Liquid Secondary Ion Mass Spectrometry

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Introduction

Liquid secondary-ion mass spectrometry (liquid SIMS) is an obsolescent ionization technique that revolutionized the analyses of large biological molecules in the 1980s. The term includes fast atom bombardment mass spectrometry (FAB-MS). The technique has been largely superseded by electrospray ionization. This account will cover the history of the technique, the principles of the operation, and the development of continuous flow systems allowing a degree of liquid chromatography–mass spectrometry operation and cover the application of the technique, especially to polar and large molecules such as peptides and small proteins.

The Development of Liquid SIMS

Liquid SIMS grew out of the development of secondary-ion mass spectrometry. In this technique, ionized atomic or molecular particles are analyzed following their emission from a surface after bombardment with a beam of energetic primary particles. This primary beam may be composed of electrons, ions, fast neutrals, or photons. Secondary ions were observed as early as 1910 by J.J. Thomson after bombarding a metal surface with a beam of ions, but the use of SIMS as an analytical tool came into common use in the 1970s. In this mode of operation, a high-energy beam of ions was directed at a surface to obtain a high yield of secondary ions. The surface itself was eroded quite rapidly. The technique was used especially to study the surface layers of metals and silicon wafers in the growing electronics industry. This technique is known as dynamic SIMS.

To analyze material absorbed on a surface required a less energetic primary beam that would not disrupt the crystalline structure of the substrate. This technique was described as static SIMS. Problems arose inasmuch poorly conducting substrates would become charged, disrupting the primary beam and the emission of secondary ions. Vickerman and co-workers showed in 1979 that the use of a fast atom beam, a stream of energetic neutrals, reduced the charging problem and improved the stability of the secondary ion beam. They called their technique fast atom bombardment SIMS or FAB SIMS. The technique was taken up by Barber and co-workers for the analysis of intractable organic molecules, and rapidly evolved thereafter into liquid SIMS.

Barber was working at a time when it was required to have a sample in the gas phase before ionization, which is not possible for biologically important materials that are involatile, thermally unstable, and often charged. The most obvious classes are peptides and proteins that are massive, fragile, and often very polar. Some techniques existed for studying these types of samples by introduction in the solid phase and the most used techniques in the 1970s were probably field desorption, static SIMS, and LASER-induced desorption. All these techniques had difficulties with their use and often gave irreproducible results. Barber used static SIMS by coating targets with a thin layer of deposited sample and subjecting this surface to a beam of argon ions with 2–5 keV energy. Both positive and negative ion spectra could be obtained but the sputtered ions leaving the surface had considerable energy spread reducing resolution, and surface charging was a continuing problem. Barber developed a small source producing a beam of fast argon atoms with 2–8 keV energy to strike the sample. Spectra were obtained by depositing a solution of sample on a copper target and introducing this into the fast atom beam. A chance observation showed that spectra could be much improved if the target was covered with an involatile oil (typically glycerol) and the sample dissolved in this. Not only were more stable spectra obtained but also the sample lifetime was considerably extended. Spectra were obtained in both positive and negative ionization modes. The technique was called fast atom bombardment mass spectrometry.

It was quickly realized that it was not essential to use a ‘pure’ atom beam. Acidified glycerol was a sufficiently good conductor where charging of the surface did not occur and several groups, such as M-Scan Ltd. in the UK, developed simpler sources relying on generating beams of fast ions for the primary beam. Initially, gas sources were used for generating beams of xenon ions with energies of 8–10 keV. It was shown that the intensity of the secondary beam depended on the atomic weight of the primary ions, i.e., xenon > argon > neon. Later sources used cesium to generate a primary beam of cesium ions with very high energy (20–30 keV).

The high voltages used to generate the primary beam and hence the secondary beam plus the high concentration of sputtered neutral molecules made magnetic sector instruments the most ideal mass analyzer to use with liquid SIMS sources. Linear
instruments such as quadrupoles are affected by noise from neutrals whilst other analyzers requiring higher vacuum (such as FT-ICR–MS) are badly affected by the relatively high gas pressures.

The use of ion beams precluded the use of the term FAB and the family of techniques became known as liquid SIMS.

**Structure and Operation of the Liquid SIMS System**

The liquid SIMS system comprises a target stage mounted on the tip of an introduction probe, a source for the primary ionization beam (commonly known as a ‘gun’), and a source comprising beam focusing, extraction, and exit lenses to shape the secondary ion beam and inject it into the mass analyzer (Figure 1).

The target stage originally comprised a thin steel plate mounted on two contact legs in early sources, although later more robust solid blocks were used. In operation, the target would be covered with a film of involatile oil known as the ‘matrix’ in which the sample was dissolved. Matrix materials became an art with special recipes for some selected applications but in the main most samples could be analyzed using a matrix of acidified glycerol. For some biomolecules, thioglycerol or a mixture of thioglycerol and glycerol gave better results whilst \( m \)-nitrobenzyl alcohol was useful for some apolar compounds that did not dissolve in the hydrophilic matrices. Acidification was usually accomplished using acetic or hydrochloric acid, although occasionally thioacetic was used. In order to obtain good spectra it was essential to ensure that the sample remained in solution within the matrix. Samples were usually added dissolved in a suitable solvent; experience showed that alcohols (methanol, ethanol) and dimethylsulfoxide were useful. Halocarbon solvents were unsuitable as they created flashovers and generally gave weak, noisy spectra.

The primary beams could be generated using three types of guns. Initial work by Barber used a fast atom generator, but later authors used ion guns either using xenon or cesium.

The fast atom generator is a device (Figure 2) in which argon gas is first ionized and accelerated towards the target, then passes through a collision cell in which charge exchange occurs between the fast-moving argon ions and slow argon atoms. This produces a mixed beam of fast and slow argon atoms and ions. After ejection from the gun the remaining ions are deflected from the beam whilst the slow argon atoms diffuse and are lost. The accelerating and exit electrodes are aluminum and erode during use and this type of gun requires cleaning and replacement of these parts every so often. The beam of mainly fast argon atoms hits the surface of the matrix causing sputtering and the expulsion of sample ions (see below).
The need for a fast atom beam was not necessary as the target was connected into an electrical circuit and the matrix was sufficiently conducting so that surface charging effects caused by using a fast ion beam no longer occurred. Consequently, an alternative primary ion device was developed that used a simple charged nozzle to ionize a jet of gas ions. Gas at a flow rate of 0.5 ml min\(^{-1}\) passes through a nozzle with a narrow orifice that is held at high voltage to a cap (counter electrode) with an exit hole. Experiments with various gases showed that the intensity of the secondary ion beam depended on the mass of the bombarding gas and therefore xenon was used as the gas of choice. However, the flow of gas into the source chamber was higher than that from the fast atom gun, and care was required to avoid tripping the high vacuum (Figure 3).

The final development of a primary ion source was the cesium ion gun. A pellet of cesium iodide molded around a pin is heated to allow the salt to flow. A high voltage is applied (25–30 kV) and a beam of fast moving cesium ions is formed, which is extracted and focused onto the target through a series of lenses. The great advantage of this type of device is the very low load of gas in the source chamber. The disadvantage is the very high voltages employed, which require special generators and insulators to prevent electrical breakdowns.

All three types of guns generate a beam of high-velocity particles (either atoms or ions). These hit the surface of the matrix with considerable energy and disrupt the surface layers. Any ions contained within the surface layers are sputtered away and, as the target is held at the accelerating voltage for the mass spectrometer, are accelerated through conventional beam shaping optics into the mass analyzer. Holding the target with a positive charge allows the analysis of positive ions, while a negative charge allows the analysis of negative ions. With ion guns the accelerating energy of the incident beam needs to be greater than the accelerating voltage applied to the target. Thus, in a typical sector instrument with an accelerating voltage of 6 or 8 kV, the incident beam needs to be at least 8 keV. Cesium guns usually operate at much higher voltages so no problems arise with these sources.

The amount of energy released by the incident beam is sufficiently high in the vicinity of the point of impact that sample molecules are ionized by proton transfer from a protonated matrix. Typically, the spectrum obtained shows cluster ions formed from the matrix. In particular, the positive ion spectrum of glycerol (mw 92 u) shows a series of ions 92\(n + 1\) where \(n\) can be 1 up to 12 or more. This spectrum can be used to calibrate the mass spectrometer and also provide internal lock masses for accurate mass measurement, described in applications below.

A modified target is used with capillary high-performance liquid chromatography systems, developed by Ito in 1985. In this target, a fused silica line brings the effluent from the liquid chromatogram to a diffuser in the target (Figure 4). This was a difficult technique to operate because flows to the tip had to be kept very low (<1 \(\mu\)l min\(^{-1}\)) and the matrix (usually glycerol) had to be added postcolumn. The effluent from a capillary LC system was mixed with a solution of matrix and the combined solution, containing perhaps 10% glycerol by volume, passed through a splitter to keep the flow to the mass spectrometer within limits. The complete system was described as dynamic FAB. The simple system was developed by various manufacturers, especially JEOL and Micromass. The ultimate probe tips had a diffusing mesh across the end of the capillary with an annular wick of tissue outside this to trap excess liquid. The tip was warmed slightly to aid evaporation of liquid from the wick. Results were obtained and published using these systems but it was difficult to retain chromatographic resolution and the quality of the spectra was not great. Data were obtained on tryptic digests of proteins using these techniques, which allowed accurate mass measurements using some polyethylene glycol as internal calibrant. This study showed that dynamic FAB could produce a stable ion beam but better results were obtained by collecting chromatographic fractions offline, concentrating these, and running as normal samples. This aspect of liquid SIMS has been completely superseded by capillary electrospray sources, introduced in the 1990s. Electrospray gave better sensitivity and the generation of multiply charged ions allowed the analysis of much larger molecules than FAB, which usually tends to give protonated molecules.

All liquid SIMS sources work in the same fashion. The primary beam impacts the surface of a liquid

![Figure 3 Schematic for an ion gun.](image_url)
matrix. At the point of impact considerable energy is released, disrupting the lattice of molecules of the matrix making up the meniscus. Indeed, the energy at the point of impact is sufficient to cause molecular fragmentation, so for glycerol successive losses of water are observed (the protonated molecule for glycerol has \( m/z \) 93 u; fragments are observed at \( m/z \) 75 and 57 u). The impact sputters protonated species away from the surface, both from the matrix and from any analyte molecules contained within the surface layers. If the analyte exists as a protonated species within the matrix then these cations are analyzed directly. Otherwise the energy of the impacting beam allows proton transfer between the protonated matrix and neutral species just above the surface of the matrix. Further away from the impact point clusters are sputtered with insufficient energy to break apart. This is clearly observed for glycerol where a sequence of cluster ions is observed having mass \( 92n + 1 \), \( n \) ranging from 2 up to 15–20. The sequence is sufficiently stable to act as an internal calibrant for accurate mass measurements (see below).

The matrix may contain traces of metal cations, especially alkali metals such as \( \text{Li}^+ \), \( \text{Na}^+ \), and \( \text{K}^+ \). In these cases, cluster ions are observed for analytes of the type \( \text{M} + \text{Na}^+ \). These are often accompanied by \( \text{M} + \text{K}^+ \), giving a sequence of ions \( \text{M} + 1^+ \), \( \text{M} + 23^+ \), and \( \text{M} + 39^+ \).

Negative ions can also be observed by reversing the polarities within the ion source and the target. The primary beam remains positive. Deprotonated species are sputtered in a manner analogous to the cationized species. Clusters are sometimes observed such as \( \text{M} + \text{Cl}^- \).

The elegance of liquid SIMS is that after such disruptive events the surface is restored by the flow of liquid matrix. Further, the conducting properties mean that any charge buildup in the meniscus is conducted away to the electrically connected target stage. Thus, a sample can be analyzed for as long as liquid remains on the target, often for minutes on end. Spectra can be accumulated by a data system improving the signal-to-noise ratio.

Sample intensity depends on the atomic mass of the bombarding gas. In experiments with both fast atom and ion guns the intensity of the signal was in the approximate ratio of 1:0.28:0.15 for xenon, argon, and neon, respectively (the ratio of atomic masses is 1:0.30:0.15). The approximately linear relationship between atomic mass and intensity is as expected.

Accurate mass measurement could be carried out using suitable calibrants. A popular calibration compound was polyethylene glycol, a mixture of polymers of the form \( \text{H} (\text{OCH}_2\text{CH}_2)^n \text{OH} \). This commonly used oil has a series of protonated molecules with 44 u spacing, e.g., 151, 195, 239, 283, 372, etc. It also gave a similar negative ion series (149, 193, 237, etc.). Other calibrants were glycerol itself, relying on a series of cluster ions of mass \( 92n \) in positive ion mode, and \( 92n - 1 \) in negative ion mode.

High mass calibration was carried out using various alkaline iodide salts, especially cesium iodide with clusters of \( (\text{CsI})_n \text{Cs}^+ \) in positive mode and \( (\text{CsI})_n \text{I}^- \) in negative ion mode.

**Some Applications of Liquid SIMS**

From the start liquid SIMS was used for the analysis of important biological molecules that, due to their polarity or their mass, were not amenable to existing mass spectral methods. This was exemplified in Barber’s original presentation at the Chemical Society Symposium on Soft Ionization Biological Mass...
Spectrometry held at Imperial College, London, in July 1980, which was the first announcement of the FAB technique. Barber’s examples included a glycoside antibiotic (bluensomycin), a dinucleotide, some penicillins, and some peptides, including gastrin with a molecular weight of almost 2000 Da. These examples set the main application areas for liquid SIMS. The development of high-field magnetic sector instruments with mass ranges up to 10,000 u, and fitted with liquid SIMS sources, revolutionized the analysis of peptides and proteins in the 1980s at a time when the young biotechnology industry required these analyses to be done.

Confirmation of the primary structure of peptides and proteins generated using recombinant DNA was rapidly identified as an important application. Indeed, using a magnetic sector instrument, accurate mass measurements could be carried out. In an early case using peak matching and a resolution of 10,000 the mass of a recombinant octapeptide (Ac-thr-glu-phe-ser-glu-leu-lys-OH) was determined as 952.4691. The calculated mass for C_{42}H_{66}N_{9}O_{16}, the protonated molecule, was 952.4628.

For larger engineered proteins a combination of enzymatic digestion and analysis of the mixture of peptides formed was used to confirm the primary structure of engineered proteins. Simple programs were written to predict the expected masses of the peptides formed and any overlaps due to partial cleavages. The technique was termed ‘FAB mapping’ in 1981. It was especially useful in determining whether any point mutations had taken place in the sequence of a recombinant protein or if partial cleavage of N- or C-terminal amino acids had taken place, a phenomenon known as ‘ragged ends’.

In a typical experiment a protein would be subjected to enzymatic digestion using an enzyme such as trypsin, which cleaves the amino acid chain on the C-terminal side of lysine or arginine. This produces a

![Diagram of peptide bond cleavages](image)

**Figure 5** The fragment ions formed by cleavages involving the peptide bond from protonated peptides following ionization using a liquid SIMS source.
mixture of peptides comprising not only the peptide chains between arginine and lysine residues but also lesser amounts of chains comprising two or more residues due to partial cleavage. The probability of two peptides having the same mass is low, so the 'map' produced is an accurate quality control tool for monitoring the presence of the correct components in the protein sequence. If any discrepancies occur, further maps, using different enzymes giving different mixtures of peptides, can be used to identify a point mutation. Additionally, microchemical manipulation such as methylation or acetylation can be carried out on the probe tip to give additional information. In these cases, it is often useful to use a mixed stable isotopically labeled reagent to give a clear marker signal, for example, acetylation with a 1:1 mixture of CH₃CO and C₂H₃CO markers. The mass shift and isotopic pattern immediately highlights the number of added acetate groups for each tryptic peptide. This technique was used in the 1980s for monitoring the quality of recombinant insulins and interferons.

Similar techniques were used for determining the structures of unknown peptides and proteins but in these cases the individual peptides were isolated and the primary sequence determined. The mass spectral fragmentation of peptides occurs principally at the peptide linkages and can give rise to six fragment ion types. A nomenclature for this fragmentation (see Figure 5) was proposed by Roepstorff and Fohlman in 1985, and has been accepted as a standard since. In the early 1980s, many reports appeared describing the primary structures of peptides that had been derived using a combination of liquid SIMS sources and magnetic sector mass spectrometers. Spectra were produced using MS/MS techniques as well as the spectra from single sector analysis. These spectra were marked by a series of ions spaced with the residual mass of the amino acid residue lost and were easy to read. Only two pairs of amino acids had the same residual mass, namely leucine and isoleucine (isomers with residual mass 113), and glutamine and lysine (different formulae but same nominal mass 128). An example is the spectrum of the C-terminal y-ions from α-endorphin generated using an ion-gun and a mixed glycerol/thioglycerol matrix (Figure 6).

The revolution in the use of mass spectrometry was extended to other biopolymers, especially sugars. Most enzymes consist of a protein molecule that is modified by the attachment of polysaccharides. The structure of these sugar residues can be vital for the activity of the enzyme and liquid SIMS was used, often in the negative ion mode, to study these. An example showing the fragmentation of two isomeric sugars is shown in Figure 7.

Although the combination of liquid SIMS and magnetic sector mass spectrometry gave rise to the

**Figure 6** The fragmentation of α-endorphin protonated molecule after FAB ionization (1984; example provided by M-Scan Ltd.).
use of mass spectrometry as, arguably, the most important analytical tool for biochemistry, by itself this technique is almost obsolete. The development of atmospheric pressure electrospray (ESI), then microelectrospray sources, and of analyzers such as orthogonal axis time-of-flight (oa-ToF) has allowed analysis of biopolymers to become a routine in most biology research laboratories. Amenable to the application of sophisticated computing programs to control the whole system, from separation and introduction devices to complex MS/MS experiments, the ESI-oa-ToF combination is capable of automated analyses on very small quantities of material, far exceeding the sensitivity of liquid SIMS.

See also: Mass Spectrometry: Overview; Electrospray; Peptides and Proteins. Surface Analysis: Secondary Ion Mass Spectrometry of Polymers.

Further Reading

Matrix-Assisted Laser Desorption/Ionization

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Introduction

Matrix-assisted laser desorption/ionization (MALDI) is a relatively new ionization technique that is capable of ionizing a large variety of compounds, particularly large proteins, for analysis by mass spectrometry. Its introduction, together with electrospray ionization in the late 1980s, extended the mass range of molecules that could be examined by mass spectrometry into the low megadalton range and provided a new tool that could be used for analysis of both biopolymers and synthetic polymers. The technique is comparatively simple to use; the sample is mixed with an excess of a suitable matrix, usually a solid, and ionized with pulses from a laser (Figure 1). Ion detection is usually, but not exclusively, achieved by use of a time-of-flight (TOF) mass spectrometer. The purpose of the matrix is mainly to dilute the sample and dissipate the laser energy; however, the details of the ionization process are still poorly understood and subject to intensive research. The technique is capable of high-throughput spectral recording and typical target plates, such as those used for proteomics, can accommodate several hundred samples. Since its introduction, MALDI has become one of the major techniques for ionizing organic molecules and has largely replaced older, less sensitive, methods such as fast-atom bombardment. This article briefly reviews the technique and describes applications to several compound types.

Two variations of MALDI mass spectrometry were invented almost simultaneously in the late 1980s. In