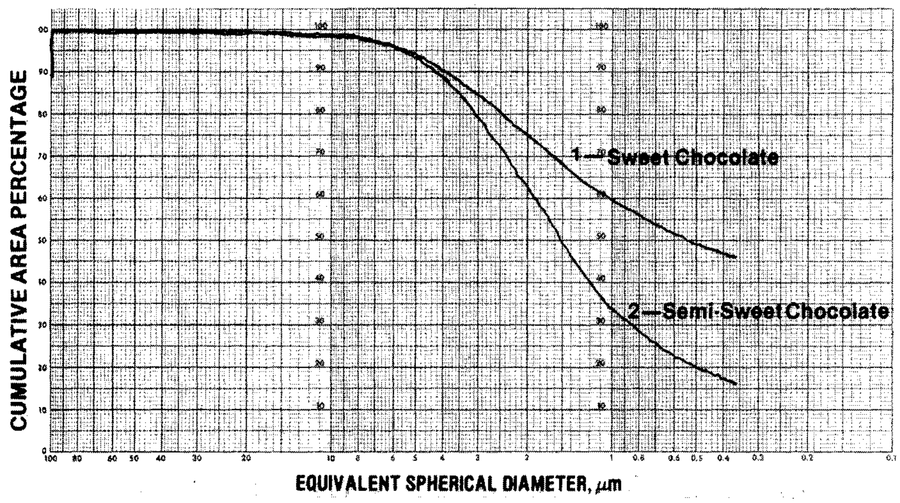


Particle size analysis of chocolate

These data, taken by the photosedimentation method, show that the sweetness of chocolate is controlled by the size of cocoa particles. Sweet chocolate has more small particles than semi-sweet. [*Source: Micromeritics Instrument Corporation*]

formula that describes the process. As they settle, the particles block a collimated x-ray or white light beam. Electronic circuitry continuously executes the operations prescribed by the mathematical formula as the particles settle and less and less light is blocked. In the end, a graph giving the fraction of particles at each particle size is generated. In the earliest versions of such instruments, the time required for this analysis could be several hours or even days. Nowadays, the collimated light beam is programmed to scan from the bottom to the top of the container, thereby reducing the time to a half hour or less. Jack Smithwick of Micromeritics Instrument Corporation, Norcross, Georgia, cited as one example of the timesaving possible with automated machines the analysis of particle size in clay. Where it once took 200 hours to track 0.25-micrometer clay particles, it now requires only 67 minutes.

Two companies, Micromeritics and Microscal Ltd. of London, were at the Pittsburgh Conference offering sedimentation instruments. The Micromeritics x-ray version handles particles in the size range 0.1 to 100 micrometers and costs $20,250. The company also manufactures a similar instrument based on the blockage of a visible light beam by settling particles that are transparent to x-rays. This machine covers the same size range and costs $14,950. The Microscal Photosedimentometer, which uses visible light, is designed for particles from 2 to 75 micrometers. Prices start at $5500 for a nonscanning version, the least expensive of three models. Microscal also makes a scanning x-ray instrument that measures particles having diameters from 1 to 75 micrometers and that costs $23,000.

A variation of the sedimentation method is the use of a centrifuge. Two firms exhibiting particle-sizing instruments based on centrifuging were Joyce-Loebl of Marquisway in the United Kingdom and Shimadzu Scientific Instruments, Inc., in Columbia, Maryland. Joyce-Loebl's device covers particles with sizes from 100 angstroms to 60 micrometers and costs $35,000. A data analysis terminal is also available. The Shimadzu instrument measures particles with diameters from 0.1 to 150 micrometers and is priced at $9000. Microscal did not show it at the conference, but the firm does offer a centrifuging x-ray sedimentometer that can measure particles as small as 100 angstroms in size.

Another well-established particle measurement technique does not rely on optical phenomena. Particles are dispersed in an electrolyte containing two electrodes. Between the two electrodes is an orifice. The resistance to current flowing in the electrochemical cell formed by this system depends in part on what is happening at the orifice. In particular, if a particle fills the orifice and displaces some of the electrolyte, the resistance is momentarily increased. The volume of the particle in the orifice, and hence its size, can be calculated from the change in the resistance.

Two companies exhibited particle measuring instruments of this type. One is Particle Data, Inc., Elmhurst, Illinois, whose device measures particles having sizes from 0.3 to 450 micrometers. The company sells four versions of its instrument ranging in price from $9,000 for a manual system to $30,000 for a highly automated one. The second manufacturer is Coulter, whose Coulter Counter is

(Continued on page 152)

Rough Going for Lasers at the Pittsburgh Conference

Lasers have never been a big hit at the Pittsburgh Conference. In part, this may be because the emphasis there has been on instruments that are as rugged and inexpensive as possible and, above all, can be operated by relatively unskilled technicians. Most lasers fit none of these categories. The J-Y Optical Systems Division of Instruments SA, Inc., for example, has exhibited for several years a Raman scattering spectrometer, an instrument that is practical only with a laser as the light source, but J-Y sales representatives have told *Science* that this equipment has never generated much interest. Well-known laser manufacturers, when they come to the conference at all, display other lines of instruments, such as chromatography equipment. Probably the biggest use of lasers has come in instruments that measure the size of minute particles by light scattering (see story, p. 146). These devices use helium-neon gas lasers that are rugged and inexpensive, and for the most part the operator does not need to know whether there is a laser or a light bulb in the machine. This is called making the laser transparent to the user.

Transparency may be the key to lasers penetrating the analytical chemistry market. One company that is trying this approach is the Laser Analytics Division of Spectra-Physics. Several years ago Laser Analytics developed a solid-state infrared laser whose wavelength could be varied by adjusting its temperature within the range from 15 to 100 K. Three such Laser Analytics introduced a microcomputer-controlled isotope ratio company has been marketing a complete infrared spectrometer system built around these sources. This year, Laser Analytics introduced a microcomputer-controlled isotope ratio measurement system that is based on measuring the absorption of light from a tunable solid-state laser at two different wavelengths. A six-page brochure mentions the word laser only four times and emphasizes that a technician can operate the instrument.

The device is intended to measure

the ratio of carbon-13 to carbon-12 isotopes and is meant to be an alternative to mass spectrometer–based systems that can cost from two to four times as much as the $89,000 price of this instrument. Expected applications include looking at breath samples of patients, after they have eaten food laced with carbon-13, in order to obtain information on metabolic rates that may signal any of several disorders. In the industrial world, the same ratio is used by geologists in their mapping of potential oil fields.

Thus, the object is to sell not a laser but a solution to a problem. Three other exhibitors were on hand at the Pittsburgh Conference this year that were not as far along this road as Laser Analytics, but they were thinking the same way. Tachisto Incorporated made a stab at introducing analytical chemists to the carbon dioxide gas laser 5 years ago but withdrew when little interest was shown. These infrared sources are heavily used in physical chemistry research, but are not the sort of thing one turns on just by flipping a switch. This year Tachisto was back with a compact and tunable (9 to 11 micrometers) carbon dioxide laser system for measuring the concentrations of components in gases and liquids. The laser itself with an optical bench costs about $12,000. For an additional $7000, one can get the required optical components and a detector for a complete system.

Lasermetrics, Inc., exhibited a high-power, solid-state, infrared laser (ruby, neodymium-doped glass, and neodymium-doped yttrium aluminum garnet are available). A representative said that the company in its first visit to the conference was mainly interested in getting some exposure and learning about applications that analytical chemists are interested in. Complete systems for specific applications could be expected in the future. The ruby laser shown at the Pittsburgh Conference was priced from $13,000 to $25,000, depending on its performance. It was powerful enough to create a miniature lightning bolt as its beam passed through the air, much to the consternation of a salesman in a neighboring booth.

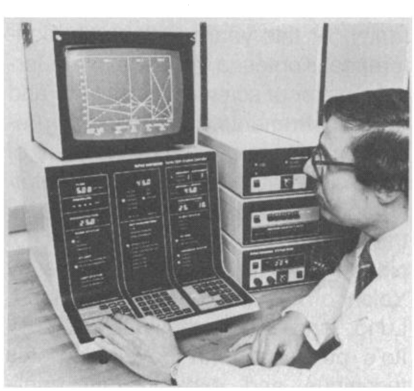
Finally, Hammamatsu Corporation held a seminar on, but did not have on hand in its booth, its excimer, ultraviolet lasers. Excimer lasers have gases like krypton fluoride as their active medium and are similar in complexity to carbon dioxide lasers. A spokesman for the company said that he hoped to have a complete ultraviolet spectrometer system in the future.

—A.L.R.

How Many Solvents Is the Limit for HPLC?

High-performance liquid chromatography (HPLC) continues to be the fastest growing of the myriad analytical techniques exhibited at the Pittsburgh Conference. The goal of HPLC is to separate a mixture of (usually) known components so that their concentrations can be measured or so that they can be collected for further study or such uses as the synthesis of compounds. Ideally the peaks in a chromatogram corresponding to each component will not overlap, so that the separation is complete; and they will not be too far apart, so that the separation does not take any longer than necessary (a few minutes).

The key to achieving this devoutly to be wished for situation is the composition of the solvent in which the multicomponent sample is dissolved. This year the DuPont Company introduced a microcomputer-controlled HPLC module that mixes as many as four solvents, thus stepping ahead of other manufacturers who have been touting three-solvent instruments in the last year or so. The module, which consists of a gradient controller, a graphics display, and a three-piston pump, costs $16,200. The four-solvent capability can be added to DuPont HPLC's that are already in the field for $5000.



DuPont four-solvent module

Among other capabilities, the display portion of the module allows the chromatographer to see the changes in the concentrations of each of the four solvents with time. [Source: DuPont Analytical Instruments Division]

The reason for having more than one solvent at all is that the solvent composition best suited for separating components A and B from one another may not serve well for separating C from A or B, and so on. By changing the solvent composition (gradient elution) as the chromatogram is being run, this deficiency can be remedied. And the more solvents there are to play with, the more likely it is that the ideal composition for separating each pair of components will be found, at least in principle. Last year, when ternary solvent mixers were being ballyhooed, scoffers complained that three solvents provided too many variables and that chromatographers would not be able to handle the resulting complexity efficiently. Four solvents would seem to exacerbate the problem greatly. One competitor said DuPont was just getting caught up in a solvents race that is reminiscent of a similar contest in the 1960's to see which manufacturer could produce the instrument operating at the highest pressure. (The P in HPLC once stood for pressure.)

DuPont's argument is that the company picked four as the number of solvents because their theoretical work had shown that four was the optimum number. Presumably, then, the next Pittsburgh Conference will not see a five-solvent HPLC introduced. A paper presented at the conference by Joseph Glajch, Jack Kirkland, Karen Squire, and James Minor, all of DuPont, also demonstrated a practical way of dealing with the complexity presented by four solvents.

The DuPont chromatographers recommended picking three solvents with very different properties (acid or base character and strength of dipole-dipole interactions) and a carrier solvent. The recommended four were methanol, acetonitrile, tetrahydrofuran, and water in the case of reversed-phase HPLC, the most popular form. With the data from as few as seven test runs with different solvent compositions, a minicomputer could generate a map showing the ability of every possible solvent composition to separate each pair of components in the mixture. In this way, solvent compositions that can separate all the components can be spotted. It is this opti-

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mum solvent composition that is used in subsequent separations.

Obviously, since even ternary solvent mixing in HPLC is new, it remains to be seen if four solvents will catch on.—A.L.R.

Concentrations Measured by Delayed Lasing

An unusual new way to measure the concentrations of components in dilute solutions has been developed by J. Michael Ramsey and William B. Whitten of the Oak Ridge National Laboratory. Their approach transforms a concentration determination from a measurement of the intensity of transmitted light to a measurement of a time difference, which can be performed more accurately. The measurement is made with optically pumped lasers—that is, lasers that emit light at one frequency when stimulated by a laser operating at a different frequency.

The active material in some pumped lasers exhibits a relatively slow rise in optical gain after the injection of energy by the pumping laser. As a result of this slow rise, there is a significant time delay between the initiation of pumping and the onset of lasing. The delay corresponds to the time required for the optical gain of the active medium to increase above the optical losses of the laser cavity. Absorption of light by a sample injected into the laser cavity increases these losses, and the increase in delay time is directly proportional to the concentration of the sample.

Ramsey and Whitten use a Nd:YAG laser pumped by a frequency-doubled Nd:YAG laser. In their original experiments, which had a completely different goal, they observed two light pulses from the system, one from the pumping laser and the other from the pumped laser. They further observed that the amplitude of the second pulse could be increased and the delay time reduced virtually to zero by increasing the pumping energy. Since anything that would absorb energy from the system would increase the delay time, they reasoned that it could be used to measure concentrations. Experimenting with copper sulfate, which absorbs at the wavelength of the Nd:YAG laser (1054 nanometers), they found this to be the case. By increasing the stability of the pumping laser, reducing cavity losses, and reducing the size of the cavity, they estimate that concentrations as low as 10^{-5} molar can be measured.

The chief advantage of this approach is that it involves only determination of a time period, which can easily be measured in nanoseconds. The response is linear over most of the concentration range of interest, which is also an advantage. And perhaps most important, there is the potential for a zero-background measurement, which leads to high sensitivity.

The chief disadvantage, says Ramsey, is that "few things absorb at 1054 nanometers." This problem can be partly overcome by using other media for the pumped laser: Er:YLF lasers emit at 850 nanometers, Ho:YLF lasers emit at 750 nanometers, and alexandrite ("almost a ruby") emits between 700 and 800 nanometers, which gives some potential for tuning. The lanthanides also have bands in the near-infrared which could be useful. Any wide application of the technique, though, would clearly require development of new media that emit in the ultraviolet and visible regions.
—T.H.M.

Persistence Pays Off in the Form of a New Product

The popular high-performance liquid chromatography (HPLC) is a specific instance of the more general class liquid-liquid partition chromatography. At this year's Pittsburgh Conference, Kontes, a Vineland, New Jersey, maker of scientific glassware and other instruments, introduced a preliminary version of another kind of liquid chromatograph. Called a centrifugal countercurrent chromatograph, the device is based on a concept pioneered over the last 15 years by Yoichiro Ito of the National Heart, Lung, and Blood Institute (NHLBI). Ito's persistence in developing this technique and exploring its applications may be on the verge of paying off. In addition to its impending commercialization, countercurrent chromatography was also featured in a special technical session at the conference and was the subject of a workshop sponsored by Kontes.

Ito and several co-workers first published on countercurrent chromatography in 1966. Since that time, the NHLBI group has engineered several types of devices, some that are improvements on the original design and some that are variations for specific applications. In fact, earlier versions of countercurrent chromatographs have been marketed for some time by the Tokyo Rikakikai Company Ltd. of Tokyo. The firm, which sells two models called a droplet countercurrent chromatograph and a rotation locular countercurrent chromatograph, respectively, first exhibited at the Pittsburgh Conference last year, but has been selling to U.S. analytical chemists directly from Japan for a longer time. Each instrument costs $9300, excluding a detector. Pharmaceutical companies and schools of pharmacy have been the major customers, presumably because countercurrent chromatography seems best suited for the separation of bioactive natural products, which HPLC finds hard to handle.

Kontes plans to start slowly in developing a market for its apparatus, which has the long-winded name horizontal flow-through planet centrifuge, according to a representative, John Babashak. By placing a dozen or so instruments in certain key laboratories, the company hopes that a body of applications literature will be built up and that sales will follow. The cost to the first selected customers will be $7500.

In HPLC instruments, one of the two liquid phases, the stationary phase, adheres to micrometer-sized solid particles, giving rise to the term bonded phase chromatography. In contrast, boosters of countercurrent chromatography call their technique a true liquid-liquid partition chromatography because there is no solid support for the stationary phase. In the version of the instrument being sold by Kontes, the second liquid phase, the mobile phase, flows through several helical glass tubes containing the stationary phase. The entire assembly rotates, and the combination of gravity and centrifugal force from the rotation promotes mixing of the two liquid phases and thereby the partitioning of the components of the sample between them. Whereas HPLC machines typically operate at several thousand pounds per square inch pressure, the countercurrent chromatograph requires only a few hundred pounds per square inch. The lower pressure does, however, extend separation times from minutes to hours.

There are two modes of operation. The first is analytical work involving samples of 1 gram or less. For this, a cluster of small-diameter helical tubes is used. The second is preparative liquid chromatography in which the object is to collect larger quantities of material for subsequent use. One large-diameter helical tube is sufficient for this purpose. The instrument houses clusters of both large- and small-diameter tubing, so both modes can operate simultaneously.

In the technical session, several applications of countercurrent chromatography were discussed, including separation of prostaglandins, plant hormone analysis, and isolation of pure pesticides. But the most enthusiastic comment of all was heard at the workshop, where a participant exclaimed, "The EPA should put 15 of these in each of its laboratories."

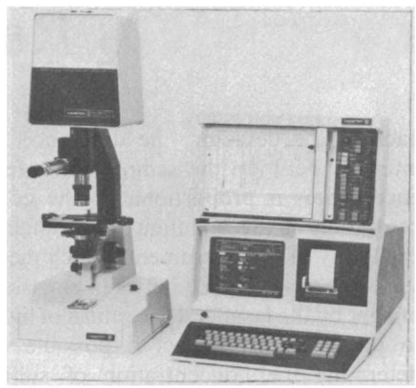
—A.L.R.

IR Spectrophotometer Wed to a Microscope

An infrared microspectrophotometer that combines the virtues of a research microscope with those of an infrared spectrophotometer was displayed for the first time at the Pittsburgh Conference by Nanometrics Inc. of Sunnyvale, California. Called the NanoSpec/20IR, the instrument can obtain infrared spectra of objects as small as 20 micrometers square.

A conventional Nernst glower provides both visible and infrared light that are focused by an all-reflecting optical system. The visible image of the sample is magnified 150-fold and transmitted to an eyepiece for precise positioning of the sample. The infrared beam is focused on a liquid-nitrogen-cooled mercury cadmium telluride photodetector that is at least 100 times more sensitive than a conventional thermal detector. A microprocessor controls a circular variable filter that provides wavelength scanning or analysis at a fixed wavelength; the microprocessor also digitizes the signal from the photodetector. Data are displayed on both a cathode-ray tube and an X-Y recorder. As many as eight complete spectra can be stored in the microprocessor memory, manipulated arithmetically, and displayed. An optional floppy disk memory allows storage of large numbers of spectra and other useful information.

The NanoSpec/20IR has a large number of potential applications in the forensic sciences. It is also useful for



analysis of trace amounts of organic materials. Some possibilities include organic contaminants on silicon wafers in the semiconductor industry, inclusions in thin films of various sorts, single crystals of drugs, particulate matter in intravenous fluids, and any other material that is present in only very small amounts. The device can, however, also produce spectra of larger samples in conventional infrared and gas cells. The purchase price of the NanoSpec/20IR is $49,500.

—T.H.M.

IBM Now Making Analytical Instruments

It may not be fair to describe other instrument manufacturers as quaking in their booths, but there was certainly a fair amount of apprehension about the entrance of International Business Machines Corporation into the analytical instruments arena. At the 1980 Pittsburgh Conference, IBM had a small contingent demonstrating an interface for coupling analytical instruments to computers and a color sensor. This year, IBM Instruments, Inc., of Danbury, Connecticut, a subsidiary formed only 10 months ago, occupied a dozen booths and three seminar rooms and exhibited six lines of instruments—the device coupler and five new ones. The company is known to be developing other product lines also, and at least one reliable source reports that IBM intends to be the dominant force in the analytical instrument field by 1990.

In some respects, IBM's entry is a logical one. The company had a reasonable amount of free cash to invest in new fields as a result of its profitable computer and business equipment operations. Furthermore, IBM has for a long time manufactured silicon chips and microprocessors of the type that are in instruments manufactured by other companies. Since the microprocessor is the most sophisticated part of many instruments, according to one competitor, IBM undoubtedly decided that it would be a relatively simple matter to market a complete instrument.

IBM obtained a hard core of instrument specialists by acquiring a noncontrolling interest in Bruker Spectrospin, which is among other things a well-known maker of nuclear magnetic resonance (NMR) spectrometers with manufacturing facilities in West Germany. They also recruited a number of instrument specialists from other companies, but the exact number remains a matter of contention. There are estimates that at least 150 Ph.D.'s have been hired from companies along the eastern seaboard in recent months, but an IBM spokesman says that total employment at the Danbury site is only 200, many of them support personnel.

What is not a matter of contention is that the company has introduced an impressive array of new instruments. In addition to the NMR spectrometers already manufactured by Bruker, IBM is offering the PC/20 line of tabletop NMR analyzers for process control in industry.

They also have the ER/100 and ER/200 lines of electron paramagnetic resonance spectrometers, the IR/80 series of Fourier-transform infrared spectrophotometers, and the LC/9500 line of liquid chromatographs and accessories. Finally, for electrochemical analyses, IBM has the EC/200 series, which includes a stripping voltammeter, a voltammetric analyzer, and a sequencer for automating the whole procedure. Some of the new instruments will not be available for delivery until later this year.—T.H.M.