Many compounds, however, must be sprayed with a specific reagent. Alkaloids, for example, can be visualized with iodoplatinate; carbamate pesticides with *N*-2,6-trichloro-*p*-benzoquinone imine; steroids with concentrated sulfuric acid in ethanol. One of the greatest arts in TLC is finding an appropriate visualization technique.

Several new ones were reported at the conference. Joseph C. Touchstone and his colleagues at the University of Pennsylvania, for example, reported that unsaturated lipids can be visualized with a solution of 3 percent cupric acetate and 10 percent cupric sulfate in 8 percent phosphonic acid. After the plate is sprayed, it is heated at 180°C for 10 minutes to char the lipids. The heating must be uniform, and Touchstone achieved good results only when his group constructed a narrow opening in the oven door so that heat is not lost when the plate is inserted.

James E. Gagnon and Arthur Mendel of the 3M Company in St. Paul, Minnesota, reported that perfluorinated chemicals could be detected at levels as low as 0.1 microgram with either of two approaches. One involves spraying with tris(4-amino-3-methylphenyl)methane, also known as new fuchsin. The second is a method originally developed by Frank F. Wong of Antioch College for visualization of sulfur-containing compounds; the chromatogram is sprayed with chloroplatinic acid and then with starch solution. Finally, it is immersed in an iodine vapor chamber.

The final step in the HPTLC process is generally quantitation of the amount of material in each spot. Most commonly, a densitometer is used to monitor the difference in ultraviolet or visible absorption between the background and the spot, or the difference in phosphorescence, to determine the relative sizes of the spots. Instruments for this purpose are sold by several companies, including Camag; the Joyce Loebl division of Vickers Instruments, Inc., of Walden, Massachusetts; Kratos Analytical Instruments of Westwood, New Jersey; and Shimadzu Scientific Instruments, Inc., of Columbia, Maryland. Typically, the chromatogram is also photographed to provide a permanent record.

In certain cases, retrieval of the sample may be desired. This can be accomplished by scraping off the layer of adsorbent containing the spot or by extracting the spot in situ. For extracting the spot, Camag has a relatively new instrument called the Eluchrom. A small head is clamped over the spot and a suitable solvent is pumped through it, extracting the sample. A sample can be eluted with as little as 50 microliters of solvent in less than 2 minutes.

Fancy equipment notwithstanding, the heart of the TLC process is the adsorbent. Beyond the decrease in particle size, the most significant recent improvement was the introduction by Whatman of an adsorbent designed for reversed-phase TLC, a procedure in which the solvent is of greater polarity than the adsorbent. This is generally accomplished by binding linear hydrocarbons to the surface of the silica to make it more hydrophobic. It is estimated that as much as 80 percent of HPLC is now performed on reversed-phase columns because they are simply more versatile, and it seems likely that the new plates will have a similar impact on TLC. Significant growth has occurred in the last 2 years and now every manufacturer offers reversed-phase plates.

Preparative-scale TLC has been a difficult problem because the sample spots tend to become "smeared out" when large amounts are loaded onto the plate. Herman R. Felton of Analtech reported on a new approach to preparative chromatography using adsorbent applied in a gradient. Unlike previous attempts by others, however, Felton's gradient has the thin portion of the adsorbent wedge on the bottom of the plate and the thick portion at the top. In this manner, he says, solvent flow is better regulated and smearing is minimized. If the sample is banded across the bottom of a 20 by 20 centimeter plate, he says, it is possible to separate as much as 1.5 grams, a massive amount in TLC terms. The plates are not yet commercially available because of difficulties in producing the gradient reliably, but he hopes they will be in the near future.

A new type of adsorbent was reported by K. Srinivasulu and A. K. Sonakia of Vikram University in Ujjain, India. The adsorbent is scolecite, a crystalline hydrated calcium aluminum silicate that is a naturally occurring zeolite mineral. The pair had reported at previous Pittsburgh Conferences that many organic acids, amines, and amino acids can be separated on plates coated with scolecite. This year, they reported that many carbohydrates can also be separated on the plates faster than on conventional plates and with higher resolution.

In a highly technological world, it is often easy to overlook the simpler approaches to problems because they lack "glamor." A growing legion of thin-layer chromatographers, however, argue forcefully that they would prefer good separations to glamor anytime.

—THOMAS H. MAUGH II

Weight Limit for Mass Spectroscopy Raised

The mass spectrometer has been called the most sensitive of weighing machines. It identifies unknown chemical compounds by separating individual molecules and fragments of molecules according to their mass. But in the past, chemists could not analyze compounds whose molecules were too massive. They were either not volatile enough to be introduced as separate, gaseous molecules, or the energy required to volatilize them tore them into fragments, leaving no clue as to how large the whole molecule had been.

Within the past year, the wide availability of fast atom bombardment (FAB) as an option on mass spectrometers has pushed this weight limit upward. Most instruments had been capable of separating molecules having masses of 1 to about 1000 atomic mass units (amu). FAB allows the routine identification of molecules up to 2000 amu. Compounds up to about 4000 amu are being determined in many laboratories. Chemists now consider the analysis of vitamin B_{12} (molecular weight 1355), once impossible without chemical modification to make it more volatile, to be rather mundane. A host of other biochemical compounds, which seem to be particularly suitable for the method, can now be readily determined with FAB, even in complex mixtures. FAB is not satisfactory for every compound, but no one yet sees any obvious limits on the size of molecules that might be analyzed.

Researchers developed FAB while looking for a way to analyze a quite different sort of sample, the surface of inorganic insulators. John Vickerman

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and Michael Barber, both at the University of Manchester's Institute of Science and Technology (UMIST), were bombarding samples of insulators with high-speed ions inside a mass spectrometer. Using a technique called secondary ion mass spectroscopy, they were attempting to knock off and ionize a bit of the insulator's surface so that it might be swept up by a magnetic field and carried into the analyzer section of the instrument.

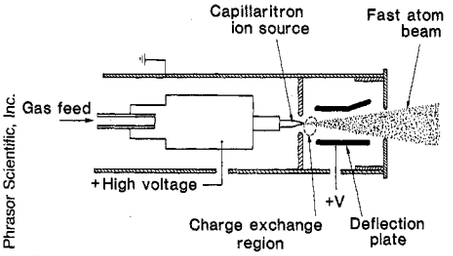
But they had a problem. The insulator allowed an electric field of 3 to 5 kilovolts to build on it. That tended to obstruct the incoming ion beam and to suppress the desired emission of secondary sample ions. If they used a beam of neutral atoms they might avoid these problems, but it was the charge on the beam ions that allowed magnetic fields to accelerate the ions to high energies and to focus them on the sample.

Their solution was to accelerate and focus argon ions and then to remove their charge before the argon atoms hit the sample. To do this, they put a chamber filled with neutral argon in front of a conventional ion gun. At the relatively high pressure of the chamber (10^{-5} torr), the speeding argon ions collided with the neutral atoms, transferred their charges to the slower, randomly moving gas, and continued on their way to the target as a beam of neutral, fast atoms.

Barber and Vickerman continued work on FAB by applying it to the analysis of organic compounds. Such a method of "soft ionization" might be useful in vaporizing and ionizing heavy compounds without destroying them, but there was another problem. A sample did not survive more than about 10 seconds under the FAB gun, too short a time for thorough analysis. To protect the sample, they dissolved it in a film of glycerol. Apparently, enough molecules of the sample are exposed on the surface of the film to produce a steady supply of ions for hours; a reservoir of sample below can resupply the surface.

Kratos Analytical Instruments first introduced FAB at last year's Pittsburgh Conference. It is now offered by most major suppliers as a standard component or as an option at an additional cost of $20,000 to $40,000. Older mass spectrometers can be modified to accept FAB as well. FAB guns are available from various suppliers, including Ion-Tech, Manchester, England, and Phrasor Scientific, Inc., Duarte, California.

Examples of FAB applications abound. K. L. Rinehart and M. L. Moore at the University of Illinois at Urbana-Champaign and A. N. Tyler at UMIST have determined the sequence of amino acids in a peptide by using FAB mass spectroscopy. The compound was antiamoebin III, one of a family of linear peptide antibiotics. The mass spectrum of the negative ions produced by FAB contained a strong peak representing the 1654-amu molecule minus a hydrogen atom. The positive-ion FAB spectrum showed distinct peaks representing fragments of the molecule resulting from the successive loss of specific amino acids. This information, plus an amino acid analysis of the hydrolyzed peptide by combined gas chromatography–mass spectrometry, allowed the group to determine an unambiguous sequence for the 15 amino acids of antiamoebin III.



A fast atom source

A diagram of the Phrasor Scientific, Inc., fast atom capillaritron source. Unlike other designs, this source forms ions, accelerates them, and removes their charge in a single small area. Some of the argon atoms passing through a 25- to 50-micrometer capillary become ionized under a high voltage applied to the capillary and are accelerated by a concentric electrode. The ions can transfer their charges to neutral argon atoms exiting from the same capillary by colliding with them. A charged plate deflects those ions that escape neutralization.

An active center of FAB work has been the Middle Atlantic Mass Spectrometry Laboratory at Johns Hopkins University. The laboratory is a regional facility funded by the National Science Foundation to analyze samples for the staff as well as academic and industrial researchers who need the most sophisticated, and expensive, techniques available. Robert Murphy and Rodney Matthews of the University of Colorado Medical School in Denver, Joshua Rokach of Merck-Frosst Laboratories of Pointe-Claire, Canada, and Catherine Fenselau of Johns Hopkins Medical School used the facilities there to help confirm the reported structure of leukotriene C_4 derived from murine mastocytoma cells. This compound (molecular weight 625) has been implicated in allergy and inflammation processes (*Science*, 12 March, p. 1380). The reported structure was based on chemical degradation experiments and ultraviolet absorption characteristics of natural and synthetic samples, but alternative structures had been suggested. Three consecutive chemical derivatizations had been required to increase the volatility of the compound for conventional mass spectroscopy. Still, the ionization techniques that were used fragmented the molecule so much that the proposed alternative structures could not be refuted.

Using FAB, Murphy's group did not have to derivatize the sample, which avoided the possibility of undetected alterations. On the basis of a strong quasi-molecular ion (from the loss of a single hydrogen) and significant peaks in both positive and negative ion spectra, they could support the reported structure. In addition, they suggested that one of the proposed alternatives may have been the product of unintended oxidation.

In other FAB work at the regional instrument facility, biochemical compounds having molecular weights up to 2000 are being analyzed routinely, according to Fenselau. Compounds in the 1800-to-2000 range that are being investigated at the facility include phosphoglycolipids, gangliosides, hexadioxynucleotides, and triantennary decasaccharides. Biochemical compounds having molecular weights up to about 4000 are also being analyzed, but with greater difficulty, she says. Samples in this weight range include glycopeptides, polyacylglycosides, and, among other peptides, glucodon, a peptide of 29 amino acids having a molecular weight of 3482. This is about the same size as insulin and the endorphins, she notes.

FAB, for all of its attractive features, is not the perfect method. A major competitor is the 10-year-old field desorption (FD) technique. "FAB won't replace FD," says Fenselau, "but fortunately it will replace much of FD." FD can be as effective as FAB with compounds of low volatility, but FD is notorious for the great effort and sophisticated technique needed to grow tiny carbon whiskers on the sample filament, the key to the method. Desorption chemical ionization is another soft ionization technique that competes with FAB, but its upper limit seems to be a molecular weight of about 2000. Opinion varies, but FAB seems best suited for ionic or relatively polar compounds. That still covers a lot of ground in biochemistry.

No one sees an obvious upper limit to FAB. There is talk about "when we get to 40,000." But no one has used it yet to examine a compound with a molecular weight over 5000. Mass spectroscopists have yet to analyze what the biochemists consider truly heavy biomolecules.

—RICHARD A. KERR