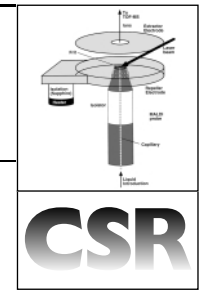
Interfaces for on-line liquid sample delivery for matrix-assisted laser desorption ionisation mass spectrometry



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Recent advances in on-line interfacing of liquid separation methods with matrix-assisted laser desorption ionisation (MALDI) mass spectrometry are reviewed. A number of concepts for this purpose are described, including pneumatic nebulisation creating an aerosol, use of continuous flow probes, and mechanical introduction with a rotating ball inlet or a vacuum deposition interface. Additionally, a MALDI source operating at ambient pressure has recently been introduced, opening a very simple and reliable way to obtain on-line MALDI measurements. Advantages and limitations of these interfaces are discussed.

1 Introduction

The introduction of matrix-assisted laser desorption ionisation (MALDI) and electrospray ionisation (ESI) as soft ionisation techniques in mass spectrometry have revolutionised the analysis of a wide variety of high mass compounds, including biochemically important polymers.[1] MALDI is a method that allows the production of intact gas-phase ions from large, nonvolatile, and thermally labile compounds such as proteins, oligonucleotides, and synthetic polymers. In the thirteen years since its introduction, MALDI has become a standard method for the mass spectrometric analysis of large biomolecules. Molecules with molecular weights in excess of one million Daltons can be desorbed and ionised intact.[2]

An analysis by MALDI mass spectrometry may be divided into two steps. The first step involves preparing a sample by mixing the analyte with a molar excess of matrix. The typical matrix is an aromatic acid that strongly absorbs ultraviolet wavelength laser light. The second step of the MALDI process involves desorption of bulk portions of the solid sample by intense, short pulses of laser light. The matrix is believed to serve three purposes: isolation of the analytes from each other, absorption of energy from the laser light to desorb the analytes, and promotion of ionisation. The laser light causes a small fraction of the matrix and analyte sample to be volatilised and ionised. The molecular masses of the resulting gas-phase ions

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Henrik Orsnes received his education in molecular and cell biology at Odense University (Denmark) where he obtained his bachelor degree in 1996. Since then he has been working for his PhD degree in biotechnology and has carried out doctoral research in several countries, including Denmark (Odense University), Hungary (Hungarian Academy of Sciences), USA (Emory University) and Switzerland (the Swiss Federal Institute of Technology). His research interests, include applying mass spectrometry to determine trace amounts of organic compounds in aqueous solutions and monitoring bioreactor products. Furthermore he invented and holds a patent on the Rotating Ball Inlet (ROBIN) in collaboration with Hans Degn and Thomas Graf from Odense University. This method has been developed for monitoring solutions in the remarkably short analysis time of one-tenth of a second or less. He has implemented the ROBIN technique for MALDI mass spectrometry and is currently developing this. He has received several funding supports in order to finance his international research visits, including a Danish Oil and Nature Gas Research Grant in 1998.

Renato Zenobi is Professor of Analytical Chemistry at the Organic Chemistry Laboratory of the ETH Zürich. He is also one of the founders and directors of the center of excellence in analytical chemistry at the ETH Zürich. Renato Zenobi was born in Zürich in 1961. He received his MS degree from the ETH Zürich in 1986. He completed his PhD at Stanford University in the USA in 1990, then followed two postdoctoral appointments at the University of Pittsburgh (1990–1991) and at the University of Michigan (1991). He returned to Switzerland in 1992 as a Werner Fellow at the EPFL Lausanne, where he established his own research group. He became assistant professor at the ETH in 1995 and was promoted to associate professor in 1997, and to full professor in 2000. His research areas include laser-based analytical chemistry, laser-assisted mass spectrometry, laser-surface interactions, and near-field optical microscopy and spectroscopy. Renato Zenobi has received several awards and fellowships for his scientific work, among them the Thomas Hirschfeld Award (1989), an Andrew Mellon Fellowship (1990), the Ruzicka Prize (1993), and the Heinrich Emanuel Merck-Prize (1998).

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are usually determined by time-of-flight (TOF) mass spectrometry.

In the electrospray interface, liquid sample is sprayed at atmospheric pressure from a capillary exit kept at a high potential (1–4 kV). The electrically induced spray of charged microdroplets desolvates into multiply charged ions, which are then transferred into the high vacuum area of the mass spectrometer. It is more difficult to analyse liquid samples with MALDI. Samples are generally first dried on a solid surface before insertion into the mass spectrometer. As the solvent evaporates, the analyte and matrix co-precipitate to form a solid crystalline layer of analyte and matrix. Conventional MALDI sources are operated under high vacuum, and hence, changing the sample holder for renewed sample deposition requires breaking the vacuum. This severely limits sample throughput and generally requires user intervention. For these reasons, there have been a number of efforts towards on-line coupling of MALDI with liquid sample introduction.

An ultimate goal of bringing MALDI mass spectrometry on-line is to be able to continuously analyse the effluents from liquid separation devices, including high performance liquid chromatography (HPLC) and capillary electrophoresis (CE). In both of these techniques a complex mixture containing many analytes may be separated into its single components. The utilisation of one of these techniques is often necessitated when analysing biological samples, as no analytical device is capable of detecting all the compounds contained in such a sample during one measurement. Hence a two-dimensional analysis has to be performed. In molecular biology, mass spectrometry is generally the method of choice for the determination of protein identity. The direct coupling between a separation device and the mass spectrometer is therefore of great interest. The combination of HPLC and electrospray ionisation mass spectrometry is commercially available and widely used. The combination of liquid separation methods with MALDI is not yet commercially available, but several research groups have demonstrated the ability of MALDI MS to analyse flowing liquid streams and HPLC effluents. These attempts to develop a versatile on-line MALDI interface have shed light on a number of problems. The main problem inherent with the on-line coupling of liquid samples and MALDI mass spectrometry arises because analyte and matrix need to be co-crystallised, giving rise to clogging of the interface.

Despite the widespread use of ESI combined with liquid separations, electrospray ionisation has several drawbacks. First, an ESI mass spectrum includes multiply charged ions. On one hand, their presence allows the detection of large ions at relatively low *m*/*z*, but on the other hand, it complicates the interpretation of the spectra of incompletely separated peaks in a chromatogram. Second, the sensitivity is severely compromised by the presence of salts, impurities, and organic buffers which are often required in chromatographic separations. Finally, ESI sources are often combined with quadrupole mass analysers that are slow in scanning the whole mass range. MALDI MS overcomes these disadvantages. Since it is a pulsed technique, it is natural to use it with a TOF analyser, resulting in a complete mass spectrum from every single laser shot. MALDI TOF MS therefore allows a high speed of analysis which is especially important in a continuous-flow interface for rapidly eluting peaks.

The off-line and on-line couplings of MALDI MS to liquid chromatography and capillary electrophoresis have been comprehensively summarised in recent review papers.[3,4] There are several reports of off-line coupling of CE or LC separation techniques with MALDI MS.[5–10] The effluents were either collected in fractions in an array of cups,[6] on a coaxial fraction collector,[10] on a moving-belt-like system[5] with the matrix solution being subsequently added to the sample, or they were continuously spotted onto a MALDI target precoated with matrix.[7–9] The collection of CE fractions poses a greater problem than that of LC fractions because electrical contact to the capillary must be maintained during separation and the eluent volume is as small as a few nanoliters. The analysis of synthetic polymers with broad molecular weight distributions can be performed by coupling gel permeation chromatography (GPC) with MALDI MS. In this case, GPC fractions, each with a narrow polymer distribution, are investigated by MALDI TOF MS. Off-line combinations of MALDI MS with thin layer chromatography (TLC) have also been reported.[11–13]

The on-line delivery of liquid samples to MALDI MS is continuously being improved and several novel approaches have emerged recently. The intention of this review is to highlight proven or promising advances in on-line MALDI. Over the past three years the new developments in on-line MALDI have expanded the number of alternative approaches from initially only two viable interfaces to now at least six very promising on-line interfaces. This recent literature is emphasised here.

2 Continuous flow probe

A continuous flow (CF) probe, similar to a CF fast atom bombardment (FAB) interface, has been used for the analysis of a flowing sample with MALDI MS.[14] A schematic diagram of

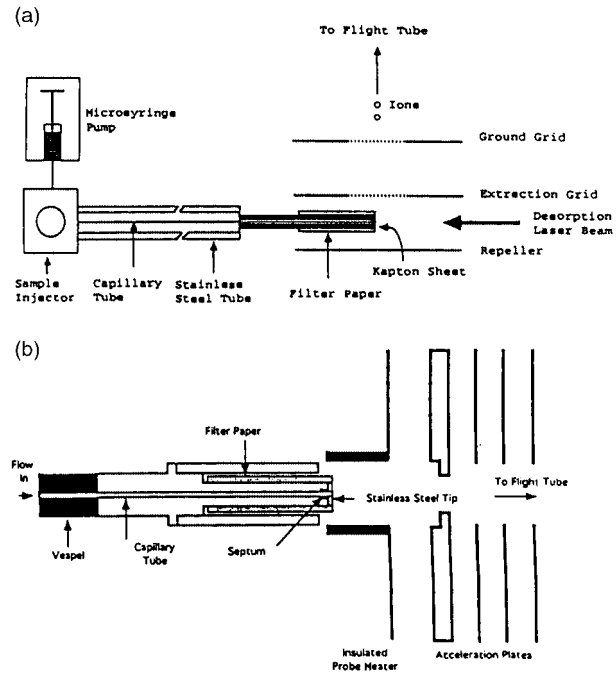

Fig. 1 (a) Schematic of a CF-MALDI probe. (Reprinted with permission from Nagra *et al.*[15] Copyright 1995 Elsevier Science). (b) Schematic of a CF-MALDI with parallel ion extraction. (Reprinted with permission from Whittal *et al.*[16] Copyright 1998 Elsevier Science).

the CF-MALDI is shown in Fig. 1a. A mixture of analyte and liquid MALDI matrix is delivered through the CF probe. The mixture of analyte and matrix is desorbed directly from the probe tip by a 266 nm laser at a repetition rate of 10 Hz. In this original design the probe was positioned orthogonally to the ion acceleration direction. This configuration limited the mass resolution to around 10. In order to improve mass resolution the ion optics were later configured to parallel ion extraction[15] (Fig. 1b), which additionally increased the sensitivity of the instrument by a factor of 10 (low picomol detection limits).[16] Modifying the orientation of the probe to parallel ion extraction along with the adaptation of delayed ion extraction greatly improved the mass resolution, typical values were of the order of 500–700. Until recently applying a liquid matrix has been the

only way to cope with clogging problems in CF probes. In CF-MALDI, the choice of liquid matrix is very limited at present. There are only two known liquid matrices, 3-nitrobenzyl alcohol and 2-nitrophenyl octyl ether, that are suitable for UV MALDI analysis. Due to the limited number of suitable liquid matrices for MALDI applications and the rather poor detection limits obtained with the interface, this approach has only had limited success.

Recently, a new method employing IR MALDI (2.8 μm) and 0.1% glycerol in ethanol as a matrix has been developed for continuous liquid introduction.[17] One potential advantage to IR MALDI is the ability to employ protic solvents as matrices, using the OH stretch absorption near 3 μm. A large number of potential liquid IR MALDI matrices are available compared to UV MALDI. Water is an obvious choice as an IR matrix because it absorbs strongly at around 3 μm, and it is very interesting as a MALDI solvent because it is the natural medium for biomolecules. Also, water plays a role as a solvent in separations of biomolecules, and could therefore be directly employed in a hyphenated liquid separation–MALDI MS system. It has been demonstrated in off-line mode that water ice may be utilised as solvent matrix.[18] However, for on-line applications, a great deal of work needs to be done in order to cope with water freezing at the CF capillary end leading into vacuum. Strong material ablation associated with IR MALDI may reduce this problem, but experimental evidence is needed to demonstrate the applicability of water as a matrix for CF IR-MALDI.

A semi-continuous interface making use of a capillary for sample introduction has also been demonstrated for MALDI MS.[19] The term semi-continuous is used here in order to distinguish this approach from other continuous flow techniques, because in the present version matrix and analyte are allowed to co-crystallise on a porous frit connected to the high

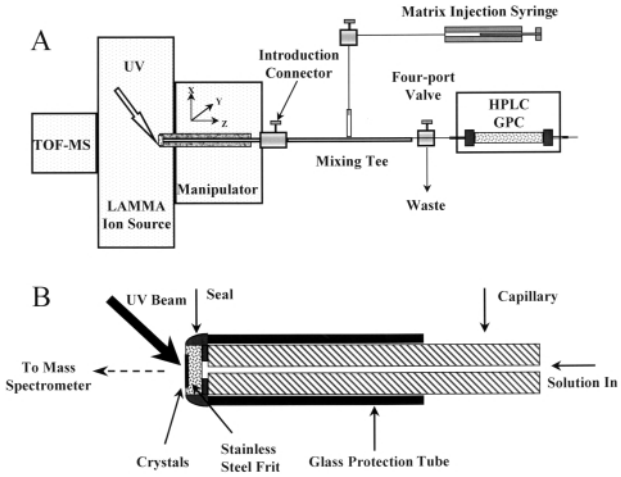

Fig. 2 On-line MALDI interface with semi-continuous analyte–matrix crystallization. A: instrument schematic; B: on-line probe. (Reprinted with permission from Zhan *et al*.[19] Copyright 1999 Wiley Interscience).

vacuum end of a CF-capillary (Fig. 2). The liquid solution containing analyte and matrix flows through the frit into the vacuum of the mass spectrometer. The volatile solvent of the sample evaporates rapidly, leaving a crystalline layer of matrix and analyte on the vacuum side of the frit. Regeneration of the interface is achieved by a combination of flushing the frit with pure solvent and laser ablation. The authors claim that the interface should allow the direct on-line coupling of liquid capillary chromatography with MALDI-MS, but the study offers only cursory examination of such a potential.

Yeung and coworkers[20] coupled capillary electrophoresis to a linear TOF mass spectrometer *via* an interface consisting of a capillary that reached directly into the ion source. An aqueous solution of 0.5 to 1 mM $CuCl_2$ was used for three different purposes at once: as running buffer, as a UV absorbing liquid which assists in analyte desorption, and to prevent freezing of the expanding solvent. It is not known whether the copper salt was also involved in analyte ionisation. A stable flow was maintained by optimising the laser repetition rate and the flow rate. CF experiments with a flow rate of 150 nL min^{-1} and using serotonin (178 Da) as analyte gave mass spectra with a resolution of 100 and a detection limit of 10^{-7} M. When analysing a mixture of serotonin and tryptamine by CE coupled to TOF MS by this interface, chromatographic peak broadening was observed and sensitivity was worse than in the CF mode. Only analytes up to *m*/*z* 200 could be investigated, and the method is limited to solvents that can dissolve the copper salt.

The interface of Amster and coworkers[21] included two capillaries that terminated flush with the probe surface. One of them delivered the analyte, the other a solution of MALDI matrix. In the CF mode with a flow rate of 1.5 μL min^{-1} into the linear TOF mass spectrometer, a detection limit of 10^{-5} M and a mass resolution of 130 in the linear mode were obtained for the peptide bradykinin. Insulin could not be observed. No further results on this approach have since been published. This method did not appear very promising because the solid matrix used may crystallise at the end of the capillary and block the flow.

A related, but more promising strategy is to extend the concept of two-phase MALDI matrices to the frit material terminating a continuous-flow capillary. A two-phase MALDI matrix consists of solid particles that absorb the laser energy and assist in analyte desorption, and a liquid for dissolving and ionising the analyte. This concept offers more flexibility compared to a laser energy absorbing liquid matrix or a solid matrix, where both properties must be combined in one chemical species. An important requirement is that small particles (1 μm diam. or less) are used, presumably because small particles have a low thermal mass and will be heated to higher final temperatures upon laser irradiation. Several combinations of particulates and liquids have been successfully used to analyse proteins, oligosaccharides, synthetic polymers, and dyes up to a molecular weight of over 10000 using the two-phase MALDI approach.[22–27]

A comprehensive study was recently undertaken in our group[28] to develop immobilised two-phase MALDI matrices for use in an on-line interface. The novelty of this concept is that the solid particulates are compressed or sintered into a porous frit that is placed at or near the MALDI probe tip, and that the eluent from the liquid separation continuously delivers sample to such a "particle frit". There are a number of additional requirements for using the two-phase MALDI methodology in a CF interface. For the UV-absorbing substrate, these are mechanical stability, chemical inertness, porosity, low dead volume, and, through proper packing of the particulate phase, the possibility of achieving the high energy densities necessary for desorption/ionisation. A variety of materials, including TiN, CuO, graphite, and other forms of carbon were tested, mostly in off-line mode. Disks with a diameter of 2.5 mm and a thickness between 0.4 and 0.7 mm were prepared and were fixed onto the surface of a normal MALDI probe tip. The TiN frits performed best among all of the laser absorber materials tested. These frits were fabricated by either pressing TiN powder or by pressing Ti powder with subsequent nitridation of Ti. For good results it was necessary to condition the frits with liquid before use. It was possible to generate ions from compounds with molecular weights greater than 10000, as shown in Fig. 3.

We also designed a special probe tip for on-line measurements where a fused silica capillary of 50 μm i.d. delivered the sample solution to the outer side of the frit through which the sample penetrates to the vacuum side facing the ion source of the mass spectrometer. The ionisation of the analyte is promoted by the liquid matrix. In the CF experiment, the flow of sample

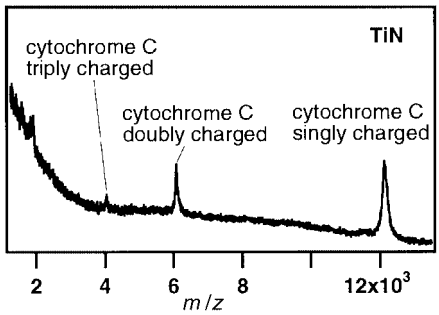

Fig. 3 MALDI mass spectrum of cytochrome c using a TiN frit (off-line mode). The frit was conditioned in a 3:7 (v:v) mixture of glycerol and methanol.

solution can either be hydrodynamic, *i.e.* driven by the difference in pressure between the mass spectrometer and atmosphere, or be controlled with a syringe pump. In order to prevent freezing of the liquid, an electrically insulated heating stage for the MALDI probe tip was designed and added as

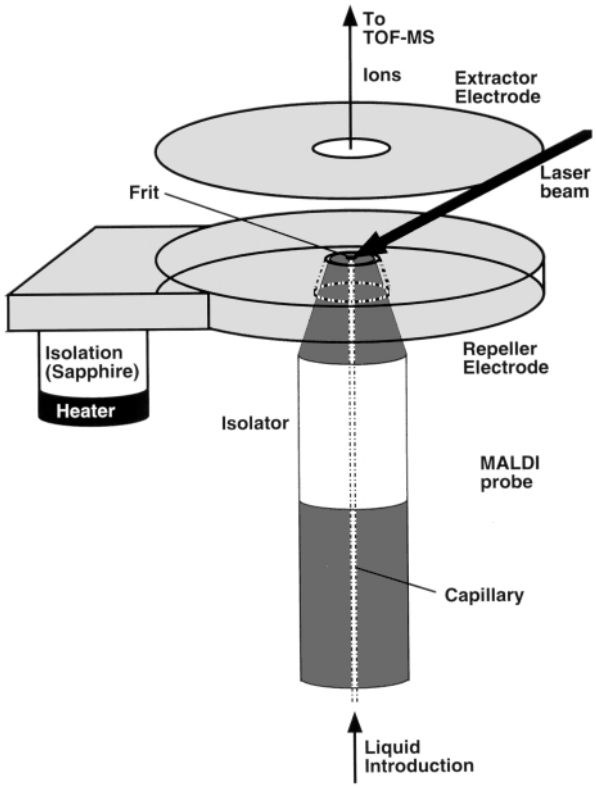

Fig. 4 Schematic view of the continuous flow probe tip along with the ion extraction region, including heated repeller plate and extractor electrode.

shown in Fig. 4. A resistor heated the repeller plate and consequently the probe tip. As the repeller plate had to be at high voltage, it was not possible to mount the resistor on this plate. Hence, it was attached on a copper plate which was isolated from the repeller plate by a sapphire cylinder. Sapphire is characterised by low electrical and high thermal conductivity.

The problem that was encountered in all on-line and CF mode experiments, though, was that the mechanical stability of the frits was not satisfactory. With one exception, they could not be tested in CF mode where the frits had to support themselves between the end of the capillary and the vacuum side of the interface. The exception concerns a CuO frit fabricated by oxidation of a copper grid that was then glued over the outlet of a capillary which reached up to the MALDI probe tip. This CuO frit was found to perform well with substance P as an analyte, transported in a methanol–water mixture. However, in the case of CuO, the fabrication process was not reproducible due to difficulties in controlling the oxidation process. Important aspects such as memory effects, dead volume, *etc*. were therefore never studied in CF mode, either for TiN or for CuO. Nevertheless, we believe that the concept of using sintered two-phase matrix frits holds much promise, because of its simplicity, and because few restrictions exist for the use of a wide variety of eluents for chromatographic separations. An extended search for optimised frit materials and research directed towards increased mechanical stability should ultimately result in a widely usable particulate frit for MALDI ion production.

Porous silicon fabricated electrochemically from silicon wafers has been investigated for use as a "smart" sample support by Wei *et al.*[29] This new approach has been named Desorption Ionisation On Silicon (DIOS). These authors found that it was possible to efficiently generate mass spectra from surface modified porous silicon, up to a molecular weight of *m*/*z* 3000. The authors successfully performed UV laser desorption–ionisation experiments in static mode on this material without the addition of a matrix. One of the advantages with a matrix-less technique is that the MS analysis may be carried out on low molecular weight compounds without matrix interference, as shown in Fig. 5. It may therefore be a promising direction to fabricate a MALDI-active frit directly using porous Si. Such a device might be produced using microchip technology, and it is tempting to speculate about combining chip-based separation methods directly with a porous Si frit outlet for on-line MALDI MS detection.

3 Aerosol liquid introduction

In the aerosol MALDI method, the solution containing matrix and analyte is sprayed into the mass spectrometer where the solvent evaporates.[30] The dried aerosol particles are ionised with a pulsed laser and analysed by time-of-flight MS. Fig. 6 presents the schematic of an aerosol MALDI instrument configured for on-line coupling to gel-permeation chromatography (GPC). Additionally the instrument was equipped with a reflector time-of-flight tube.[31] The mass resolution for aerosol MALDI in TOF MS is often hampered by the large ion spatial distribution in the acceleration region of the ion source, but incorporating a reflector in the flight tube partly compensates for the spread in ion energies. Typically high flow-rates, *e.g.* 0.5 mL min^{-1}, are applied in the aerosol MALDI technique. This inefficient sample utilisation has partly been solved by introducing and ionising single aerosol particles.[32] Utilising a pneumatic nebuliser with a flow rate of only 5–10 μL min^{-1} efficient aerosol generation could be obtained. By irradiating single aerosol particles with a 337 nm pulsed nitrogen laser, the ions produced were accelerated perpendicularly to the particle beam into a reflector time-of-flight mass spectrometer. The mass resolution was 400–500 FWHM for most analytes, but the instrument was only able to detect masses below *m*/*z* 2000. Due to inefficient particle transmission typical detectable analyte amounts were in the upper pmol level. The aerosol interface is currently limited by these rather poor performance characteristics, but instrumental improvements, including improved particle transmission efficiency, may result in a reliable on-line interface for MALDI analysis.

4 Atmospheric pressure MALDI

Conventional MALDI sources are operated under high vacuum in order to achieve unrestricted ion motion in the mass

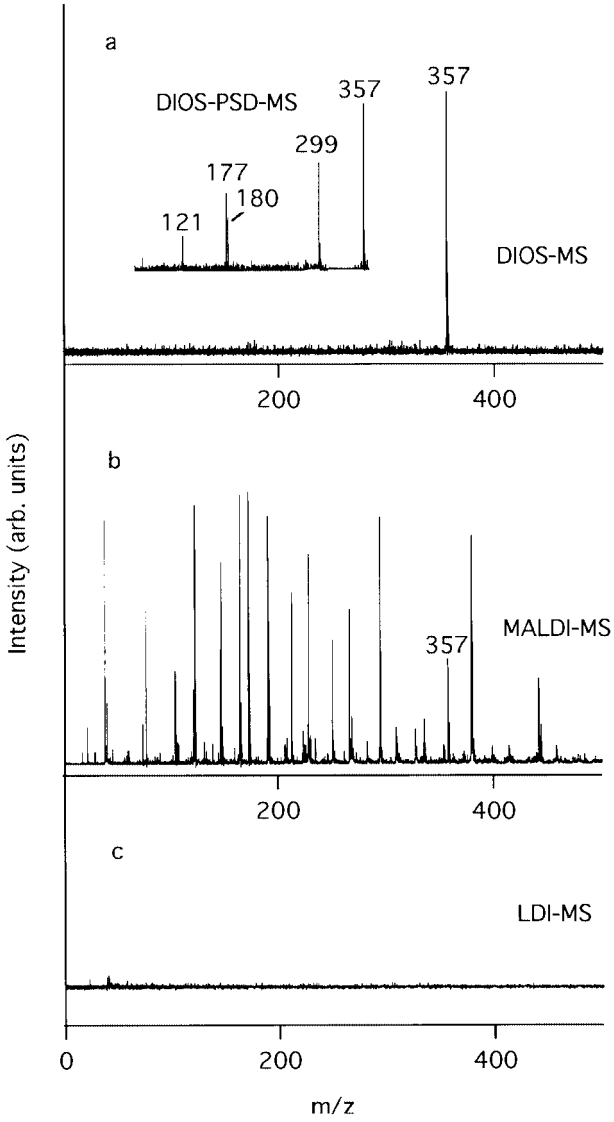

Fig. 5 Analysis of an antiviral drug using different desorption/ionisation techniques. (a) DIOS mass spectrum using porous Si surface. The inset shows MS–MS data generated by post source decay (PSD) of the same sample. (b) MALDI mass spectrum of the same amount of antiviral drug using a conventional matrix (α-cyano-4-hydroxycinnamic acid). (c) No signal was obtained from any of the compounds tested using desorption–ionization off a bare gold sample platter, without any matrix added to the antiviral drug sample. (Reprinted with permission from Wei *et al.*[29] Copyright 1999 Nature © Macmillan Publishers Ltd.)

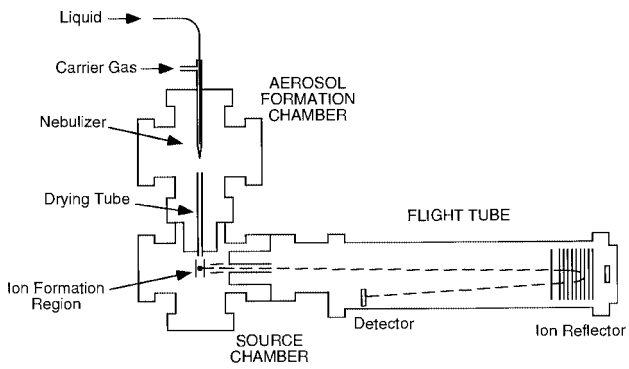

Fig. 6 Aerosol MALDI instrument for on-line GPC-MALDI. (Reprinted with permission from Fei *et al.*[31] Copyright 1996 American Chemical Society).

spectrometer. Hence, sample introduction into the MS requires an arrangement that ensures that the high vacuum will not be breached. Generally the sample probe is evacuated in a separately pumped chamber before insertion into the high vacuum chamber of the MS through a vacuum interlock.

Laiko *et al.*[33] recently discovered that a MALDI source may be effectively operated at atmospheric pressure (AP-MALDI). The ions generated by the laser irradiation on the sample are transported into the vacuum of the mass spectrometer by means of a carrier gas or electric fields. The interface is schematically

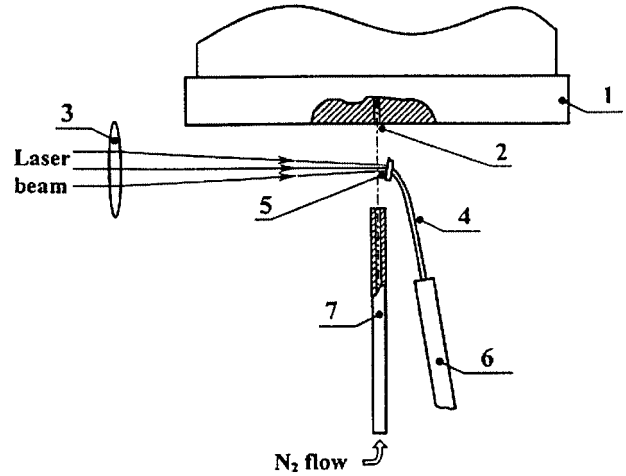

Fig. 7 Schematic view of the AP MALDI source. 1, atmospheric pressure interface of the mass spectrometer; 2, inlet nozzle to MS instrument; 3, quartz lens to focus laser beam onto the sample target; 4, replaceable probe tip; 5, stainless steel MALDI target plate; 6, target holder; 7, stainless steel capillary gas nozzle. The ions are subject to a gas-assisted flow directed towards the nozzle 2. Furthermore the pressure gradient established in this nozzle orifice drags the ions into the mass spectrometer (Reprinted with permission from Laiko *et al.*[33] Copyright 2000 American Chemical Society).

shown in Fig. 7. Due to the decoupling of the desorption/ionisation step from the subsequent mass separation, and the almost continuous generation of ions, AP MALDI may also be used in combination with other types of mass spectrometers, including sector field mass spectrometers, quadrupoles, ion-traps, and FT-ICR instruments. Tandem mass spectrometry is not easily implemented in time-of-flight instruments, but triple-quadrupoles, ion-traps and FT-ICR instruments may be utilised for this purpose.

Many problems associated with conventional MALDI sources may be solved with the AP MALDI source. As the novel approach basically introduces the ions continuously into the MS, a much higher sample throughput is obtained and automation can be more easily applied. It should be noted that real on-line experiments have not been demonstrated in the literature, but the interface has an obvious potential to analyse a trace of sample deposited as a lane along the sample holder. Laiko *et al.* mentioned this potential and it will only be a question of time before on-line measurements will be demonstrated.

The laser intensity is often attenuated in conventional MALDI time-of-flight instruments in order to reduce the spatial and energetic spread of the ablated MALDI plume. A lower laser intensity is obviously less efficient in producing ions. Due to rapid thermalisation of the ions, by collision with ambient gas before fragmentation may occur, higher laser energies may be utilised in AP MALDI to produce more ions per unit time. Additionally, the positional accuracy and geometry of the MALDI probe and associated ion optics is not critical for mass accuracy and resolution as it is for conventional MALDI. Furthermore AP-MALDI is capable of analysing samples not compatible with high vacuum conditions, including electrophoresis gels and polymer membranes which shrink when exposed to low pressures.

Less fragmentation is seen in AP MALDI spectra, but in-source fragmentation may be induced by elevating the nozzle-skimmer potential. Clustering between matrix and analyte ions is typically seen in the AP-MALDI spectra, especially at laser energies around the ionisation threshold. These unwanted adduct ions may be partly eliminated at high irradiation intensities.

5 Continuous vacuum deposition interface

Recently Preisler *et al.*[34] demonstrated that a device for continuous vacuum deposition of matrix and analyte from a solution onto a moving surface inside the mass spectrometer can be used for generating MALDI spectra of a flowing liquid sample. The device makes use of a rotating quartz wheel onto which the liquid is deposited through a narrow fused silica capillary that is kept in contact with the wheel. When the wheel is rotating, deposited sample is transported into the ion source

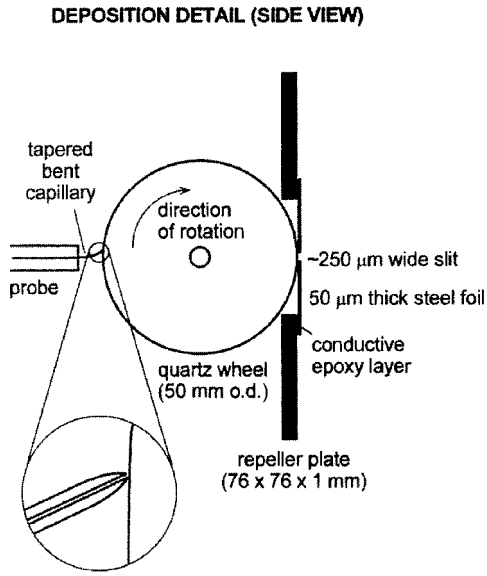

Fig. 8 Enlarged view of the liquid deposition wheel. (Reprinted with permission from Preisler *et al.*[34] Copyright 1998 American Chemical Society).

region where MALDI takes place (Fig. 8). Promising results have been obtained, and the system is compatible with crystalline matrices because clogging at the capillary exit is prevented due to the physical contact with the rotating wheel. The sample consisting of analytes and matrix was deposited in the form of a uniform narrow trace. The design resulted in excellent spot-to-spot reproducibility and attomole sensitivity. Capillary electrophoresis (CE) was directly coupled with the interface. Fig. 9 shows the capability of the interface to analyse the rapidly separated peptides from a CE column. As can be seen, 11 of the 12 peptides were resolved in the MS electropherogram. A major disadvantage of the system is the limited operation time, because cleaning of the wheel is needed after it has made a 360 degrees cycle lasting about 3 min. However, the authors have solved this problem in a second generation interface, where the solution from the infusion capillary is deposited onto a disposable Mylar tape.[35] With a total tape length of 80 meters, uninterrupted deposition for about 24 hours has been demonstrated. Additionally, a multiplex system utilising a 12-capillary array has been adapted to the interface. The beam of the MALDI laser was scanned across the tape with 12 deposited traces providing multiplex MS for high throughput analysis without compromising data quality.

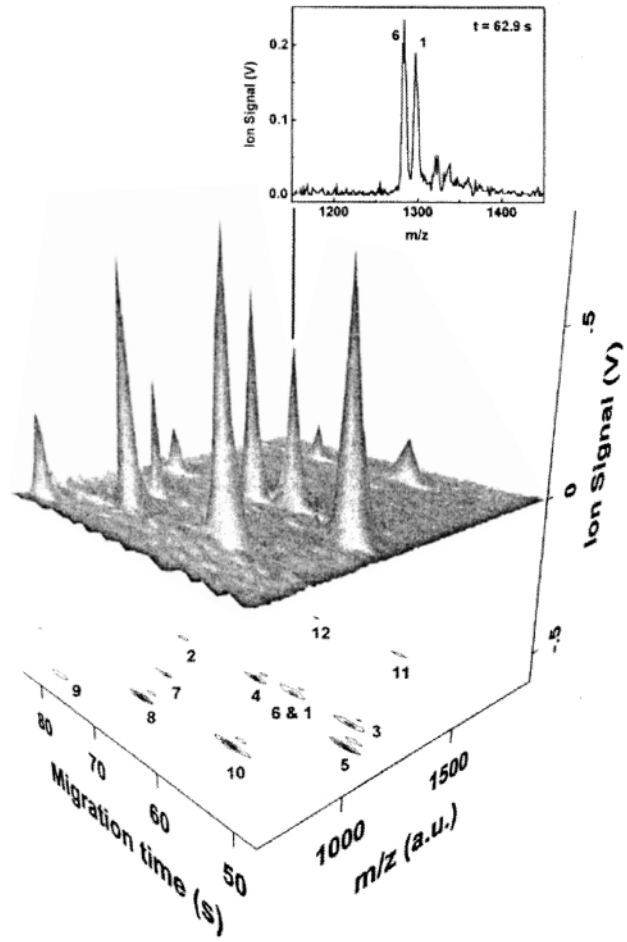

Fig. 9 On-line CE-MALDI MS of a mixture of 12 different angiotensin peptides. (Reprinted with permission from Preisler *et al.*[34] Copyright 1998 American Chemical Society).

6 Rotating ball inlet

The rotating ball inlet is an alternative means of mechanical introduction of liquid samples. The ROBIN MALDI interface[36] represents a development of ROBIN which was originally designed for on-line analysis of volatile compounds.[37–39] The principle of the inlet is that sample adhering to the surface of a ball is continuously carried past a polymer gasket into the vacuum chamber of the mass spectrometer. Volatile components evaporate from the surface of the ball when exposed to the vacuum. Non-volatiles, including crystalline matrix and biopolymers, may be desorbed and ionised by laser irradiation of the ball surface in the vacuum of the mass spectrometer. This new interface was recently adapted to on-line MALDI. Here, the matrix and analyte solution is delivered through a capillary to a polymer gasket held tightly against the rotating ball (Fig. 10). When the ball rotates it drags sample solution into the MS, where the solvent evaporates leaving a thin crystalline deposit of analyte and matrix on the surface of the ball. Using 2,5-dihydroxybenzoic acid (DHB) as the matrix and 355 nm laser radiation the ROBIN MALDI interface showed its ability to perform flow injection analysis of protein samples. Fig. 11 shows the flow injection ion profile of three 1 μL injections of 10 pmole insulin. Since the liquid sample was introduced as a very thin layer there was not enough material on one spot to form macrocrystals of matrix or solutes. Thus there is no risk of clogging the interface because of crystal formation.

The major limitation of the system is the mass resolution (*ca.* 300), which preferably should be one order of magnitude higher. Achieving a lower pressure and more parallel field lines in the ion-source region should improve the mass resolution.

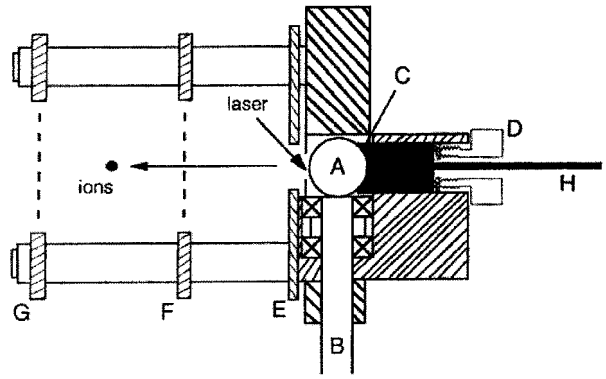

Fig. 10 Diagram of the on-line ROBIN MALDI interface. A, 10 mm in diameter stainless steel ball; B, drive shaft; C, gasket; D, adjustment screw; E, repeller; F, extraction grid; G, ground grid; H, capillary. The ball is rotated through the shaft, which is connected to a gear motor positioned outside the vacuum chamber (not shown). (Reprinted with permission from Orsnes *et al.*[36] Copyright 2000 American Chemical Society).

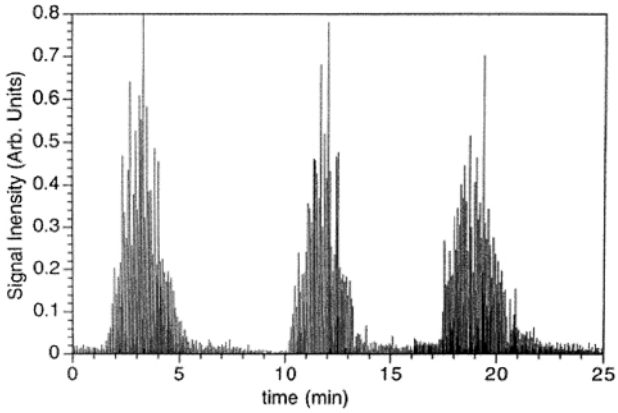

Fig. 11 Flow injection ion profile of three repeated injections of 10 pmole bovine insulin. The laser was operated at a repetition rate of 10 Hz. Dihydroxybenzoic acid was used as matrix, dissolved in both the mobile phase and the analyte solutions to a concentration of 10 mg mL^{-1}. (Reprinted with permission from Orsnes *et al.*[36] Copyright 2000 American Chemical Society).

Additionally the sample utilisation should be more efficient, which can be improved by reducing the contact area between the sealing gasket and the ball. We are currently working on a second generation ROBIN MALDI interface in order to overcome these limitations.

A notable difference between the second generation ROBIN MALDI interface and the previously published one is the orientation of the ball relative to the vacuum of the mass spectrometer. As shown in Fig. 12 the major part of the ball is outside and not inside the vacuum chamber as in the first design. Furthermore we have implemented a flow-through channel in the new design in order to continuously clean the ball surface. Additionally, the analyte stream, premixed with matrix, is deposited onto the rotating ball through a narrow fused silica capillary, whereas the previously published ROBIN MALDI approach delivers sample to the ball surface through a capillary surrounded by a polymer gasket held tightly against the rotating ball. A major advantage of the new system is that the solvent evaporates outside of the vacuum. As a consequence, a very good vacuum pressure can be maintained, comparable to the pressures obtained in conventional MALDI instruments. The present approach is conceptually similar to that of the moving belt interface, where the deposition of sample likewise occurs at atmospheric pressure.[40] A notable difference is the vacuum sealing, as we introduce the crystallised sample directly into the

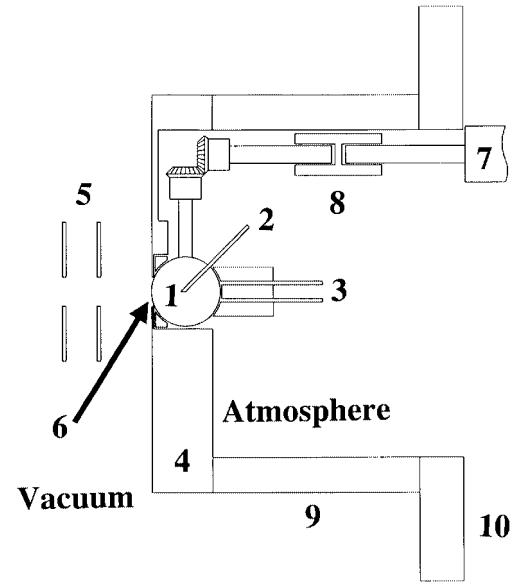

Fig. 12 Diagram of the second generation ROBIN MALDI interface. (1) 20 mm diameter stainless steel ball; (2) capillary for sample deposition onto the rotating ball; (3) flow-through channel for cleaning the ball surface; (4) plate for repelling the ions towards ion extraction optics; (5) ion extraction optics; (6) laser beam; (7) motor for rotating the ball; (8) electrically insulating joint; (9) electrically insulating spacer; (10) flange for mounting the interface on the mass spectrometer.

vacuum of the mass spectrometer, whereas the moving belt makes use of differential pumping between the vacuum of the mass spectrometer and the atmosphere due to the poor vacuum seal inherent with this interface.

The new ROBIN MALDI interface has not yet been tested thoroughly, but we have carried out some measurements which shed light on the performance characteristics of this system. Of particular importance is the ability to perform flow injection

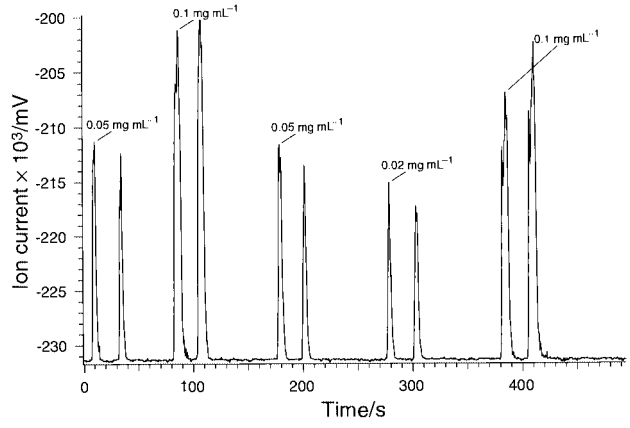

Fig. 13 Flow injection ion profile of repeated 100 nL injections of ACTH. Analyte concentrations ranged between 0.02 and 0.1 mg mL^{-1}; dihydroxybenzoic acid (DHB) was used as matrix dissolved in the analyte sample. In all injections the concentration of DHB was 5 mg mL^{-1}. The flow rate of the mobile phase was 1 μL min^{-1}.

analysis FIA. Fig. 13 shows the flow injection ion profiles of samples with different concentrations the peptide adrenocorticotropic hormone (ACTH). The ion current was measured by integrating the peak area of the pseudo-molecular ion peak of this compound. The FIA peaks indicate that the new version of the ROBIN MALDI interface does not introduce any memory effects, and the injected volume gives rise to a peak width in agreement with the flow-rate in the capillary and the injected volume. It should be noted that the first version of the ROBIN MALDI interface introduced FIA peaks that were broader than

would be expected from the injected volume as seen in Fig. 11.

7 Conclusions and future perspectives

Several variations of the continuous flow probe have been presented in this review. Basically all of these probes are governed by the same principles, only differing in frit design and purpose of the frit. One of these does however work differently, in that the matrix and analyte are allowed to co-crystallise on the vacuum side of the frit; consequently clogging occurs and the flow is stopped until the crystalline layer is removed, either by solvent flushing of laser ablation or combinations thereof. Therefore we have denoted this technique semi-continuous. Very rapid response changes in analyte composition or concentration will not be observable with this semi-continuous CF probe. A major advantage of this novel approach is the compatibility with crystalline matrices which are still by far the most popular laser absorbing compounds used in MALDI mass spectrometry.

The approach with a laser absorber made from pressed silicon powder may be pursued further. Alternatively, the use of porous silicon from silicon wafers, which have been investigated by Wei *et al.*[29] may be another promising path. The authors successfully performed UV laser desorption–ionisation experiments in static mode on this material without the addition of a matrix. A porous silicon surface may be used directly as a frit in CF MALDI.

The potential of developing an on-line AP MALDI interface may be simply realised by providing a mobile sample holder. By continuosly depositing the liquid stream to be analysed on a moving sample holder the system should provide real-time monitoring of the analytes if the sample trace subsequently is moved into the target area of the laser.

The vacuum deposition interface has already shown its capability to analyse the eluent from a capillary electrophoresis column. By adapting a disposable Mylar tape, the system could be operated for up to 24 hours without interruption. Furthermore, the inventors of this novel approach have demonstrated a multiplex system using a 12-capillary array for simultaneous MALDI analysis. The latter system obviously gives rise to increased sample throughput rates, which is particularly important in proteome studies. A notable drawback of the system is the introduction of solvent into the mass spectrometer, which inevitably gives rise to higher vacuum pressures in comparison with the new version of the ROBIN MALDI interface, where the solvent is evaporated prior to sample introduction into the mass spectrometer. So far, ROBIN MALDI has only been used as a flow-injection technique, but we will move to couple the interface with solution based separation methods, such as liquid chromatography and ultimately capillary electrophoresis.

Due to the inefficient sample utilisation the aerosol MALDI technique does not seem to offer any advantages in the analysis of very small quantities of biopolymers. However, the system is simple and robust which are major requirements for industrial applications. Hence, routine analysis of synthetic polymers may be a very potential application. The interface suffers from rather poor detection limits and mass resolution, but these drawbacks may be negligible from an industrial point of view because the interface offers several advantages such as ease of automation and low service requirements.

Most of the devices that have been presented here were developed in research laboratories and still need to see general acceptance before being manufactured commercially. The efficiency and stability of these on-line interfaces need additional improvement before they will reach the status of established and commercialised electrospray (ESI) sources, which currently constitute the dominant interfaces for on-line coupling solution based separations with mass spectrometry. These new and required developments can be expected to enhance sampling efficiency, sensitivity, mass resolution, reproducibility, and robustness.

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