Two other forms of HPLC are ion exchange chromatography and size exclusion chromatography. In ion exchange chromatography, ion exchange resins in the form of small or porous particles serve as the column packing material. This technique is generally restricted to such specialized uses as amino acid analysis where the materials to be separated are ionic species. In size exclusion chromatography, the dimensions of the pores in the packing material are small enough to physically prevent the passage of large molecules such as polymers, and the technique is primarily used for polymer analyses, such as determinations of molecular weights.

In principle, any HPLC instrument could accomplish, with the appropriate column and detector, any of these forms of liquid chromatography. In practice, not all can, and, in particular, size exclusion chromatography is usually done by a special chromatograph designed specifically for this purpose; part of the reason for this is that the column must often be kept at a high temperature for this type of chromatography.

Besides a column and a pump, the other essential part of a liquid chromatography system is the detector. The three most common detectors measure refractive index, ultraviolet absorption, or fluorescence emission. Detectors are therefore essentially conventional optical instruments modified to incorporate special flow cells through which the solution passes continuously. Since the volume of a flow cell is quite small (a few microliters), the optics must be adjusted to handle samples much smaller than normal.

Of the three types of detectors, the refractive index instrument—which detects the presence of a component if its refractive index is different from that of the solvent—is the closest thing to a universal detector for all substances, but it is not as sensitive as the other detectors (minimum detectable sample is 5×10^{-7} gram per milliliter). The ultraviolet absorption detectors are a thousand times more sensitive, but they record absorption at only one wavelength (usually 254 nanometers) and not all substances have absorption bands in this region of the spectrum. Recently, the use of variable wavelength ultraviolet spectrophotometers has become popular. But because the intensity of light from sources that emit light over a broad range of wavelengths is much lower than that from sources that emit at only a few discrete wavelengths, the sensitivity of these instruments is lower. Fluorescence detectors are more sensitive yet, but they are usable only if the analyzed substance is intrinsically fluorescent or if it can be made to emit light by the addition of fluorescent moities or fluorophores.

Instruments that incorporate these three ingredients (pump, column, and detector) along with the now almost obligatory microcomputer to control their operation and a second computer for data handling come in a wide variety of sizes, capabilities, and costs. The cost, in fact, is rather modest—at least when compared to some other types of instrumentation, and it is difficult to pay more than about $40,000 for a "Cadillac" model HPLC. According to David Banks of Hewlett-Packard, the most rapidly growing segment of the liquid chromatography market is that devoted to more or less routine separations, as opposed to frontier research. Much of the motivation for this trend appears to lie with government environmental and safety regulations that have driven companies to monitor their chemical processes more closely. Another motivation stems from the need to control the properties of chemical products. As simple a thing as the tartness of grapefruit juice can be fine-tuned by adjusting the concentration of a certain acid. In any case, the HPLC instruments shown at the Pittsburgh Conference seemed to be oriented toward users interested in testing large numbers of samples with minimum operator attention.

Several manufacturers thus exhibited instruments with automatic sampling devices. In most cases, these are carousels holding (depending on the manufacturer) approximately 60 test tubes or vials. Usually three or more sample injections could be taken from each vial, so that about 200 samples could be taken automatically. (One company, Altex Scientific, demonstrated an autosampler in which the vials were stacked vertically rather than in a carousel.)

A complication that arises for multicomponent samples is that the best solvent (mobile phase)—that is, the solvent that will provide evenly spaced peaks separated sufficiently so that complete resolution is achieved but not so widely that too much time is wasted—can vary from one pair of components to another. A partial solution to this problem is to continuously vary the composition of the solvent as the chromatogram is being run. Generally two, but in some cases three, solvents can be mixed in any desired proportion that changes with time. This procedure is called gradient elution.

Thus, the microcomputer that controls the operation of the liquid chromatograph must be able to set the basic operating parameters of each separation, such as pressure limits, flow rate, and column temperature, and at the same time manage the gradient program appropriate to each sample. For a hundred or more samples, it is well beyond the microcomputer's capability to remember

Three-Dimensional Fluorescence Spectroscopy

The first commercial instrument designed to record automatically three-dimensional spectra of fluorescence intensity as a function of both excitation and emission wavelengths made its debut at this year's Pittsburgh Conference. The Fluorocomp total luminescence spectroscopy (TLS) system introduced by Baird Corporation combines a high-resolution ratio spectrofluorometer with a minicomputer, a floppy disk file system, a digital plotter, and a software package, all at a cost of about $45,000.

Fluorescence spectroscopy is an established method for qualitative and quantitative analyses of substances, such as aromatic organic compounds, that fluoresce—that is, absorb light at some wavelengths and reemit it at others. The traditional approach is to pick a wavelength of light that excites the sample, and then to measure the intensity of light emitted by the sample as a function of wavelength (an "emission" spectrum). Alternatively, the intensity of emitted light at a given wavelength can be measured while varying the wavelength of light in the exciting light beam (an "excitation" spectrum). One problem with these approaches is that a sample containing more than one fluorescent compound produces composite spectra, and it may be difficult to determine how much of a given peak is due to each of the compounds.

With a "total luminescence" (TL) spectrum, fluorescence intensity is measured as a function of both excitation and emission wavelengths. Just as a topographic map of the earth's surface shows elevation as a function of latitude and longitude, a contour plot of the TL spectrum can be made showing fluorescence in-

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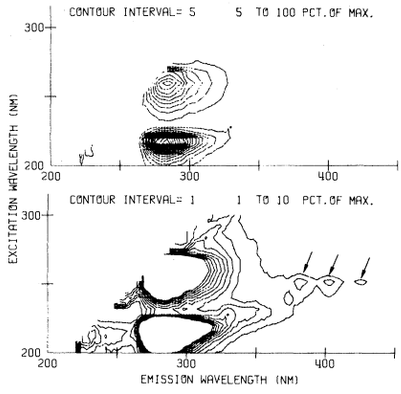
a separate program for each sample, but samples that will be treated alike can be grouped; microcomputer-controlled chromatographs can generally store about ten programs. And instruments can, if necessary, be connected to mini- or larger computers for further direction.

At the other end of the chromatography process are the data, usually in the form of a spectrum giving the response of the optical detector as a function of time. Peaks in the output at specific times provide the identities of the components of the sample, whereas the areas under the peaks are a measure of the quantity of each component present. At a minimum then, data systems report the elution time of each peak and the peak area. Often there are mathematical routines stored and these enable overlapping peaks to be deconvoluted and permit the average peak parameters and the variances for multiple runs to be calculated. And, if errors are detected that

tensity over a range of excitation and emission wavelengths. Compounds with overlapping spectral maxima for a given excitation wavelength may be readily distinguishable at other excitation wavelengths. Also, nondescript bumps and wiggles in an emission spectrum may suggest trace amounts of impurities when the TL spectrum is examined. In the figure, two different contour intervals are used in plots of the TL spectrum of undecylbenzene. With the 1 percent contour interval, three small peaks (indicated by arrows) are suggestive of an anthracene-like contaminant.

Almost any spectrofluorometer can produce TL spectra under manual



control, but the process is rather tedious. With Baird's system, the operator converses with the computer initially, telling it how to produce the desired spectrum. Then the computer takes over. It takes about $1^1/_2$ hours to record a complete TL spectrum, much less time than would be needed with an ordinary spectrofluorometer under manual control. One of the floppy disks is dedicated to system software, the second is used to store TL spectra. Typically, between 10 and 20 spectra will fit on a single diskette. If desired, data stored on the disk can be processed to produce TL contour plots, as well as detailed excitation and emission spectra. More sophisticated routines for processing TL spectra are currently under development.

Baird's TLS system is expected to be a practical addition to research and industrial labs engaged in hazardous materials searches or chemical quality assurance programs. In addition, pharmacologists and clinical and forensic chemists might find such an instrument a valuable asset.—F.F.H.

New Tool for Mass Spectrometry

A new instrument that was not physically present at the Pittsburgh Conference, but that was nonetheless discussed with great interest was the Lamma 500, a *l*aser *m*icroprobe *m*ass *a*nalyzer developed by Leybold-Heraus GmbH of Cologne, Federal Republic of Germany. In simplest terms, the instrument is a mass spectrometer in which a laser beam is used to ionize the sample, but several refinements in design are claimed to give it much better spatial and analytical resolution than other types of commercial spectrometers and similar instruments constructed in research laboratories.

The basic components of the system are a laser, an optical microscope, and a time-of-flight mass spectrometer. Thinly sliced specimens similar to those used for microscopy are placed in the evacuated entry port of the spectrometer under a cover slide that functions both as an optical window for the microscope and as a vacuum seal for the port. The microscope serves both to image the specimen and to focus the laser on a small area (less than 1 micrometer in diameter) of the specimen chosen for analysis. A short pulse of the laser vaporizes about 10^{-13} gram of sample and simultaneously ionizes it. The microplasma thus created is drawn into the spectrometer by magnetic fields. Each laser pulse produces a complete mass spectrum of either positive or negative ions in about 1 second. Because more than half of the ionized sample is drawn into the spectrometer, detection limits are quite low, on the order of 10^{-18} to 10^{-20} gram. This is comparable to or better than any other commercial instrument, the company contends.

For analysis of elements, Lamma spectroscopy is thus quite similar to electron probe microanalysis (EPM) (*Science*, 22 July 1977, p. 356), with the obvious difference that the detection techniques are different. Lamma can identify the isotopic composition of a sample, whereas EPM cannot. Lamma can also be used for organic materials; EPM cannot. The new technique is particularly useful for identifying organic compounds, the company says, because the parent ion (from which the molecular formula of the compound can be obtained) is produced in abundance and because mass spectra produced by Lamma are generally much simpler than those produced by other techniques. Polymeric material generally breaks down into ionic species characteristic of its chemical structure.

Many of the applications are similar to those of EPM. Lamma spectrometry has been used, for example, to study the distribution of lithium in brain cells, of fluorine in teeth, and of calcium in muscle cells. It has also been used to detect individual manganese-containing bacteria in heterogeneous populations of bacteria, microdistribution of preservatives in wood, and uranium in carbon microspheres. The technique can also, however, presumably be extended beyond EPM to study the subcellular distribution of organic materials, such as drugs, metabolites, and so forth.

The Lamma 500 now sells for the equivalent of $200,000 in Germany, but will probably be somewhat more expensive in this country because of import duties.—T.H.M.