and fluidic connections.

A variety of chip materials, such as silicon,\textsuperscript{80-82} glass,\textsuperscript{83,84} quartz,\textsuperscript{85,86} fused silica,\textsuperscript{87} and various polymers,\textsuperscript{88-92} have been used for miniaturization of LC, but no single material has yet taken the lead. Separation columns on microchips have been fabricated on the basis of various principles and have included particle-packed columns,\textsuperscript{88,89,91} monolithic columns, micropillar structured channels,\textsuperscript{81,85,93} and even open channels.\textsuperscript{82,94} Both detection methods integrated on chips and methods relying on external detectors have
been employed with miniaturized LC devices. These include amperometric,\textsuperscript{82} electrochemical,\textsuperscript{89} and conductometric detection and detection with fluorescence or \textsuperscript{UV}\textsuperscript{86,95} instruments. Although some of these methods may be highly sensitive or specific, none can provide the performance, versatility, and identification capability of mass spectrometry.

Until now, the only ionization method to have been employed with miniaturized LC devices is ESI, particularly nanoelectrospray.\textsuperscript{83,90,91,96} Agilent’s high performance liquid chromatography (HPLC) chip is the best known of the LC-ESI microchips; it is available with a number of stationary phases and has been used in applications for the analysis of proteins and peptides,\textsuperscript{91,97-102} antocyanins,\textsuperscript{103} oligosaccharides,\textsuperscript{104} glycosaminoglycans,\textsuperscript{105} and pharmaceuticals.\textsuperscript{106} Because of the limitations of ESI, sensitive detection of nonpolar compounds is not possible with current microchip LC devices. In addition to polar and ionic compounds, APCI and APPI can provide efficient ionization of less polar compounds and APPI also of nonpolar compounds. As discussed earlier, APCI and APPI also allow the use of nonpolar solvents and suffer less than ESI from ion suppression.

### 1.4 Ambient mass spectrometry

Mass spectrometric analysis of a sample typically requires at least some and usually a considerable amount of sample preparation. Ideally, analysis would be done directly from a sample in ambient atmosphere within a very short time. Instruments for rapid and simple collection and analysis of trace residues, such as drugs and explosives, were commercially available as early as the 1980s. However, these instruments were largely forgotten by the 1990s.\textsuperscript{107} A commercial but poorly-known instrument for direct ambient analysis has been available since the early 2000s and used for the detection of drug traces on money.\textsuperscript{108} The widespread interest in methods categorized under the term “ambient mass spectrometry” began in 2004 with the introduction of desorption electrospray ionization (DESI) by Takats et al.\textsuperscript{109}. Since then, the need for ambient MS analysis has been addressed by the presentation of a wide variety of new atmospheric pressure desorption/ionization methods. Currently there are over 15 different methods for desorption and ionization of samples directly from surfaces in ambient conditions. With these methods, a wide variety of surfaces can be analyzed in seconds with no sample preparation. The methods and their applications have recently been reviewed by Van Berkel et al.,\textsuperscript{107} Venter et al.,\textsuperscript{110} Ifa et al.,\textsuperscript{111,112} Chen et al.,\textsuperscript{113} and Weston.\textsuperscript{114} The newest ambient MS method not included in the reviews is microplasma discharge ionization presented by Symonds et al.\textsuperscript{115}

In DESI the spray from a typical electrospray nebulizer is directed on a surface for desorption and ionization of compounds. A vast number of applications for DESI have been presented and many are covered in the general desorption/ionization reviews.\textsuperscript{107,110,111} As an extension of ESI, DESI allows the analysis of polar compounds ranging from small molecules to proteins. The desorption and ionization mechanisms of DESI have been studied experimentally and by simulations.\textsuperscript{116,117} DESI has been commercialized and is available from Prosolia, Inc. under the name Omni Spray.\textsuperscript{118}
Desorption sonic spray ionization (DeSSI)\textsuperscript{119} is a method closely related to DESI, just as SSI is related to ESI. In DeSSI, desorption and ionization are accomplished with the spray from a pneumatic nebulizer with high velocity gas stream, and neither voltage nor heating is applied. DeSSI is claimed to be the simplest and most easily implemented ambient desorption method providing very gentle ionization due to the lack of high voltage and thus clean mass spectra.\textsuperscript{120} The technique was recently renamed easy ambient sonic-spray ionization (EASI)\textsuperscript{120}.

In direct analysis in real time (DART),\textsuperscript{121} a stream of gas with excited species is used for desorption and ionization. The source employs an electric discharge in atmospheric pressure to create metastable excited atoms or molecules from gas, typically helium. The gas stream can be heated to enhance desorption, but desorption is also possible simply by bombardment with excited or ionized species. Ionization of analytes takes place through gas-phase reactions initiated by the excited atoms or molecules. A clear difference from other ambient desorption/ionization methods is that Penning ionization, by energy transfer from neutral excited species to analytes, is possible. A wide variety of surfaces and materials can be analyzed and, unlike DESI, DART also allows the analysis of nonpolar compounds. DART is commercially available from JEOL\textsuperscript{122} and Ionsense.\textsuperscript{123}

The atmospheric pressure solid analysis probe\textsuperscript{124} (ASAP) employs a heated jet of gas and APCI. Desorption is effected with a hot nitrogen jet from a normal ESI or APCI nebulizer and ionization takes place in gas phase by APCI. ASAP can be used with many ESI and APCI interfaces by making modifications to the ion source housing. The analysis capability in regard to analyte polarity and MW is similar to that of APCI. ASAP is commercially available from Waters.\textsuperscript{125}

Methods under the name desorption atmospheric pressure chemical ionization (DAPCI) have been presented in two versions, by Cooks et al.\textsuperscript{126,127} and Williams et al.\textsuperscript{128,129} The first device has a high voltage discharge needle in a gas tube in which carrier gas is flowing. Solvent vapor may be added to the gas and no heating is required. The gas flow is directed onto a surface, causing analyte desorption and ionization. The second setup utilizes a normal heated nebulizer as a source for heated vapor for desorption and corona discharge for APCI ionization. In principle, this version of DAPCI is the same as ASAP.

Desorption atmospheric pressure photoionization (DAPPI)\textsuperscript{VI} was developed in this work. In DAPPI, a heated jet of solvent vapor from a HN microchip is directed onto a surface for analyte desorption, and this is followed by ionization in gas phase by photoionization. The desorption and ionization mechanisms of DAPPI have been studied\textsuperscript{130} and it has been applied for the detection of several types of illegal pharmaceuticals and drugs\textsuperscript{131,132} and the analysis of food and environmental samples.\textsuperscript{133} MS imaging with DAPPI has also been demonstrated.\textsuperscript{134}

\section{1.5 Temperature and fluidic measurements in microscale}

The performance of API sources in terms of ionization and ion collection efficiency depends on the design and operational principle of the source.\textsuperscript{135} Performance is affected
by the fluidic properties, including temperature, flow rate, linear speed of gas flow, and other characteristics of gas and vapor flows. Understanding of these parameters is critical in the further development of ion sources. APCI and APPI sources produce a heated jet of vaporized solvent, which is ionized after vaporization. The behavior of the vapor jet is largely unknown, not least because the measurement of gas jets is challenging. In the case of heated nebulizer microchips, where dimensions are small, fluidic characterization becomes even more challenging.

Visual characterization of sprays or jets is difficult since they consist of tiny droplets or are vapor and usually exhibit low or no contrast against the ambient atmosphere. Sprays created by ESI sources can be visualized by fluorescence if a fluorescent sample is sprayed, or simply by photography, with or without a microscope since ESI sprays consist of droplets that reflect and scatter light. In APCI and APPI sources the sample is completely vaporized, and vapor, unlike droplets, is transparent to visible light and not directly observable. Conventional fluorescent imaging is impossible as well, since common fluorescent dyes are fluorescent in liquid phase only. Advanced methods, such as planar laser induced fluorescence, have been applied to visualize vapor-phase sprays, but the equipment is expensive and rarely available in laboratories. In the case of vaporized jets, the shape is determined by velocity distribution of the jet, but measuring the velocity of a relatively small gas flow expanding in free space is very difficult. However, in gas flows the distribution of velocity is closely interconnected to that of temperature, and temperature distribution can be used to describe velocity distribution. Furthermore, measurement of temperature is considerably easier than measurement of velocity.

The simplest temperature sensor is the thermocouple, in which two dissimilar metal wires are joined together to a loop. When the two joining points are at different temperatures, voltage is produced. This phenomenon is known as the thermoelectric effect. The voltage depends solely on the temperature difference between the junction points and not on the temperature of the wires. Thus the sensing element of a thermocouple is the joining point of the two wires. The size of this point depends on the wire size and the method of joining and in commonly used thermocouples is roughly from 0.5 to 2 mm. Since a thermocouple only measures the temperature of the tip and not of the wires, the smaller the wire diameter is, the smaller is the sensing part. Thus a sensor capable of spatial resolution of less than 100 μm is easily constructible. A small tip also means that the response time of the sensor is decreased since the response time is dependent on the thermal capacitance of the tip. Miniature thermocouples can therefore be used to measure temperatures of gas flow with high spatial and temporal resolution. Thermocouples down to 13 μm wire diameter are available commercially, and sub-micrometer thermocouples have been manufactured and used for measuring temperature on optical fibers. A method for thermal characterization of miniature gas jets with a miniature thermocouple is presented as a part of this work.

Another method capable of high spatial resolution in temperature measurement is infrared (IR) imaging. An IR camera is essentially an IR thermometer, which measures temperature at many points and generates a temperature image of the object. Modern IR cameras can measure temperatures across an IR image with high accuracy. Moveover, as
with normal cameras, lenses can be used to adjust the optical properties, and macro photography can be performed. This easily allows spatial resolution down to a few tens of micrometers, and IR cameras can be used for detailed temperature measurement of miniature objects. In the present work, IR imaging was used to measure the surface temperature of HN microchips. High performance IR cameras, it needs to be added, are at a totally different cost level than thermocouples and the meters used with them. Moreover, IR cameras can only measure the temperature of solid objects of high emissivity; gases cannot be measured.
2 AIMS OF THE STUDY

The overall aim of this study was to develop applications for heated nebulizer microchips for mass spectrometry and to enhance the performance, structure, and usability of the chips.

The detailed aims of the research were

- to develop a thermal measurement method for characterizing microfluidic gas jets and to use it to characterize the thermal and fluidic properties of heated nebulizer microchips (I)
- to demonstrate the performance of microchip APPI for petroleum analysis by high resolution mass spectrometry (II)
- to employ the heated nebulizer microchip for sonic spray ionization (III)
- to develop a robust microchip APPI source that would improve on previous versions of the chips (II, IV)
- to combine microchip APPI with capillary liquid chromatography and gas chromatography and test the performance of the methods (IV)
- to integrate a liquid chromatographic separation device and heated nebulizer onto a single microchip and demonstrate the performance of the chip (V)
- to develop a new surface desorption/ionization method by using the heated nebulizer microchips and APPI (VI)
3 MATERIALS AND METHODS

This section describes the chemicals and materials, commercial instruments, microfabrication processes, and instrumental setups used in this study. Chemicals, materials, and instruments are listed in Tables 1-3 and microfabrication processes are described in the text and visualized in figures. More detailed descriptions of the instrumental setups and parameters can be found in the original publications I-VI.

3.1 Chemicals and materials

Table 1 lists the chemicals used in the study are, with notes indicating their use. All solvents were of HPLC grade unless otherwise stated.

Table 1. Chemicals used in the study.

<table>
<thead>
<tr>
<th>Reagent/Solvent/Standard</th>
<th>Manufacturer/Supplier</th>
<th>Note</th>
<th>Publication</th>
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<td>Solvent</td>
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<td>Reagent</td>
<td>III</td>
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<tr>
<td>Angiotensin I</td>
<td>Bachem, Weil am Rhein, Germany</td>
<td>Standard</td>
<td>III</td>
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<tr>
<td>Anthracene</td>
<td>Sigma Aldrich Chemie GmbH, Steinheim, Germany</td>
<td>Standard</td>
<td>IV, V</td>
</tr>
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<td>Benz[a]anthracene (B[a]A)</td>
<td>Sigma Aldrich Chemie GmbH, Steinheim, Germany</td>
<td>Standard</td>
<td>V</td>
</tr>
<tr>
<td>Benzo[a]pyrene (B[a]P)</td>
<td>Sigma Aldrich Chemie GmbH, Steinheim, Germany</td>
<td>Standard</td>
<td>IV, V</td>
</tr>
<tr>
<td>BODIPY ® 493/503</td>
<td>Invitrogen, Carlsbad, CA</td>
<td>Standard</td>
<td>V</td>
</tr>
<tr>
<td>Chloroform</td>
<td>Mallinckrodt Baker B.V., Deventer, The Netherlands</td>
<td>Solvent</td>
<td>IV</td>
</tr>
<tr>
<td>Dimethyl sulfoxide (analytical grade)</td>
<td>Sigma-Aldrich Laborchemikalien GmbH, Seelze, Germany</td>
<td>Solvent</td>
<td>IV</td>
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<tr>
<td>Diphenylamine</td>
<td>United Laboratories Ltd, Helsinki, Finland</td>
<td>Standard</td>
<td>IV</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Altia, Helsinki, Finland</td>
<td>Solvent</td>
<td>V</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>Lab Scan, Dublin, Ireland</td>
<td>Solvent</td>
<td>IV</td>
</tr>
<tr>
<td>Fluorescein</td>
<td>Sigma Aldrich Chemie GmbH, Steinheim, Germany</td>
<td>Standard</td>
<td>V</td>
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<tr>
<td>Formic acid</td>
<td>Riedel-de-Haën, Seelze, Germany</td>
<td>Reagent</td>
<td>III</td>
</tr>
<tr>
<td>Reagent/Solvent/Standard</td>
<td>Manufacturer/Supplier</td>
<td>Note</td>
<td>Publication</td>
</tr>
<tr>
<td>-------------------------</td>
<td>-----------------------</td>
<td>------</td>
<td>-------------</td>
</tr>
<tr>
<td>Helium (99.9995%)</td>
<td>Woikoski, Vuohijärvi, Finland</td>
<td>GC carrier gas</td>
<td>IV</td>
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<tr>
<td>Hexane</td>
<td>Mallinckrodt Baker B.V., Deventer, The Netherlands</td>
<td>Solvent</td>
<td>VI</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>University Pharmacy, Helsinki, Finland</td>
<td>Standard</td>
<td>III</td>
</tr>
<tr>
<td>Methanol</td>
<td>Mallinckrodt Baker B.V., Deventer, The Netherlands</td>
<td>Solvent</td>
<td>III–VI</td>
</tr>
<tr>
<td>Methylenedioxyamphetamine (MDA)</td>
<td>United Laboratories Ltd., Helsinki, Finland</td>
<td>Standard</td>
<td>IV</td>
</tr>
<tr>
<td>Methylenedioxyethylamphetamine (MDMA)</td>
<td>United Laboratories Ltd., Helsinki, Finland</td>
<td>Standard</td>
<td>IV</td>
</tr>
<tr>
<td>Methylenedioxymethamphetamine (MDEA)</td>
<td>United Laboratories Ltd., Helsinki, Finland</td>
<td>Standard</td>
<td>IV, VI</td>
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<td>Naphtho[2,3-α]pyrene (N[α]P)</td>
<td>Sigma-Aldrich, St. Louis, MO</td>
<td>Standard</td>
<td>II</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>Woikoski, Vuohijärvi, Finland</td>
<td>Nebulizer gas</td>
<td>III</td>
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<tr>
<td>Progesterone</td>
<td>Sigma Aldrich Chemie GmbH, Steinheim, Germany</td>
<td>Standard</td>
<td>IV</td>
</tr>
<tr>
<td>SARM2, SARM3, and SARM4</td>
<td>German Sports University Cologne, Cologne, Germany</td>
<td>Standard</td>
<td>V</td>
</tr>
<tr>
<td>Synthetic air</td>
<td>Woikoski, Vuohijärvi, Finland</td>
<td>Nebulizer gas</td>
<td>III</td>
</tr>
<tr>
<td>Tenox tablets</td>
<td>Orion Oyj, Espoo, Finland</td>
<td>Standard</td>
<td>VI</td>
</tr>
<tr>
<td>Testosterone</td>
<td>Fluka Chemie, Buchs, Switzerland</td>
<td>Standard</td>
<td>III, IV</td>
</tr>
<tr>
<td>Testosterone</td>
<td>Sigma Aldrich Chemie GmbH, Steinheim, Germany</td>
<td>Standard</td>
<td>IV, VI</td>
</tr>
<tr>
<td>Tetra-N-butylammonium iodide</td>
<td>Lancaster Synthesis, Morecambe, UK</td>
<td>Standard</td>
<td>III</td>
</tr>
<tr>
<td>Toluene</td>
<td>Fisher Scientific, Pittsburgh, PA</td>
<td>Solvent</td>
<td>II</td>
</tr>
<tr>
<td>Toluene</td>
<td>Mallinckrodt Baker B.V., Deventer, The Netherlands</td>
<td>Solvent</td>
<td>IV</td>
</tr>
<tr>
<td>Toluene (≥ 99.9%)</td>
<td>Sigma-Aldrich, St. Louis, MO</td>
<td>APPI dopant</td>
<td>IV–VI</td>
</tr>
<tr>
<td>Tylenol Cold tablets</td>
<td>McNeil PPC Inc., Fort Washington, PA</td>
<td>Standard</td>
<td>VI</td>
</tr>
<tr>
<td>Verapamil</td>
<td>Sigma-Aldrich, Steinheim, Germany</td>
<td>Standard</td>
<td>III</td>
</tr>
<tr>
<td>Verapamil hydrochloride</td>
<td>Sigma Aldrich Chemie GmbH, Steinheim, Germany</td>
<td>Standard</td>
<td>VI</td>
</tr>
</tbody>
</table>

Table 2 lists the commercially available materials and products used in the study, with notes indicating their use. Common materials, such as the metals incorporated in mechanical parts, are not listed.
Table 2. Commercially available materials and products used in the study.

<table>
<thead>
<tr>
<th>Material/Product</th>
<th>Manufacturer/Supplier</th>
<th>Note</th>
<th>Publication</th>
</tr>
</thead>
<tbody>
<tr>
<td>AZ5214E photoresist</td>
<td>AZ Electronic Materials (Germany) GmbH</td>
<td>Chip fabrication</td>
<td>I–VI</td>
</tr>
<tr>
<td>Deactivated fused silica capillary glue</td>
<td>SGE Analytical Science Pty Ltd., Ringwood, Australia</td>
<td>Sample transfer capillary for HN microchips</td>
<td>I–IV, VI</td>
</tr>
<tr>
<td>Duralco 4703 epoxy glue</td>
<td>Cotronics Corp., Brooklyn, NY</td>
<td>Glue for HN chips</td>
<td>I–IV, VI</td>
</tr>
<tr>
<td>Macor ®</td>
<td>RS Components, Northants, UK</td>
<td>Material for chip holder</td>
<td>V</td>
</tr>
<tr>
<td>Nanoport connector</td>
<td>Upchurch Scientific, Oak Harbor, WA</td>
<td>Gas connector for HN chips</td>
<td>I–IV, VI</td>
</tr>
<tr>
<td>PMMA</td>
<td>Vink Finland Oy, Kerava, Finland</td>
<td>Sample surface for DAPPI</td>
<td>VI</td>
</tr>
<tr>
<td>Pyrex 7740 wafers</td>
<td>Several</td>
<td>Chip material</td>
<td>I–VI</td>
</tr>
<tr>
<td>Silicon &lt;100&gt; wafers</td>
<td>Several</td>
<td>Chip material</td>
<td>I, III, IV, V</td>
</tr>
<tr>
<td>Sintimid NT</td>
<td>Ensinger Sintimid GmbH, Lenzing, Austria</td>
<td>Material for chip holder</td>
<td>V</td>
</tr>
<tr>
<td>XBridge™ 2.5 μm solid-phase particles</td>
<td>Waters, Milford, MA</td>
<td>Integrated LC–HN chip</td>
<td>V</td>
</tr>
</tbody>
</table>

3.2 Instrumentation

Table 3 itemizes the commercial instruments and electrical and mechanical parts used in the study, with notes indicating their use. In addition to the instruments listed, some in-house built and standard laboratory instruments were employed. The most important of these was the 9.4 Tesla FT-ICR mass spectrometer. The instrument has been described in detail by Senko et al. and Häkansson et al.

Table 3. Instruments and parts used in the study.

<table>
<thead>
<tr>
<th>Instrument/Part</th>
<th>Manufacturer/Supplier</th>
<th>Note</th>
<th>Publication</th>
</tr>
</thead>
<tbody>
<tr>
<td>For microfabrication</td>
<td>-----------------------------------------------</td>
<td>-------------------------------------------</td>
<td>-------------</td>
</tr>
<tr>
<td>Plasmalab 80 Plus</td>
<td>Oxford Instruments Plasma Technology, Abingdon, UK</td>
<td>Reactive ion etching</td>
<td>I, III-V</td>
</tr>
<tr>
<td>Plasmalab System 400</td>
<td>Oxford Instruments Plasma Technology, Abingdon, UK</td>
<td>Sputtering</td>
<td>I–VI</td>
</tr>
<tr>
<td>STS ASE™</td>
<td>Surface Technology Systems plc, Newport, UK</td>
<td>Deep reactive ion etching</td>
<td>I, III-V</td>
</tr>
<tr>
<td>For GC and LC</td>
<td>-----------------------------------------------</td>
<td>-------------------------------------------</td>
<td>-------------</td>
</tr>
<tr>
<td>1100 Capillary LC system</td>
<td>Agilent Technologies, Waldbronn, Germany</td>
<td>Liquid chromatograph</td>
<td>IV, V</td>
</tr>
<tr>
<td>Capillary column butt connector</td>
<td>Sigma Aldrich Chemie GmbH, Steinheim, Germany</td>
<td>GC column to chip connection</td>
<td>IV</td>
</tr>
<tr>
<td>Instrument/Part</td>
<td>Manufacturer/Supplier</td>
<td>Note</td>
<td>Publication</td>
</tr>
<tr>
<td>---------------------------</td>
<td>------------------------------------------------</td>
<td>--------------------------------------------------</td>
<td>-------------</td>
</tr>
<tr>
<td>Deactivated fused silica capillary</td>
<td>Varian B.V., Middelburg, The Netherlands</td>
<td>GC precolumn</td>
<td>IV</td>
</tr>
<tr>
<td>FactorFour VF5-MS column</td>
<td>Varian B.V., Middelburg, The Netherlands</td>
<td>GC column</td>
<td>IV</td>
</tr>
<tr>
<td>HP 5890A gas chromatograph</td>
<td>Hewlett-Packard, Waldbronn, Germany</td>
<td>Gas chromatograph</td>
<td>IV</td>
</tr>
<tr>
<td>NanoTight fittings</td>
<td>Upchurch Scientific, oak, Harbor, WA</td>
<td>Connector for fused silica capillary</td>
<td>IV</td>
</tr>
<tr>
<td>Super Flangeless™ fittings</td>
<td>Upchurch Scientific, Oak Harbor, WA</td>
<td>Fluidic connectors for integrated LC–HN chip</td>
<td>V</td>
</tr>
<tr>
<td>SymmetryShield RP18 column</td>
<td>Waters, Milford, MA</td>
<td>LC column</td>
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**For thermal and fluidic characterization**

<table>
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<th>Instrument/Part</th>
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<th>Note</th>
<th>Publication</th>
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<td>ADS95 thermocouple amplifier</td>
<td>Analog Devices, Norwood, MA</td>
<td>Determination of thermocouple response time</td>
<td>I</td>
</tr>
<tr>
<td>CNC platform</td>
<td>Datentechnik Dr. Gert Müller GmbH, Bonn, Germany</td>
<td>Determination of thermocouple response time</td>
<td>I</td>
</tr>
<tr>
<td>GRV 3000 NC machining platform</td>
<td>Datentechnik Dr. Gert Müller GmbH, Bonn, Germany</td>
<td>Determination of thermocouple response time</td>
<td>I</td>
</tr>
<tr>
<td>KFT25-200-050 miniature thermocouple</td>
<td>Anbe SMT Co., Japan</td>
<td>Jet shape analysis</td>
<td>I</td>
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<tr>
<td>NI CompactDAQ system</td>
<td>National Instruments, Austin, TX</td>
<td>Jet shape analysis</td>
<td>I</td>
</tr>
<tr>
<td>NI9211, NI9401, and NI9205 modules</td>
<td>National Instruments, Austin, TX</td>
<td>Jet shape analysis</td>
<td>I</td>
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<td>ThermaCAM SC 3000 infrared camera</td>
<td>FLIR Systems, Inc., Boston, MA</td>
<td>Thermal imaging</td>
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<tr>
<td>XYZ linear stage</td>
<td>Newport Corporation, Irvine, CA</td>
<td>Jet shape analysis</td>
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**For mass spectrometry**

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<th>Note</th>
<th>Publication</th>
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<td>APPI ion source</td>
<td>Thermo Fisher Scientific, Waltham, MA</td>
<td>Sample ionization</td>
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<tr>
<td>APPI power source</td>
<td>University of Groningen, Groningen, The Netherlands</td>
<td>VUV lamp power supply</td>
<td>IV–VI</td>
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<tr>
<td>Drying gas extension</td>
<td>Agilent Technologies, Santa Clara, CA</td>
<td>Modification for Esquire 3000 Plus</td>
<td>VI</td>
</tr>
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<td>Esquire 3000 Plus mass spectrometer</td>
<td>Bruker Daltonics GmbH, Bremen, Germany</td>
<td>Mass spectrometer</td>
<td>VI</td>
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<td>Ion source frame for API 300/3000</td>
<td>Proxeon, Odense, Denmark</td>
<td>Frame for chip installation</td>
<td>III, IV, V</td>
</tr>
<tr>
<td>Ion source frame for Esquire 3000 Plus</td>
<td>Proxeon, Odense, Denmark</td>
<td>Frame for DAPPI setup</td>
<td>VI</td>
</tr>
<tr>
<td>Instrument/Part</td>
<td>Manufacturer/Supplier</td>
<td>Note</td>
<td>Publication</td>
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<td>-------------------------------------</td>
<td>---------------------------------------------------</td>
<td>-------------------------------------------</td>
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</tr>
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<td>PE Sciex API 300 mass spectrometer</td>
<td>MDS Sciex, Concord, Canada</td>
<td>Mass spectrometer</td>
<td>III</td>
</tr>
<tr>
<td>PE Sciex API 3000 mass spectrometer</td>
<td>MDS Sciex, Concord, Canada</td>
<td>Mass spectrometer</td>
<td>IV, V</td>
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<td>PKS 100 krypton VUV lamp</td>
<td>Heraeus Noblelight Analytics Ltd., Cambridge, UK</td>
<td>Photoionization</td>
<td>IV–VI</td>
</tr>
<tr>
<td>RF excited krypton VUV lamp</td>
<td>Thermo Fisher Scientific, Waltham, MA</td>
<td>Photoionization</td>
<td>II</td>
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<td>XYZ positioning stage</td>
<td>Proxeon, Odense, Denmark</td>
<td>Chip and sample positioning</td>
<td>III–VI</td>
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<td><strong>For laser induced fluorescence</strong></td>
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<tr>
<td>488 nm laser</td>
<td>Cheos Oy, Espoo, Finland</td>
<td>Excitation laser</td>
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<td>Pico Technology Ltd., St Neots, UK</td>
<td>LIF detection</td>
<td>V</td>
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<td>Inverted fluorescence microscope</td>
<td>Leica Microsystems GmbH, Wetzlar, Germany</td>
<td>LIF detection</td>
<td>V</td>
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<td>Photomultiplier tube</td>
<td>Cairn Research Ltd., Faversham, UK</td>
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<td>V</td>
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<td><strong>Other</strong></td>
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<td>II</td>
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<tr>
<td>dc power supply</td>
<td>Thurlby-Thandar Instruments Ltd., Huntingdon, UK</td>
<td>HN chip heating</td>
<td>VI</td>
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<tr>
<td>EPS EP-6515 dc power supply</td>
<td>Sankyo Kogyo Corp., Japan</td>
<td>HN chip heating</td>
<td>IV</td>
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<td>Flow meter</td>
<td>Aalborg, Orangeburg, NY</td>
<td>Nebulizer gas flow control</td>
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<td>Fluke 54-II thermometer</td>
<td>Fluke Corporation, Everett, MA</td>
<td>Temperature measurement</td>
<td>I, IV</td>
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<td>GCF17 mass flow controller</td>
<td>Aalborg, Orangeburg, NY</td>
<td>Nebulizer/auxiliary gas flow control</td>
<td>I, IV–VI</td>
</tr>
<tr>
<td>ISO TECH IPS-603A dc power supply</td>
<td>RS Components, Northants, UK</td>
<td>Chip heating</td>
<td>I, V</td>
</tr>
<tr>
<td>Milli-Q Plus purification system</td>
<td>Millipore, Molsheim, France</td>
<td>Water purification</td>
<td>I, III–VI</td>
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<tr>
<td>PHD 2000 syringe pump</td>
<td>Harvard Apparatus, Holliston, MA</td>
<td>Sample/dopant pumping</td>
<td>I, III, IV, V</td>
</tr>
<tr>
<td>Rotation stage</td>
<td>Newport Corp., Irvine, CA</td>
<td>DESI sprayer positioning</td>
<td>VI</td>
</tr>
<tr>
<td>Syringe pump</td>
<td>Cole Palmer, Vernon Hills, IL</td>
<td>Solvent pumping</td>
<td>VI</td>
</tr>
<tr>
<td>Whatman 75-72 nitrogen generator</td>
<td>Whatman Inc., Haverhill, MA</td>
<td>Curtain, nebulizer/auxiliary, and collision gas</td>
<td>I, III, IV, V</td>
</tr>
</tbody>
</table>
3.3 Microchip fabrication

The fabrication processes for the three chip types used in this work are presented in the following subsections. The processes are depicted with cross-section views of a chip in various stages of fabrication. The processes themselves are not visualized but rather the differences created by each processing step. Several minor changes were made in the fabrication processes in the course of the work, and similar chips were fabricated with slightly different processes. For example, both lift-off patterning and lithography and etching were applied in heater fabrication, with the same final result.

3.3.1 Silicon–glass heated nebulizer microchips

The fabrication process for the silicon–glass heated nebulizer microchips is presented in Figure 6. Fabrication started with a 380-nm-thick double-sided polished silicon wafer. The wafer was cleaned in ammonia/peroxide mix, with a HF dip, and in hydrochloric acid/peroxide mix with ultra pure water rinse cycles in between. A 1-μm-thick thermal oxide, which acted as a mask for through-wafer etching, was grown in a furnace (1). Double-sided lithography was then performed (2), and the oxide was etched with buffered hydrofluoric acid (BHF) to create the hard mask patterns (3). Silicon was etched with DRIE first from the channel side (4) and, after turning of the wafer, from the through-hole side (5). After mask removal (6) a blank 500-μm-thick glass wafer was bonded with anodic bonding (7). A 200-nm-thick platinum thin film with a chromium adhesion layer of 17 nm was sputtered on the glass side of the wafer stack (8), and heater structures were patterned by lithography (9) followed by platinum etching in diluted aqua regia (3:1:2 HCl/HNO₃/H₂O, 70 °C) (10). Finally the resist was removed (11) and the wafer stack was diced into individual chips (12).

![Figure 6. Fabrication of the silicon–glass heated nebulizer microchip.](image-url)
After the microfabrication, methyl-deactivated sample transfer capillaries of 150/220 μm (i.d./o.d.) and Nanoport connectors were glued manually with high-temperature-resistant epoxy. The capillary was inserted from the rear edge of the chip into the vaporizer channel and glued in place.

### 3.3.2 All-glass heated nebulizer microchips

The all-glass nebulizer chips were fabricated of two glass wafers bonded together. The fabrication process for the all-glass nebulizer chips is illustrated in Figure 7. First, a 400-nm silicon hard mask was deposited on a 500-μm-thick borosilicate glass (Pyrex) wafer by low pressure chemical vapor deposition (1). The silicon masking layer was patterned using double-sided lithography (2) and an isotropic silicon wet etchant (44:1:18 HNO₃–NH₄F–H₂O) (3). After resist removal (4), the glass was etched in 10:1 HF–HCl solution simultaneously from both sides (5). When the etch had proceeded through the wafer the remaining silicon mask was removed in 25% tetramethylammonium hydroxide solution at 80 °C (6). An ammonia/peroxide cleaning step was done before fusion bonding to another glass wafer in a furnace at 650 °C (7). Lithography onto the blank side of the wafer stack was performed (8) before a 17-nm chromium adhesion layer and a 200-nm platinum film were sputtered (9). The platinum heaters were patterned using lift-off patterning (10). Chips were diced with a wafer saw (11).

**Figure 7.** Fabrication of the all-glass heated nebulizer microchip.

As with the silicon–glass chips, sample transfer capillaries of 150/220 μm size (i.d./o.d.) were glued manually with high-temperature-resistant epoxy. In the older versions of all-glass chips, Nanoports were glued on as well, but in but the latest version only the capillary was glued.
3.3.3 Integrated liquid chromatography – heated nebulizer microchips

The layout of the integrated liquid chromatography – heated nebulizer (LC–HN) microchip is presented in Figure 8. The fabrication process, presented in Figure 9, is based on that for the silicon–glass heated nebulizer microchip. First, a 380-μm-thick double-sided polished silicon wafer was cleaned in ammonia/peroxide mix, with a HF dip, and in hydrochloric acid/peroxide mix with ultra pure water rinse cycles in between. Thermal oxidation at 1100 °C was used to grow a 1.5-μm-thick silicon dioxide hard mask (1). Double-sided lithography (2) was followed by etching of the silicon dioxide by reactive ion etching (3) and a dip in BHF. After preparation of the hard mask, DRIE of silicon was applied to make the fluidic channels, micropillar frit, and nozzle structures on one side (4) and nozzle outer walls and through-holes for the LC column and nebulizer gas inlets on the other side (5). The remaining hard mask was removed in BHF (6), and this was followed by ammonia/peroxide cleaning and anodic bonding with a blank 500-μm-thick glass wafer (7). A 200-nm-thick platinum thin film with a chromium adhesion layer of 17 nm was sputtered on the glass side of the wafer stack (8), and heater structures were patterned by lithography (9) followed by platinum etching in diluted aqua regia (3:1:2 HCl:HNO₃:H₂O, 70 °C) (10). Finally after resist removal (11) the wafer was diced into individual chips (12).

The LC–HN chips did not require manually attached parts such as capillaries. The LC channel was packed with 2.5 μm XBridge™ C18 particles. The particle slurry was prepared in a container (an empty HPLC column) of 1.7 mL volume by mixing a few milligrams of particles with solvent (ethanol/acetonitrile 50/50, v/v); the slurry was then vortexed and sonicated. The container was connected to the LC column inlet of the chip with tubing and the parts were placed in an ultrasonic bath. Pure packing solvent was pumped though the container with a capillary LC pump to transfer the particles into the column channel. The progress of the packing was observed though the glass cover. The solvent flow rate was initially 10 μL/min but was reduced to 6 μL/min as the packing progressed.
Figure 8. Layout of the LC–heated nebulizer microchip. (a) A silicon chip with etched structures is bonded with (b) a glass cover with heater elements. (c) Chip cross section at the column section.

Figure 9. Fabrication of the integrated LC–HN microchip.

3.4 Experimental setups

3.4.1 Thermal and fluidic measurements

IR imaging with a ThermaCAM SC 3000 IR camera was used to study the surface temperatures of the HN microchips. Nitrogen was applied as auxiliary gas, but no liquid was infused because of practical limitations on the measurements; that is, the amount of equipment transportable to another laboratory was limited. Since neither platinum of the heater nor silicon has the high emissivity required for IR temperature measurement, the
bottom sides of the silicon–glass and all-glass chips and the top side of the silicon–glass chip were painted with heat tolerant paint to increase emissivity. This allowed recording of reliable IR images.

A miniature thermocouple (Figure 10) was used for gas temperature measurements. The tip of the thermocouple is about 5 mm long, made of a thin wire of 25 μm diameter, and shaped as a sharp ‘V’. Since the thermoelectric voltage depends only on the temperature of the tip, and not on that of the wires, the resolution and response time of the temperature measurement depend mostly on the size of the tip. Measurements of thermocouple response time were made with a numerical control (NC) machining platform and thermocouple amplifier. An HN chip producing the gas jet was fixed in place, and the thermocouple was attached to the NC platform. Temperature data was acquired at a sampling rate of 1000 Hz, and the speed of the platform was 32 mm/s.

![Miniature thermocouple made of 25 μm wire.](image)

The temperature distribution of the heated gas jet of the heated nebulizer microchips was measured with the thermocouple attached to a custom-built high-precision computer-controlled xyz-positioning system. The positioning system is based on a 3D linear stage equipped with stepper motors. The measurement was controlled by a NI CompactDAQ system with in-house developed software. Thermocouple data was recorded using NI 9211 thermocouple module, and the xyz stage was controlled with 9401 digital input/output and 9205 analog input modules. The thermocouple was scanned across the jet, and both temperature and coordinate data were recorded for further data plotting and analysis. Movement velocity, step length, and temperature stabilization conditions were optimized with respect to measurement time and accuracy. A schematic view of the measurement setup is in Figure 11. The thermocouple was placed parallel to the jet to minimize the impact area and thus the effect of the thermocouple on jet shape. The thermocouple body and the measurement assembly behind the thin tip were far enough apart not to distort the jet at the tip of the thermocouple. The origin of the xyz-coordinate system used in the results was at the center point of the nozzle of the heated nebulizer microchip, and the gas jet was propagating along the positive x-axis.
3.4.2 Chromatography and mass spectrometry

In most of the experiments, general laboratory hardware and custom-made parts for mechanical and electrical connections were used to connect the heated nebulizer microchips to gas and liquid chromatographs and mass spectrometers. The following paragraphs briefly describe the experimental setups and conditions in studies II-V.

Gas chromatography

The HP 5890A gas chromatograph (study IV) was equipped with a split/splitless injector and a column transfer line, which was originally a mass selective detector (MSD) transfer line. A 2 m × 0.25 mm i.d. precolumn of methyl-deactivated fused-silica tubing was connected with a quartz column connector to the analytical column (5% phenyl–95% dimethyl, 15 m × 0.25 mm i.d. × 0.25 μm film thickness). The GC column and the transfer capillary of the microchip were connected with a stainless-steel capillary butt connector and a polyimide ferrule. The microchip was the improved silicon–glass chip presented in Figure 4 (b). In addition to the MSD transfer line, an additional in-house made heated transfer line was used to heat the end of the GC column and the transfer capillary. A photograph of the GC-μAPPI-MS setup is in Figure 12.

The power source for the additional transfer line was a programmable dc power supply, and the temperature of the heater was held at about 310 °C. The temperature was monitored with a thermometer. The carrier gas was 99.9995% pure helium with 90 kPa column pressure and flow rate of 1.7 mL/min (at 100 °C oven temperature) corresponding to a linear velocity of 58 cm/s. The GC temperature increased from 65 °C (hold for 1 min) at 20 °C/min to 320 °C (hold for 3 min). The samples were injected manually with a 1 min splitless period, after which the inlet purge valve was opened. The temperature of the injector and the MSD transfer line was 330 °C.
Liquid chromatography

The liquid chromatograph used in studies IV and V was an 1100 series capillary liquid chromatography system. In study IV, a SymmetryShield RP18 column (100 mm × 0.3 mm i.d., particle size 3.5 μm) was used. The mobile phases consisted of water (A) and acetonitrile/water (90/10, v/v) (B) (in IV) and water/acetonitrile (95/5) (A) and acetonitrile (B) (in V). The flow rates of the eluent were 9 μL/min (IV) and 3 or 4 μL/min (V). Samples were injected by an autosampler equipped with an 8-μL loop. In study IV, the transfer capillary of the microchip was connected directly to the LC column with a NanoTight fitting. The chip was an all-glass HN chip (Figure 4 (c)). In study V, a custom-made holder, as described in the Results and Discussion section, was utilized with the integrated LC–HN microchip.

Laser induced fluorescence

Laser induced fluorescence (LIF) detection (publication V) was performed with an inverted fluorescence microscope. A 488-nm laser was used for excitation, and the fluorescence emission was detected with a photomultiplier tube and recorded with an analog–digital converter and a computer. A filter cube in the microscope was equipped with filters for excitation and emission wavelengths.
**Mass spectrometry and heated nebulizer microchips**

In study II, the mass spectrometer was a 9.4 T FT-ICR MS. An rf-excited krypton discharge VUV lamp was used for ionization with the HN microchip. The conventional APPI source used for comparison was equipped with the same VUV lamp. With both sources, carbon dioxide was used as nebulizer gas. The microchip was an improved all-glass HN chip (Figure 4 (c)).

In studies III-VI, ion source frames, instead of standard ion sources, were mounted in front of the mass spectrometers. Besides bypassing the magnetic interlocks of the mass spectrometers, the frames provided a mechanical base for xyz manipulators and other parts. With the frames, the microchip ion sources could be installed to and removed from the mass spectrometers as one piece.

A PE Sciex API 300 triple quadrupole was used in study III. A Whatman 75-72 nitrogen generator produced the nitrogen used as curtain gas, and bottled nitrogen and synthetic air were used as nebulizer gas in microchip SSI. The microchip was the improved silicon–glass HN chip presented in Figure 4 (b).

The mass spectrometer used in studies IV and V was a PE Sciex API 3000 triple quadrupole. A Whatman 75-72 nitrogen generator produced the nitrogen used as curtain and auxiliary gas. A krypton dc discharge VUV lamp was used for photoionization and situated perpendicular to the microchips. The lamp was installed in a Vespel holder and powered with an APPI power source. The power source connected the high-voltage output of the MS, which normally supplies the APCI or ionspray voltage, to a repeller electrode in front of the VUV lamp in the lamp holder. The repeller voltage was needed to counteract the 1 kV positive voltage at the curtain plate of the mass spectrometer. Both silicon–glass and all-glass chips (Figure 4 (b) and (c)) were used.

In study VI, the mass spectrometer was a Bruker Esquire 3000 Plus ion trap. Nitrogen generated from liquid nitrogen was used as drying gas and auxiliary gas for the chip. The same VUV as in studies IV and V was used for photoionization. The experimental setup of DAPPI is described in the Results and Discussion section.

A mass flow controller was used to control the nebulizer/auxiliary gas flow into the microchip in all experiments except those of study II. For those, a manual rotameter was used. In all cases, syringe pumps were employed for sample and dopant delivery to the microchips. The microchips were heated with various dc power supplies.
4 RESULTS AND DISCUSSION

At the outset of this work, the newest version of the heated nebulizer microchip was a silicon–glass chip with a wet-etched silicon part, one Nanoport connector, and a platinum heater. The chip was a considerable improvement over the first version (Figure 4 (a)), which relied on three Nanoports and had an aluminum heater. Unfortunately, wet etching of silicon produces inclined channel sidewalls, and the shape and dimensions of the nozzle and fluidic inlet holes can never be ideal. Deep reactive ion etching (DRIE) of silicon offers precise dimension control and vertical sidewalls, and DRIE was used for silicon etching in the next silicon–glass chip versions. The chip shown in Figure 4 (b) is one of the models based on DRIE.

In parallel with the silicon–glass chip, a new all-glass chip was developed. Glass-to-glass anodic bonding with a silicon intermediate layer was applied in an initial version, but glass-to-glass fusion bonding proved superior in bond strength and was used in all subsequent chips. Like the silicon–glass chips, the all-glass chips rely on Nanoport connectors for gas introduction. Several versions of both basic types, with minor differences, have been manufactured.

The silicon–glass or all-glass HN microchip was chosen according to the requirements of the application; for example, silicon–glass chips were used for GC-APPI applications because of their high thermal conductivity and all-glass chips for LC-APPI and direct infusion applications because of their better durability. The all-glass chips were later modified for use with special chip holders in order to avoid the use of a Nanoport and to reduce the chip size and amount of manual (gluing) work required. The most recent improvements allow all-glass chips to be used in all applications. Several changes in the chip design and fabrication process, the improved knowledge of chip characteristics, and experience gained in actual use have led to significant improvements over the five years of research. Ease of use, range of applications, chip durability, and analytical performance have all improved. Modifications are continuing, however, and the results reported below should be seen as those of an ongoing project.

4.1 Thermal and fluidic properties of heated nebulizer microchips

Knowledge of the thermal and fluidic behavior of HN microchips is essential for their further development. The temperature and its distribution over the chip surface affect chip performance and durability. Since conventional thermometers can not be used with such miniature objects, IR imaging was chosen as the method of investigation. The fluidic properties of the chips, particularly of the produced gas jets, affect the ionization and ion transfer processes, and a new method based on a miniature thermocouple was developed for measurement of thermal jet shape. (I)
4.1.1 Performance of the thermocouple sensor

The sensor for measuring temperature distribution of miniature gas jets was a miniature thermocouple, which thanks to its small size has inherently good spatial resolution. The temporal resolution, i.e., response time, is a key factor since it affects measurement time. Response time varies directly with sensor size, but it is difficult to calculate and was thus measured in the conditions in which the thermocouple was finally used.

The thermocouple response curve is shown in Figure 13. First the thermocouple was kept in room temperature air; then it was quickly inserted into the hot gas jet from an all-glass HN nebulizer chip and similarly quickly removed from the jet. The heating and cooling times of the thermocouple were calculated from the temperature signal. The response times for rising and falling to 63% of the change in temperature were 28 ms and 60 ms, respectively. The inset of Figure 13 shows the rise and fall of the signal in detail. The rise time is shorter than the fall time because the hot gas jet leads to fast convective heat transfer to the thermocouple, whereas in the still air outside the jet the cooling is passive. Overall, the response times are insignificant relative to the time of about 0.5 seconds required to measure one data point.

![Figure 13. Response curve of the miniature thermocouple. The thermocouple is inserted into the hot gas jet at 300 ms and removed at 6800 ms.](image)

4.1.2 Thermal and fluidic characterization of heated nebulizer microchips

The temperature of the heated nebulizer microchip surface was characterized by IR imaging. Thermal images were of high resolution and accuracy, showing detailed temperature distribution on the chips. Figure 14 presents IR images of silicon–glass and all-glass HN microchips (the versions shown in Figure 4 (b) and (c)) from top and bottom sides with a heating power of 3.0 W and nitrogen flow of 150 mL/min for both chips. Note that the temperature scales in the images are different. On the pictures taken from top side,
the Nanoport connectors distort the image on the right side of the chips. This does not, however, affect the temperature reading on the heated left side of the chips.

The temperature on the silicon–glass chip is about 200–230 °C on the silicon side (top) and about 170–200 °C on the glass side (bottom). Except for the platinum heater, which has a maximum temperature of about 400 °C, the temperature is more or less uniform on the two sides. This is due to the high thermal conductivity of silicon, which spreads the heat over the chip. The full-glass chip is very different. On both sides the temperature distribution is highly uneven, due to the low thermal conductivity of glass. The heater is about 70 °C hotter than that of the silicon–glass chip, and the highest temperature on the top side is 100 °C higher. The cooling effect of the vaporizer channel can also be seen on the upside where a small dip in temperature is visible at the centerline of the chip.

Figure 14. IR images of silicon glass and all-glass heated nebulizer microchips from top and bottom sides.

The heating power ranges of these types of silicon–glass and all-glass chips are 0–3 W and 0–4.5 W, respectively. The epoxy glue used to attach the Nanoport connector limits the practical heating power of the silicon–glass chip to below 3 W in long-term operation since at higher heating power the Nanoport may detach from the chip. Detachment of the Nanoport is not a problem with the all-glass chip, because the Nanoport area remains at a lower temperature than in silicon–glass chips. The durability of the platinum heater is an issue in both chip types. The higher the heating power, the shorter is the lifetime of the heater. The upper limit of 4.5 W for the all-glass chip is a chosen limit for long-term use, but higher heating power can be used for short-term operation. In practice, a chip can usually be used for one to a few weeks.

Their small size means that the HN microchips heat up and cool down rapidly. The thermal response, i.e., the heating up and cooling down times, of the all-glass heated
nebulizer microchip was measured and the response curve is shown in Figure 15. The response was determined by connecting the chip instantaneously to a pre-set voltage corresponding to a heating power of 3 W. Once the temperature had stabilized, the power was turned off. The thermocouple was fixed in the center of the gas jet at the chip nozzle. The flow rate of the nebulizer gas was 100 mL/min, and no liquid flow was used since at low temperatures unvaporized liquid droplets exiting from the chip would hit the thermocouple and destabilize the temperature signal. The rise and fall times to a practically stable level of 95% of change in the temperature were 36 s for heating up and 78 s for cooling down. Analogous to the thermocouple response times, the temperature rise time of the chip is shorter than the fall time since heating of the chip is active with electric power fed to the chip, but cooling takes place through passive heat loss. With respect to practical applications in analytical chemistry, the rise and fall times are small enough for the temperature to be varied within an LC run in all applications but very fast LC separations. Thus, the temperature could be optimized separately for each analyte or group of analytes. Heat up and cool down times could be accelerated by applying higher heating power during the heat up phase and then active cooling methods.

**Figure 15.** Response curve of the all-glass HN chip. Heating power is turned on at 10 s and off at 155 s. Dotted vertical lines mark the start and end times for response time determination.

Next the effects of heating power and gas and liquid flow rates on the temperature of the HN chip gas jet were studied. The thermocouple was fixed in the center of the gas jet at a distance of 5 mm from the nozzle. Each data point was an average of three measurements, each a time average of temperature over a period of 30 s. Averaging was done to compensate rapid fluctuations in the temperature due mainly to movements of the surrounding room air. These fluctuations may be up to 30 °C from peak to peak within a few seconds. The measurements of temperature versus heating power (Figure 16 (a)) and temperature versus gas flow rate (Figure 16 (b)) were done without liquid. The gas flow rate was 100 mL/min for Figure 16 (a) and (c) and the heating power was 3.0 W for (b) and (c).
As seen in Figure 16 (a), the temperature depends almost linearly on the heating power. The deviation from linear response is due to nonlinearity of the different heat loss mechanisms: conduction, convection, and radiation. The expansion of gas when heated may also play a role. Moreover, the channel is not long enough for the temperature of the gas flow to fully reach the chip temperature. This partial warming-up effect is amplified with increasing temperature since hotter gas is less dense and has a higher linear speed, which reduces its residence time in the chip.

In Figure 16 (b), which shows the effect of gas flow rate on the temperature, the temperature rises rapidly, reaches a peak value at a gas flow of approximately 120 mL/min, and falls slowly when the gas flow increases further. The jet produced by the chip is less confined and the range of the jet smaller with low gas flow rates than with medium flow rates causing the heat to be spread out even at 5 mm from the nozzle. At high flow rates, the gas exits the chip before it has reached thermal equilibrium with the channel walls. In terms of jet temperature, the chip works optimally with gas flow rates from 80 to 160 mL/min, which is also the typical operating range.

Figure 16 (c) shows the effect of infusing a water/methanol mixture into the chip with flow rates 0–15 μL/min. The decrease in temperature is only slight because the amount of energy needed to vaporize and heat up the liquid is small compared with the total energy input to the chip. For example, when the heating power is 3.0 W, the whole gas jet is at an average temperature of 250 °C. At this temperature, the flow rates and heat capacities of the gas and liquid prove that about 0.5 W of power goes to heating the nitrogen (100 mL/min) and 0.3 W to heating and vaporizing the liquid (10 μL/min) and heating the vapor. The rest of the heating power is dissipated from the chip outer surface into the surroundings.

![Figure 16](image_url)

**Figure 16.** Temperature of the HN nebulizer chip jet as a function of different parameters: (a) heating power at a gas flow of 100 mL/min, (b) gas flow rate at a heating power of 3.0 W, and (c) infusion of water/methanol (50/50) with 100 mL/min gas flow at 3.0 W heating power.

Measurements of thermal jet shape were made with the all-glass chip and the silicon–glass chip. The results with the silicon–glass chip are discussed here. In all measurements, the heating power was 3.0 W, gas flow rate was 100 mL/min, and liquid flow rate was 10 μL/min. The scanning resolution was set to 75 μm along the y- and z-axes and to 200 μm along the x-axis to reduce the number of data points. In longitudinal scans, the
thermocouple was reciprocated along the y- or z-axis and in transversal scans along the y-axis.

The measurement time for a single data point was about 0.5 s on average, but it varied with the temperature region because the measurement program has a stabilization algorithm that allows the user to adjust stabilization parameters. There is a trade-off between the measurement time and accuracy: longer temperature signal averaging would allow more accurate results but would increase the overall measurement time. Too fast measurements result in a hysteresis effect, as is visible in the jet in Figure 17 (a) at 2–5 mm on the x-axis. This minor hysteresis is due to the extremely high temperature gradient (up to 450 °C/mm) within a few hundred micrometers and could be reduced by further optimizing the stabilization tolerance parameters. However, the shift in temperature between different measurement directions is merely one data point. The noise seen in the images is due to the rapid fluctuations in the surrounding gas temperature discussed earlier.

The jet shape of the silicon–glass HN chip can be seen in Figure 17, the jet shape of the all-glass chip in Figure 7 of publication I. The jet produced by the silicon–glass chip is very narrow in the xy-plane but wider in the xz-plane. At 1 mm the jet is round and very small, only about 0.6 mm wide. At 5 mm it is an upright oval and at 10 mm it is round again. The change in shape along the x-axis is explained by the chip design. The gas channel is of constant depth, but the width narrows from 800 μm to 400 μm just before the nozzle. This channel shape sets the output gas flow in a motion that gives rise to the non-round shape seen in Figure 17 (d) and to a shortened range of the jet. The successful characterization of these miniature gas jets clearly demonstrates the high resolution of the measurement method.

The maximum gas jet temperature of the all-glass chips is about 80 °C higher than that of the silicon–glass chip owing to differences in the thermal conductivity of the chip materials and the shorter channel of the silicon–glass chip. Glass is thermally insulating, causing the heat to be focused on the heater and the channel under it. By contrast, in the silicon–glass chip, the highly thermally conductive silicon acts as a thermalizing block and heat spreads out. In the all-glass chip, the longer residence time in the longer channel allows the gas to heat up closer to the chip temperature. Thus, with the same heating power, the all-glass chip heats the gas to a higher temperature.
4.2 Direct infusion studies

The high temperature of the HN microchips described above can be utilized for vaporizing compounds of high boiling point. In this work, the HN microchips were used in APPI mode and combined with high resolution Fourier transform ion cyclotron resonance mass spectrometry for the analysis of crude oil. (II) High temperature in conventional APCI and APPI devices limits their use to stable molecules of relatively low MW, and large biomolecules can not be ionized. The HN microchip is also shown to perform without heating in SSI mode, and its potential for the analysis of labile biomolecules is demonstrated. (III)
4.2.1 Microchip atmospheric pressure photoionization – Fourier transform ion cyclotron resonance mass spectrometry

One of the major advantages of the HN microchips is their versatility: the chips can be used in various ionization modes and with different mass spectrometric techniques. Conventional ion sources are typically limited to use with a few MS models from the same manufacturer and provide only one ionization mode. Here, an all-glass version of the HN microchip (Figure 4 (c)) was applied for APPI in combination with high resolution FT-ICR mass spectrometry in the analysis of crude oil.

Crude oil is an extremely complex mixture containing hydrocarbons with multiple aromatic rings, which may also contain heteroatoms such as nitrogen, sulfur, and oxygen. Knowledge of oil composition is important because variations in crude oil composition complicate oil refinery processes and sulfur-containing and many other compounds are harmful to the environment on combustion. Boiling point increases with the number of heteroatoms, double-bond equivalent (DBE, number of rings plus double bonds to carbon), and number of carbon atoms, and a large proportion of crude oil components have high boiling point. Their vaporization and analysis by MS is challenging as a result.

Naphtho[2,3-\textit{a}]pyrene (N[\textit{a}]P) was used to optimize the μAPPI parameters. The optimal distance between the nozzle of the HN microchip and the heated metal capillary inlet of the mass spectrometer was about 4 mm. The photoionization lamp was positioned close (3 mm) to the sample vapor jet to ensure maximum intensity of the photons at the surface of the jet. The optimal nebulizer gas (CO₂) flow rate was 115 mL/min. Nitrogen has been used in other applications of HN microchips, but CO₂ worked equally well. The toluene used as solvent also acted as dopant in APPI, and additional dopant was not needed. The signal stability of μAPPI was good with solvent flow rates at and above 1 μL/min, but it deteriorated at a flow rate of 0.5 μL/min, perhaps because the number of reagent ions (i.e., radical cations of toluene) was insufficient for efficient ionization at lower flow rates. The response increased with sample flow rate from 0.5 to 4 μL/min, showing that μAPPI is mass flow sensitive.

The performance of μAPPI FT-ICR MS was evaluated by infusing 40, 400, and 4000 nM N[\textit{a}]P standard solutions at a flow rate of 1 μL/min. With the same 10 s accumulation of ions in the first octopole of the MS, a factor of 10 increase in concentration (40 to 400 nM) resulted in a factor of 10 increase in signal. An even more concentrated sample (4000 nM) accumulated for 1 s, i.e., just one-tenth as long as for the 400 nM sample, gave a noisier but similar signal response to the 400 nM sample. Repeatability for different ion accumulation periods was studied for the 400 nM N[\textit{a}]P standard infused at a flow rate of 1 μL/min. The relative standard deviations (RSD) with accumulation periods of 1, 2, 4, and 10 s were 13%, 10%, 10%, and 5%, respectively, indicating good signal repeatability. The repeatability improved with longer accumulation periods, probably because fast signal variations were filtered out from the output signal. Moreover, the signal response scaled linearly with the ion accumulation period. Overall, these results showed that flow rates of 1–2 μL/min and signal accumulation periods between 2 and 10 s are optimal for μAPPI FT-ICR MS analysis of model compounds.
The stability of μAPPI for crude oil analysis was tested by infusing heavy crude oil dissolved in toluene (500 μg/mL) at a flow rate of 2 μL/min continuously for 4 h before data was collected. The total ion current over the 1 h period of data collection (Figure 3 in publication II) showed good stability indicating that the chip is suitable for crude oil analysis. The low flow rates of 1–2 μL/min provided by μAPPI mean that contamination of the ion source is significantly less than at the higher flow rates (50-200 μL/min) used in conventional APPI — an especially important advantage in the analysis of heavy petroleum samples with large fraction of high MW nonvolatile components.

Full-scan spectra of crude oil recorded with μAPPI and with conventional APPI are compared in Figure 18. Since all observed ions were singly charged, mass spectral peak positions are reported here in Da rather than as m/z. The flow rate of the crude oil sample (500 μg/mL crude oil in toluene) was 2 μL/min with μAPPI and 50 μL/min with conventional APPI. The temperature of the conventional APPI source was set to 350 °C (maximum allowable temperature for the source components, i.e., fused-silica coating and gas connections), while a heating power of 3.0 W, corresponding to roughly 300 °C in the vaporizer, was used for μAPPI. In both experiments the ion accumulation period was 3 s, and 100 acquisitions were summed for each spectrum. Although the flow rate with μAPPI was only 1/25 of that of conventional APPI, signal response was 60% of that for conventional APPI. This translates into a significant increase of about 15-fold in mass flow sensitivity for μAPPI.

The high mass resolution and accuracy allow the elemental composition of the peaks in the FT-ICR mass spectra to be calculated. With thousands of spectral peaks, this is highly beneficial for data analysis since compounds can then easily be classified into groups according to their properties (number of heteroatoms and DBE, for example). Elemental composition assignments were accomplished by converting the mass spectral data from the IUPAC mass scale to the Kendrick mass scale.147,148 Figure 19 shows the heteroatom class distributions derived from the spectra in Figure 18. The same classes were detected by conventional APPI and μAPPI, and the relative abundances for the various classes were
roughly comparable for the two ion sources. In other words, no significant information is lost at the much lower flow rate for μAPPI.

![Graph showing relative abundance of heteroatom class distributions](image)

**Figure 19.** Heteroatom class distributions derived from the mass spectra of Figure 18.

Figure 20 shows color-coded isoabundance-contoured plots of DBE versus carbon number for $S_1$ and $O_1S_1$ classes as measured by μAPPI and conventional APPI FT-ICR MS. The $S_1$ class plots are closely similar. However, the $O_1S_1$ class plots show somewhat higher DBE species with μAPPI, possibly because μAPPI at 3 W is at a higher temperature than the heated nebulizer of the conventional source, and therefore higher DBE species are vaporized and ionized more efficiently. The higher μAPPI source temperature may also explain the higher relative abundances of compounds including oxygen ($O_1S_1$, $O_1S_2$, $O_2S_1$, and $O_1$ classes) with μAPPI (Figure 19). Overall, Figure 20 shows that the performance of μAPPI in terms of DBE and carbon number distribution is closely similar to that of conventional APPI.

As Figure 18 demonstrates, μAPPI at a heating power of 3 W produces the same kind of mass spectral profile of crude oil as the conventional APPI source at 350 °C. However, the heating power of the chip can easily be adjusted between 0 and 4 W, and when the heating power is increased from 3 to 4 W, clearly more components are detected at the high-mass end of the mass spectrum (Figure 21), indicating the high vaporizing efficiency of μAPPI. The increased amount of residue that accumulated inside the microchip nozzle and vaporizer channels at higher heating powers could be removed by flushing toluene through the chip. The residue buildup mechanism may be coke formation due to the high temperature in the vaporizer channel.
Figure 20. Isoabundance-contoured plots of double-bond equivalents vs. carbon number for \( S_1 \) and \( O_1S_1 \) class ions in FT-ICR mass spectra produced by \( \mu \)APPI and conventional APPI of crude oil. The highest abundance species are colored red, with lower abundance species of different color as indicated.

Figure 21. FT-ICR mass spectra for high-mass ends of heavy crude oil ionized by \( \mu \)APPI at 3.0, 3.5, and 4.0 W of heating power. Crude oil concentration was 500 \( \mu \)g/mL and sample flow rate 2 \( \mu \)L/min.
4.2.2 Microchip sonic spray ionization

Microchip sonic spray experiments were conducted with an improved version of the silicon–glass HN chip (Figure 4 (b)). Velocity of the nebulizer significantly effects the ionization in SSI, and the best ionization efficiency is achieved at sonic speed. In this study, the velocity was controlled by adjusting the inlet pressure of the gas (N₂). The maximum signal for the M⁺ ion of tetra-N-butylammonium (10 μM, flow rate 10 μL/min) at m/z 242 was reached at 10 bar. Increasing the pressure up to 30 bar had only a minor effect on the ionization efficiency. A similar trend has been reported for an SSI interface designed for low liquid flow rates. On the basis of these findings and the fact that ionization efficiency is best at sonic speed, it is estimated that, with microchip SSI, sonic speed was reached at a pressure of 10 bar. Nitrogen and synthetic air were tested as nebulizer gas, with the same result, but since synthetic air was the more economical alternative, it was used in the rest of the experiments. In the original publication, it was reported that 10 bar corresponds to a flow rate of 1.1 L/min, but this was an estimate only. Later experiments showed that 10 bar actually corresponds to approximately 6 L/min.

A correlation between the sample flow rate and the analyte signal would help to determine the application range of the method and to characterize the ionization mechanism. To test such a correlation, 10 μM tetra-N-butylammonium was infused with flow rates ranging from 0.5 to 10 μL/min, while the pressure of the nebulizer gas was kept constant at 10 bar. Within the tested range, the M⁺ signal of tetra-N-butylammonium increased linearly (r² = 0.9995) with the flow rate. In other words, microchip SSI is a mass-flow-dependent ionization method.

Tetra-N-butylammonium was also used to test linearity, repeatability, and stability. Linearity was examined for sample concentrations from 50 nM (limit of quantification, signal-to-noise (S/N) = 10) to 5 μM. Increasing concentration caused a linear increase of the signal within 50–500 nM (r² = 0.9999), and thereafter a nonlinear increase. It is possible, however, that linearity in SSI-MS depends on the particular ionized compound and solvent composition, for linear ranges from one to four orders of magnitude have been reported. The quantitative repeatability was tested with a 50 nM solution of tetra-N-butylammonium (S/N = 10) infused at 10 μL/min. With six measurements of the peak height of the M⁺ ion, the relative standard deviation (RSD) was 16.3%. Taking into account the low concentration used, the microchip SSI can be considered a stable ion source. The long term stability of microchip SSI-MS was tested by infusing 500 nM solution at a flow rate of 5 μL/min for 2 hours. A stable ion current was produced throughout the measurement.

Finally, the performance of microchip SSI was demonstrated by recording the mass spectra (Figure 22) of five compounds of different polarity and size: verapamil, tetra-N-butylammonium, testosterone, and Angiotensin I in positive ion mode and ibuprofen in negative ion mode. A flow rate of 5 μL/min was used in positive ion mode and 10 μL/min in negative ion mode. Tetra-N-butylammonium produced the M⁺ ion at m/z 242 as the most abundant ion, whereas verapamil and testosterone produced [M + H]⁺ ions at m/z 455 and 289, respectively. Testosterone, which has lower proton affinity than verapamil,
also produced intense \([M + Na]^+\) and \([M + Na + H_2O]^+\) adducts. The spectrum of angiotensin I, an oligopeptide, showed singly, doubly, and triply protonated molecules at \(m/z\) 1297, 649, and 433, respectively. In negative mode, ibuprofen produced a deprotonated molecule at \(m/z\) 205 and a deprotonated dimer at \(m/z\) 411. The appearance of adducts and dimers and the lack of fragment in the spectra confirm that microchip SSI is a soft ionization method.

Limits of detection (LOD) for the test compounds were determined by analyzing samples at different concentrations at a flow rate of 10 \(\mu\)L/min. The concentration resulting in S/N = 3 of the most abundant peak of the analyte was chosen as the LOD. The LODs of tetra-\(N\)-butylammonium, verapamil, ibuprofen, testosterone, and Angiotensin I were 15 nM, 100 mM, 1 \(\mu\)M, 1.7 \(\mu\)M, and 4 \(\mu\)M, respectively. In all, the results demonstrate successful operation of the first SSI microchip source. Both small molecules and a peptide were ionized successfully, showing that HN microchips can be applied for both small molecules and biomolecules not ionizable with APCI or APPI.

**Figure 22.** The SSI mass spectra of 1 \(\mu\)M verapamil (a), 500 nM tetra-\(N\)-butylammonium (b), 100 \(\mu\)M testosterone (c), 100 \(\mu\)M angiotensin I (d), and 100 \(\mu\)M ibuprofen (e). The sample solution flow rates were 5 \(\mu\)L/min of water/methanol (50/50) with 0.1% of formic acid in positive ion mode (a-d) and 10 \(\mu\)L/min of water/methanol (50/50) with 0.1% of ammonium hydroxide in negative ion mode (e). Ten spectra were accumulated.

### 4.3 Connecting heated nebulizer microchips with chromatography

Besides the easy connectivity to different mass spectrometers and choice of ionization mode, the HN microchips provide easy connection of API mass spectrometers to chromatographs. The same microchips can be used for connecting a capillary liquid chromatograph or a gas chromatograph to MS — something not possible with
conventional ion sources. Here the HN microchips were used with APPI for GC analysis of PAHs and capLC analysis of steroids. (IV)

4.3.1 Gas chromatography – microchip atmospheric pressure photoionization – mass spectrometry

The HN microchip used for connecting to GC was an improved version of the silicon–glass chip (Figure 4 (b)). The high thermal conductivity of silicon, and thus high overall temperature of the chip, is beneficial for connecting to GC since cold spots must be avoided in the connection. First the auxiliary gas (N\textsubscript{2}) flow rate, dopant (toluene) flow rate, and heating power of the chip were optimized as these significantly affect the signal intensity and stability. The effects of flow rates of dopant (0–10 μL/min) and auxiliary gas (30–300 mL/min) on the sensitivity were studied with anthracene as a test compound. The maximal signal intensity was achieved with an auxiliary gas flow rate of about 90–120 mL/min, while lower or higher flow rates caused significant decreases in signal intensity. The use of dopant was necessary to improve the signal intensity. The flow rate of the dopant also had a significant effect on sensitivity: increasing the flow rate from 1 μL/min increased the signal until it reached a plateau at about 4 μL/min, above which there was no increase in intensity. Robb and Blades\textsuperscript{152} observed similar activity in a commercial APPI source.

The heating power of the chip in the GC experiments was set at 2.5 W, corresponding to a temperature of about 290 °C in the vaporizer channel. This temperature was high enough to ensure elution of the nonvolatile benzo[\textsubscript{a}]pyrene (B[\textsubscript{a}]P) (boiling point 496 °C). The mass spectra of the PAH standards acenaphthene, anthracene, and B[\textsubscript{a}]P showed intense radical cations and negligible fragmentation, indicating that the ionization mechanism was charge exchange through reactions (3) and (4) (see section 1.2.4).

Figure 23 shows selected reaction monitoring (SRM) chromatograms of the PAH standards measured with the GC-μAPPI-MS/MS setup. The injection volume was 1 μL, and concentrations of acenaphthene, anthracene, and B[\textsubscript{a}]P were 65, 56, and 40 nM, respectively. The chromatogram shows good performance with very narrow peaks of acenaphthene and anthracene, both of which have a peak width at half-height of 1.0–1.1 s. This is less than or equal to the typical peak width of PAHs obtained with conventional GC-MS methods.\textsuperscript{153} The main reason for the narrow peaks is that the internal volume of the vaporizer channel inside the chip is only about 2 mm\textsuperscript{3}, and it is flushed by the auxiliary gas in less than 2 ms. The peaks of acenaphthene and anthracene are also symmetric, with asymmetry factors (A\textsubscript{s}) of 0.98 and 0.89, indicating that no significant adsorption onto the vaporization channel of the chip occurs. However, the peak of B[\textsubscript{a}]P tails (A\textsubscript{s} = 2.95) and is broader than the first two peaks, just as in conventional GC-MS analysis.\textsuperscript{154,155} Even then, adsorption onto the microchip cannot be fully excluded since the temperature of the chip is non-uniform and some parts may be at a temperature lower than the elution temperature of B[\textsubscript{a}]P, which is 310 °C, thus causing adsorption.
Figure 23. SRM chromatograms of acenaphthene, anthracene, and B[a]P measured with GC-μAPPI-MS/MS. The amounts injected were 65, 56, and 40 fmol, respectively. SRM pairs were m/z 154/127 and 154/77 for acenaphthene, m/z 178/152 and 178/151 for anthracene, and m/z 252/250 for B[a]P.

The quantitative performance of the GC-μAPPI-MS/MS system using SRM was evaluated by determining the LOD, linearity, and repeatability for the PAH standards (Table 4). The concentration giving a S/N of at least 3 was chosen as the LOD for each compound. The LODs, ranging from 0.79 nM (B[a]P) to 13 nM (acenaphthene) with 0.79 to 13 fmol injected, were lower than or equal to those reported with conventional GC-MS/MS methods and electron ionization.\textsuperscript{156-158} In comparison with our previous GC-μAPCI-MS method,\textsuperscript{70} the new μAPPI method was over two orders of magnitude more sensitive. Although the analytes and the mass spectrometer were not the same, the new GC-μAPPI-MS method clearly performed better than the GC-μAPCI-MS method. The correlation coefficients ($r$) of the calibration curves within the concentration range examined (0.79-4000 nM) were above 0.995, indicating favorable linearity of the method (Table 4). The RSD of each analyte was below 15%, indicating acceptable repeatability. Since the injections were performed manually without the use of an internal standard, a significant proportion of the deviation was probably due to variation in the injections.

Table 4. Limits of detection, linear ranges, linearities, and repeatabilities of the compounds studied with GC-μAPPI-MS/MS.

<table>
<thead>
<tr>
<th></th>
<th>LOD (S/N = 3)</th>
<th>Linear range (nM) and linearity ($r$)</th>
<th>Repeatability (RSD, N=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>acenaphthene</td>
<td>13 nM, 13 fmol</td>
<td>32-3200, 0.9963</td>
<td>7.5% (130 nM)</td>
</tr>
<tr>
<td>anthracene</td>
<td>2.8 nM, 2.8 fmol</td>
<td>2.8-280, 0.9987</td>
<td>14% (110 nM)</td>
</tr>
<tr>
<td>benzo[a]pyrene</td>
<td>0.79 nM, 0.79 fmol</td>
<td>0.79-4000, 0.9952</td>
<td>11% (79 nM)</td>
</tr>
</tbody>
</table>

The feasibility of GC-μAPPI-MS/MS in qualitative analysis of polar compounds was tested with a set of amphetamine derivatives (methylenedioxyamphetamine (MDA), methylenedioxymethamphetamine (MDMA), and methylenedioxyethylamphetamine
(MDEA)) and diphenylamine. A mixture of dopant (toluene) and methanol (50/50, v/v) was added to the auxiliary gas at a flow rate of 8 μL/min to enhance the ionization process. The spectra of the amphetamine derivatives showed significant fragmentation, but abundant protonated molecules also formed in reactions (3), (5), and (6) with methanol as the solvent (S). The intensity of the protonated molecules increased and the degree of fragmentation decreased when the toluene/methanol mixture was used rather than toluene alone. When only toluene is used, the proton-transfer reaction occurs directly between a toluene radical cation and the analyte. The addition of methanol alters the reagent ion composition and, instead of toluene radical cations, the protonated methanol clusters act as proton donors. Since the proton affinity of methanol clusters is higher than that of a toluene radical cation,53 the exothermicity of the proton transfer reaction is higher, and the fragmentation is stronger without than with methanol. In general, the degree of fragmentation and selectivity in GC-μAPPI-MS can be controlled by adding suitable volatile reagents to the auxiliary gas in the same way as a reagent gas is chosen in conventional chemical ionization.

4.3.2 Capillary liquid chromatography – microchip atmospheric pressure photoionization – mass spectrometry

The HN microchip used for connection with capLC was an all-glass chip similar to the one in Figure 4 (c). As for the GC connection, the auxiliary gas flow rate and dopant flow rate were optimized in direct infusion experiments. Testosterone and progesterone were used as test compounds. The results for the two compounds were the same, with the optimal gas and liquid flow rates being 90-120 mL/min and 4 μL/min, respectively. The heating power of the chip substantially affected signal stability, and a stable vaporization process was achieved only at heating powers higher than 3 W. Below 3 W, the vaporization process was unstable and produced unstable LC-MS ion chromatograms. The instability is likely related to the boiling process inside the vaporizer channel of the chip. Vapor film boiling, in which the liquid is not in direct contact with the surface, is preferable to nucleate boiling, in which liquid boils on the surface.135 Owing to direct contact of the sample with the surface, nucleate boiling increases the chance for peak tailing in LC-MS through absorption of the analytes on the surface. Since heating powers that are too high may increase thermal dissociation of analytes, the optimal heating power was tested and found to be 4 W, corresponding to about 370 °C in the vaporizer channel of the chip.

The spectra of testosterone and progesterone showed intense protonated molecules and minor fragment ions. The ionization process is similar to that presented for the amphetamines. The proton-transfer reaction occurs via protonated eluent (acetonitrile) cluster molecules, which were observed as the background ions instead of the toluene radical cations. Figure 24 shows chromatograms measured in SRM mode using a 5 nM sample and a 3 μL injection volume.
Figure 24. SRM chromatograms of testosterone and progesterone measured with LC-μAPPI-MS/MS. The amount of compound injected was 15 fmol. SRM pairs were m/z 289/109 and 289/97 for testosterone and m/z 315/109 for progesterone. Water and acetonitrile/water (90/10) were used as eluents.

The chromatograms show good stability of the method with the all-glass chip since it, heated as high as 500 °C, provides efficient and stable vaporization of the eluent and the analytes. The peak widths at half-height of the testosterone and progesterone peaks are 4.2 and 7.1 s, respectively, which are typical for capLC separations. The asymmetry factors are acceptable: 1.39 for testosterone and 1.61 for progesterone. The chromatograms show some peak tailing, however, perhaps due to dead volume in the transfer capillary connection to the column. The microchip itself should not cause tailing, owing to the high temperature and small dead volume of the vaporizer channel of the chip. Peak shape is significantly improved as compared with that of the capLC-μAPCI-MS work, even though the present microchip was used without deactivation. The improvement is most likely due to the higher chip temperature allowed by the all-glass chip, better positioning of the transfer capillary inside the chip, and minimized dead volumes in the column–capillary connection.

Table 5 shows the quantitative performance of the capLC-μAPPI-MS/MS system using 1 μL injection volume. With our previous capLC-μAPCI-MS method, the LOD of testosterone was 10 nM (20 fmol injected). Here it is 1 nM, and 2 nM for progesterone. Considering that the mass spectrometer was different, the new capLC-μAPPI-MS method can be considered more or equally sensitive. The linearity of the method is good for both compounds, as shown by correlation coefficients (r) of about 0.999. The repeatabilities are below 10% at 50 nM concentration and below 20% at a concentration near the limit of quantification (about 5 nM). Overall, the results show good quantitative performance.
Table 5. Limits of detection, linear ranges, linearities, and repeatabilities of the compounds studied with capLC-μAPPI-MS/MS.

<table>
<thead>
<tr>
<th></th>
<th>LOD (S/N = 3)</th>
<th>Linear range (nM) and linearity (r)</th>
<th>Repeatability (RSD%, 10 nM, 1 μL, N = 6)</th>
<th>Repeatability (RSD%, 50 nM, 3 μL, N = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nM</td>
<td>fmol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>testosterone</td>
<td>1</td>
<td>1</td>
<td>1-500, 0.9989</td>
<td>11</td>
</tr>
<tr>
<td>progesterone</td>
<td>2</td>
<td>2</td>
<td>5-1000, 0.9992</td>
<td>16</td>
</tr>
</tbody>
</table>

4.4 Integration of chromatographic separation and heated nebulizer

This section presents the first microchip combining an LC column and a heated vaporizer. (V) Previous integrated LC-ionization microchips have relied on ESI and heating has not been exploited. Like the HN microchips, the integrated chip has the advantage of connectivity and can be operated in different ionization modes.

4.4.1 Microchip structure and operation

The integrated LC–HN microchip was used with custom-made holders for fluidic and electrical connections. The holder for fluidic connections was made of a plate of Macor and a piece of aluminum with a milled slot for the chip. A polyetheretherketone (PEEK) liquid inlet capillary of 1/32 inches o.d. and fluorinated ethylene propylene (FEP) gas tubing of 1/16 inches o.d. were connected with flat-bottom fittings to the liquid and gas inlets on the microchip, respectively. The electrical connection for heating the vaporizer section was accomplished with a separate holder, which also provided water cooling to the column section of the chip. The holder was made of Sintimid NT polymer with spring-loaded electrical connectors and aluminum. Cooling water (2.5 mL/min) was pumped through the aluminum block. The chip with the holders and connectors is shown in Figure 25.

The micropillar frit at the end of the column channel is manufactured as an integrated part of the chip, which means that, unlike one with a polymeric frit, the chip is fully ready for particle packing after microfabrication. The pillars on the mask are oval shaped (6μm × 9μm), and the gap between adjacent pillars is 3 μm. The structure of the micropillar frit after fabrication has an aspect ratio close to 50, which is difficult to achieve even with advanced DRIE etchers. A structure of this type requires a highly optimized anisotropic etch process that minimizes undercut. Even with a fine-tuned process, the pattern of the mask is not transferred to the silicon without alteration. The gap between the pillars on the mask is 3 μm, but the actual gap at the top of the pillars after etching is about 6 μm. Similarly, the width of the pillars at the top is reduced from the initial 6 μm to about 3 μm. This occurs because the etching process is not perfectly anisotropic and some etching also takes place in the lateral direction. Figure 26 shows a scanning electron microscope (SEM) image (a) of the frit and a photograph of a packed channel (b). Even though the particle
size is clearly smaller than the gaps between pillars, the frit holds the particles in place. Even the first row of pillars keeps the particles inside the column channel after numerous LC runs. This is due to the keystone effect, which causes particles considerably smaller than a hole to be retained. The pillars missing in the third and fourth row were likely damaged during the washing steps of the fabrication, but this has no effect on the frit performance.

Figure 25. Integrated LC–HN microchip in its holder with fluidic connections, and the water cooler block with electrical connections.

Figure 26. (a) SEM image of the micropillar frit before bonding of the glass cover. (b) End of the LC column in a packed microchip showing the micropillar frit holding 2.5 μm particles.

On the other side of the frit, the liquid channel continues at 50 μm wide for 5 mm, allowing optional optical detection; it then joins with the nebulizer gas channels. The sample liquid is vaporized and mixed with nebulizer gas and the vapor is sprayed out from the nozzle. The vaporizer channel can be heated up to 400 °C, providing efficient vaporization of nonvolatile analytes. The nozzle forms a confined jet similar to that of the HN microchip characterized earlier (Figure 17).

Heater elements are separate for the LC column and the vaporizer parts to allow independent temperature control. However, maintaining high temperature gradients is difficult, owing to the high thermal conductivity of silicon. Excess silicon was removed
around the vaporization channel to reduce heat conduction to the cooler LC column section, but heat transfer from the vaporizer section to the column section was nevertheless considerable. With no cooling applied, the temperature in the column section rose above 50 °C when heating power of the vaporizer section exceeded 1.5 W. At the same time, stable vaporization of the LC eluent required a minimum of 3 W heating power. Additional measures for thermal isolation between the column and vaporizer sections were therefore needed. A water-cooled aluminum block proved to be the best solution: it easily allowed 4 W heating power while maintaining the column section at about 25 °C. The block was positioned after the column section so that, even with cooling, the column could be heated with the separate platinum heater. (Column heating was not used here, however.)

**4.4.2. Liquid chromatography**

Performance of the microchip for liquid chromatography was tested with various samples, with LIF or APPI-MS used for detection. A gradient elution was designed to focus the analytes at the beginning of the column to avoid the effect of possible dead volumes in the connections before the column. Column capacity for high injection volumes, up to four times the column internal volume (1.2 μL), was tested by comparing 1 and 5 μL injections, and no peak fronting, increase in peak width, or shift in retention time was observed. The tested eluent flow rates of 3 and 4 μL/min correspond to linear velocities of 1.7 and 2.2 mm/s, which are close to the optimal velocity corresponding to minimal theoretical plate height. Although the back pressure was as high as 150 bar during the column packing and LC analysis, no leaks or other problems on the chip were observed. This indicates good mechanical stability, particularly of the micropillar frit and silicon-glass bonding and good pressure tolerance of the eluent connection. The extremely smooth silicon surface of the chip provides a tight connection with the flat-bottom connectors, sufficient to tolerate the high solvent back pressure. Also, the column stability was good after packing; no voids were visible anywhere in the column when it was viewed with a stereo microscope after packing or about 100 LC analyses.

Figure 27 shows a chromatogram of fluorescein and BODIPY 493/503 separated with the chip and detected by LIF. The fluorescence signal of BODIPY is approximately 10 times as high as that of fluorescein, mainly due to the high fluorescence quantum yield of BODIPY under the conditions of the LC analysis. Repeatability (RSD) of the microchip LC-LIF method with 0.1 μL injections of 1 μM samples was tested with six injections. The RSDs of peak areas were 0.6% and 1.0% and the RSDs of retention times 0.6% and 0.5% for fluorescein and BODIPY, respectively. RSDs of at most 1% are good for a chip-based system and show that, in terms of repeatability of signal response and retention time, the performance of the LC part of the chip with LIF detection is good. Plate numbers for the fluorescein and BODIPY peaks calculated from peak width at half-height are 5500 and 79000, respectively.
Figure 27. Chromatogram of fluorescein and BODIPY® 493/503 separated and detected by microchip LC-LIF. Concentrations were 1 μM and injection volume 0.1 μL.

The feasibility of the LC–HN chip with APPI-MS for the determination of nonpolar and polar compounds was demonstrated with PAHs and selective androgen receptor modulators (SARM). Figure 28 (a) shows chromatograms of three PAHs (anthracene, benz[a]anthracene (B[a]A), and B[a]P) and Figure 28 (b) presents chromatograms of three SARMs (2, 3, and 4) measured by microchip LC-APPI-MS/MS in SRM mode with toluene as a dopant. The retention and separation are acceptable. The nonpolar PAHs produced radical cations through charge exchange (reaction (4), and the more polar SARMs formed protonated molecules by proton transfer with protonated solvent (acetonitrile) clusters (6). The experiments show that nonpolar as well as polar compounds can be efficiently ionized with the integrated LC–HN chip.

Performance of the LC–HN microchip with MS was tested using APPI-MS/MS and PAHs. Table 6 shows the values for LOD (S/N = 3), repeatability, and linearity. Repeatability of seven injections was measured at 100 ng/mL concentration, and two injections were done at each concentration in the linearity measurements. Considering the observed chromatographic performance, for which improvement is anticipated, the LODs, ranging from 5 to 30 ng/mL, are at an acceptable level. The RSDs of peak areas and retention times are less than 4% and 0.5%, respectively, indicating good repeatability and stability of the chip. The linearity is good for all compounds with correlation coefficient $r \geq 0.9990$. 

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Figure 28. (a) SRM chromatograms of PAHs measured by microchip LC-APPI-MS/MS. Concentrations were 1 μg/mL and injection volume 1 μL. (b) SRM chromatograms of SARMs measured by microchip LC-APPI-MS/MS. Concentrations were 10 μg/mL and injection volume 0.5 μL.
### Table 6. Limits of detection, repeatabilities of peak areas and retention times, and linearity for PAHs analyzed by microchip LC-APPI-MS/MS.

<table>
<thead>
<tr>
<th>PAH</th>
<th>LOD (ng/mL), 1 μL inj.</th>
<th>Repeatability of peak area (RSD %)</th>
<th>Repeatability of retention time (RSD %)</th>
<th>Linearity (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthracene</td>
<td>5</td>
<td>3.4</td>
<td>0.3</td>
<td>0.9990</td>
</tr>
<tr>
<td>B[a]A</td>
<td>10</td>
<td>4.0</td>
<td>0.3</td>
<td>0.9992</td>
</tr>
<tr>
<td>B[a]P</td>
<td>30</td>
<td>2.6</td>
<td>0.5</td>
<td>0.9992</td>
</tr>
</tbody>
</table>

Several factors contribute to the shape of the LC peaks. The effect of precolumn dead volumes was overcome with the use of LC gradient profiles that concentrate analytes at the beginning of the column. The quality of the stationary phase depends on the selected particle type and size and on the density and uniformity of the packing. The rectangular channel cross-section may lead to issues with flow and packing uniformity, especially at the corners. Surface effects in the channels may also play a role in the chromatographic performance. Adsorption and desorption of analytes may take place on the glass and silicon surfaces in the LC column, optical detection channel, and the vaporizer. The efficiency and stability of the vaporization are of concern, too. Since peak tailing was also observed in LIF measurements in which the detection point is before the heated nebulizer section, tailing is probably mostly due to interactions between analytes and the walls of the separation channel or to improper packing at the corners of the rectangular column channel.

### 4.5 Desorption atmospheric pressure photoionization

The most widely used desorption/ionization technique is DESI, which works like ESI in terms of analyte polarity and cannot be used for nonpolar compounds. A new method for ambient MS analysis based on APPI was developed to overcome this deficiency. (VI) Desorption atmospheric photoionization (DAPPI) allows the analysis of both polar and nonpolar compounds, directly from surfaces, without sample pretreatment.

The ion trap MS used with DAPPI was equipped with an inlet extension, which extends the ion inlet for approximately 5 cm. The DAPPI setup consists of an all-glass HN microchip (Figure 4 (c)), a VUV photoionization lamp, and a sampling mount. A schematic view of the DAPPI system is shown in Figure 29. The sampling mount and MS inlet extension are in horizontal position, and the lamp is aligned perpendicular to them. The microchip nebulizer is at an angle of ~45° from horizontal. Positions of the microchip and the sampling mount in relation to the inlet of the mass spectrometer can be adjusted with two independent manual xyz-positioning stages. The entire apparatus is mounted on a frame, which is attached to the mass spectrometer instead of a standard ion source.
In initial experiments, the positions of the components in the DAPPI setup were adjusted to achieve maximum sensitivity and stability. The vapor jet exiting from the chip and the spot where the jet hits the surface (later referred to as the sampling spot) were positioned on-axis with the MS inlet. The distance between the chip nozzle and the sampling spot was not a crucial parameter since the vapor jet is highly confined, and the distance between the sampling spot and the MS inlet was not critical within the range of 2–8 mm. The distances used in further experiments were ~10 mm and ~3 mm, respectively. The photoionization lamp was positioned ~10 mm above the sampling spot. The exact radial distribution of the VUV light emitted by the lamp is unknown, but the internal structure of the lamp means that the distribution is inherently nonconfined. Thus, the VUV light illuminates not only the sampling spot but also the end of the incoming vapor jet and the analytes desorbed from the surface.

To verify the exact position of the sampling spot, a piece of polystyrene was placed on the sampling mount. The sampling spot became visible in a few seconds, when the heated vapor jet caused the polystyrene to become deformed. The deformed area was roughly 1.5 mm in diameter, which confirmed the localized nature of the surface heating. One benefit of using the nebulizer chip in DAPPI is the possibility for rapid adjustment of temperature, so that different temperatures can be applied for samples on the same sample plate without prolonging the analysis time.

The effect of the vapor jet temperature on the desorption/ionization of anthracene and testosterone was tested by varying the heating power of the chip in the range of 2–5 W corresponding to vapor jet temperatures of ~130–240 °C at a distance of 10 mm from the nozzle. The intensity of the molecular ions or protonated molecules of the analytes increased with the temperature. The higher the boiling point of the analyte, the higher was the temperature needed for efficient desorption. Figure 30 shows the effect of temperature on the ion chromatograms of anthracene and testosterone when spots of 50 pmol of anthracene and 10 pmol of testosterone were analyzed with vapor jet temperatures of
The signals for both compounds were more stable and intense with a temperature of 220 °C than of 130 °C, and the signals lasted longer with the lower temperature. In general, the analyte signal lasted from a few seconds to 20 s depending on the analyte and the temperature of the vapor jet. The lower the boiling point of the analyte, the narrower was its signal. This effect is clearly seen in Figure 30, where the peak of anthracene is significantly narrower than that of testosterone. A second analysis of a previously analyzed sample plate verified that the analytes had been completely desorbed from the surface.

Figure 30. Extracted ion chromatograms of (a) 50 pmol of anthracene and (b) 10 pmol of testosterone with DAPPI-MS at vapor jet temperatures of 220 and 130 °C. The extracted ion was m/z 178 for anthracene and m/z 289 for testosterone. The flow rate of the nebulizer gas was 180 mL/min and that of the solvent (toluene) 10 μL/min.

Study was made of the effects of nebulizer gas and spray solvent flow rates on the ionization. The flow rate of the nebulizer gas was varied from 50 to 300 mL/min while the intensity of the molecular ion of anthracene was monitored. The highest intensity was detected at a flow rate of ~180 mL/min; below and above that value the intensity was decreased. The optimal flow rate is somewhat higher than in other applications of HN microchips, probably because in DAPPI the jet has to heat the sampling surface instead of carrying the analytes. The effect of solvent flow rate on the ionization efficiency was tested with toluene as the solvent and anthracene, MDMA, testosterone, and verapamil as
the test compounds. No analyte signal was detected without solvent, but a flow rate of even 1 μL/min led to considerable ionization. In general, solvent flow rates at and above 8 μL/min gave the highest signals; above 8 μL/min the signals of all compounds remained roughly constant. This finding is in good agreement with reports on dopant-assisted APPI.152,161

The analytical potential of DAPPI was evaluated by analyzing the test compounds with use of various solvents. The solvents tested were pure toluene and acetone, toluene/acetone (50/50, v/v), toluene/methanol and acetone/methanol (50/50 and 10/90, v/v), and pure methanol. The most intense signals were achieved with pure toluene and acetone and with toluene/acetone (50/50). No significant signal was seen for any of the analytes with pure methanol, and the signal was considerably lower with mixtures of methanol and toluene or acetone than with pure toluene or acetone. All four analytes were ionized with toluene, with neutral anthracene and testosterone giving the strongest signals. With acetone, the signals for testosterone, MDMA, and verapamil were intense, but no signal was observed for anthracene.

Figure 31 (a) presents a mass spectrum of the four test compounds with toluene at 10 μL/min as the solvent, vapor jet temperature at ~220 °C, and nebulizer gas flow rate at 180 mL/min. The amounts of anthracene, testosterone, MDMA, and verapamil on the spots were 10, 1, 10, and 10 pmol, respectively. Signals of all compounds are clearly visible, with anthracene as the highest peak in the spectrum. Taking into account the smaller amount of testosterone, it was desorbed and ionized most efficiently. Anthracene produces a molecular ion at m/z 178, while testosterone, MDMA, and verapamil produce protonated molecules at m/z 289, 194, and 455, respectively. The ion at m/z 303 was identified as a fragment of verapamil. Fragmentation of verapamil has also been detected with conventional APCI and APPI.66,162 The molecular ion of anthracene was concluded to have formed through charge exchange with the molecular ion of toluene (reaction (4)). The protonated molecules of the other three compounds were probably formed by proton transfer from the toluene molecular ion (or protonated acetone where acetone was used).

Figure 31 (b) shows a mass spectrum of the four test compounds measured with DAPPI with acetone as the solvent. No ions from anthracene are observed, but the other analytes produce intense protonated molecules. Since the ionization energy of anthracene is 7.44 eV and that of acetone 9.70 eV, charge-exchange reaction between anthracene and the molecular ion of acetone would take place if the latter were present. However, the background spectrum of acetone showed only the protonated molecule and protonated dimer formed by self-protonation. In APPI, acetone works best for polar compounds that can be ionized through proton transfer; ionization of nonpolar compounds through charge exchange is usually not achieved.50 A comparison to DESI with the same compounds can be seen in Figure 4 of publication VI.
The sensitivity of DAPPI was tested by determining the LODs for MDMA, testosterone, and verapamil in SRM mode and the LOD for anthracene in full-scan MS mode without background subtraction. The SRM mode did not improve the signal of anthracene owing to inadequate fragmentation. Toluene was used as the solvent for anthracene and testosterone, and acetone for MDMA and verapamil. The S/Ns values were determined by measuring the S/N value of eight spots and calculating the average. The LODs (S/N = 3) for anthracene, testosterone, MDMA, and verapamil were 670, 83, 56, and 56 fmol, respectively. The sensitivity of DAPPI was mainly attributed to the efficient desorption of the whole sample spot by the hot vapor. Efficient desorption also resulted in good stability of the signal, which sometimes is difficult to achieve with surface ionization techniques.

On the basis of the results, it can be concluded that the desorption/ionization mechanism of DAPPI is a combination of thermal and chemical processes. Since the intensity increases with the temperature of the vapor jet, the desorption is probably largely thermal. The effect of the nebulizer gas velocity in the desorption differs in DAPPI and DESI, since the gas velocity in DAPPI is only a fraction of the velocity of solvent droplets in DESI. In DAPPI, with a gas flow rate of 180 mL/min, the average linear velocity at the
chip nozzle is 30 m/s, and lower further in the jet, while in DESI the mean velocity of the solvent droplets, which have a crucial role, is typically 120 m/s. In DAPPI the high temperature of the chip vaporizes the solvent efficiently and it is improbable that droplets exist in the heated vapor jet. The ionization in DAPPI is similar to that in APPI, where photons emitted by the VUV lamp initiate the ionization process, for no signal of any of the analytes was detected with the lamp switched off. Additionally, the presence of dopant-like solvent (toluene or acetone) was necessary for the ionization of the analytes in DAPPI, which suggests that the ionization is initiated by the photoionization of the dopant, just as in APPI. Also similarly to APPI, the selectivity of ionization in DAPPI can be controlled by choosing a solvent that promotes either charge exchange or proton transfer.

Finally, the applicability of DAPPI was demonstrated for the qualitative analysis of pharmaceuticals from a tablet surface. Nebulizer gas flow rate was 180 mL/min, toluene flow rate 10 μL/min, and vapor jet temperature ~ 220 °C. Figure 32 presents the mass spectrum of a Tenox tablet (20 mg of temazepam). The spectrum shows a base peak at m/z 301, which was verified by MS/MS as the protonated molecule of temazepam.

Figure 33 presents the mass spectrum of a Tylenol Cold tablet (80 mg of acetaminophen, 0.5 mg of chlorpheniramine maleate, 2.5 mg of dextromethorphan HBr, and 7.5 mg of pseudoephedrine). The experimental parameters were as above. The spectrum shows intense ions at m/z 151, 166, 272, and 275, which were verified by MS/MS to be the molecular ion of acetaminophen and the protonated molecules of pseudoephedrine, dextromethorphan, and chlorpheniramine, respectively. Both examples show that DAPPI-MS is well suited for rapid and easy analysis of tablets.

![Figure 32. Analysis of a Tenox tablet with DAPPI-MS and the product ion spectrum of m/z 301. Solvent toluene at 10 μL/min. Background not subtracted.](image)
Figure 33. Analysis of a Tylenol Cold tablet by DAPPI-MS (a) and the product ion spectra of acetaminophen (b), pseudoephedrine (c), dextromethorphan (d), and chlorpheniramine (e). Solvent toluene at 10 μL/min. Background not subtracted.
5 CONCLUSIONS

In the course of this work, a new integrated LC–HN microchip was presented, a new ambient MS method was developed, and the first microchip SSI source was introduced. Physical properties of HN microchips were characterized, and their performance in analytical applications was evaluated. In addition, a new method for miniature thermal jet shape measurement was developed.

The HN microchips have several advantages over conventional ion sources. HN chips can be used with any API mass spectrometer and they can be connected with GC and LC. They can be used in APCI, APPI, SSI, APTSI, and ionspray modes, thereby covering the most widely used API techniques. The HN chips allow low flow rates to be used, which is seldom possible with conventional APCI and APPI sources. Unlike ESI, APPI with the HN chips provides high sensitivity for both polar and nonpolar molecules, allowing efficient analysis of a wide range of compounds with low flow rate separation methods such as capillary LC. Other major benefits derive from the microfabrication. With batch fabrication, all chips are identical and perform equally. The cost of fabrication of one chip could be under 50 euros counting all expenses, which is 1/100 or less of the price of conventional API sources.

The development of the first integrated LC–HN chip was envisioned a few years ago, and the chip has now been introduced. Although APPI was used here, integrated HN also allows the use of other ionization modes. With APCI and APPI, the application of chip-based LC devices is extended to nonpolar compounds. Moreover, LC-API-MS with the chip is automated, like conventional LC–MS, and the chip can also be used with optical detection. In future, study will be made of factor contributing to the analytical performance of the chip. Deactivation of the channel sidewalls will perhaps be the most straightforward improvement. Heating of the LC column to allow high temperature LC is an intriguing prospect.

The new DAPPI method opens up fresh possibilities in ambient ionization of surfaces and broadens the range of compounds that can be analyzed by desorption/ionization–mass spectrometry techniques to nonpolar compounds. Applications include direct analysis of plant and other natural surfaces and forensic analysis of contraband drugs, confiscated material, and material evidence. Although DAPPI has so far been performed only with HN microchips, the principle could easily be extended to other conventional or miniaturized heated nebulizers. MS imaging with DAPPI could be a method with potential in biological analysis.

Certainly there is work to be done. The lifetime of the platinum heaters is typically just one to a few weeks. One way to extend the lifetime could be to move the heater from the chip surface to between the two wafers. A second problem is residue deposit in the vaporizer channel when the chips are used for complex samples. This needs to be studied and solutions found to allow chips to be used in long term analysis of complex samples. Thirdly, chips are currently being used in open ion source configurations prone to suffer from the laboratory environment, and associated with instability and increased background. A closed ion source would not only allow a stable and controlled environment but also increase safety at work by decreasing the risks workers to of
solvents, high voltages, and UV radiation. Challenges also exist in fabrication of the chips, but these are technical in nature and can likely be solved by improved or new processing methods.

All in all, the versatility of the HN microchips could make them a universal alternative to conventional ion sources. In future, HN microchips could serve as multimode ion sources for field-portable miniaturized MS instruments. Moreover, combination of integrated LC–HN with a miniature mass spectrometer could offer the basis for full LC-MS analysis with a highly compact instrument.
REFERENCES


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