Mass Spectroscopy: Adaptation for Nonvolatile Samples

Mass spectroscopy, a powerful tool for the determination of molecular structure, can generally be used only when the substance to be examined can be vaporized for ionization by electron impact or other techniques. It can be used only with great difficulty—or it cannot be used at all—for certain types of compounds that are inherently nonvolatile. Such compounds include nucleotides, nucleosides, polysaccharides, neurotoxins, and polypeptides containing polar amino acids.

Spectra of nonvolatile compounds can sometimes be obtained by converting them into volatile derivatives. Amino acids and sugars, for example, are often made more volatile by the attachment of silicon-containing moieties. In many cases, however, there is not enough of the compound available for making such derivatives. In other cases, derivatization produces undesirable or undiscernible rearrangements in the substance to be examined. And in still other cases, suitably volatile derivatives simply cannot be made. The full potential of mass spectroscopy has thus not been achieved for many applications, particularly the sequencing of polymers.

A new technique for volatilization of such materials may enable mass spectroscopy to fulfill more of that potential. The technique, developed by Ronald D. Macfarlane and David F. Torgerson of Texas A & M University, involves a unique application of nuclear chemistry to overcome the limitations of mass spectroscopy. In the technique, fission fragments from californium-252 are used for volatilization and chemical ionization of the substance to be examined.

In their system, the sample is deposited on a thin (10^{-3} millimeter) sheet of nickel foil that is then placed close to a small amount of californium-252 (Fig. 1). Fission fragments (a mixture of elemental ions) from the californium pass through the nickel foil, creating localized temperatures of 20,000° to 30,000°C for about 10^{-11} second. The high temperatures vaporize ion impurities in both the sample and the nickel, principally hydrogen, sodium, and hydride ions. These secondary ions react with heated sample molecules to produce quasimolecular ions (ions whose molecular weight is equal to that of the sample plus that of the secondary ion) that are accelerated by an electric potential into an 8-meter tube leading to an ion detector. At a constant electric potential, the time of flight through the tube is then proportional to the mass of the ion. Each fission of a californium-252 atom produces a complementary fission fragment that is detected by a scintillation counter located on the opposite side of the source from the sample. Detection of the complementary particle establishes the starting time for the time of flight measurement. The complete system, Macfarlane says, can determine masses as accurately as commercial high-resolution mass spectrometers.

Unstable Amino Acids

The potential of the new technique can be illustrated by its application to the nonvolatile, thermally unstable amino acids arginine and cystine. $(M + 1)^+$ quasimolecular ions—where M is the mass of the sample—cannot be obtained for these amino acids with conventional mass spectroscopic techniques. More sophisticated techniques, such as field desorption, produce quasimolecular ions that are very weak in intensity, with the most intense spectral peaks corresponding to the loss of NH_3 for arginine and the loss of CO_2H_2 for cystine. With Macfarlane and Torgerson's system, in contrast, the $(M + 1)^+$ peaks are the most intense, and the fragmentation peaks—which correspond to the loss of NH_3, CO_2, COOH, and CO_2H_2 from the quasimolecular ion—are all at least an order of magnitude smaller in intensity. Thus, they argue, the quasimolecular ion represents an unambiguous signature for the sample.

By reversing the sign of accelerating voltage, negative ion spectra can also be recorded. In the absence of a sample, the most intense peak in the spectrum corresponds to hydride ion (H^-). The investigators thus suggest that $(M - 1)^-$ quasimolecular ions arise by proton abstraction from sample molecules by the strongly basic hydride ion. Such quasimolecular ions are the most prominent peaks in negative ion mass spectra of arginine and cystine, and the rest of each spectrum is qualitatively similar to the corresponding positive ion spectrum.

The potential applications of this new type of mass spectroscopy have only begun to be explored. Macfarlane and Harry Mosher of Stanford University have, for example, obtained spectra of several different neurotoxins, complex molecules with masses in the range 300 to 400 daltons. The spectra of some of these, such as tetradotoxin and atelopidtoxin, have never been available before, Mosher says. Had the spectra been available, he adds, the time required for determination of their structures might have been reduced by as many as 3 or 4 years. Mosher is now using such mass spectra in the determination of the structures of other neurotoxins.

Macfarlane and Torgerson have performed other experiments to test the utility of the system. They have, for instance, obtained a spectrum showing the quasimolecular ion of vitamin B_{12}, a feat not previously accomplished. They have shown that it is now possible to identify many components in blood and urine samples from patients with uremia. And they have obtained many polypeptide spectra which suggest that amino acid sequencing might be feasible with the technique. But the main thrust of their work so far has not been the solution of chemical problems. It has, rather, been an attempt to show that the technique is applicable to many different problems and to stimulate interest in the technique among other investigators.

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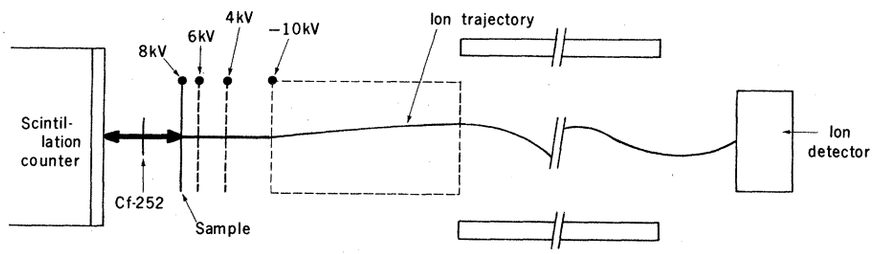

Fig. 1. Scheme of Macfarlane and Torgerson's time-of-flight mass spectrometer. The − 10 kilovolt potential contains ions within the transit tube.