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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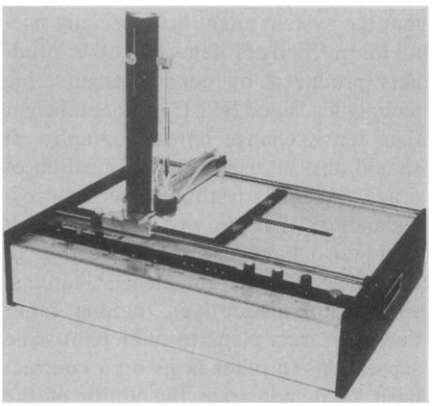
dimensional HPLC. An adsorbent similar or identical to that in an HPLC column is plated onto the surface of a glass sheet in a layer 200 to 300 micrometers thick. About 80 percent of analyses are performed on plates coated with silica gel or modified silica; most of the rest are performed on plates coated with alumina, celluloses, or modified celluloses. Samples for analysis are typically applied in a row near the bottom of the plate in spots 3 to 6 millimeters in diameter, with solvent volumes of 1 to 5 microliters. The plate is then placed in a closed chamber with the bottom resting in a shallow pool of eluent. As the eluent or mobile phase works its way up the plate by a capillary or wicking action, separation of components occurs in a manner similar to that occurring in HPLC.

The two techniques are so similar, in fact, that TLC can be used to identify the optimum conditions for HPLC. In general, says Stahr, "you shouldn't do HPLC until you do TLC," and for many routine applications, you shouldn't do HPLC at all. On a conventional TLC plate, about 20 samples can be chromatographed in about the same time required for one HPLC analysis, and at less cost. Standards are run at the same time on the same plate, so there is greater assurance of the identity of separated components. TLC is especially useful, says Thomas E. Beesley of Whatman Chemical Separation Inc. of Clifton, New Jersey, when a sample might foul a much more expensive HPLC column; it can also be used to clean up a sample before it is injected onto an HPLC column.

For many years, the silica used for TLC plates had a wide range of particle diameters, and results were occasionally unpredictable or erratic. About 6 years ago, however, plate manufacturers began using the ultrafine silica particles, between 2 and 5 micrometers in diameter, that were developed for HPLC with capillary columns. The introduction of these new plates marked the advent of what is now called high-performance TLC or HPTLC. With the small particle diameters, separations take place in much smaller distances, with higher resolution, and in shorter times. Sample volumes are typically 0.1 to 0.2 microliters, and spots are only about 1.0 millimeter in diameter. Thus the samples can be placed much closer together. A conventional TLC plate (20 by 20 centimeters) can handle about 12 samples, whereas a much smaller HPTLC plate (10 by 10 centimeters) can handle 32. Also, HPTLC plates give more reproducible separations and the detection limits are much lower. Since much smaller sample volumes are used, however, the actual detection limits are only about a factor of 2 better.



A new spotter

The Camag Linomat III applies samples in thin bands for TLC.

Because sample size is so important for quantitation in HPTLC, a whole new line of automatic spotting equipment has been developed, primarily by Camag of Muttenz, Switzerland. Camag products are distributed in the United States by Applied Analytical Industries of Wilmington, North Carolina. Samples of uniform size can be spotted onto plates by hand, but it is a tedious business that requires skill and patience. The automated spotters, in general, do a very good job of applying a precise amount of sample exactly where it is wanted. Their chief disadvantages, says Stahr, are that they are often difficult to clean and require preconcentration of the sample.

These problems are at least partially overcome with a new instrument, the Camag Linomat III, exhibited this year at Atlantic City. In the Linomat III, the sample is held in a 500-microliter syringe that can be easily removed for cleaning. The spotter is unusual in that it can apply the sample in thin bands as well as spots. In this manner, relatively large samples—up to 99 microliters for analytical purposes or 495 microliters for preparative purposes—can be used while still obtaining good resolution.

An alternative approach to spotting that has also been introduced recently is the use of a preadsorbent layer on the plate. This is a thin (about 2 centimeters wide) strip of inert diatomaceous earth applied to the bottom of the plate. Sample volumes many times larger than those normally recommended for HPTLC can be applied to the preadsorbent layer with inexpensive applicators. The procedure takes little time and no special skills are required. When elution begins, everything in the sample spot moves with the solvent front and collects at the edge of the silica; development then proceeds normally. Use of a preadsorbent layer is equivalent in many cases to applying the sample in an exceptionally narrow band, thus ensuring good resolution.

The introduction of HPTLC has also permitted unusual types of plate development. In one type, called circular development, the samples are placed in a small circle around the center of a circular TLC plate. The chromatogram is then developed by applying eluent to the center of the plate with an automatic syringe. Because of geometric considerations, the resolution is generally increased compared to development on a linear plate with the same solvent system. Sample overloading problems are minimized because diffusion of the sample tends to occur perpendicular to the direction of development—where there is plenty of room—and thus does not affect resolution.

In the alternative configuration, called anticircular development, the sample is spotted in a ring around the edge of the circular plate and eluted toward the center. The eluent is provided by a circular wick in contact with the edge of the plate. Anticircular development provides the fastest separation for a given solvent, and the geometry produces a constant apparent flow rate toward the center. It is also the only practicable TLC mode for studying compounds that elute near the solvent front. Camag manufactures systems for developing both circular and anticircular chromatograms.

One interesting adaptation of circular development was reported by D. A. McCamey, R. L. Wade, and K. Y. Lee of the Procter & Gamble Company, Cincinnati, Ohio. They constructed a development chamber in which the circular plate is spun around its primary axis at a high speed. The centrifugal force developed thereby speeds the flow of the mobile phase, decreasing a typical development time from 12 minutes to only 3. The shorter development time limits diffusion of samples, improving the resolution and increasing the sensitivity.

Once the chromatogram is developed, it is necessary to visualize the normally colorless spots. The time-honored way of doing this is to place the plate in a bottle containing iodine crystals, whose vapor will turn many compounds reddish-brown. Another common technique is to impregnate the plate with inorganic phosphors. Any compound that absorbs light will quench the phosphorescence, appearing as a black spot against a green background. Radiolabeled compounds can be detected by autoradiography.

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

(*Continued on page 166*)

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