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-

cal shift of a nucleus from the coupling to other nuclei, thereby simplifying the spectrum and making it easier to assign each resonance to a specific nucleus.

▶ Nuclear Overhauser effect spectroscopy (NOESY) is a technique to measure the interaction of nuclei through space rather than through chemical bonds. It is thus a good technique for determining distances between nonadjacent residues in a peptide chain, for example.

▶ Multiple quantum transitions is a technique in which molecules in a sample are forced to absorb or emit several quanta of energy at one time. This is a very powerful technique which can be used, for example, to determine which carbon atoms in a molecule are connected to which other atoms. This is one of the least used applications, says Ad Bax of the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, "because of the inherent low sensitivity of the experiment."

The use of 2D-NMR for small molecules is now "almost routine," says Bax, particularly in the pharmaceutical industry, where identification or confirmation of unknown molecules is very important, and among synthetic chemists. But perhaps the area of greatest excitement now, he adds, is in biochemical applications. About five or six groups in the United States and three or four in Europe are using the technique to study the conformations of polypeptides, proteins (up to masses of about 15,000 daltons), transfer RNA's, and so forth.

Two-dimensional NMR is the only alternative to x-ray crystallography for determining structures, says Bax. If the assignment of spectral resonances to individual nuclei within a protein has already been accomplished, the three-dimensional structure of the protein can be determined, "in principle, overnight." If the resonances are not assigned it may take much longer, but potentially still less time than is required for x-ray crystallography. The problem is simplified if there are histidine residues, for example, in the active site, since they are easier to assign.

The same is true for other types of materials. Brian Reed of the University of Washington argues that 2D-NMR will almost completely replace x-ray crystallography for determining structures of tRNA's. Unfortunately, adds David Cowburn of the Rockefeller University, a lot of the best work on polymers has been performed by industrial scientists who have not been able to publish all of their results.

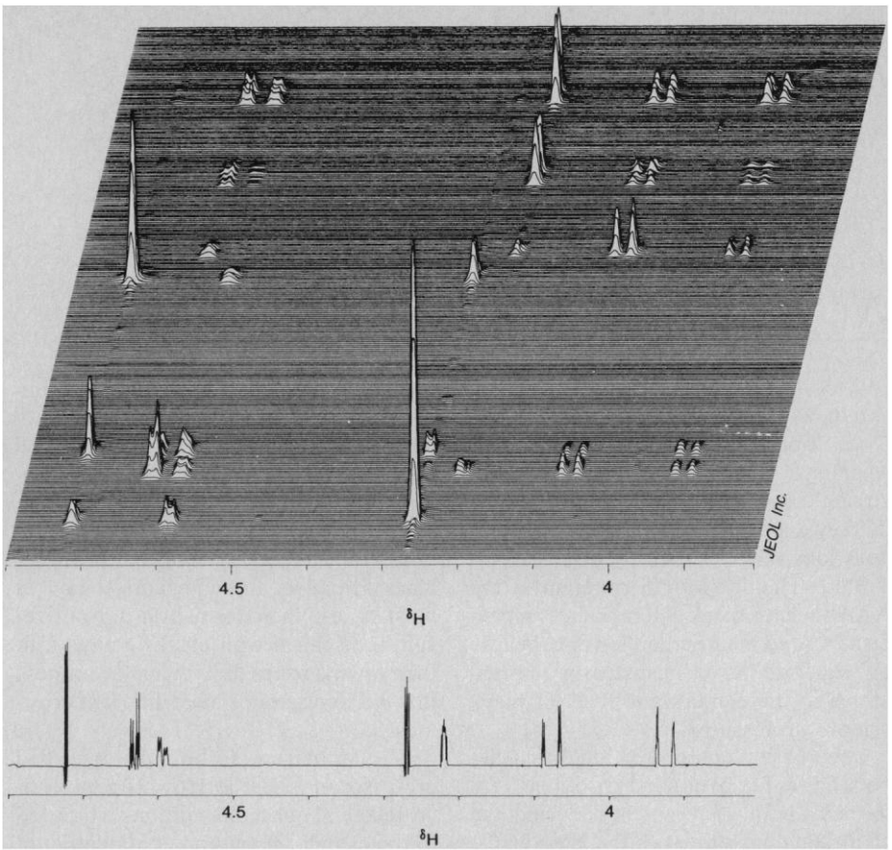
As versatile a technique as 2D-NMR is, there are still several problems associated with its use. The most important is the time required for data acquisition and workup. Where a conventional FT-NMR spectrum might have 2000 data points for each of 256 or 512 scans accumulated for a spectrum, a 2D spectrum will have a 2000 × 2000 array of data points for each. Quite obviously, this requires a lot of data storage space. The collection time for this data may range from an hour to a whole weekend. For 2D-NOE, furthermore, it is often necessary to perform each experiment three to four times.

Once the data is collected, each of the 512 scans must undergo a Fourier transform, which is a very time-consuming process. If the NMR instrument has only one central processing unit (CPU), the transform process must be interrupted each time a data point is collected, which further lengthens the time required. Many investigators now prefer to perform the transform on a larger computer separate from the NMR. One step to help alleviate this problem is the introduction by Varian of an NMR containing two 32-bit CPU's that operate independently, one for data collection and one for data processing.

Another problem is the size of the memory associated with the CPU. Most 2D-NMR spectrometers have only 128 to 256 kilobytes of memory, so only a small portion of the data matrix can be worked on at any time; this result must then be written onto a disk and new data read. The read/write process takes much more time than the actual computation. The Varian instrument has a 16-megabyte memory, so that the CPU must access the disk only about a tenth as often. This means that a computation that takes hours on another instrument might take only minutes on the Varian unit. Even this may not be sufficient, however. Many users argue that the instruments should use Winchester hard disk drives; these are not only faster but they also store more information. Many investigators also complain that the programs produced by the instrument makers for running a program of second pulses are not optimum for many of the less common uses.

In sum, says Cowburn, 2D-NMR has many benefits in addition to those already cited. Because the instruments have increased sensitivity, spectrometry of unusual nuclei and of solids will also become easier. "Many barriers," he concludes, "will simply disappear."

—THOMAS H. MAUGH II



A new dimension

A conventional NMR spectrum of a sugar in $CDCl_3$ (bottom) and a 2D-NMR spectrum (top). The 2D spectrum can also be plotted as a contour map with intensities denoted by color.