The 1982 Pittsburgh Conference: A Special Instrumentation Report

The 1982 Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy was somewhat like a circus, with three magicians, a man in an alligator suit, a 10-kilometer race, lots of free apples, and other accoutrements. The circus atmosphere reflects the robust health of the instrument industry, even at a time when other industries are losing ground. The instrument industry as a whole is growing at the rate of 12 percent a year, and some segments, such as high-performance liquid chromatography and ion chromatography, are growing at twice that rate. Everyone, be they in industry or academia, needs instruments, and they need not take out a high-interest loan, even for the most expensive ones. The Pittsburgh Conference is also robust; attendance is up 15 percent to 19,884, the number of exhibitors is up 12.5 percent to 560, and the number of booths is up 15 percent to 1380. This good health is straining the facilities of the Atlantic City Convention Center. Meeting rooms that were on the convention floor have been moved to adjacent hotels and even part of the restaurant space has been co-opted for exhibits. Conference officials predict that next year will be the last in which the meeting will be able to squeeze into the hall, and the 1984 meeting is scheduled for Washington, D.C.—if that city's new convention center is completed in time. Successive years will see the meeting in Atlanta and New Orleans before it comes back to Atlantic City in 1987, by which time an addition to the convention hall should be completed. Meanwhile, the happiest people around are the makers of cathode-ray tubes, microprocessors, and keyboards, for these accessories appeared on just about every major instrument at the meeting. Perkin-Elmer Corporation introduced about 23 brand new instruments at the meeting, most of which had video displays. User manuals are disappearing from laboratory shelves and making their way into computer memories, whence they are displayed on the video unit to assist the neophyte technician. "User friendly" is the buzzword signifying ease of use engendered by this approach. Many instruments also feature "soft" keys whose function is changed by changes in the programming, thereby reducing the number of buttons facing the user. Truly, the path to the consumer's heart now seems to be through his television screen.

New Applications, Accessories for HPLC

High-performance liquid chromatography (HPLC) continues to be one of the most actively growing areas of the instrument industry, with sales growing by about 23 percent per year by one estimate. This growth reflects not only new users purchasing instruments and established users upgrading their chromatographs, but also new applications, several of which were evident at the Pittsburgh Conference. Among the more visible trends:

Chiral columns. The separation of optical isomers has always been one of the most difficult problems encountered by the synthetic or analytical chemist. The traditional approach has been to combine a racemic mixture of the desired compound (that is, a mixture containing an optically active compound and its mirror image in more or less equal quantities) with a second optically active compound to form compounds with two chiral (optically active) centers. These compounds can then often be separated by fractional crystallization or chromatography, after which the two chiral compounds are separated and the desired stereoisomer is purified.

Many investigators have attempted to short-circuit this time-consuming process by chromatographing the racemic mixture on columns packed with a chiral stationary phase. Among the many materials that have been tried are modified celluloses, sugars, polypeptides, wool, quartz crystals, and potato starch. Successes with this approach have been very limited.

One of the most successful practitioners of chiral chromatography has been William H. Pirkle of the University of Illinois. Pirkle and his colleagues began by studying chiral agents that would bind with both members of a racemic pair to shift their NMR spectra apart so that absolute spatial configurations and relative proportions of stereoisomers could be determined. It was then a natural progression to apply what they had learned to HPLC.

Their first attempts involved chiral fluoroalcohols bonded to a silica support. These were used to separate, among other things, 3,5-dinitrobenzoyl derivatives of amino acids. They soon realized, however, that a more versatile column could be obtained with the amino acids as the stationary phase. The best proved to be (*R*)-*N*-3,5-dinitrobenzoylphenylglycine. This compound has a chiral center surrounded by three different bonding groups—a π-electron center and two hydrogen-bonding regions, one a donor and the other an acceptor. Steric effects may also be important.

This configuration provides three different sites to which the appropriate chiral substrate can bind. The substrate's mirror image, however, binds to only two and thus passes through the column faster. Pirkle's group has shown [*J. Am. Chem. Soc.* **103**, 3964 (1981)] that such columns can resolve a large number of racemic mixtures, including secondary benzyl alcohols, aryl phosphonates, aryl sulfoxides, cyclic alcohols, and nitrogen heterocycles, among many others.

At Atlantic City, both the Regis Chemical Company of Morton Grove, Illinois, and J. T. Baker Research Products of Phillipsburg, New Jersey, introduced commercial analytical columns in which the Pirkle reagent is ionically bonded to a silica support. Regis also introduced a second column in which the reagent is covalently bonded to the support. Separations on the covalent column are not as good as those on the ionic, but it has the

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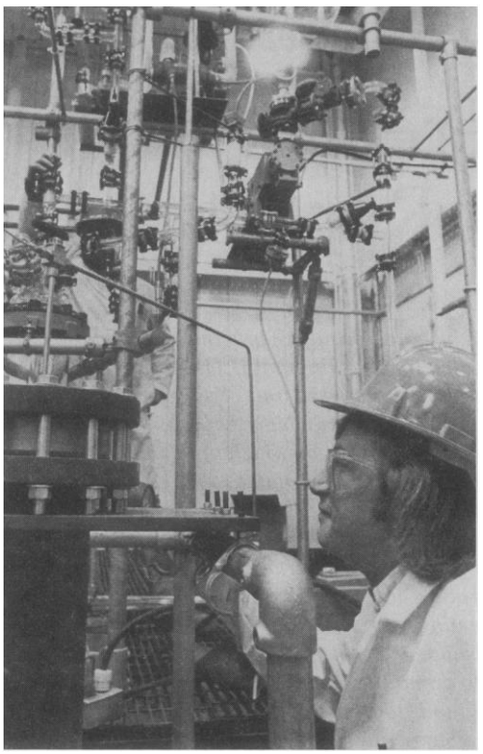

This is HPLC?

Process-scale HPLC columns are much larger than conventional columns.

advantage that water can be used as a solvent for reversed-phase HPLC.

Other columns will probably be available soon. Pirkle and his students have sold a small number of fluoroalcohol columns to help support their research and it seems likely that this will be the second commercial product. Furthermore, Pirkle says, the design of such columns is at present in "a state of flux" and those available now will probably be superseded in a few years by much better ones.

Supercritical fluids. One of the more unusual aspects of HPLC is the use of supercritical fluids as the mobile phase. The critical temperature (T_c) of a liquid is the temperature (for a given pressure range) at which the liquid and gas phases have the same density, so that they are neither liquid nor gas, but simply fluids; this condition is identified by the absence of a meniscus separating the phases. Supercritical fluids exist in a range of temperatures and pressures above the critical point in which a low density fluid (gaslike) can be compressed to a high density fluid (liquid-like) without a discontinuity in density—that is, without a gas-to-liquid condensation. In oversimplified terms, a supercritical fluid might be considered a liquid with the viscosity or diffusivity of a gas, or a gas with the density of a liquid.

This unusual combination of characteristics proves to be almost ideal for chromatography of certain types of compounds. The high diffusivity of supercritical fluids relative to liquids permits much higher linear velocities through the column than can be obtained with liquid chromatography, so there is less peak broadening. The low viscosity of supercritical fluids reduces the pressure drop across the column for any given flow rate, making it possible to use longer columns packed with particles of smaller diameter, again increasing separation. Finally, the solubility of organic compounds in supercritical fluids is a strong function of the fluid's density, which can be altered by changing the pressure. The elution pattern of a series of peaks can be altered substantially by changing the column pressure or by operating a pressure gradient instead of the more normal concentration gradient.

Chromatography with supercritical fluids is thus intermediate between HPLC and gas chromatography (GC), and complementary to both. Many materials that can be volatilized for GC only with difficulty or that are thermally labile can be chromatographed readily in supercritical fluids. Many compounds that can be separated by HPLC can be separated in one-fifth to one-tenth the time, as well as more cleanly, in supercritical fluids. And for preparative chromatography, isolation of the desired material is very easy, since lowering the pressure causes the mobile phase to expand, condensing the sample.

Among the supercritical fluids most often used for chromatography are carbon dioxide (T_c, 31.3°C), dichlorotetrafluoroethane (T_c, 146.7°C), *n*-pentane (T_c, 196.6°C), and isopropanol (T_c, 253.3°C). The critical pressures for these fluids range from 33.3 atmospheres for *n*-pentane to 72.9 atmospheres for carbon dioxide.

Chromatography with supercritical fluids was first suggested by the independent British scientist James Lovelock in 1958; it was pioneered in the early 1960's by Ernst Klesper of the University of Freiburg in West Germany, who used it for thermally labile materials. But columns and instrumentation were really not up to the technique then, and it was soon overshadowed by the then-new technology of HPLC. Advances in instruments and the recent development of new columns packed with small particle diameter stationary phases have renewed interest in the technique and several groups of investigators have constructed chromatographs especially designed to use supercritical fluids.

In a series of papers at Atlantic City, Dennis R. Gere, Robert Board, Harry Weaver, and Douglass McManigill of the Hewlett-Packard Company of Palo Alto, California, described the easy conversion of a commercial HPLC, the Hewlett-Packard 1084, for supercritical fluid chromatography with carbon dioxide. The most important modifications included cooling the pump heads to permit efficient pumping of liquid carbon dioxide; adding a restriction downstream from the detector to control the pressure; modification of the optical flow cell of the ultraviolet detector to permit high pressure operation; and addition of extra thermostating to ensure that the temperatures of the injector, detector, and transfer lines remain above the critical temperature.

With these modifications, which can be readily made in any laboratory, the instrument can use supercritical carbon dioxide at temperatures between 32°C and 100°C and pressures between 75 and 400 atmospheres. The instrument can be reconverted for conventional HPLC simply by removing the restriction and reconnecting the pumps to the conventional solvent reservoirs. With this apparatus, they were able to separate, for example, aromatic hydrocarbons ranging from toluene up to coronene (which has seven fused aromatic rings and a boiling point of 600°C) in 20 minutes. They were also able to separate alkaloids, azo dyes, and many other classes of materials.

Paul A. Peaden, Milton L. Lee, and John C. Fjeldsted of Brigham Young University reported that they had produced a supercritical fluid chromatograph by combining a Varian HPLC pump with a Hewlett-Packard gas chromatograph oven. The oven was needed to maintain the high temperatures necessary for use of supercritical *n*-pentane. Stephen R. Springston and Milos Novotny of Indiana University constructed a similar system to study supercritical fluids ranging from ethane to pentane. Both groups used capillary columns rather than the conventional columns employed by the Hewlett-Packard group. They thus had to design special small-volume injectors and detectors. Both groups have spent considerable time working out the mathematical details of supercritical fluid chromatography, but Peaden reported separating "dozens of components" in a coal tar sample by using a pressure gradient of pentane.

The Hewlett-Packard group also confirmed the observation of other investigators that polar solvents can be added to the supercritical fluids in small quantities to change the elution patterns. Careful examination suggested that the polar modifiers, such as methanol at concentrations up to 1 percent, do not signifi-

cantly affect the mobile phase. Rather, they seem to bind to the stationary phase, altering its interaction with the samples; for some chemicals, the polar solvent changed retention by a hundredfold or more. They observed that some compounds that had been thought to be insoluble in supercritical carbon dioxide simply bind very tightly to the stationary phase; these compounds can often be readily eluted with polar modifiers. This technique is particularly good for high molecular weight materials with polar side groups. Among the classes of compounds they were able to separate in this manner were ubiquinones with side chains containing as many as 66 carbons, steroids, oil-soluble vitamins such as E and D_2, triglycerides, and glycolipids. These results suggest that supercritical fluid chromatography may find a great deal more use in the future.

Process chromatography. For analytical purposes, the trend in HPLC has been toward ever smaller column diameters, with some now as small as 30 micrometers. At the other extreme, preparative columns have been growing to the point where grams of material can be isolated at one time. Now, Waters Associates of Milford, Massachusetts, has taken this trend one step further. By refining technology to produce a 10- to 100-fold increase in throughput, they have built a pilot plant for process-scale chromatography that can separate 1 to 10 kilograms of material per hour. The system uses several columns, each 6 to 10 inches in diameter and 2 feet long, connected in series. Solvent flow varies from 3 to 20 liters per minute, and any conventional stationary phase and solvent can be used. The solvent is recycled so that there is little waste.

Carl W. Rausche of Waters speculates that the system might be especially useful for purifying proteins and other products produced by genetic engineering techniques, since HPLC is more efficient than ion exchange chromatography. It should also be useful for purification of drugs—particularly those, such as prostaglandins, that do not crystallize. It might also have use in purification of catalysts, specialty chemicals, vitamins, and photographic dyes, among other things. Waters plans to offer pilot-scale separations to other firms on a contract basis to demonstrate the utility of the system.

Other accessories. Last year, the DuPont Company of Wilmington, Delaware, introduced a system for using four solvents in HPLC (*Science*, 10 April 1981, p. 149); this system is based on research by DuPont's Lloyd L. Snyder which indicates that four is the optimum number of solvents for maximizing separations of complex mixtures. Development of a separation technique for a given mixture, however, might take as long as a month because of the number of different solvent combinations that must be tried. This year DuPont is attempting to overcome that problem by introducing Sentinel, an automated system that can find the optimum separation conditions in as little as 12 hours. The optimization procedure requires a minimum of seven elutions using different solvent combinations. Sentinel uses the results from the first elution, a gradient of methanol in water, to predict the optimum parameters for the next elution, then performs the elution. If the prediction is not verified, it uses the new results to make a new prediction and performs a second elution with the same solvents; if the result is verified, Sentinel uses all the available data to predict parameters for the next solvent combination. This process is repeated automatically until the ultimate conditions are obtained.

Beckman Instruments, Inc., of Fullerton, California, has introduced a new family of liquid chromatographs, called Series 340, that is based on the concept of distributed intelligence. Each major component of the system is microprocessor controlled so that it can operate independently of the central controller and monitor its own performance. The company says that this concept minimizes the need for attention by an operator.

Micromeritics Instrument Corporation of Norcross, Georgia, introduced the 788 Dual Variable Wavelength Detector for HPLC. By monitoring samples at two wavelengths, the company says, it will be possible in many cases to characterize chemical species, determine the purity of individual peaks in the chromatogram, and identify the amount of material in each of two overlapping peaks.

A more unusual detector was introduced by Applied Chromatography Systems of Bedfordshire, England. The Model 750/14 directs eluent from the chromatography column into a heated column that vaporizes the solvent, leaving the sample behind as a cloud of fine particles that can be detected by light scattering. When pure solvent is evaporated, the resulting vapor leaves no residue and there is no light scattering. The system was designed specifically for detection of polymers, but can be used for a large number of other materials. It can be used with any solvent that is not buffered or does not contain ions. The detector is distributed in this country by Combined Sciences Corp. of Darien, Connecticut.—THOMAS H. MAUGH II

TLC: The Overlooked Alternative

Most chemists over the age of 40 can probably remember dipping microscope slides into beakers of silica gel suspended in chloroform to make plates for thin-layer chromatography (TLC). They went to that trouble because TLC was a simple and inexpensive tool for identifying the components of a mixture, monitoring the course of a preparative reaction, estimating the purity of a product, or isolating trace amounts of a material. Some scientists still make their own plates for specialized purposes, but most investigators were overjoyed in 1961 when Analtech, Inc., of Newark, Delaware, introduced the first commercially prepared plates for TLC.

Since then, other companies have introduced their own plates, special equipment for applying samples and eluting them, and densitometers for analyzing the results. The dollar volume of sales of TLC supplies is still substantially lower than that of, for example, high-performance liquid chromatography (HPLC); there are, after all, fewer microprocessors and no video display units. Nonetheless, the number of analyses performed by TLC each year is at least as large as the number performed by HPLC. The technique is the workhorse of the pharmaceutical industry for determining drug purity, and it is widely used in medical laboratories and in the chemical industry. But this growth has occurred in relative obscurity. Says H. Michael Stahr of Iowa State University: "TLC is so simple that nobody pays much attention to it, and so complex that we're lucky we get any separation at all."

TLC might arguably be called two-

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