Automating Wet Chemistry with FIA

Amid the glamour of $400,000 NMR spectrometers and $300,000 x-ray analyzers, it is often easy to forget that the majority of laboratory analysis is conducted with much cheaper apparatus. Yet some of that less expensive instrumentation can have as large an impact for its own applications as the $400,000 spectrometer does for NMR. One good example is flow injection analysis (FIA). FIA uses the modern techniques of miniaturization and microprocessor control to automate repetitive chemical analyses for quality control, process control, and any other application where large numbers of chemical tests must be performed.

FIA was invented in 1974 by J. Ruzicka and E. H. Hansen at the Technical University of Denmark and the technique caught on rapidly in Europe. U.S. scientists were slower to adopt the technique, but sales have been increasing in the last couple of years to the point where they now total about $1 million per year. Considering the utility of FIA for many applications, it seems likely that sales will continue to grow rapidly.

FIA is a method for automating wet chemistry. It is based on the introduction of a defined volume of sample into a carrier stream that moves through the apparatus. This results in formation of a sample plug bracketed by carrier. The carrier stream is then merged with a reagent stream to bring about a chemical reaction between the sample and reagent. After passage through a manifold, during which time reaction occurs, the stream then flows through a detector cell. The detector is most often a colorimeter, but just about every other detection technique has also been used.

In some ways, FIA is similar to the technology of the clinical analyzers produced by Technicon Instruments Corporation. The Technicon instruments, however, incorporate a segmented flow design in which samples are separated by air bubbles. Because air compresses, says Karl Schick of FiAtron Systems, Inc., the stream tends to pulsate; the streams also have to be debubbled before they enter the flowcell, and it is necessary to use a wetting agent.

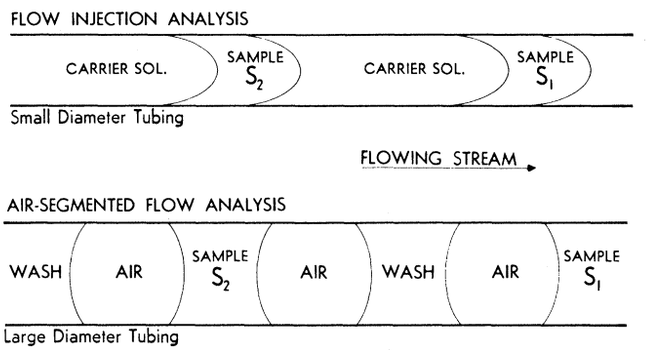
In FIA, adds B. I. Karlberg of Tecator AB, the pump provides constant pressure and "the residence time of the sample in the system is absolutely constant." The amount of dispersion (dilution) of the sample can be controlled by varying a number of factors, including length and diameter of mixing coils, sample volume, and flow rate. When the dispersed sample zone reaches the detector, neither the chemical reaction nor the dispersion process will typically have reached a steady state. Because the residence time is constant, however, both dispersion and reaction will have proceeded to the same extent for each sample and each standard, so that highly reproducible results are obtained. If a reaction requires more than the 15 to 20 seconds flow time that is standard in most instruments, the flow can be halted for a predetermined period of time, typically with the sample in the flowcell. For conventional analyses, though, a single instrument can analyze as many as 300 samples per hour.

FIA can be performed on a conventional high-performance liquid chromatograph (HPLC) by simply replacing the packed column with an open tube, says Gilbert Pacey of Miami University, but better results are usually obtained with a dedicated system. In general, HPLC pumps pulse too much, while the ideal in FIA is to have completely smooth fluid flow. FIA systems have generally been better at this than HPLC's, but even they have not been as good as could be desired.

Two new approaches to pumping were demonstrated at the Pittsburgh Conference this year. FiAtron introduced a "pulse free, high precision, Teflon piston pump" that is suitable for both FIA and low-pressure liquid chromatography. Control Equipment Corporation introduced a system in which air pressure is the driving force for the liquid, so that flow is also pulse-free.

Beyond the need for a very good pump, says Pacey, the instrumentation is actually rather simple. Virtually all of the instruments available are now microprocessor-controlled, for example, and there seem to be very few differences in handling characteristics. Perhaps the major differences among companies is how they position their products for various marketplaces. FiAtron, for example, emphasizes the utility of its SHS-200 for industrial process control and quality control. It thus has produced Teflon-lined tubing and pumps that can withstand concentrated acids and bases and other corrosive substances. The company has also developed new valving arrangements that allow injection of extremely small samples, on the order of 2 microliters. It is thus possible, says Schick, to get a 500-fold dilution in one step, a necessary requisite for many applications.

Tecator, which along with FiAtron and Lachat Instruments is one of the largest FIA vendors, emphasizes its ability to supply instruments configured for specific types of applications. Tecator's FIAstar 5020 has been widely used in water chemistry (both for analysis of impurities in intake water and for analysis of toxic pollutants in effluent), food chemistry, beverages, and fertilizer manufacturing.



Two separation methods

A schematic showing how sample separation is maintained in flow injection analysis (top) and segmented flow analysis.

Lachat emphasizes the modularity of its QuikChem system, which allows a user to pick out only the components needed. The company also argues that its software is better than anyone else's; it will, for example, allow analysis of a particular sample to be repeated if the results are not satisfactory, allow relinearization with standards, and provide complete data reduction and report generation. Lachat's most advanced model has 12 pumping channels and will handle as many as four different analyses simultaneously.

One of the main applications for Control Equipment's AMI-103 is as a sample preparation system for atomic absorption spectroscopy. All of the FIA instruments can be used for this purpose, and they are quite good because they perform required dilutions and additions of reagents and the continuous flow of solvent into the nebulizer enhances flame

stability. Pulsation in the liquid flow can be a problem, however, particularly with inductively coupled plasma (ICP) nebulizers. Control Equipment's gas displacement pumping system eliminates this source of variability. The company has also developed a new glucose detector, designed for beverage, biotechnology, and food industries, that also requires a pulse-free flow.

Alpkem Corporation's RFA-300 is unique in that it can be used for both FIA and air-segmented continuous flow analysis. It is thus possible, the company says, to use whichever of the two techniques is best suited for any particular application. American Research Products Corporation is a new entry in the field whose AMFIA-2200 uses an Apple IIe computer both to control the instrument and to provide data workup. The system is also modular.

FIA does have some limitations. It does not work well with nonaqueous solvents, for example, and it is not very good for reactions that take longer than about 15 minutes to go to completion. It is also not very good for reactions that require multiple reagents because the dispersion can become too great; such reactions are better handled by segmented flow analysis. And finally, very sensitive detectors are required because the flow volumes are quite low.

Despite the versatility of FIA, the instruments are relatively cheap. Prices range from a low of about $9700 for the computer-compatible, but not microprocessor-controlled, AMI-103 to a high of about $40,000 for a four-channel Lachat system. A typical price is about $20,000. For any kind of repetitive analysis where the procedures are "extraordinarily tedious and time-consuming," in the words of one salesman, FIA seems an ideal way to relieve boredom and improve productivity.

—THOMAS H. MAUGH II

A New Dimension in NMR

Two-dimensional nuclear magnetic resonance (2D-NMR) spectrometry may well be the fastest growing area of spectrometry today. While there are, in theory, few things that can be done by 2D-NMR that cannot be done by conventional NMR, in practice there are vast differences. In general, 2D-NMR does things more simply and more quickly (when interpretation time is considered); in many cases, furthermore, interpretation of the resultant data can be performed by a novice rather than by the skilled spectroscopist required to interpret conventional NMR studies.

NMR can be performed on any atom having a nucleus with nonzero spin angular momenta or, equivalently, a magnetic dipole moment; typically, that is an element with an odd atomic number, such as hydrogen, carbon-13, nitrogen-15, oxygen-17, and so on. The sample is inserted into a high magnetic field, which orients the dipoles. The dipoles are then perturbed by a radio-frequency (r-f) pulse, and their subsequent collective behavior monitored as a function of time. Fourier transformation of the resulting amplitude-time function produces the characteristic NMR spectrum, with each resonant nucleus giving rise to a peak at a position on a frequency scale. The position of this peak relative to a standard is called the chemical shift and is characteristic of the electronic environment of the nucleus. The signal for each chemical shift, furthermore, is split into two or more peaks as a result of coupling between nuclei transmitted through bonds; in effect, the dipole of one nucleus "senses" the orientation of the dipole in an adjacent nucleus.

For simple molecules, the spectrum is easy to interpret. As the molecule becomes more complex, individual peaks begin to overlap and interpretation becomes more difficult. For very large molecules and polymers, interpretation becomes almost impossible without the use of sophisticated techniques that aid in assigning resonances to specific nuclei. 2D-NMR is a technique that separates many of the magnetic interactions that are jumbled together in a conventional spectrum. It was developed in the early 1970's by Jean Jeener of Université Libré in Belgium and by Ray Freeman and Richard Ernst at the Varian Corporation; Freeman is now at Oxford University and Ernst is at Eidgenössische Technische Hochschule in Zurich.

Perhaps the best analogy that can be used in explaining the difference between conventional and 2D-NMR is the difference between one-dimensional and two-dimensional thin-layer chromatography (2D-TLC). In conventional TLC, a specific solvent system is used to elute the samples along one dimension of a TLC plate. In 2D-TLC, the plate is rotated 90° after the first elution and eluted with a second solvent system to separate components that were not separated by the original solvent system.

In 2D-NMR, the second dimension can be achieved by several methods. The simplest way, perhaps, is to plot the spectrum for one nucleus against that for a second type of nucleus in the same compound. A proton spectrum might be plotted against the carbon-13 spectrum, for example. When this is done, the plot gives a direct indication of which protons are coupled to which carbon-13 atoms.

In this and other cases, the second dimension is achieved by introducing a second r-f pulse (or set of pulses) before relaxation from the first pulse is complete. By varying the timing of the pulses, it is possible both to tune (in effect) the spectrometer to each of the couplings in the sample molecule and to perform different types of experiments. A Fourier transform of the collected data provides a conventional NMR spectrum. A second Fourier transform of the same data—that is, a transform of the transform—provides the second dimension.

The result is a two-dimensional plot such as that in the accompanying figure. The diagonal represents the actual one-dimensional spectrum of the sample. As is apparent, many pairs of data points on the diagonal are accompanied by symmetrical data points on both sides of the spectrum, arranged so that the four points form the corners of a square. This arrangement indicates that the nuclei which produced the two resonances are correlated. In a proton spectrum, for example, this might mean that the protons responsible for the signals are attached to adjacent carbon atoms.

Applications of 2D-NMR can be broken down into four general categories, all dependent on the same type of mathematics:

▶ The most common application is known as correlation spectroscopy or COSY. This approach involves correlation of groups that are thought to be coupled to each other to prove that they are, in fact, coupled. A variant that provides similar information is called spin echo correlation spectroscopy or SECSY.

▶ The second most common technique is called J-resolved spectroscopy. It provides a way to separate the chemi-