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Timing the flight of biomolecules: a personal perspective

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Abstract

Some developments in time-of-flight (TOF) mass spectrometry in the last half century are described. Emphasis is placed on work in the author's laboratory at Manitoba, as well as developments elsewhere that had a significant bearing on it. The account is mainly concerned with TOF techniques, so applications are only considered incidentally. (Int J Mass Spectrom 200 (2000) 597–610) © 2000 Elsevier Science B.V.

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1. The early years: introduction

Time-of-flight mass spectrometry (TOFMS) is now over 50 years old. The technique was first proposed by William E. Stephens (a nuclear physicist at the University of Pennsylvania) in 1946 to take advantage of wartime advances in electronics, which could provide "microsecond pulses . . . every millisecond", and could be well suited to "composition control, rapid analysis, and portable use" [1]. TOF spectrometers have in fact been useful in such applications. However, Stephens failed to anticipate the widespread use of the technique for analysis of biomolecules (not surprisingly), and consequently did not anticipate features of the technique that are now among the most important in biological applications: "unlimited" *m/z* range, and simultaneous observation of the entire *m/z* spectrum without scanning.

Several TOF mass spectrometers based on Stephen's ideas were constructed in the next few years, and a commercial model was introduced by Bendix in the 1950s that achieved considerable popularity. However, the quadrupole mass filter was discovered about the same time, and it cut TOF instruments out of the "low-end" market for which they were deemed most suitable. "High-end" applications were monopolized by sector machines, so by the early 1970s TOF mass spectrometers had been largely relegated to applications in which their rapid time response was important.

Nevertheless, several developments that were to be important in future TOF machines had already occurred in this period, notably the use of a reflecting electrostatic field (the "reflectron") by Mamyrin et al. [2], time-lag focusing by Wiley and McLaren [3], and the first applications of orthogonal injection [4]. However, the advantages of these techniques for TOF measurements were still largely unrecognized, partly because of limitations in the existing ion sources.

Progress in TOFMS in the succeeding years is described in the following. Emphasis is placed on work in the author's laboratory at Manitoba, as well

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as progress elsewhere that had a significant bearing on it. The account is mainly concerned with TOF techniques, so applications are only considered incidentally. Readers wishing to obtain a broader view of the subject may consult the book by Cotter [5], which gives a comprehensive account of the field up to the last few years.

2. TOF milestone I: the discovery of plasma desorption mass spectrometry

2.1. Fission fragment bombardment

The first of several developments that were to bring TOFMS into the mainstream of biomolecular analysis occurred in the mid-1970s, when Ron Macfarlane and his collaborators (nuclear chemists) announced the surprising result that bombarding a thin solid target by fission fragments could produce ions from labile and involatile biomolecules [6,7]; examples are shown in Figs. 1 and 2. They named the technique plasma desorption mass spectrometry (PDMS). The discovery created great interest, especially as the analytes included molecules of masses greater than 1000 Da [8]. This result was the byproduct of an investigation that measured the masses of short-lived nuclides by their flight times, a technique that is well suited to examining individual particles. Therefore it was natural to apply TOF analysis also to the biomolecular ions, particularly since the complementary fission fragment provided a convenient start signal.

Indeed, the discovery of desorption/ionization by fission fragments represented a true milestone in TOFMS. Not only was the phenomenon completely new and unexpected, the methods of measurement that Macfarlane and his collaborators introduced were a departure from conventional practice in mass spectrometry. A schematic diagram of their spectrometer "Maggie" is shown in Fig. 3 [9]. Instead of measuring ion currents, individual ions were observed, as in the nuclear experiments. Microchannel plates were used as detectors, enabling the application of digital time measurements, rather than the analogue methods commonly used previously. Such techniques, using time-to-digital converters, made it possible to view the complete m/z spectrum without scanning, thus greatly increasing the spectrometer efficiency. In addition, ions were produced from a suitably oriented equipotential surface, a procedure that removed variations in flight time caused by an initial spatial spread. The net result was the ability to see molecular ions from biomolecules of masses up to a thousand daltons and more, which had not hitherto been accessible to mass spectrometry. The subsequent introduction of a commercial PDMS instrument by BIO-ION in 1984 made the technique available to more than 50 laboratories across the world.

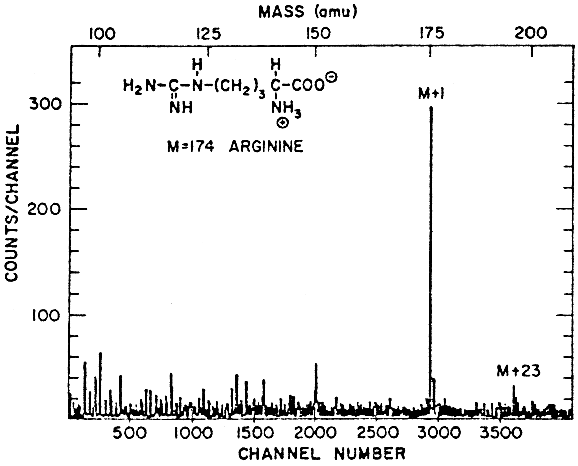

Fig. 1. PDMS spectrum of arginine [6].

2.2. Static secondary ion mass spectrometry

A little later, Benninghoven showed that involatile organic ions could also be desorbed/ionized by bombardment with a low flux of ions in the kiloelectron volt energy range—so-called static secondary ion mass spectrometry (static SIMS) [10,11]. However, Benninghoven measured the secondary ions in a quadrupole mass filter (using a continuous beam), so he was able to report results only for ions of masses up to a few hundred daltons, because of the m/z limitations of this instrument. It was therefore not clear whether or not the method would be effective for

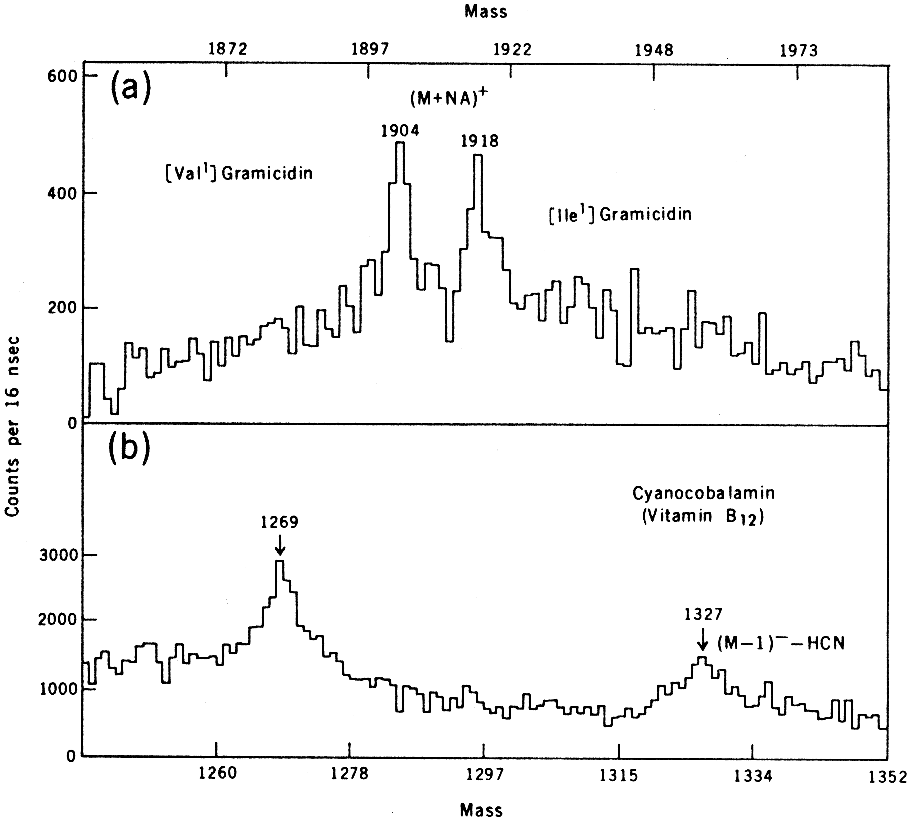

Fig. 2. PDMS spectra of gramicidins and vitamin B12 [8].

the ions of larger mass that had been measured by PDMS.

2.3. The Manitoba laboratory

Time-of-flight mass spectrometry began for me early in 1977. Brian Chait and I (both nuclear physicists) had been working on a method of analyzing grain for total protein (actually nitrogen) content by activating the short-lived isotope of oxygen ^{14}O through a (*p*,*n*) reaction on ^{14}N, but by that time we had realized that it was important to obtain more detailed information about the structure of the proteins. Thus we became interested in the PDMS technique, and I made arrangements to visit Ron Macfarlane's laboratory, where he and his colleagues welcomed me cordially, and succeeded in inoculating me with enthusiasm for PDMS. Neither Brian nor I had any experience in mass spectrometry, but we had done some nuclear TOF measurements, and of course were familiar with the digital techniques that Ron had adapted to TOFMS. Any funds at my disposal were already committed, but we were successful in obtaining a grant of $5300 from our graduate school, and on that rather slim basis we were brave (or foolhardy) enough to start building a TOF mass spectrometer.

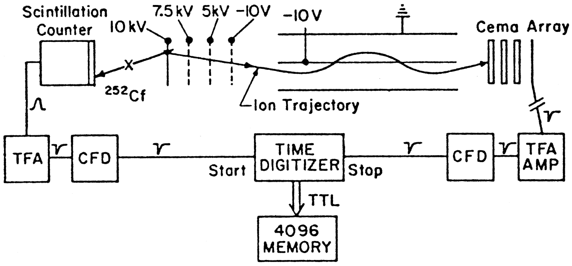

Fig. 3. Schematic diagram of Macfarlane's spectrometer.

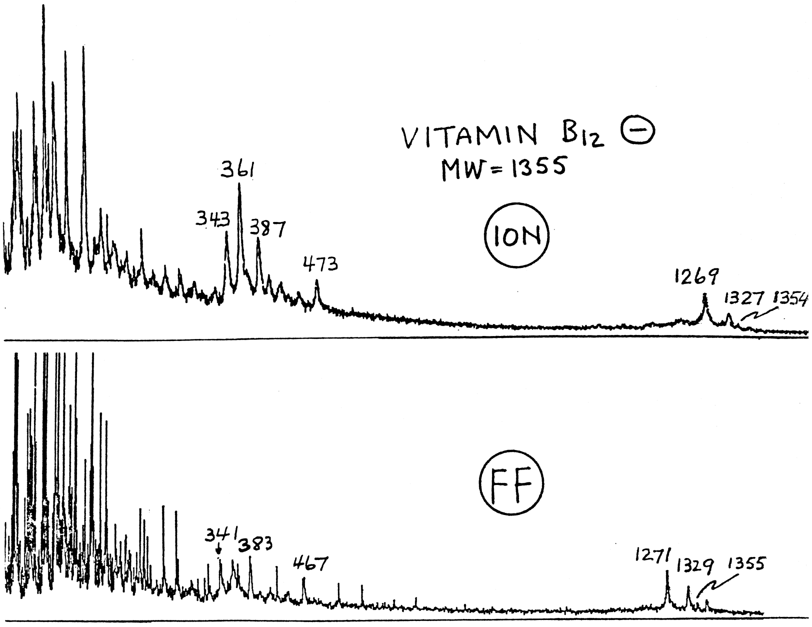

Fig. 4. TOF negative vitamin B12 spectra measured from bombardment by 18 keV Cs^+ ions at Manitoba (top), and fission fragments at Rockefeller (bottom) [14].

We fully intended to follow Macfarlane's example and produce ions by fission fragment bombardment, and we in fact purchased a Cf source. However, during construction of the spectrometer we became aware of Benninghoven's results with SIMS [10,11]. As mentioned previously, it was not clear if his technique would be effective for the larger ions that Macfarlane had observed. Also, his ion source gave a continuous beam, so it was not suited for TOF measurements. Nevertheless, we found the idea of a controllable ion beam compelling, so we decided to construct a pulsed ion source (alkali metal) for use with our new spectrometer. Building the spectrometer and exploiting the TOF-SIMS technique occupied all our attention for the next few years, so the Cf source was never used!

A description of our TOF-SIMS spectrometer was published in 1981 [12], after early results obtained with the instrument on involatile organic molecules had been presented at the Paris [13] and Münster [14] workshops in 1980. In particular, a direct comparison between SIMS and PDMS showed comparable results up to mass ~1000 Da (Fig. 4) [13–15]. Thus at that time it seemed likely that TOF-SIMS would become a junior partner of PDMS, and the two would be the favored methods for the analysis of such "large" molecules.

2.4. Fast atom bombardment

An important discovery by Michael Barber and his collaborators soon upset this assumption [16–18]. This was another SIMS-related technique [(fast atom bombardment (FAB)] whose key element was the use of a matrix (usually glycerol) mixed with the analyte. The use of the matrix minimized the effect of radiation damage on the analyte material, and so made it possible to use much larger bombarding fluxes than static SIMS. The correspondingly larger secondary ion beams were well suited to existing mass analyzers (mainly quadrupoles and sector machines), and most workers saw little reason to adopt TOF methods. Macfarlane has described how much of the interest in PDMS disappeared almost immediately after the introduction of FAB [19]. Indeed, FAB and the related

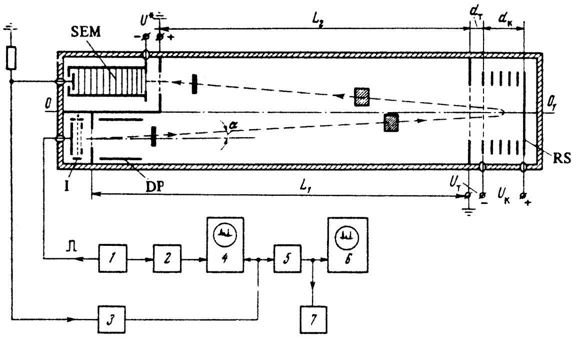

Fig. 5. Mamyrin's spectrometer [2].

liquid-SIMS technique dominated the field for the next few years.

3. TOF milestone II: the electrostatic reflector

In addition to taking over many applications, the introduction of FAB served to inhibit other workers from entering the TOF field, but those already in the field continued to believe that it offered many advantages, and remained active. In particular, the 1980s saw a number of laboratories improve TOF resolution by the use of a reflector[2,20,21], one of the ideas mentioned previously that had been lying fallow for more than 10 years. The original spectrometer built by Mamyrin et al. (Fig. 5) used a two stage reflector [2]. However, the simplest implementation of the concept is a single stage mirror, as shown in the schematic diagram (Fig. 6) of a reflecting instrument built at Manitoba [22]. This instrument has a resolving power ~10 000. In fact a single stage electrostatic mirror corrects the ion initial velocity spread for resolving powers up to about this value, at least when the ions originate from a suitably oriented plane surface. Consequently this reflector configuration has been widely adopted, as well as various two-stage, gridless, and nonlinear geometries [5].

3.1. Metastable decay in the flight tube

The adoption of reflectors stimulated the investigation of the behavior of ions in the flight tube. At first sight it seems surprising that biomolecular ions produced by PDMS or SIMS last even long enough to be observed, because of the apparently violent nature of the process ("like digging potatoes with a nuclear explosion" [23]). Hence it is not surprising that many

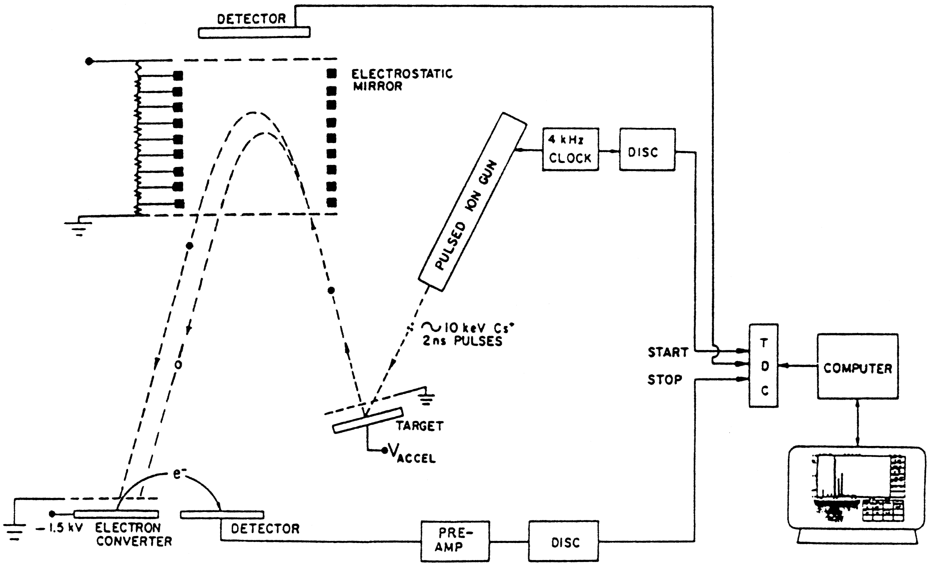

Fig. 6. Manitoba reflecting SIMS-TOF spectrometer [22].

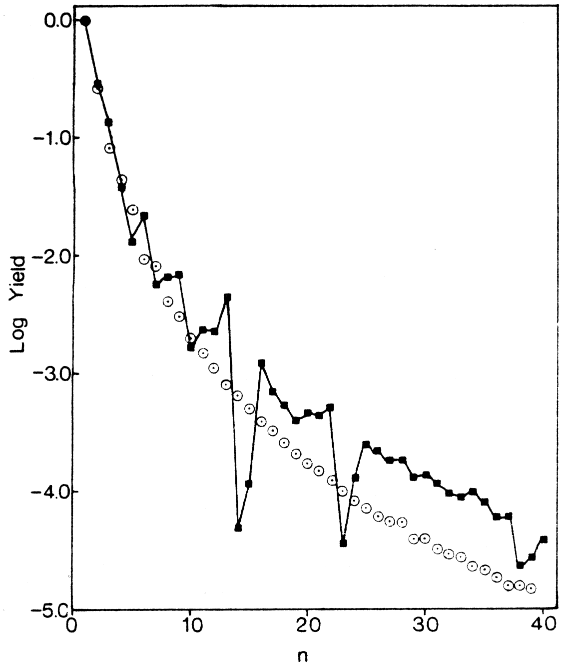

Fig. 7. Relative yields of $[(CsI)_nCs]^+$ ions in a TOF spectrometer (open circles) and in a sector instrument (filled circles) [25].

ions acquire enough excitation to suffer metastable decay as they pass through the spectrometer [12,24].

A particularly graphic example of the phenomenon is provided by the spectrum of CsI cluster decay shown in Fig. 7 [25]. This case illustrates an interesting property of TOF spectrometers—as a result of conservation of momentum, both neutral and charged products of the decay arrive at a detector at the end of the first leg of the flight path (e.g. the upper detector in Fig. 6) at approximately the same time as the parent ion would have arrived. Some spread is introduced by the energy liberated in the decay, so the TOF spectrum of the products in this detector is a broadened version of the parent ion spectrum. On the other hand, the daughter ion spectrum after reflection is quite different—the total flight time for daughter ions depends on the time they spend in the mirror, which in turn depends on their energy, and consequently on their mass [22]. Thus the total flight time of a daughter ion determines its mass.

Della Negra and LeBeyec realized that this property provides a means of determining the structure of

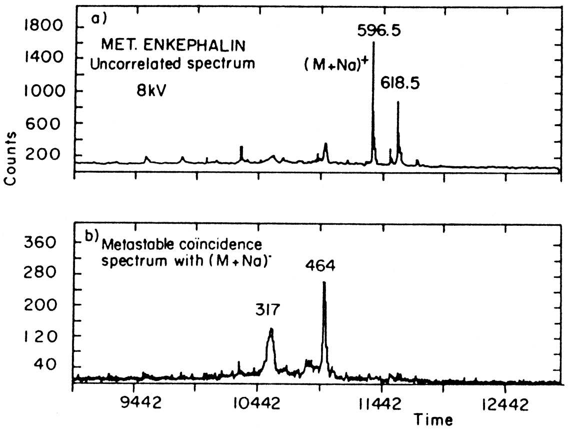

Fig. 8. (a) Uncorrelated spectrum of met-enkephalin. (b) Daughter ion spectrum correlated with neutrals from $(M + Na)^+$ parent ion decay [27].

the parent ion without using a tandem spectrometer [26,27]. The arrival time of the neutral fragment in the first detector defines the parent ion (with somewhat limited resolving power, usually <100), whereas the time of arrival of the daughter ion in the second detector measures the daughter ion mass. Fig. 8 shows an early example [27]. This “correlated reflex” technique can also be applied to SIMS measurements, provided the counting rate is not too high [28,29]. In this method it is important to realize that the optimum mirror voltage for observing the parent ion is no longer optimum for the daughter ions, and must be set separately for each daughter ion mass [22,29]. A simple example of the improvement in the resolution that is obtainable by using the correct voltage setting is shown in Fig. 9 [29].

4. TOF milestone III: matrix-assisted desorption/ionization

The breakthrough that finally brought TOF mass analyzers into the MS mainstream was the discovery of matrix-assisted desorption/ionization (MALDI) in 1987 and 1988. Laser desorption was already well known, particularly for microanalysis [5], but it had not been successful in producing ions from molecules larger than a few hundred daltons. It was the discov-

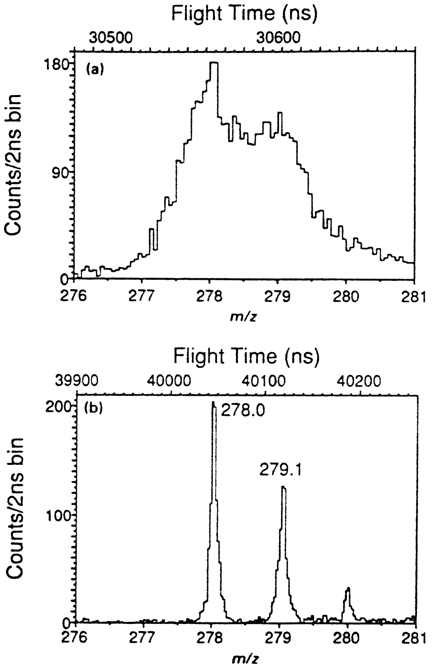

Fig. 9. Mass spectra of daughter ions of *m/z* 278/279 from decay of $(M + H)^+$ parent ions of leucine-enkephalin: (a) with the mirror optimized for the parent ion; (b) with the mirror optimized for the daughter ions [29].

ery of suitable matrices by Karas, Hillenkamp, and co-workers that transformed the field. These workers had obtained encouraging results from the use of nicotinic acid as a matrix for examining relatively low mass analytes [30], and were stimulated to try the same technique at higher masses by the experiments of Tanaka et al. [31], news of which was brought to the IFOS IV meeting in Münster in September 1987; (Tanaka's method also used a matrix—ultrafine Co particles in glycerol—but that technique has not been widely adopted). Even the first measurements of Karas, Hillenkamp, and co-workers, reported at the Bordeaux International Conference in August/September 1988 were strikingly successful [32], as shown in Fig. 10. At last it was possible to examine analytes of mass 100 000 Da and beyond by mass spectrometry [33], and the technique was equally effective for biomolecules of lower mass.

Consequently the MALDI-TOF technique immediately captured the imagination of mass spectrometrists and brought TOF to the forefront of current MS research. The next few years saw an explosion of activity in the field, aided considerably by the availability of a number of commercial instruments. In addition to many applications, particularly to proteins

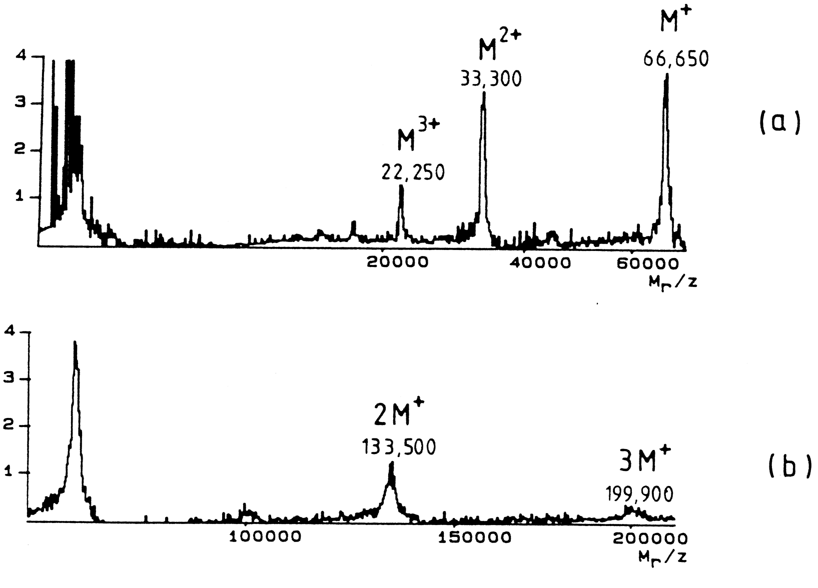

Fig. 10. MALDI spectra of bovine albumin: a single shot; b 20 accumulated spectra for high mass range [32].

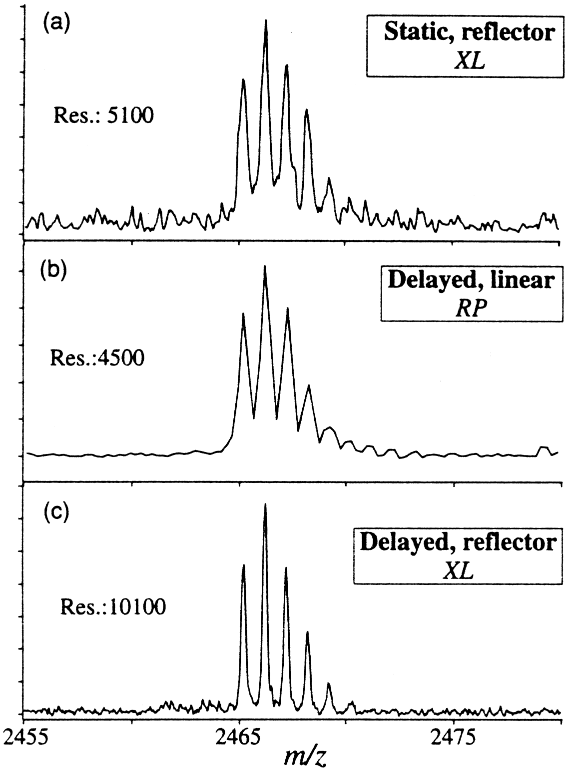

Fig. 11. Mass spectra of ACTH clip (18–39) MH^+ ion (2465.2 Da) obtained with PerSeptive spectrometers of various effective flight paths in static and delayed reflection modes: (a) static reflector XL (6.6 m); (b) delayed linear RP (1.3 m); delayed reflector XL (6.6 m) [36].

and peptides, there were a number of investigations that explored the fundamental properties of the MALDI process.

4.1. Delayed extraction

A significant development in MALDI came a few years later, with the rediscovery [34,35] of the benefits of the delayed extraction technique of Wiley and McLaren [3] (the second relic of the 1950s mentioned previously). The technique yields considerable improvement in MALDI resolution, particularly when ions are produced on a suitable oriented equipotential surface. Fig. 11 shows an example [36].

4.2. Postsource decay

Many ions produced by MALDI suffer metastable decay as they pass down the flight tube, just like those desorbed by PDMS or SIMS, and the ions may also be broken up by collisions in that region. Thus a daughter ion spectrum can be obtained by examining the overall flight time spectrum after reflection, as in the correlated reflex technique described above. However, in this case it is not possible to identify the parent ion by observation of a correlated neutral product, because MALDI produces a very large number of ions in each individual pulse, and no individual neutral can be singled out as the correlated partner of a given daughter ion. Thus it is necessary to apply the more straightforward method of selecting the parent ion before it decays by insertion of a “gate” part way down the first leg of the flight tube [37–39]. A resolving power for parent ion selection up to a few hundred has been obtained with a single gate, and dual gates in series have more recently been developed to give a net resolving power ~1000 or more [40, 41].

As mentioned previously (see Fig. 9), the voltage on the mirror should be separately adjusted for each mass to obtain optimum results. Kaufmann and Spengler have made the procedure more systematic by stitching together a succession of spectra, each of which is optimized for a given mass, and have called the overall phenomenon “postsource decay,” to distinguish events taking place in the first leg of the flight path from those occurring earlier in the ion source [37–39]. This method has been extensively applied for measurements of molecular structure in the last few years.

5. TOF milestone IV: orthogonal injection

5.1. TOF with electrospray

The second ionization method that has revolutionized the analysis of biomolecules in recent years is electrospray ionization (ESI) [42], developed independently by Fenn and co-workers [43], and by a group at St. Petersburg [44]. In contrast with MALDI, ESI produces a continuous beam of ions. Like other continuous ion sources, it is most compatible with mass spectrometers that operate in a similarly continuous fashion, such as quadrupole mass filters. The combination of an ESI source and a quadrupole mass filter has become a popular and satisfactory configuration, but it has some defects. First, most commercial quadrupoles are restricted to *m/z* values below ~4000. Although this is not a problem when examining electrosprayed ions from most species because of the high charge states usually produced, some entities, notably noncovalent complexes, may have *m/z* values up to 10 000 or more. Second, the quadrupole mass filter is a scanning device; it examines ion species in the spectrum one at a time, which implies a reciprocal relation between resolution and sensitivity. Thus an increase in sensitivity requires a decrease in resolution—a significant handicap in cases where the whole mass spectrum must be examined from a limited amount of sample.

The above remarks suggest that coupling an ESI source to a TOF spectrometer might overcome the problems just mentioned. However, such a configuration has its own problems, since there are difficulties in coupling any continuous source to a TOF instrument. The most straightforward technique is to inject ions along the spectrometer axis (the *z* axis), but this procedure involves a tremendous loss in sensitivity. However, it is fortunate that TOF instruments can tolerate a relatively large spatial or velocity spread in a plane perpendicular to the spectrometer axis, as witnessed by the large sources ($\lesssim$1 cm in diameter) typically used in PDMS. This tolerance can be exploited by injecting electrospray ions into the TOF instrument perpendicular to the *z* axis, i.e. "orthogonal injection." Such a geometry provides a high efficiency interface for transferring ions from a continuous beam to a pulsed mode. Another advantage is the small velocity spread in the *z* direction that is usually observed, making high resolution easier to obtain.

Orthogonal injection, mentioned earlier, was another "lost" technique, this one dating from the 1960s. In 1964 O'Halloran et al. at the Bendix Corporation reported the first use of orthogonal injection into a TOF instrument, in their case injection from an atmospheric-pressure plasma ion source [4]. A schematic diagram of their apparatus (Fig. 12) clearly shows the main features of the method, but the report was evidently unknown to most workers, so the technique was independently reinvented by a number of groups 20 or more years later, using various types of continuous ion source. Dodonov and co-workers were the first to couple an ESI source to an orthogonal injection TOF instrument, which made it particularly useful for observation of biomolecules, and also the first to use a reflecting geometry in such a device, which improved the resolution [45–48]. Fig. 13 shows their instrument, together with a spectrum of multiply charged ions of insulin. These results were first reported in 1987 [45], but were not widely known until Dodonov's report at the Amsterdam mass spectrometry conference [46], and his subsequent publications [47,48]. By this time there was some awareness of orthogonal injection, particularly after a paper by Dawson and Guilhaus appeared describing plans for constructing an orthogonal injection instrument

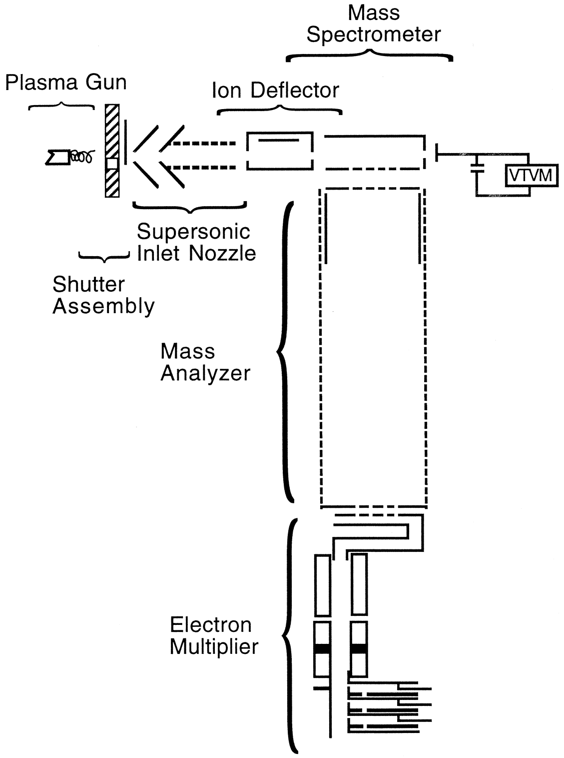

Fig. 12. Orthogonal injection TOF spectrometer used by O'Halloran et al. in 1964 [4].

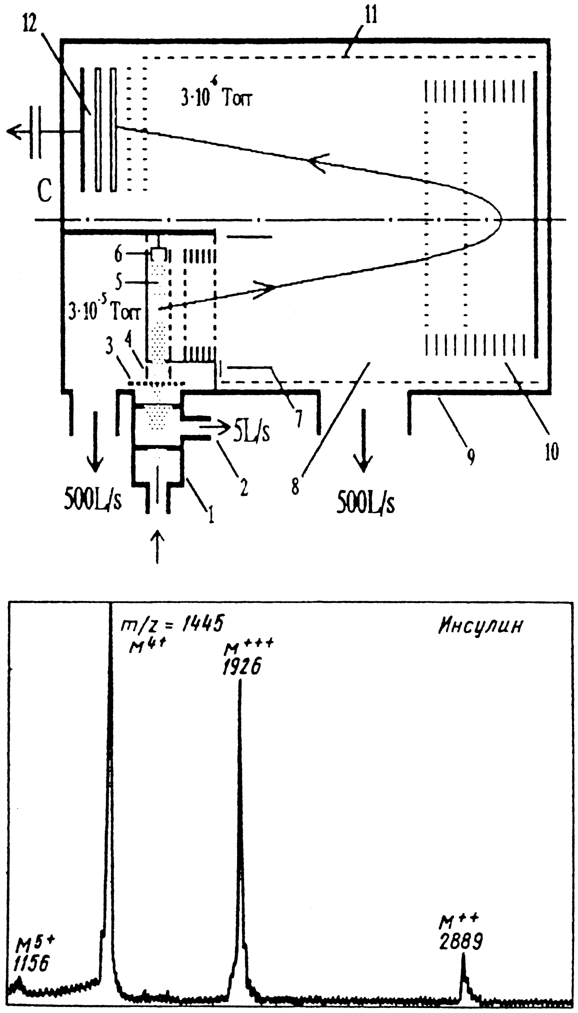

Fig. 13. (Top) Schematic diagram of the reflecting TOF mass spectrometer with orthogonally injected electrosprayed ions developed by Dodonov et al. [48]. (Bottom) The first spectrum of multiply charged ions from bovine insulin obtained with the instrument [45].

using electron ionization [49]. However, it was Dodonov's Amsterdam report that kindled our interest in the technique for the study of large molecules.

At this time we were fortunate to have Anatoli Verentchikov in our laboratory at Manitoba, since he had worked with the Russian electrospray group at St Petersburg. We also had a reflecting TOF instrument similar to the one shown in Fig. 6, which was not currently being used. We therefore decided to convert this spectrometer to an orthogonal injection instrument with an electrospray source. Within a few months (by January 1993) Anatoli had the machine running and was making significant measurements [50]. Since then it has been in constant use, especially for studies of noncovalent complexes [51].

5.2. Collisional cooling

Until recently ions have usually been introduced into the TOF spectrometer more or less directly from the ion source through an interface that only provided collimation to define the beam shape, and differential pumping to transfer the beam from the source pressure to high vacuum. As a result the performance of the instrument has been limited by the characteristics of the ion beam produced by the source. As the ions usually diverge from the source, significant losses due to collimation are necessary to produce the narrow beam cross section required for high resolution, or else resolution may have to be sacrificed for sensitivity. A second limitation of direct introduction comes from the velocity distribution of the ions in the direction of injection, which may lead to significant *m/z* discrimination.

It is possible to overcome these limitations by collisional damping of ions in a rf-quadrupole ion guide at relatively high pressure (0.01–1 Torr), as introduced by Douglas and French [52] and Xu et al. [53] for quadrupole mass spectrometers. The rf field in the quadrupole focuses ions onto its axis, whereas collisions with the molecules of the ambient gas reduce the ion velocities to near-thermal values [54]. Thus the beam leaving the ion guide has a small spatial spread in the *z*direction, and small velocity spreads in both *y*and *z*directions, and these properties are almost independent of the original parameters of the beam delivered by the source. This leads to improved performance. Indeed, a collisional cooling ion guide added to the Manitoba ESI-TOF instrument just described reduced the *m/z* discrimination in that instrument significantly, and improved both sensitivity and resolution [54]. The instrument then yielded a resolving power ($M/\Delta M_{\mathrm{FWHM}}$) between 8000 and 10000 for peptides with masses 1000–6000 Da. This resolution is sufficient to distinguish separate isotopic peaks for most peptides, and hence to determine the individual charge states. Such a capability is particularly useful when interpreting complicated spectra from mixtures such as tryptic digests, or spectra resulting from fragmentation of multiply charged ions.

5.3. Orthogonal injection of MALDI ions

TOF spectrometers have always been favored for measuring MALDI ions because of the pulsed nature of the lasers normally used for ion production. In the usual geometry, ions are ejected from the target along the axis of the TOF instrument normal to the sample surface, and the flight-time measurement is correlated to the laser pulse (or to the extraction pulse if delayed extraction is used. Although this configuration has been highly successful, it does have limitations, such as peak broadening from the ion energy spread, and difficulties in obtaining accurate calibrations. Optimum focusing conditions depend on laser fluency, the sample matrix, the sample preparation method, and even the location of the laser spot on the sample. Moreover, it is not possible to use long-pulse lasers without a corresponding deterioration in resolution.

Orthogonal injection of MALDI ions into a TOF spectrometer could, in principle, solve some of these problems, but orthogonal injection without collisional cooling is extremely inefficient because of the large momentum spread of the MALDI ions. As mentioned above, collisional cooling gives considerable benefits even for electrosprayed ions, but it appears that such damping is much more important to obtain optimum results with MALDI, again because of the large momentum spread.

In addition, collisional cooling has a property that is uniquely important for injection of an initially pulsed beam into a TOF instrument. Collisions in the ion guide spread out the ion pulse along the quadrupole axis, transforming the pulsed beam into a quasi-continuous beam, as illustrated in Fig. 14 [55]. The beam then behaves just like an electrosprayed beam. In this case the start time for the TOF measurement is no longer correlated with the laser pulse, so the beam can be injected into the spectrometer at a high repetition rate (up to 10 kHz) as in electrospray, even with a laser running at 20 Hz. Effects of peak saturation are removed, and the count rates are compatible with pulse-counting methods using a time-to-digital converter. Installation of a MALDI source on the TOF instrument of Fig. 6 gave encouraging results [55], and paved the way for installation of a MALDI source on a QqTOF spectrometer, as described in the following.

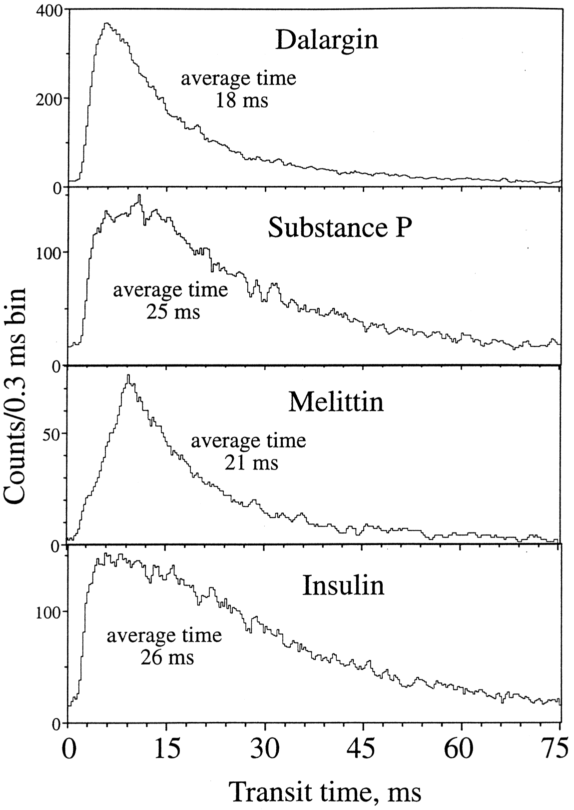

Fig. 14. Distribution of transit times through the collisional damping interface for different MALDI ions [55].

5.4. Tandem instruments

Tandem MS (MS/MS) has become a valuable means for determining molecular structure. In a true tandem measurement the parent ion is selected in one mass spectrometer, and the daughter ions are measured in a second spectrometer after breakup of the

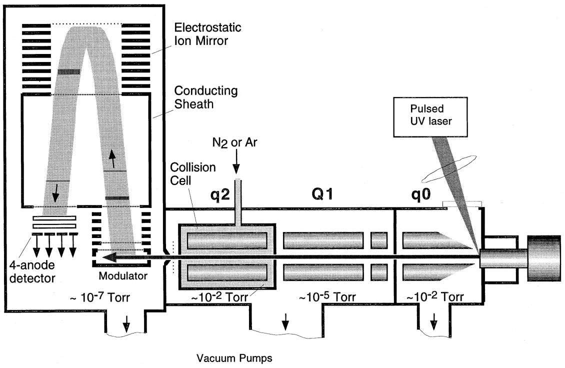

Fig. 15. Schematic diagram of the MALDI QqTOF mass spectrometer [60].

parent ion, usually in a collision cell. Similar information can be obtained in a reflecting TOF instrument (“a poor man’s tandem” [5]), by the correlated reflex and the PSD methods described previously, but these methods are limited in resolution, mass accuracy, and speed. They also lack the control over collision conditions that is obtainable in a true tandem instrument, and the results tend to be difficult to interpret.

At present the most popular tandem instrument is the triple quadrupole QqQ, in which both mass analyzers, designated Q, are quadrupole mass filters, and the collision cell lies within a rf quadrupole, designated q. A limitation of this configuration is the need to scan the daughter ion mass spectrum in the final quadrupole, which considerably reduces either sensitivity or resolution. This suggests that it would be worthwhile to replace the final quadrupole by a TOF spectrometer, in which case the entire daughter ion spectrum can be measured in parallel.

The first instrument of this type was reported by Glish et al. [56,57], but the ions were injected into the TOF spectrometer along the axis, so its performance was limited. Again, orthogonal injection provides a remedy for the problem. Recently two such tandem spectrometers with orthogonal injection of electrosprayed ions have been reported. One has the collision cell in a rf hexapole (QhTOF) [58], and the other has the cell in a rf quadrupole (QqTOF) [59]. Commercial versions of both instruments are now available, and several hundred are in the field, so the instruments have already been remarkably successful.

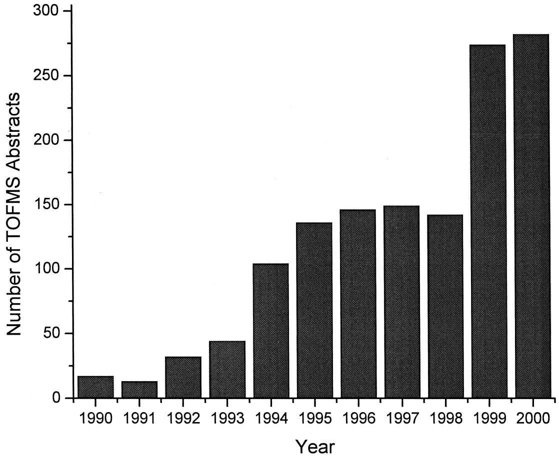

Fig. 16. Number of TOF articles presented at the Annual Conferences, American Society for Mass Spectrometry1990–2000. Results for the years 1999 and 2000 were obtained by scanning the full text. Results for earlier years were obtained from the keyword index, so they should probably be increased by a factor ~1.7 (according to our rough estimates). Thus the increase from 1998 to 1999 is likely much less than it appears.

The QqTOF spectrometer has also been interfaced to a MALDI source [60], and this configuration is shown in Fig. 15. It consists of the MALDI source, three quadrupoles (q0, Q1, and q2) followed by the TOF spectrometer. The additional quadrupole q0 is used for collisional cooling of the MALDI ions, so it is always operated in the rf only made, as is the collision cell q2. This instrument appears to be well suited for high throughput structural measurements [61], and seems likely to be as successful as the ESI version. Even more benefits can be expected from such instruments that allow both ESI and MALDI measurements, with easy changeover between them [55,62].

Progress in orthogonal injection has recently been covered in two review articles [63,64]. Other hybrid tandem spectrometers with TOF components have also been reported—a sector-TOF combination [65], again with orthogonal injection, and a TOF–TOF instrument [66]—so the field is still evolving, and the comparative merits of the various configurations must still be explored.

6. Time of flight at age ~50

A rough idea of the popularity of the technique may be gained by examining the variation with time of the number of TOF articles presented at the Annual Conferences of the American Society for Mass Spectrometry. This is shown in Fig. 16. Clearly TOFMS is alive and well. It will be interesting to see how the technique fares in the next 50 years.

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