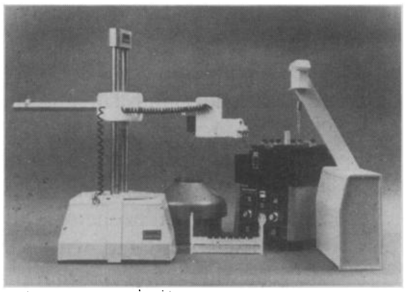
Sample Preparation by a One-Armed Robot

It never failed to draw a crowd at the exposition—its lone arm whirling over the table, plopping test tubes into racks, squirting liquids into them, and passing tubes on to the next chore. The demonstration had obvious visual appeal, but the folks at Zymark Corporation of Hopkinton, Massachusetts, believe that their computer-controlled robot has a serious role to play in the modern laboratory. Sample analysis and data handling have become highly automated, but now it is time to improve the efficiency of sample preparation, they say.



The central actor of the Zymate Laboratory Automation System is the robot, a horizontal arm running on two vertical pillars set in a base that is bolted to the table. By whirling in a circle, bobbing up and down, and extending itself, the arm can use its tonged "hand" to manipulate test tubes or vials anywhere within a few feet of its central pillar. The robot can change its own hand by leaving the tonged version on a stand and attaching a syringe-equipped one.

The Master Laboratory Station helps prepare samples that the arm brings to it. It dispenses liquids through three syringes and collects liquid fractions in a turntable rack after filtration or extraction steps. The brain behind the whole operation is the Controller, a microprocessor that directs the actions of the arm, master station, and any additional pieces of equipment. The human operator, with no knowledge of computer languages, can direct the robot to prepare multiple samples by entering plain-language commands into the controller keyboard.

At the exposition, the robot was adding reagents to samples with its syringe hand, transferring them to a heat-shaker with its tong hand, and delivering them to the master station, which forced them through a disposable packed column. Because the Zymate system manipulates the sample in much the same way that a human technician does, a variety of common laboratory equipment can be included in the preparation process, Zymark officials say. In addition to the heater-shaker, they expect to include a centrifuge, a homogenizer, a balance, and a device for direct injection into a gas chromatograph. Such flexibility and the ability to handle samples containing suspended solids set this system apart from continuous-flow analysis systems, they say. The two simply complement each other, they note. The system as displayed costs about $20,000.—***R.A.K.***

Real-Time Identification of Unknown Mass Spectra

Until now, the identification of mixtures of complex organic compounds by combined gas chromatography–mass spectroscopy (GC-MS) has been a two-step process. First, the mass spectrometer produced spectra, perhaps as many as 100 per hour, as the gas chromatograph churned out separated compounds for analysis. Then, the chemist searched computer files in order to compare the unknown spectra with the spectra of the tens of thousands of known compounds in the reference file.

Now, a new computer program that was demonstrated at the exposition for the first time performs a search in 5 to 20 seconds on the GC-MS computer; that is fast enough to keep up with the flow of samples to the mass spectrometer. A group at Cornell University (Fred McLafferty, In Ki Mun, and Douglas Stauffer) and a group at Hewlett-Packard Company of Palo Alto, California (James Serum and David Peterson) collaborated on development of the program under funding from the National Science Foundation's Industry-University Cooperative Research Activity. On the same computer, the two groups have also implemented a program to assist in the interpretation of spectra that are not satisfactorily identified by the real-time search.

The Probability Based Matching (PBM) system speeds the reference file search because it identifies the reference spectra most likely to provide a match and then compares only those spectra with the unknown spectrum. Instead of having reference spectra filed by molecular weight, PBM searches a file arranged by the most important peak of each spectrum, the peak most characteristic of that compound.

A peak's importance is determined by a weighting system, a technique borrowed from search strategies used to retrieve library documents. Each peak, representing the molecular ion or a fragment of the molecule, is weighted according to its "mass uniqueness" and its abundance or prominence in the spectrum. Mass uniqueness is a measure of the usefulness in the search of a particular fragment mass/charge number—the less commonly the mass/charge number appears in the reference file, the greater the weight that is given to it. Peak weightings are used to divide the reference file into 732 categories. Instead of searching the entire file to match an unknown, only about 20 categories need be searched. Those searched are the ones whose mass/charge number is the same as that of the most important peaks of the unknown.

The new PBM system is not only fast but reliable. In a search of 41,429 reference spectra for matches with 431 randomly selected test spectra, all of the possible 934 matches, including independently determined spectra of the same compound, were found. An average of only 7.5 percent of the reference spectra had to be searched, and computer search time dropped proportionately. Using less stringent search standards, as few as 1.6 percent of the file was searched, but the success rate dropped to 97.5 percent. Even a component representing one quarter of an unknown mixture could be identified more than 97 percent of the time. This is achieved by reverse searching, which permits possibly extraneous peaks in the unknown spectrum to be ignored.

The best search strategy will not find the correct reference spectrum if it is not in the file. The Self-Training Interpretive and Retrieval System (STIRS) helps the chemist to narrow the field when PBM does not have an answer. STIRS combines information about how molecules tend to fragment with the ability to search many reference spectra for fragmentation behavior similar to that of the unknown. STIRS provides indications of which structural elements most likely appear in the unknown molecule as well as a molecular weight. Its choice of a molecular weight was right 91 percent of the time in the case of randomly selected unknowns.

The originators of the programs caution that both PBM and STIRS are aids to the trained interpreter of mass spectra, not a replacement for him. Both programs were originally implemented on the Cornell IBM 370/168 computer, where they are accessible to outside users over the TYMNET and TELENET computer networking systems.—***R.A.K.***

New Magnet Speeds Mass Spectrometers

A complete structural analysis of a complex organic molecule in 3 seconds would seem to be a sufficient accomplishment for any instrument. It was for mass spectrometers until analytical chemists began placing them in tandem with other instruments such as gas chromatographs. Keeping up with the flow of separated compounds from a high-performance gas chromatograph necessitated switching from a magnetic sector mass spectrometer to a quadrupole version. That move sacrificed the precise mass measurement capability of the magnetic sector instrument for the quadrupole's higher speed.

Now, modifications to the magnet have allowed the magnetic sector instrument to match the quadrupole's speed, reportedly without losing any of its mass resolution. The problem had been with the behavior of the magnet when it was driven faster in order to catch individual sample peaks as they came from the gas chromatograph. Approaching the required scanning speed of one decade (say, a mass range of 50 to 500) in less than 1 second, strong eddy currents would appear in the magnet. These oppose the required change in the magnetic field and reduce control of the ion beam. The solution has been to slice the magnet into smaller and smaller pieces. A magnet composed of smaller subsections generates weaker eddy currents.

The ultimate product of this approach is the fully laminated magnet. As an example, the laminated magnet of VG Analytical's Micromass 7070 series is composed of slivers less than 1 millimeter thick. The original magnet body is sliced, shaped, fitted with coils, and reassembled with each slice electrically isolated. The lamination extends from one tip through the core to the other tip. This particular magent can scan at the rate of 0.1 second per decade (50 to 500) and repeat such a scan every 0.3 second. On the Micromass 7070E, modification of the magnet geometry and the optics controlling the ion beam has allowed a total mass range for the instrument of 1 to 2500. A fully laminated magnet is also available from Kratos Analytical Instruments.

—***R.A.K.***

"Time-of-Flight" Optical Spectroscopy

Time-of-flight analysis is a frequently used technique in mass spectrometry; heavy molecules travel faster than lighter ones, so that the length of time required by a molecule to pass through the instrument is a measure of the molecule's mass. Surprisingly, "time-of-flight" techniques can also be applied to optical spectroscopy, say William B. Whitten and John R. O'Lear of the Oak Ridge National Laboratory. This possibility relies on the fact that light of different wavelengths travels through glass fibers at different speeds, making it possible to disperse the optical spectrum in time rather than in space as is done with a conventional monochromator. The concept was first advanced by Harley Ross of Oak Ridge several years ago, but it has only been recently that acceptable fibers were available.

Near wavelengths of 400 nanometers, the temporal dispersion is about 1 nanosecond per nanometer per kilometer, with longer wavelengths traveling faster. Thus, if a short light pulse is admitted to one end of a glass fiber 1 kilometer long, wavelengths that are 10 nanometers apart will arrive 10 nanoseconds apart. For practical spectroscopy in the region 400 to 900 nanometers, Whitten and O'Lear use a fiber that is about 500 meters long. The light source is a pulsed neodymium glass laser focused on a carbon tetrachloride cell to produce a wavelength continuum. The resultant light is passed through a beam splitter, half of the light going to a sample and half to a reference cell. Light from the sample is passed through the 500-meter fiber to a photodiode that detects the spectrum sequentially. Light from the reference is passed through a second fiber to the same detector. The second fiber is about 25 meters shorter than the first, so that the two light pulses arrive at the detector separately.

The output of the detector is displayed on an oscilloscope. The entire process of obtaining a spectrum requires less than 3 microseconds, and most of that time is required for passage of the light through the fiber. Resolution is limited by the length of the light pulse, about 10 picoseconds, but is more than adequate to obtain a good spectrum.

Such a dual beam spectrometer could have several different applications, says Whitten. A series of light pulses, for example, could be used to monitor the course of a reaction over time. It could also be used to obtain the spectrum of short-lived intermediates in a reaction. Emission spectra from fast discharges could also be monitored. And finally, he says, a finished instrument could be very rugged, since it would have no monochromator or other moving parts. It would thus be good for use in the field.

At shorter wavelengths, the absorbance of the fiber itself begins to be a problem, but the temporal separation becomes greater so that shorter fibers can be used. For spectra between 300 and 400 nanometers, Whitten says, a 50-meter glass fiber is adequate.

With the laser and most other light sources, the dual beam construction is a necessity because consecutive pulses are not sufficiently consistent. With a synchrotron light source, however, this would not be a problem. In fact, he concludes, "the high intensity and repetition rate of the synchrotron should make it possible to collect large amounts of data in very little time."—***T.H.M.***