
A SOFT TOUCH

New developments in mass spectrometry allow the 'weighing' of larger, more complicated molecules

BY IVARS PETERSON

A fast-moving argon atom plows into a mixture of large organic molecules floating in glycerol, like a stone striking a pond covered with autumn leaves. Unlike the pond leaves, however, one of the organic molecules occasionally flies off, now charged, to be nudged and focused by electric and magnetic fields into a detector.

This method of creating molecular ions, called fast atom bombardment (FAB), is one of several developments that are exciting analytical chemists who work with mass spectrometers. With FAB, they can identify substances, analyze mixtures and find the structures of high-molecular-weight compounds hitherto beyond the capabilities of conventional mass spectrometric techniques.

Fred W. McLafferty of Cornell University's chemistry department says, "FAB has really earned a special place, in part because it's such a convenient technique. Even more promising is that there's a great deal of momentum in research in this area."

The FAB method was first studied by Michael Barber and his associates at the University of Manchester Institute of Science and Technology in England. In the Sept. 24 NATURE, Barber and his group discuss the method and present preliminary results and spectra for compounds including peptides, glycoside antibiotics and vitamin B₁₂. His group was the first to obtain a mass spectrum of the coenzyme of vitamin B₁₂.

A mass spectrometer is essentially a sophisticated weighing machine. A source produces ions, which are then separated according to their charge-to-mass ratios. For a given charge, ions of different mass are deflected to different degrees. The technique's basic principles date back to the turn of the century and J.J. Thomson's experiments into the nature and structure of atomic particles.

In 1913, Thomson predicted, "There are many problems in Chemistry which could be solved with far greater ease by this than by any other method. The method is surprisingly sensitive — more so even than that of Spectrum Analysis — requires an infinitesimal amount of material, and does not require this to be specially purified."

Yet, it was not until the early 1940s, and the need to analyze mixtures of hydrocarbons from the output of catalytic crackers in the petroleum industry, that chemists began to use the technique analytically. Now, mass spectrometers are used in almost all analytical laboratories with applications including the tracking of environmental pollutants and, in forensic science, the detection of drugs and the characterization of paints and fibers.

Henry M. Fales, chemistry laboratory chief at the National Heart, Lung and Blood Institute, says, "We think of the mass spectrometer as a first line of analysis in organic chemistry. When we have an unknown sample, the first thing we do is try to get a mass spectrum. It doesn't take much sample. It's quick and easy."

Initially, mass spectrometry was limited to molecules that were volatile and thermally stable. In the traditional electron impact method, the compound under study enters the ion source as a gas, where it is bombarded with electrons. The positive ions created are accelerated out of this source into a high-vacuum region. Here, the stream of ions is deflected and focused by a combination of electric and magnetic fields. The ions, according to their masses, fall on photographic or electronic detectors producing a mass spectrum of the material. The organic compound ethane (C₂H₆), for example, may form a number of fragment ions, including CH⁺, C₂H₅⁺, CH₂⁺ and others, each of which would have a specific place in the mass spectrum.

The electron impact technique, however, is like battering a watch with a

sledgehammer. It works for small, simple molecules, but large, delicate molecules tend to shatter into a confusing array of pieces. This is the problem that the two most important recent developments are aimed at solving.

One significant breakthrough, about 12 years ago, was the use of the first of a series of "soft" ionization techniques. This method, field desorption (FD), became a powerful tool for analyzing high-molecular-weight, nonvolatile chemicals.

Field desorption requires careful sample preparation. The analyst dips the "emitter," consisting of a fine tungsten wire, much thinner than a hair and bushy with carbon needles, into a solution of the compound to be analyzed. After the solvent evaporates, the emitter is placed into a special ion source. A high electric field ionizes the molecules and draws them away from the emitter. When this method is used, the initial molecular ions do not fragment very often so the spectra show strong peaks for the molecular ions and generally only traces of fragment ions. Among its many applications are the analysis of chemical mixtures, such as the products from the pyrolysis of deoxyribonucleic acid (DNA), and the detection of impurities in commercial polymers.

However, FD does not work for many biologically interesting compounds. Researchers are still puzzled why some compounds will desorb and others will not. Another problem is that preparing samples is mechanically difficult, and the technique is not suitable for all mass spectrometers.

In 1974, while doing research in nuclear chemistry, Ronald D. Macfarlane at Texas A&M University in College Station, Tex., stumbled upon another potential soft ionization technique. He was interested in seeing what happened when high-energy fission fragments from radioactive nuclei (²⁵²Cf) banged into the back of a thin metal

film. He expected to see ions of the metal. Instead, he detected high-molecular-weight organic ions from contaminants on the metal surface. In later experiments, by depositing organic matter on the metal film, he was able to get intact molecular ions of complex biological materials that would decompose even on steam baths.

Macfarlane says his discovery stimulated a great deal of activity among many researchers to find other means of desorbing large organic molecules following the same general principle but without using radioactive sources. Within a few years, researchers adapted two older techniques (secondary ion mass spectrometry and laser desorption) to organic molecules and, about a year ago, developed FAB.

In all the methods, a beam of particles or light strikes a surface coated with the material of interest. Given the large beam energies involved, scientists expected the impact to demolish any organic molecules present. Instead, they found the impact sends molecular ions flying from the surface. Several research groups are trying to unravel the mysterious process that results in the emission of the large ions.

Macfarlane thinks a theoretical model, recently developed by C. D. Claussen and R. H. Ritchie, gives a nice analytical representation of what is going on in his method. The fission fragments deposit a lot of energy into the film to generate a hot spot where a plasma is formed. It reaches temperatures of 10,000K for periods of a ten trillionth of a second. During that time, fast chemical reactions take place, and some of the charged products fly off as intact molecular or fragment ions. "It's effectively a high-temperature, short-time-domain chemistry that is taking place at the surface," says Macfarlane.

All the methods seem to give similar mass spectra. The main difference is in the yields of the big ions. "We think that these methods are all somehow related," says Fales. "This seems crazy because they

ought not to be. The californium system, for example, uses million-electron-volt particles of high mass while the FAB method uses thousand-electron-volt particles." The lower energy particles plow into surfaces and transfer their energy by elastic collisions between nuclei. The higher energy particles lose their energy through electronic excitation.

Robert J. Cotter, manager of the Middle Atlantic Mass Spectrometry Laboratory in Baltimore, is one of the many researchers trying to figure out what is going on. "We think that there's a possibility that the desorption methods have a temperature base to them as well," says Cotter. "The impact rattles the lattice, and thermal effects may actually cause the overcoming of the energy barrier."

Fales says, "The FAB system is especially attractive because it can be applied to all the ordinary mass spectrometers that are in use in the field." This is not true of other ion sources, like the californium system.

"There is a remarkable uniformity of feeling that all the methods are probably going to be useful in the end," says Fales. "Some are more complicated than others in one way but simpler in another way. At the moment, I would say it is unwise for anyone to claim that their system is the best."

Meanwhile, at the University of Illinois, Kelsey D. Cook is continuing work begun by Charles A. Evans Jr. on an even softer ionization method. His system, called electrohydrodynamic ionization, uses high electric fields to draw ions out of a solution contained in a fine capillary tube directly into the high vacuum of the mass spectrometer.

Cook used polyethylene glycol dissolved in glycerol for his early experiments. Because the polymer was not ionic in solution, he added sodium iodide to the glycerol. "We were able to obtain mass spectra for ions that were the product of associations between polyethylene glycol

and sodium," Cook says. "Furthermore, the mass spectra we obtained looked like molecular weight distributions for synthetic polymers."

Cook believes he has found a useful way of characterizing polymers and for studying solution chemistry. "We can do some things that FD and other mass spectrometric techniques can't touch," he says. "I expect that eventually we will be able to demonstrate to everyone's satisfaction that the method really ought to be used in more places than here at this lab and in one place in Russia."

McLafferty predicts, "There is a very good chance that we'll see in the next few years even more techniques that will take us to even higher molecular weights."

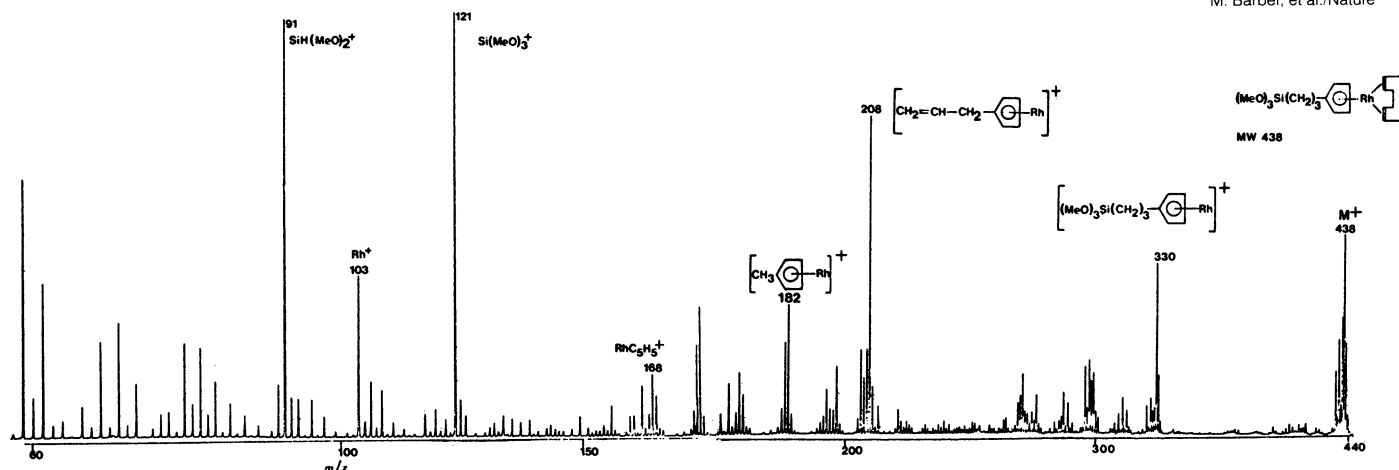
While new ionization methods are attracting attention, there is a second exciting and promising development in mass spectrometry, particularly for finding targeted compounds in complex mixtures.

The method is tandem mass spectrometry, the use of two mass spectrometers in series. Out of all the ions present in the first spectrometer, the analyst can select a particular mass and send those ions into a second mass spectrometer. Thus, an analyst can look for the presence of specific environmental pollutants or other components in a variety of samples.

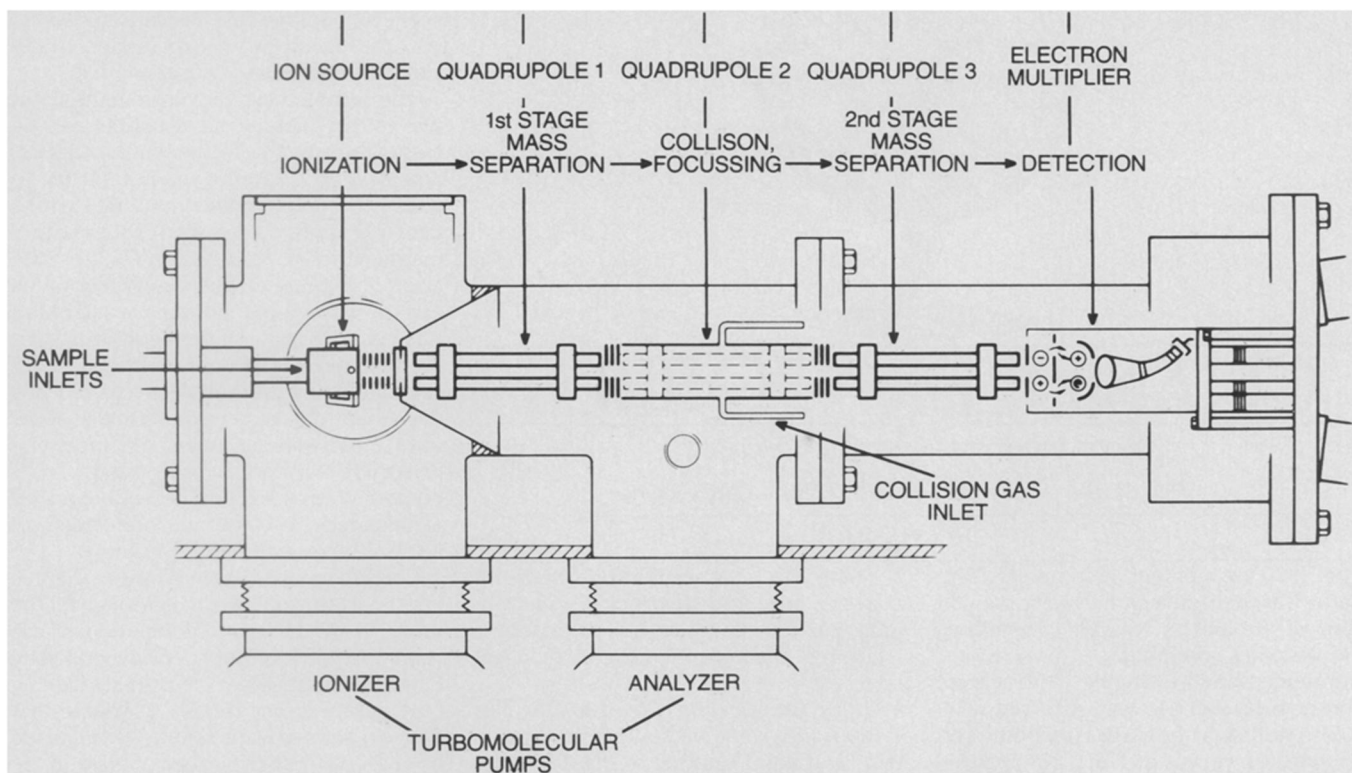
McLafferty is confident that as the cost of the instrument goes down, it will be possible to run routine analyses such as blood and urine screening in laboratories and hospitals. "Mass spectrometers are so specific and so fast. They can do a variety of analytical tasks," says McLafferty.

McLafferty is also excited about the possibility of using tandem mass spectrometry for determining the structures of complicated molecules. "Using the second mass spectrometer to find the structure of the fragments that separated in the first mass spectrometer gives us an even better chance of finding the structures of large molecules," says McLafferty.

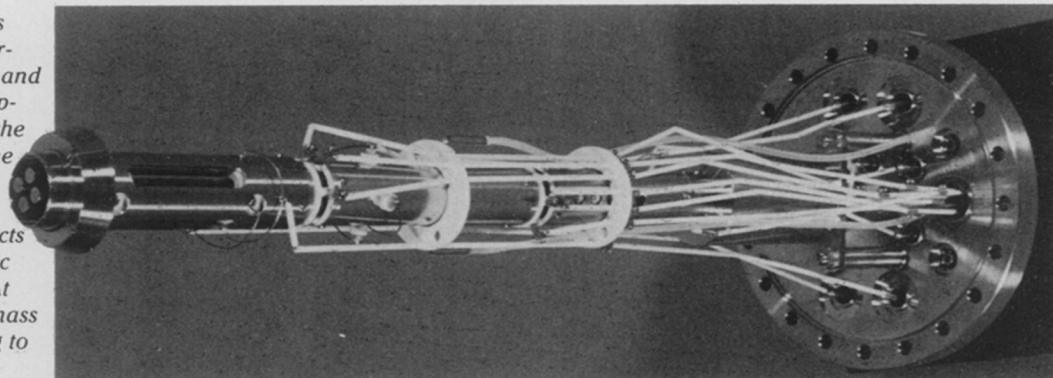
M. Barber, et al./Nature



Positive ion FAB mass spectrum of a water-sensitive, thermally unstable rhodium complex. This molecule gives no mass spectrum of any worth with conventional methods.



Schematic of a tandem mass spectrometer, above. The targeted compound is ionized, and its characteristic ions are separated from most others in the mixture in the first stage. The selected ions are then decomposed by collision, and the final mass analyzer selects secondary ions characteristic of the targeted compound. At right, the inner core of the mass spectrometer, corresponding to the schematic diagram.



Finnigan MAT

The structures of some large molecules are relatively simple to deduce, even when only one mass spectrometer is used. One example is determining the sequence of amino acids in a peptide. Cleavages occur at the peptide bonds, so the differences in mass between the main peaks in the mass spectrum correspond to the masses of individual amino acids. There are only 20 amino acids and 19 different masses.

Say there is a peak at 58 mass units away from the molecular ion peak. This means the first amino acid in the sequence is glycine. If a second peak is 58 mass units further away, then the second is glycine, too. If it's 72 mass units away, instead of 58, then it's alanine.

The actual situation is not quite this simple, but researchers have had success in finding amino acid sequences.

In the case of steroids, for example, the fragments are not as simple as the 20

possible amino acids in peptides. A second mass spectrometer is needed to characterize the structure of the fragment first. Then, it is possible to put together the entire structure from all the pieces of evidence. However, the technique has yet to be applied to a steroid of unknown structure.

The computer also plays an important role in mass spectrometry. "The mass spectrometer turns out so much more information than most other analytical methods," says McLafferty. "Until the computer came along to handle the data, you not only couldn't train enough people, but also these people didn't have enough hours in the day to do the analysis."

One of McLafferty's projects is to increase the speed of computer searches so that researchers have a better chance of matching an unknown spectrum against a file containing as many as 40,000 spectra.

This will make it much easier for investigators to use the information available in mass spectra, he says.

The problem with mass spectrometry is that it costs too much, and more things can go wrong than with most other instruments, says McLafferty. Mass spectrometry must become more reliable and less expensive in the future.

Despite these difficulties, mass spectrometry has proved to be very useful. Fales says some fields are particularly well suited to the technique. The study of insect pheromones, for example, was crawling along at a bug's pace until entomologists discovered the mass spectrometer. Now, they've had to add several new journals to cover all the data that are coming out.

As new techniques like FAB develop, more and more applications of mass spectrometry become possible. □