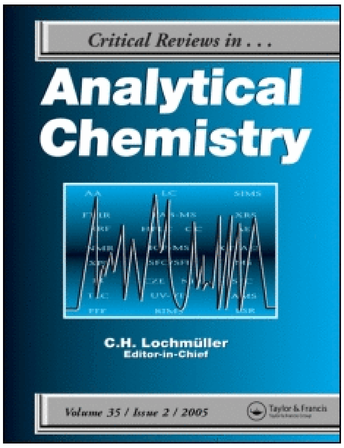

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Principal Instrumentation for Modern Automated Liquid Chromatography

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Principal Instrumentation for Modern Automated Liquid Chromatography

Vern Berry

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I. INTRODUCTION

A. Objective

The first "high-performance" liquid chromatography (LC) was begun about 20 years ago, spurred strongly on by the availability of improved equipment (reviewed through 1975 by Veening).[1] Many of the more recent generations of chromatographers are not aware of the instrumentation or the important innovations that led to the evolution of the highly automated instruments of today. Consequently, this review includes some earlier discontinued instrumentation that were milestones leading to the more recent developments. Each section begins with earlier instrumentation and discusses the importance of these innovations. Consequently, the most recent instrumentation will be found near the end of each section. As each instrument is described, the important new features over earlier instruments will be emphasized, perspective as to why these new features are important will be given, and, finally, the limitations of the instrument is noted.

Trying to produce a review that is current is like trying to hit a moving target, since new instrumentation is constantly being introduced. Hence, this review covers LC instrumentation through early 1988. However, the background developed in each section by descriptions of the then current instruments, and of significant discontinued instruments, will educate the reader on what factors distinguish components from different companies. One reassuring factor is that LC is now relatively mature, and totally new directions for instruments are less frequent than in the first few years. For example, gas amplifier pumps (Haskell), flow amplifier pumps (Micromeritics), and solenoid pumps (Problematics) have disappeared from LC.

However, this author predicts a genealogy that will probably feed new types of instruments back into LC, such as injection by electromigration or micromanipulators, and detection with lasers. Conventional LC with 4- to 5-mm id columns led to developments in micro-LC with 0.2- to 2-mm columns, which in turn led to developments in capillary electrophoresis with 0.025- to 0.100-mm columns (especially with better detection). Capillary electrophoresis has provided the biotechnology field with the capabilities for (1) detection of less sample, (2) resolution of more complex mixtures, and (3) isolation of less material. This author predicts that these new capabilities plus the improved instrumention (injection, detection, gradient generation, etc.) from capillary electrophoresis will feed back into LC and make possible open tubular LC with 0.002- to 0.005-mm (2 to 10-μ) id columns. The enormous complexity of the biotechnology problems that are ahead of us will need the different selectivities that the two methods provide.

This review has limited its discussion to only several of the key components that are so important to modern automated LC, because of the very wide variety of LC-related products. The components discussed here include autosamplers, pumping systems (and pump gradient controllers), and diode array detectors) (DADs). Not included are some very important components such as manual injectors, columns, other detectors, and data systems.

The 1987 prices give relative costs. Approximate current costs are found by increasing these prices by about 20% per year.

The reader should recall that most of the instruments discussed here are the products of companies that have been in the field for 10 to 20 years, or longer. And all of these companies and products offer some important mix of features, such as product performance, price, company support, or service, that appeals to some important market. This review considers only performance features and sometimes gives (1987) prices. However, to many users, the less tangible criteria of customer relationship, applications support, instrument reliability, and instrument repair may be far more important than specific product capabilities or price. Every reader will find different aspects important to him today, and as their jobs change, as LC methods evolve, and as products change, users will find that products from other manufacturers have become desirable.

B. Background

Conventional LC before about 1965 was "normal phase" LC with silica particles of large diameter (100 to 200 μ) packed into open glass columns. Gravity was used to produce flow of organic eluants such as hexane and chloroform. Separations required several hours to several days and could be achieved with moderately large nonpolar molecules such as steroids and plant pigments. These are retarded by the silica by specific interactions of the silica groups with polar groups of the sample molecules. With the large particles, speeding flow beyond gravity flow often produced broader peaks and less sensitivity, so there was little incentive to speed flow. Because gravity flow took many hours, it was easy to use off-line manual detection by catching samples in vials and checking them for absorbancy, pH, dry weight, etc.

Modern LC began around 1965 to 1970 with the realization from theory that sharper peaks for more sensitive detection could be obtained by using particles of smaller diameter. Gravity

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alone could no longer produce fast enough flow through columns packed with 30- to 50-μ particles, so various pumping systems were used. With pumped flow and smaller diameter particles, runs could be made many times faster than by conventional methods. Indeed, it was no longer possible to manually collect and measure fractions and keep up with the flow. Also, because peaks were sharper, the fractions had to be smaller and taken more frequently. This led to the need for on-line flow-through refractive index and UV/visible spectrophotometric detectors.

Only after about 1975 did the first reversed-phase packing start to receive interest. These consisted of aliphatic molecules such as C-18, C-8, and C-2 chemically bound by a hydrolytically stable Si–C bond onto porous silica particles. These "reversed-phase" supports received great attention because weak to strong elution came from progressing from water to organic solvent such as alcohols and acetonitrile. Thus, these packings were compatible with biologically important molecules such as drugs, nucleic acids, and urine and blood components. This compatibility to biologically and medically important materials led to modern high-performance reversed-phase chromatography becoming one of the leading instrumental methods of analysis today.

C. General Instrument Criteria

Before discussing some of the individual components of a liquid chromatograph, it is worth considering some general criteria that are desirable in all components.

High "up-time" — A concept from the computer industry, up-time is the fraction of time that an instrument is operating reliably when it is expected to operate. "Operating reliably" involves operating within the required specifications of accuracy and reproducibility. Up-time can be high if (1) the instrument rarely breaks down or (2) the instrument failure can be quickly diagnosed and quickly repaired.

Built-in diagnostics — The instrument that clearly signals when and where it is malfunctioning is highly desirable. Today, many instruments have an internal diagnostics routine that is initiated each time the instrument is turned on.

Easy repair — Instruments designed for easy repair by the operator, clear instructions for performing the repair, and a system for stocking the repair parts or quickly obtaining repair parts is highly desirable.[2]

Reasonable costs — More sophisticated managers are probably less concerned today about the "simple costs" of the initial capital outlay. The "true costs" of an instrument are much more complex, involving maintenance and repair (often 10% of initial costs per year) and more subtle but more important costs, such as lost opportunity costs or alternate opportunity costs. Lost opportunity costs are such items as the costs of lost product or production that might have been saved with a more advanced instrument, instruments simpler to operate, or better trained personnel. Alternate opportunity costs would consider how problem response might have been improved had the money invested in particular instruments, personnel, etc. been invested in other instruments, employees, training, etc. Building up a file of production problems that were solved by modern instruments can lead to cost justification of even the most expensive instruments, such as mass spectrographs.

High automation — Automation can mean instruments and components able to function in an unattended manner. However, with the microprocessor-controlled instruments of today, automation also means instruments that can perform self-diagnostics and calibration routines, instruments that are "foolproof" in that they are self-teaching, require the simplest operation, and may substitute a microprocessor "help" key for an instruction book. Initial startup "default conditions" can be set to give the most probable conditions to speed use.

Portability — Rugged, lightweight, small components or instruments that can be readily moved between applications or instruments are receiving increased attention, just as calculators, computers, and music systems have become more portable. The need for ruggedness in portable instruments implies that such instruments can maintain high up-time despite being moved and despite hostile environments. Moving toward the limit of portability comes battery-operated, pocket-size instruments (such as electronic thermometers, pH meters, and colorimeters of today). In the limit, the focus should, perhaps, be on the *function* desired and not the *device* to achieve it. For example, the function might be to produce a continuous change in eluant composition, not the device, a gradient generator consisting conventionally of two pumps, a pump controller, and a mixer. The function, gradient generation, can be far smaller than the devices we currently envision, such as new gradient generation approaches described by Berry et al.[3]

D. Problem Throughput

The high costs of poor sample throughput is receiving much attention today, now that LC is out of the discovery era, when simply achieving separation at any cost was valuable. Throughput can be measured in many ways, such as samples per day, samples per dollar of overhead (salaries, employees benefits, instrument costs, materials costs, building rental, etc.), or "net wealth" per year, "with" vs. "without" the added method (this includes the more difficult-to-assess factors such as the effect of a method to decrease bad product, improve production, decrease worker turnover, etc.).

Factors that can greatly improve throughput include:

1. Autosamplers — to permit operation during closed hours
2. Optimization programs — to reduce methods development time
3. Separation simulation computer programs — to translate methods quickly between columns of different dimensions or supports
4. Expert systems — computer programs to bring the knowledge and logic of experts to the aid of the novice for method development, instrument troubleshooting, etc.

5. "Value engineering" of instruments — to provide the most performance per dollar by maintaining operation while minimizing costs of components making up the component[4]
6. "Fast-LC" column configurations — to increase the speed of analysis, reduce costs of materials, etc.[5,6]

The last of these items for improving throughput, fast-LC, has received much attention recently from Perkin-Elmer. Conventional LC usually uses columns 100 to 250 mm long, with inside diameters between 4 and 5 mm packed with particles 5 to 10 μ in diameter. Recalling that resolution is proportional to the square root of the column length (plate number), if the column length can be halved (and sufficient resolution remains), then the time for the separation can also be halved, thus doubling the throughput (e.g., the number of samples per hour).

Fast-LC, used by Perkin-Elmer, takes this shortening of the column further by using a column about one seventh as long (33 mm) as conventional columns. Using its "3 × 3" column (33 mm long, 4.6-mm id packed with 3-μ particles) with compatible injector, detector, and special "serpentine" connecting tubing to minimize peak spreading, Perkin-Elmer has shown very fast separations. For example, as shown in Figure 1, it required only 12 s and 700 μl of eluant to provide sufficient resolution in this very short column as found with the conventional 250-mm-long column packed with 10-μ silica. The short column is packed with 3-μ silica.

These 3-μ columns are not easy to work with, and moving into fast-LC may require a considerable commitment to learn new LC techniques. The columns are easy to clog, but they can be inexpensive (about $40 each vs. $200 to $300 for conventional columns). Approaches to protect them from particle clogging and silica dissolution[7] have been addressed recently. Thus, there is a great trend today to use shorter columns.

Costs per sample potentially can be decreased also by decreasing the costs of solvents, both their purchase and disposal. In the previous example of cutting the column length in half for a specific separation, solvent costs are also halved. By decreasing column diameter from the conventional 4.6 mm to 2- to 1-mm, "narrow-bore" columns, the consumption of solvents falls in the ratio of 21:5:1; the ratio of their cross-sectional areas. That is, by using a 250-mm-long × 1-mm diameter column vs. a 250- × 4.6-mm column, only about 1/21 (approximately 5%) of the solvent is required.

Gant and Dong[8] estimated the costs per analysis by some of these columns (based on a lab overhead of $100,000):

Conventional	250 × 4.6 mm, 10 μm particles	$12.63/test
Narrow bore	250 × 1.0 mm, 10 μm particles	$12.50/test
Fast-LC	33 × 4.6 mm, 3 μm particles	$ 0.84/test

Note that compared with conventional columns, the reduced solvent consumption of narrow-bore columns was found to be not nearly so important as the 15-fold increase in the sample with the fast-LC column. Because of the cost savings on number of samples per hour, the decreased solvent consumption, and the faster response to on-line production problems, fast-LC methods using smaller particles in short columns are likely to continue to grow in importance. Papers recently by Verzele et al.[9] show that columns only 10 mm long packed with 1-μ particles may give greater advantages for large protein molecules than for smaller molecules (<5000 mol wt).

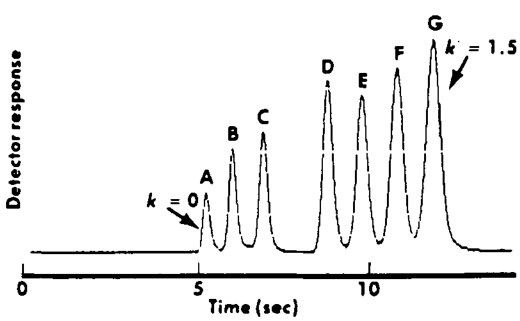

FIGURE 1. An example of fast LC using a Perkin-Elmer "3 × 3" column (33 × 4.6 mm id with 3 μ C-18 silica). Eluant is 90% acetonitrile in water at 3.5 ml/min with 254 nm detection. Peaks: A = uracil, B = phenol, C = nitrobenzene, D = isotoluene, E = ethylbenzene, F = *n*-propylbenzene, and G = *t*-butylbenzene. Generated plates range from 3000 to 4000. (From Dong, M. and Gant, J., *LC Mag.*, April 2, 1984. With permission.)

E. Low-Dispersion Methods

The two techniques described above are part of a greater group of methods called low-dispersion methods. These include:

Narrow-bore columns	250 × 1.0 mm, porous	10-μm particles,
Fast-LC columns	33 × 4.6 mm, porous	3-μm particles,
Pellicular columns	50 × 4.6 mm, nonporous	1-μm particles,
Microcolumns	250 × 0.1 mm, porous	5-μm particles,
Fast narrow columns	100 × 0.2 cm, porous[10]	5-μm particles,

All of these low-dispersion methods put great demands on the injector, connector tubing, and detector to preserve the sharpness of the peaks. They also put great demands on the injectors[11] and pumps for generating slow flows and gradients of small volumes.[12] In this review of autosamplers, pumps, pump controllers, and DADs, compatabilities with low-dispersion methods are pointed out.

II. AUTOSAMPLERS

A. General Functions

Autosamplers in their most basic function are able to reliably and reproducibly inject a number of different samples in an unattended manner on a liquid chromatograph. These auto*SAMPLERS* are distinguished from auto*INJECTORS*, which can make multiple automated injectors, *but only of the same sample*. Autoinjectors are useful for some limited applications, for example, to determine if a system has reached equilibrium or for evaluating changes in column performance vs. the number of injections.

Besides injecting different samples, "robotic autosamplers" of today can perform many more-advanced functions, such as:[13,14]

1. Dilutions
2. Standard addition
3. Precise timing
4. Derivatizations
5. Liquid-liquid extractions
6. Reinjections for out-of-specification samples
7. Variable size injections for autocalibration

As the capabilities of these autosamplers have advanced beyond simply injecting samples, it has become necessary to define some terms related to those capabilities. Generally, the microprocessor controlling these autosamplers gives them one or more of the following capabilities:

1. "Random-sample sequencing" is the possibility to inject samples in a sequence different from what it was in the autosampler rack. In earlier autosamplers, the sequence in the sample holder determined the injection sequence.
2. "Variable-injection number" indicates the possibility to inject different numbers of injections from each vial. Often the program can permit up to 3, 9, or even 99 injections. Enough sample must be available to make the injections. In earlier autosamplers, the number of injections was fixed at the beginning of the run to 1, 2, or 3 injections and much sample was wasted in washing connecting lines. Recent autosamplers use no sample to wash lines and can often make an injection of 1 μl from a 5-μl sample.
3. "Variable-sample volume" indicates the possibility to inject different volumes of sample with each vial change and/or with each injection number. This is convenient in some cases for permitting the autosampler to do periodic calibration runs by injection of, for example, 1, 3, 5, 10, etc. microliters of standard. In earlier instruments the sample volume was fixed by the mechanical size of the side loop in the six-port valve, or the mechanical setting of the injector (e.g., the H-P 1080). Variable-sample volume capability usually requires some special injector design or "partial-loop injections".
4. Vial-to-vial transfer indicates the possibility to move specific sample volumes from one vial into any other vial. This capability permits such functions as: (1) serial dilution of samples (to improve calibration plots by always injecting the same volume of sample); (2) addition of internal standard to sample to improve quantification; and (3) automated precolumn derivatization, for example, to mix precise volumes of sample, buffer, derivatizing agents, etc. For vial-to-vial transfer to be most useful, the autosampler should have the capability of precisely fixing the time at which reagents are added, reproducing the time the reagents remain together before injection, and mixing the components, often by rapidly aspirating and dispensing the mixture. Such devices must also be able to wash the lines between samples and, perhaps, thermostat the vials, since derivatization efficiency and product stability are often dependent on temperature.

"Full programmability" is defined here to indicate the ability to perform all of the four functions described above: random-sample sequencing, variable-injection number, variable-sample volume, and vial-to-vial transfer. Autosamplers with full programmability are actually automated chemistry stations, and sometimes are referred to as "robotic autosamplers". Today, there is a great interest in these for sample preparation in LC.

It should be noted that while many modern autosamplers formally may have all of the four capabilities described above, the programming to achieve those capabilities may be so difficult and time consuming as to make the capability nearly useless. Future improvements in ease of programming are certain to give some autosamplers clear advantages.

Temperature control available in some autosamplers can be very important in several ways. If biological samples are to be run, it may be necessary to cool the samples to prevent them from decomposing, or to prevent microbes (bacteria, mold, etc.) from changing the samples, especially important with the newer generation of very high capacity autosamplers (e.g., a new Gilson robotic autosampler can handle 540 vials).

A cooled sample area also makes it possible to load samples into uncovered vials, since cooling can make evaporation minimal. Using open vials can greatly reduce the costs of sample preparation not only in terms of materials (septa), but, more importantly, in terms of the costs of operator time used to seal the vials.

More versatile than having a cooling unit in the autosampler is having liquid-thermostated coils or fluid jacketing so subambient or above-ambient temperatures might be used. For example, it may be necessary to control closely the temperature above ambient to obtain high and/or reproducible yields from precolumn derivatizations. The need may even exist to both cool and heat different sections in an autosampler. For example, proteins might be cooled to preserve them in one section, but heated in vials in another section for hydrolysis or derivatization of amino acid constituents. This requirement suggests that separate racks that can be temperature controlled by circulating fluids to give different temperatures is a more flexible configuration than simply a cooled chamber.

B. Specific Autosamplers

1. Varian 8000 Autosampler

One of the first autosamplers, the Varian 8000, is an adaption of a gas chromatography injector and was introduced in about 1975 (Figure 2).[14] As with other early injectors, capabilities were very limited. This 60-position, round carousel autosampler used septum-capped 2-ml vials and permitted one or two injections. A dual septum-piercing set of needles sampled the vials. One needle extended to the bottom of the vial, and the other needle merely inserted through the septum. Positive gas pressure (approximately 60 psi) from the second needle forced sample through the needle extending to the vial bottom and down a long 16-in. length of Teflon® tubing (0.010-in. id) to flush out the line and the side loop of a six-port valve. In the first such valves the pressure of the LC was limited to about 5000 psi. With this early autosampler, electromechanical thumbwheel settings or remote contacts were used to set parameters such as analysis cycle time or injection number. Varian still offers this injector and a fully computer-programmable autosampler (Model 9090), described below.

A limitation to this early Varian autosampler was the long connecting tube (and little knowledge of refined flushing techniques) that required that much of the sample be thrown away during the flushing step. Thus, only two injections could be made with the 2-ml sample volume. Recent innovations with "serpentine" or knitted connecting lines reduces the volume required for flushing such lines. In the early designs, the gas pressure would give different flush volumes depending on the sample viscosities. Additionally, the septum had to be intact or the air pressure would not force sample through the connecting line; thus, vials could not be reused many times, as is often done in method development. Sometimes either of the two needles would clog with bits of septa, and many runs would be lost. Finally, a supply of gas pressure was required to operate the pneumatics.

2. LDC/Milton Roy 713 Autosampler

Currently, LDC/Milton Roy 713 autosampler is similar to the earlier Varian 8000 autosampler, having a round carousel holding 60 samples (0.8- or 0.3-ml tubes) with up to three injections each (Figure 3).[15] Air displacement time to force sample through the transfer tubing can be set for various flush

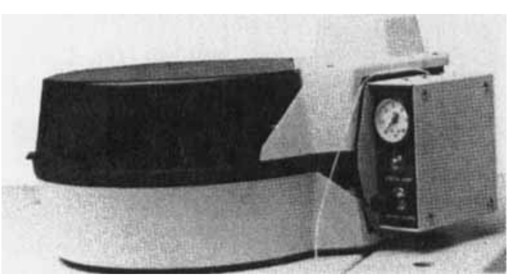

FIGURE 2. Varian 8000 autoinjector. (With permission.)

FIGURE 3. LDC/Milton Roy 713 autoinjector. (With permission.)

times to accommodate samples of various viscosities. The autosampler automatically bypasses empty locations, distinguishes sample or wash vials, and stops after the last sample. By using different Rheodyne injection valves, standard size samples (ten to hundreds of microliters) or microsamples (0.2, 0.5, or 1 μl) can be injected. Reproducibility is better than 1% RSD with cross-contamination below 0.3%.

3. Micromeritics 728 Autosampler

Introduced in 1976 as the 725, this early autosampler design is still currently available in a more advanced form as the 728 autosampler (see Figure 4).[16] Based on a design from Eli Lilly,

FIGURE 4. The Micromeritics 728 autosampler. The older 725 looked and operated similarly, but the 728 is more versatile. (With permission.)

this autosampler uses small vials (approximately 25 × 4 mm, 0.5 ml) with straight parallel sides. A tight-fitting polyethylene cap can slide inside the vial, turning the vial into a miniature syringe piston (Figure 5). During sample "load", a single needle pierces the polyethylene "plunger cap" (Figure 5, left). A mechanical "push rod" then pushes the cap down about one third into the valve, displacing a fraction of the vial volume through the side loop in the six-port valve (Figure 5, left center). At "inject", the valve is rotated (Figure 5, right center). After up to three injections, the push rod and needle are pulled out of the vial (Figure 5, right).

A major advantage to this "piston" vial displacement over earlier approaches is that many atmospheres of pressure can be produced to displace the sample out of the vial, so very narrow (0.01-in. id) tubing can connect the vial to the valve, and little sample is wasted in flushing this connecting line. Thus, up to three samples can be loaded from a single 0.5-ml vial.

Besides the uniquely simple method for displacing the sample, other innovations were introduced with the 728:

1. Between runs, the valve has an unusual "third" position that permits eluant to flush the valve to waste. After the wash, the valve is turned back to the load position, the flow through the columns allowed to equilibrate, and the next sample is loaded.
2. The 728 permits up to 64 different samples to be injected, with the important capabilities of random-sample sequencing and variable-injection number.
3. A submicroliter electrically actuated Valco valve for injections of 0.2 or 0.5 μl gave area reproducibilities at below 0.5% RSD after normalizing for retention time variations.[17]
4. A modification of this injector has a double row of vials in a circular track, and this permits simultaneous coinjection of the components of the two vials for orthophthaldehyde (OPA) derivatization of primary amino acids. A dual septum-piercing injection system simultaneously pierces one row of vials containing OPA-derivatizing reagent and the other row containing the sample, so the reagents are flow mixed.

Limitations to the Micromeritics system are that only three or fewer samples can be injected from one vial, and, since the plastic pistons cannot be easily removed, sample vials cannot be reused (although industrial laboratories rarely reuse vials).

4. Hewlett-Packard 1080 Autosampler

In 1977 Hewlett-Packard introduced its innovative 1081A LC as the "first LC with built-in processor" (Figure 6). This included a novel and very clever new auto*INJECTOR*.[18] This autoinjector was an important improvement to autoinjectors because of its unique and clever syringe design that greatly conserved precious sample and permitted the number of injections from each vial to be changed by programming from the keyboard.

Figure 7 shows the unique design of this injector and how it works. This injector can be viewed as letting eluant flow continuously down the plunger and out through the needle of the syringe through a small metal frit filter and onto the head of the column (Figure 7, left). One key innovation to this injection approach is the use of a rotary valve to momentarily

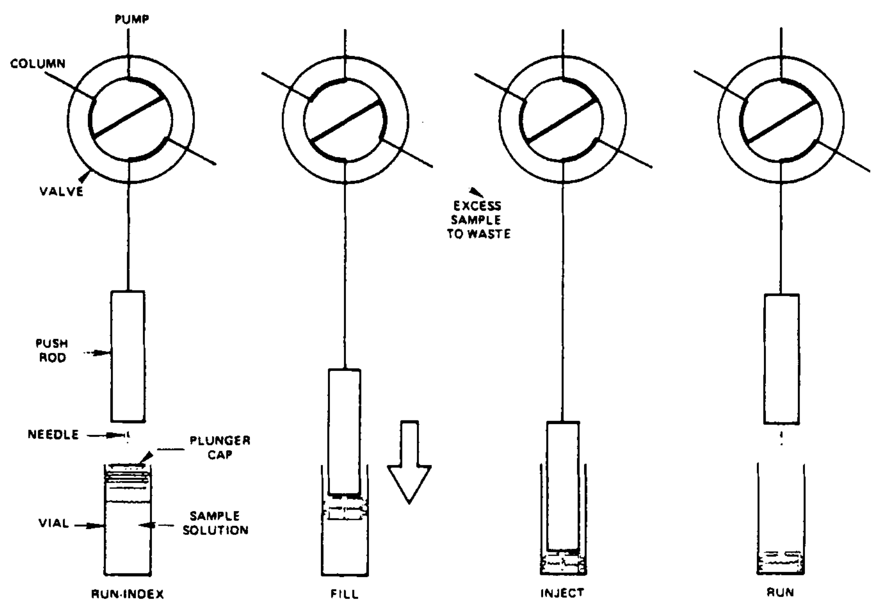

FIGURE 5. Operation of the Micromeritics 725 or 728 autosampler showing the vial cap acting as a syringe plunger (details in text). (With permission.)

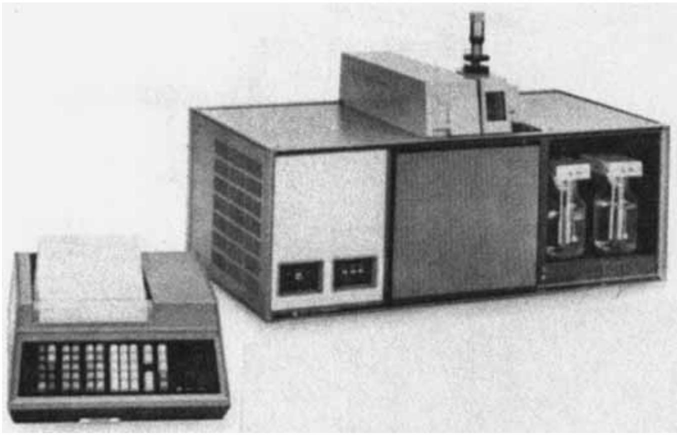

FIGURE 6. Hewlett-Packard 1080 series liquid chromatography, the "first LC with built-in processor", showing autoinjector (top center of the main instrument), but not showing the autosampler that extended to the right as a multitube loop on top of the main instrument. (With permission.)

divert eluant flow directly onto the column top, thus letting the syringe operate at ambient pressures during injection. The syringe is then mechanically lifted from the top of the column and the plunger depressed to make room for sample to be picked up (Figure 7, middle). The steel syringe is then dipped into the sample vial, the plunger is withdrawn to pull up a preset volume of sample (Figure 7, right). The syringe is then repositioned onto the head of the column and the injection made at the moment when the rotary valve redirects flow through the syringe (Figure 7 left, again). A second key innovation of this injector is having the heavy-gauge metal syringe "needle" seal against a metal seat to form a metal-to-metal seal. This eliminates rubber septa and soft polymer seats that can wear. Leakage usually is very low.

Other innovative features of this autosampler are

1. Precious samples are conserved in that no sample is used to wash lines or valve loops.
2. Only a little sample is required to make an injection, e.g., 1 μl can be injected from only 20 μl.
3. The volume range of samples is broad, from approximately 5 to 200 μl.
4. Up to nine repeat injections can be made from each vial.
5. Variable-injection number can be programmed from the keyboard and preprogrammed to change automatically during a run.
6. Reproducibility is very good (0.1 to 0.5% RSD for retention time and below 1% RSD for area).

Disadvantages to the 1080 autosampler are that injection volume has to be set manually, and random-sample sequencing or variable-sample volumes are not possible. The metal-to-metal seal between the needle and needle-seat sometimes leaks if a misinjection bends or roughens the needle tip.

5. Waters 710 and 712 WISP Autosamplers

A major impact in the market of autosamplers has been the WISP (acronym for Waters Intelligent Sample Processor), introduced about 1978. This very successful autosampler has evolved through several upgraded versions from the initial 710 to the current 712 (Figure 8, showing the sample tray partially withdrawn). The WISP is a self-contained microprocessor programmable autosampler that originally could also be controlled from the keyboard of a now-discontinued controller.

Key design concepts in the WISP seemed to be to avoid the use of conventional high-pressure rotary valves and to use on/off valves of their own design. The WISP operates as follows.[19] "Normal flow" is shown in Figure 9 (top), in which the eluant is split in about a 95:5 ratio, with the major flow going up through the sample loop, several feet of 0.02-in. id stainless

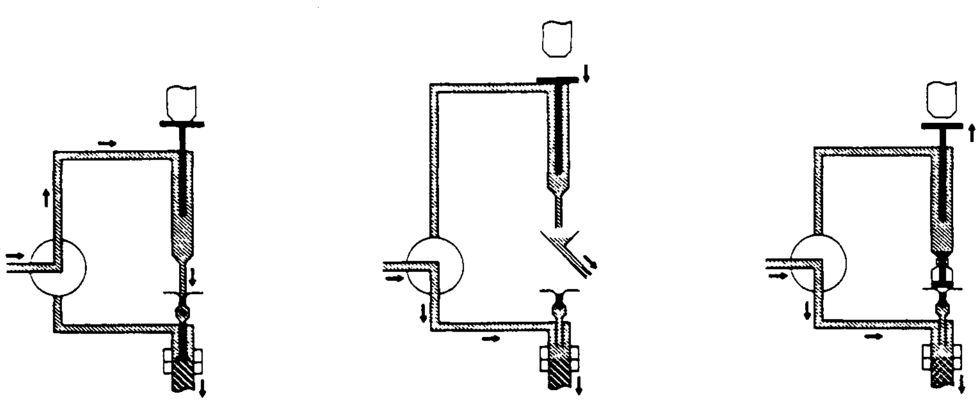

FIGURE 7. Operation of the autoinjector in the Hewlett-Packard 1080 liquid chromatograph (see text for details). (With permission.)

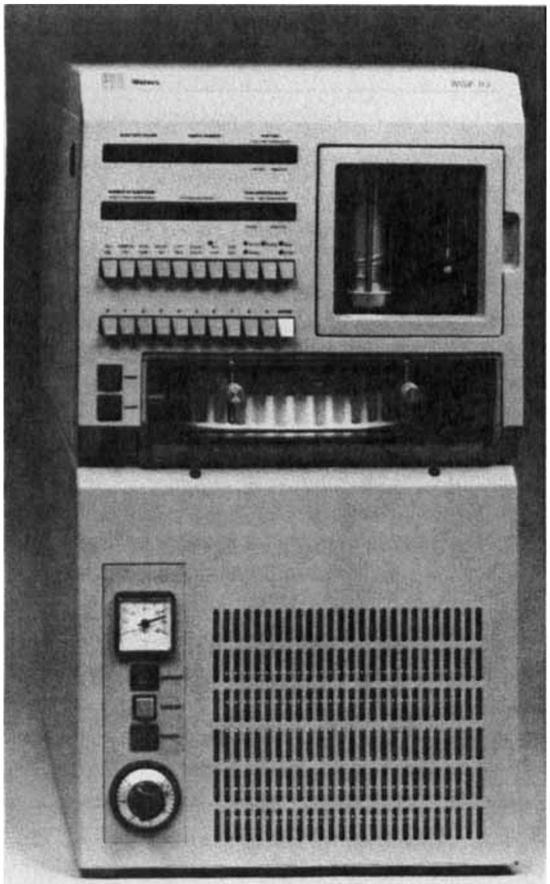

FIGURE 8. Waters-Millipore WISP 712 autosampler (top) with the sample tray door open, showing the 96-position circular rack. An optional refrigerator unit is shown (bottom). (With permission.)

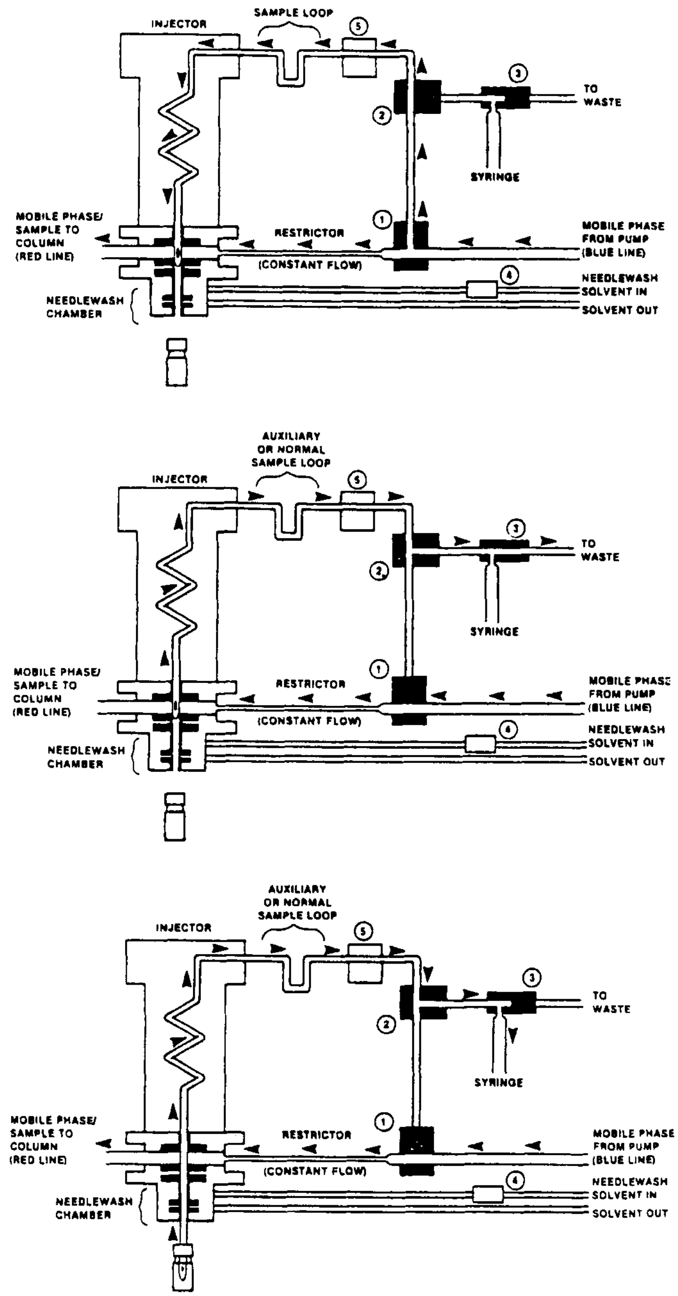

FIGURE 9. Operation of the autoinjector in the Waters-Millipore WISP autosampler (see text for details). 1, a high-pressure inject valve; 2, a high-pressure syringe valve; 3, a low-pressure waste valve; 4, a low-pressure needle wash valve; and 5, a pressure transducer. (With permission.)

steel tubing. The minor flow goes through a restrictor of 4 ft of 0.009-in. id tubing. ''Loading'' begins with valve 1 closing and valve 2 opening (Figure 9, middle). The needle pneumatically inserts into a sample vial; the plunger of the glass syringe is stepped back to draw the appropriate volume of sample into the sample loop (from 10 to approximately 600 μl) (Figure 9, bottom). For volumes above 600 μl, the 250-μl syringe pulls in one to three more aliquots of sample. For ''injection'', valves 1 and 2 are opened to allow flow to return to normal with the 95:5 split ratio to inject the sample (Figure 9, top). This split will reduce the peak height by about 5%, rarely important with conventional columns.

If requested by the operator, the ''bubble check'' command can determine if entrained air bubbles are present that would lead to an incorrect injection. For this, the needle is partially retracted to seal it, and the piston is moved forward a fixed number of steps. If the pressure transducer (at 5 in Figure 9) does not show the expected pressure (30 psi) because of entrained air bubbles compressing, the sample is expelled and injection attempted again with another sample.

In the early versions, the sample sequence was determined by the order in the sample carousel, but recent versions of the WISP (710B and 712) are very flexible, with these features:

1. Random-sample sequencing and vial-to-vial transfer is possible with precision timing of events.
2. Variable-injection number from 1 to 99 is possible (provided sufficient sample is available).
3. Variable-sample volume from 1 to 2000 μl can be set for any vial.

4. Derivatizations are possible by taking material from more than one vial into a holding tube, such as amino acid samples and OPA-derivatizing agents. Although the rate of reaction varies with each amino acid, and the OPA-derivatized products decompose with half-lives of tens of minutes to a few hours, reproducibilities below 3% can be obtained with this automated system.
5. A cooling module can rapidly cool samples in the WISP chamber from −5 to 20°C for preserving biological and derivatized samples. Waters cites examples where its "Pico-tagged" amino acids at 42 h remained above about 94% at 4°C, whereas at ambient temperature many had fallen to 80% and even to 65% in 24 h.[20] The cooling unit sits under the WISP so as to take up no additional bench space. (Figure 8, bottom.)
6. The split-flow concept, used earlier in the manual U6K injector from Waters, reduces pressure pulses introduced when usual valves are activated, and this may prolong column life.
7. A built-in pressure transducer permits checking the aspirated sample for entrained air bubbles (due to misinjection, low sample in the tube, etc.).
8. Normally a 250-μl syringe is used to inject from 10- to 2000-μl samples, but recently syringes of about one tenth this volume have been shown to permit injection volumes from about 0.1 to 10 μl.[21]
9. The inside of the needle is continuously cleaned by mobile phase.
10. An auxiliary test in the system permits leaks in the sample loop to be detected.
11. An alternative set of valves permits the outside of the needle to be washed between runs, and the side loop to be flushed with eluant.
12. The chart recorder signal can be marked with a momentary signal of different spacings that number the injection.
13. Sample injection is delayed if other parameters in the system are not ready.
14. A number of internal diagnostic messages are built into the system.
15. Sample capacity is variable: 48 4-ml vials holding up to 2.7 ml, 48 4-ml vials with "limited volume inserts" permitting injection volumes from about 6 to 240 μl, or 96 2-ml vials holding about 1.7 ml.
16. Peak spreading due to the WISP is less than 10%.
17. With the needle-wash function, carryover of one sample to the next is less than 0.1%.
18. Injection volume reproducibility is below 1% in the 5- to 50-μl range for aqueous samples.

Limitations of the WISP include the upper pressure limit (4500 psi for routine operation, with momentary 5500 psi capability). Compressed air for operating the needle and valves is sometimes inconvenient, but a small 65-psi air compressor is sufficient. Additionally, there were early problems with malfunctions of the "fluid pack" part of the injector (the parts contacting eluant and sample); however, these problems have been eliminated.

6. Perkin-Elmer ISS-100 Autosampler

Perkin-Elmer introduced a sophisticated autosampler in about 1983 (Figure 10).[22] A septum-piercing probe moves in a X, Y, Z square array over the tubes, positioning above a tube and then lowering a septum-piercing needle into the sample vials. A built-in Teflon® sampling pump (without check valves) is preceded by a "flushing pump" that does have inlet and outlet check valves (Figure 11). This autosampler also has a "flushing pump" that delivers 1400 μl/cycle. This flushes the sample port (flush volume of 240 μl) and the needle (flush volume of 1400 μl). However, the flushing pump is neither a precise metering pump nor is it under variable control in which the filling and emptying speed can be varied (see the Gilson autosampler below). However, both the flushing valve and sampling valve are inert (Teflon® and glass).

The clever design of the injector combines a six-port rotary valve with a low-pressure glass syringe, a design used in many later autosamplers. Injection is as follows. The valve assembly on the left of Figure 11 shows the normal position, so the flushing pump can clean the inside and outside of the needle (when the needle is lowered in the "flush port"). For taking up a sample, the needle inserts into the proper vial, and the sample pump withdraws sample into the Teflon® tube between the needle and sample pump (this Teflon® tube must be long enough to contain the sample). Then the needle moves to the loading port of the high-pressure six-port valve. Before loading, the valve moves to the valve position shown on the right of Figure 11, and sample is loaded into port 4 (the needle being sealed by a septumless polymer collar). For "total-loop filling", an excess of the loop volume is used. For "partial-loop filling", then, the proper volume is metered into the sample loop. Note that the tip of the needle very closely contacts what becomes the outlet of the sample loop. This facilitates low peak spreading with partial-loop filling (i.e., the sample does not traverse the unfilled part of the loop). For injection, the sample valve moves back to position A. (Figure 11, left.)

Perkin-Elmer stresses that the design of this six-port valve is not the regular six-port valve with every two holes interconnected, but rather one of their own design and manufacture (however, pressure is limited to 5100 psi).

This Perkin-Elmer autoinjecter offers a number of advantages over earlier autosamplers:

1. The square-array rack of sample vials makes better use of space compared with the earlier circular carousel-type racks.
2. The vial rack can hold 100 vials (all of the same outside dimensions, about 5 × 30 mm) but of different volumes (10, 325, or 2000 μl).

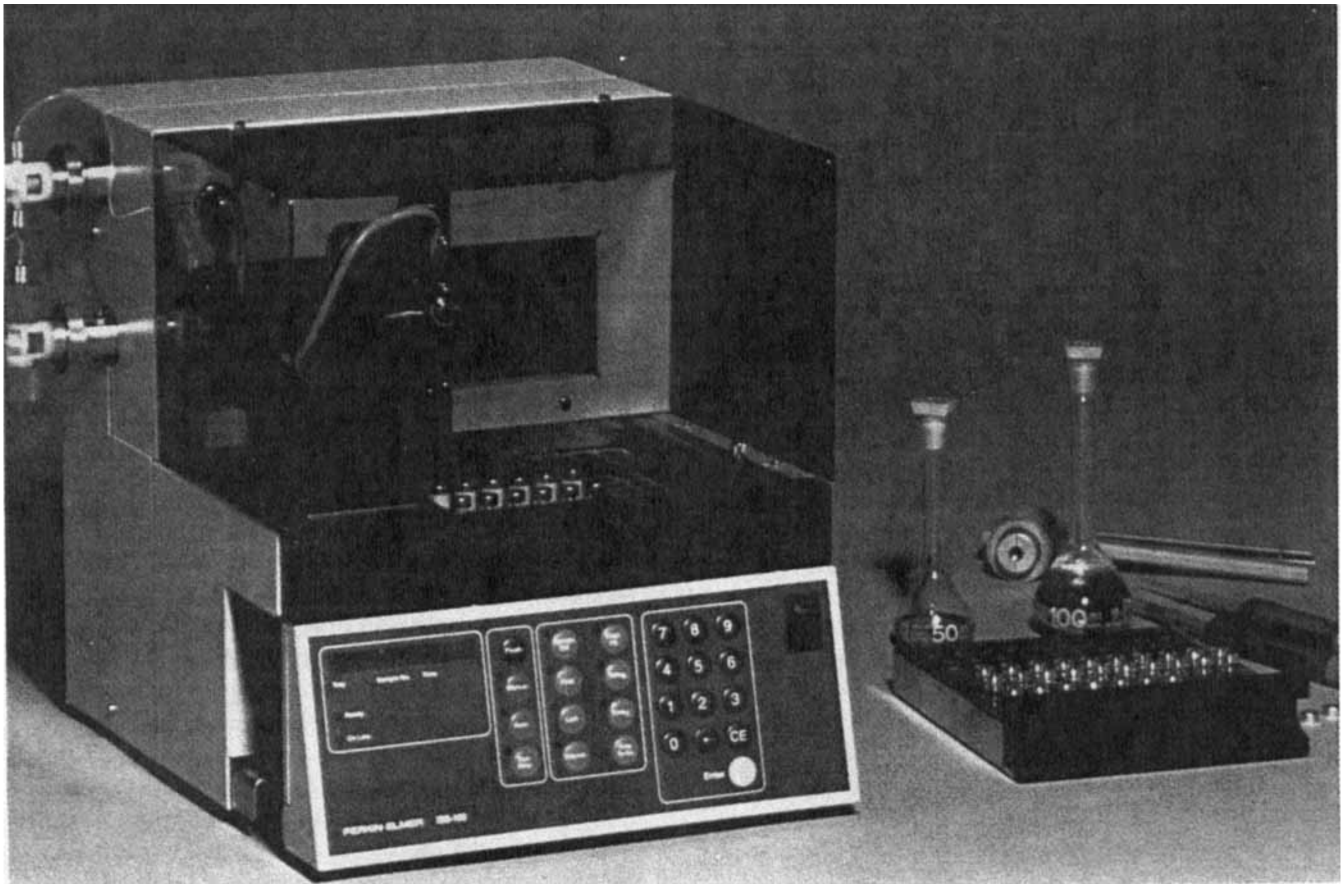

FIGURE 10. Perkin-Elmer ISS-100 autosampler showing the square-array rack to the right. (With permission.)

3. By changing the size of the sample loop, this autosampler can operate in the analytical (1 to 150 μl) or semipreparative (to 2000 μl) sample range.
4. The precision of the sampling pump determines the final reproducibility of sample volume, which is 5% RSD for 1 to 5 μl, 2% for 5 to 10 μl, 1% for 20 μl, and 0.5% for 50 μl of sample.
5. The flushing syringe reduces cross-contamination to below 0.02%.
6. A thermostated tray is available as an accessory, permitting a water bath for operation from −10 to +100°C.
7. The autosampler has considerable internal diagnostics to indicate if parameters are improperly entered, programming errors are made, a rack is not inserted, vials are not in the local position, or the transport robotic arm is not reaching the correct position.
8. Programming is versatile: if "chain" programming is used, the 100-vial rack can be broken into as many as five sections, each with its own injection volume and number of replicates.

7. Gilson 231-401 Robotic Autosampler

Gilson Medical Electronics in 1982 introduced an innovative autosampler, the 231-401. Instead of using a simple direct syringe to draw and inject sample, a "diluter" is used (see Figure 12, left).[23] This is a low-pressure glass and Teflon® syringe followed by a two-position valve that can connect the syringe via a long piece of Teflon® "transfer tubing" to a syringe needle or to an external reservoir of diluent. A typical application is to draw in through the needle 10 μl of sample, and then eject this followed by 990 μl of water from the dilutor for a 1:100 dilution. Much more complex manipulations can be programmed in. Using the definition of a "robot" as a machine that can be reprogrammed to provide new functions, this autosampler was the first of a generation of "robotic autosamplers". These new functions include the following:

1. Internal programming and memory permit vial-to-vial transfer, setting the "wait" time between all transfers, and loading sample into a high-pressure injection valve.
2. A "robotic arm" can be programmed to access any position (X, Y, or Z axis) below the arm, permitting unusual-sized containers to be used.
3. A compact "rectangular array" is used for arranging samples vs. the earlier circular array.
4. The autosampler can be controlled by an outside computer.

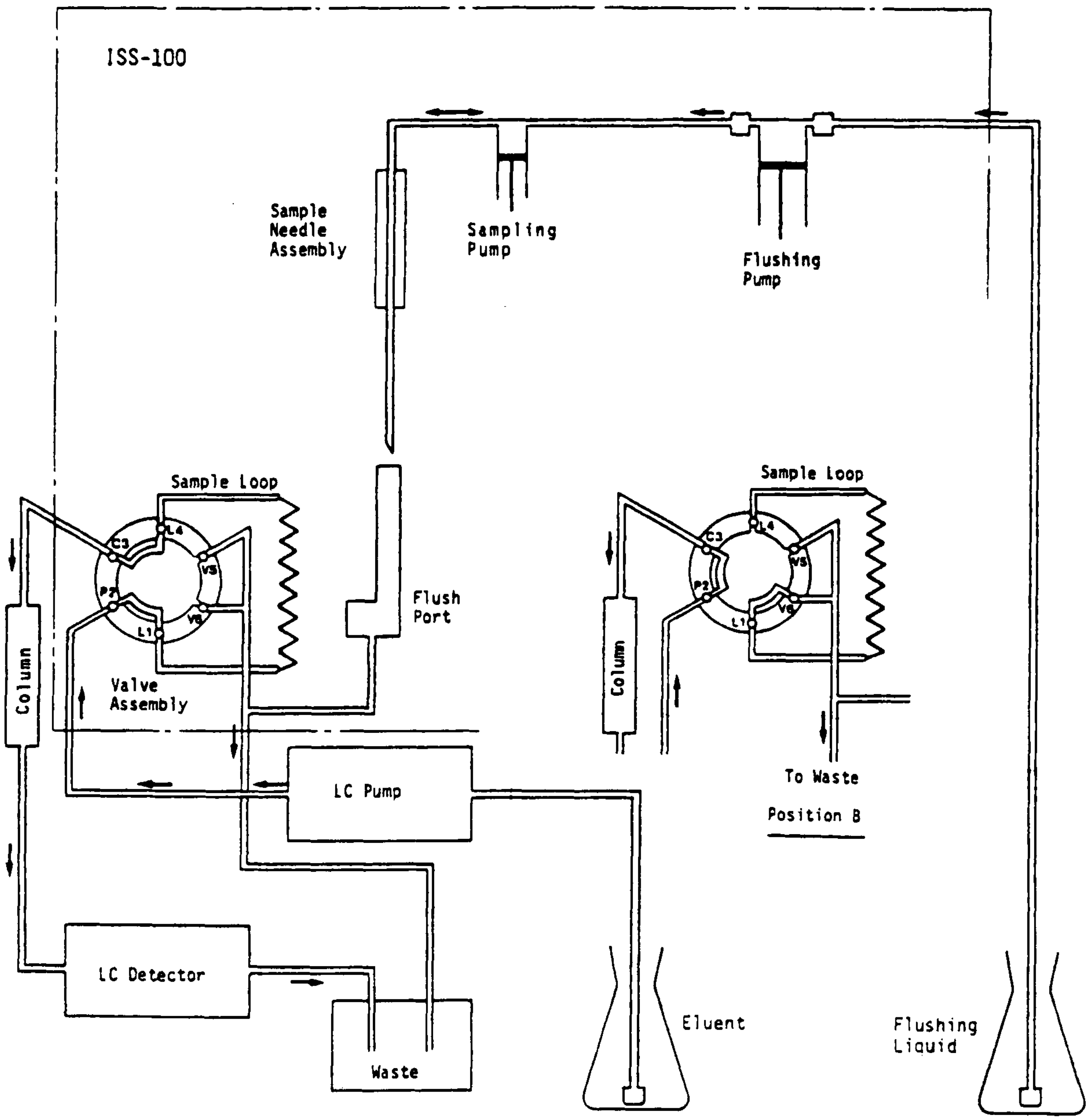

FIGURE 11. Operation of the Perkin-Elmer ISS-100 autosampler (see text for details). (With permission.)

5. The syringe unit (without the vial probe) can be used alone as a sample diluter, if configured for stand-alone use.

This "diluter" capability for accessing large volumes of an outside solvent gives very important flexibility to this autosampler, and the idea has since been used in other autosamplers.

The Gilson autosampler is currently one of the more clever autosamplers available. The needle position for the X, Y, and Z axes is fully programmable. Presently, 21 racks are available that can hold various containers (from 120 microtubes through 14 scintillation vials). Also, racks usable with a water bath are available for cooling or heating tubes. A single program code number instructs the robotic arm about the location of the tubes in each different rack. Additionally, the needle position can be freely programmed to any position in space, for accessing any kind of container. Currently, this "position programming" or the easy use of various racks is not found in many other autosamplers. It is also possible to program "sections" in a rack.

The diluter portion of the autosampler (Figure 12, the smaller unit with the syringe) is an unusual function of the Gilson autosampler. At any step in the program the volume picked

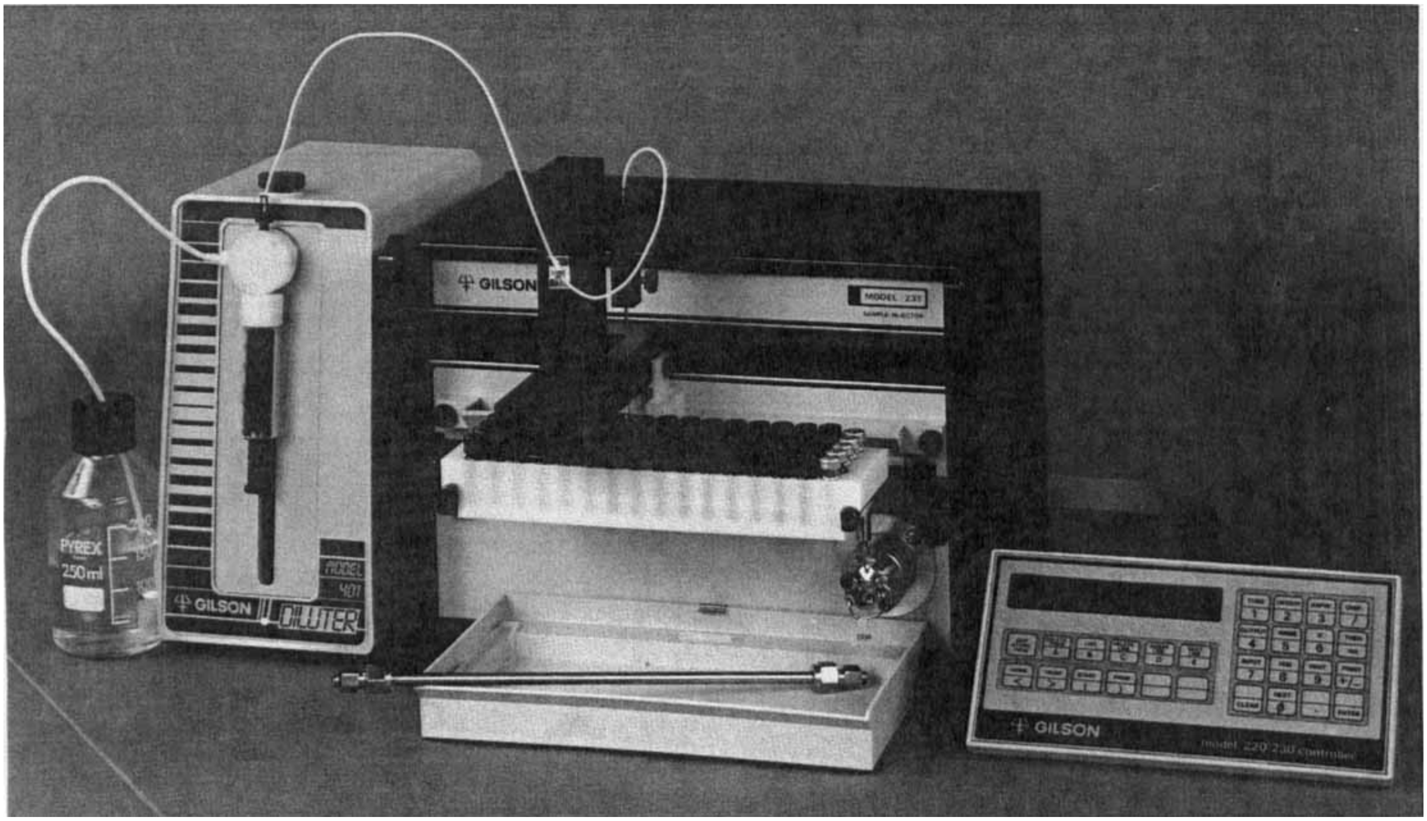

FIGURE 12. Gilson 231 "robotic" autosampler (right) and 401 diluter (left) (see text for operation details). (With permission.)

up and the volume delivered can be varied as well as the location where sample is picked up and where it is delivered. Also, the intake and delivery flow rates can be independently set over a very broad flow range (from 3 to 1200 μl/s). The diluter permits adding microliter to liter volumes of solutions to dilute samples, or to wash transfer lines, needle, and sample loops.

These diluters are inert (Teflon® and glass only) and can be used independently of the vial selection capability as simple automated pipettes or diluters (by purchasing a switch). Diluter syringes of various sizes (0.5, 1, 5, and 10 ml) can be used to obtain different precisions of delivery or speeds of delivery.

Since the racks can be divided into programming sections, a typical application might be to take a precise volume from 60 roughly loaded vials, for dilution in another set of vials, addition of internal standard, and then injection of this onto the column. With a rack containing 96 vials of about 2 ml each, this feature permits half the rack, 48 samples, to be run in an unattended manner. The autosampler is used to measure precisely samples from the roughly loaded (first 48) vials into the empty reaction vials (last 48).

Addressing the need for unattended sample injections and manipulation, Gilson has recently introduced a new version of this autosampler, the 232-401, that can do similar manipulations of up to 540 samples.[24] This would permit, for example, samples to be run unattended for a 4-d weekend, i.e., 96 h. This autosampler has provisions for cooling or heating the five racks independently. Thus, sample-containing racks might be cooled, and derivatization tubes might be heated.

The disadvantage of the Gilson autosampler is that random-sample sequencing, variable-injection number, or variable-sample volume would be extremely complex to program, and sufficient memory may not be available (although the new version, version 3.0, has an expanded memory of 8K).

8. Hewlett-Packard 1090 Autosampler

As an improvement on its very successful 1080 instrument, in 1984 Hewlett-Packard introduced its fully automated 1090 liquid chromatograph as a "family of integrated LC modules", part way between an integrated instrument and stand-alone components (Figure 13).[25] Various components can easily be added to the basic unit, all in a common housing, to upgrade the instrument readily. For example, an isocratic one-solvent instrument can be made into a two-, three-, or four-solvent gradient system; or a fixed wavelength detector can be replaced with a DAD. Although partly modular,the components cannot be used with components from other manufacturers, an important distinction from the usual "modularity" in LC. (However, the more recent 1050 system components, not reviewed here, are fully usable with other instruments and readily portable.)

The 1090 autosampler incorporates some of the principles used in the earlier Model 1080 series of liquid chromatographs

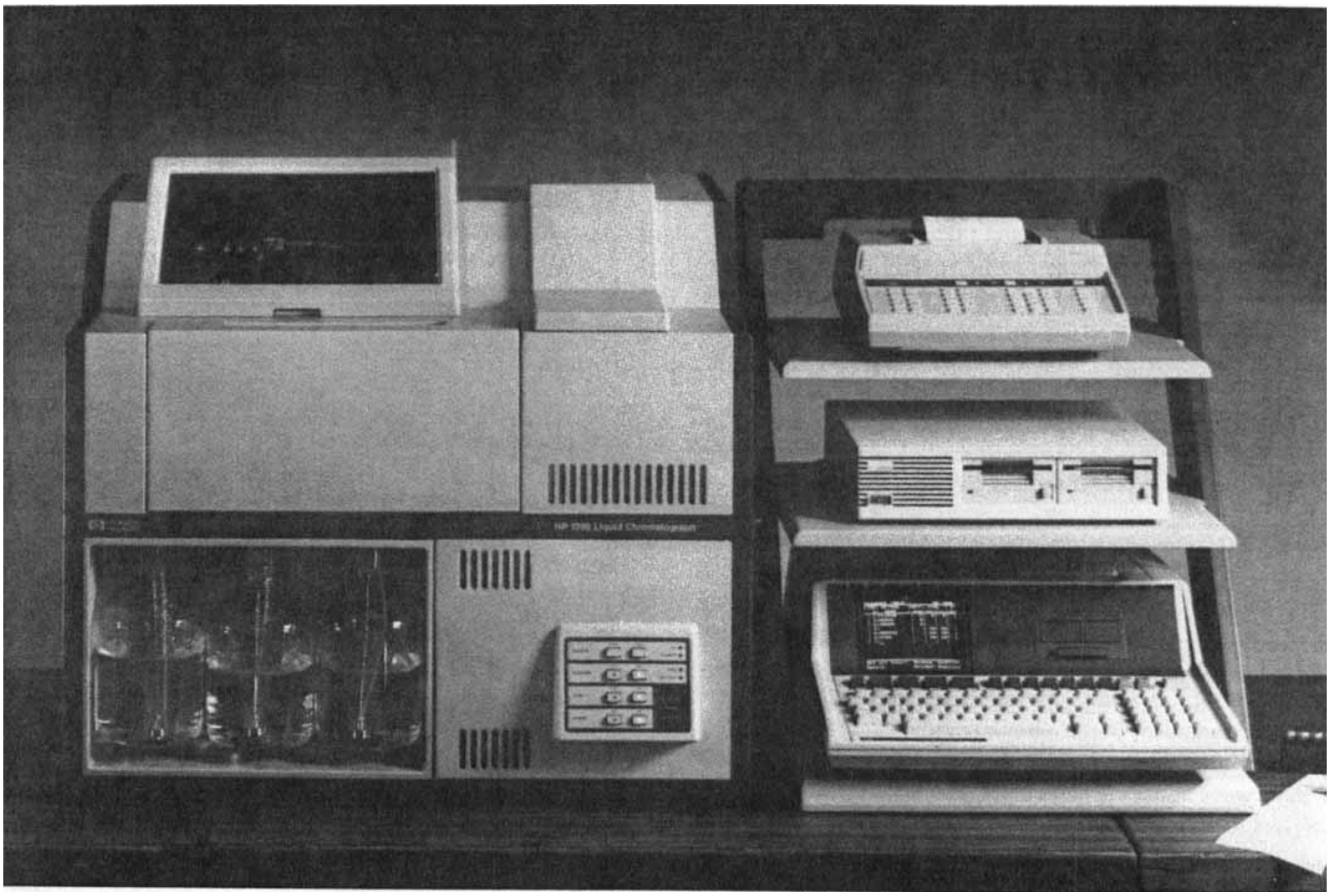

FIGURE 13. The Hewlett-Packard 1090 liquid chromatograph (left); the HP-85 PC (right, bottom); HP9121D disk drive (right, middle); and HP3392A Integrator (right, top). (With permission.)

and the principle used in the Perkin-Emer ISS-100. Eluant is usually running through a heavy needle (Figure 14, left). A six-port high-pressure valve simultaneously diverts flow from the septum-piercing needle and makes a low-pressure connection to the glass syringe. A "magazine" containing ten vials is then positioned under the needle, which then lowers to pierce the 2-ml vial (Figure 14, middle). A computer-controlled microstepping motor on the syringe then draws sample into the needle, the needle is withdrawn from the vial, the magazine returned to the storage position, and the needle dropped back to the column head position. Finally, the valve is switched back to the inject position, flushing the sample onto the column (Figure 14, right). Although similar in principle to the injector used in the Perkin-Elmer design, in which the low-pressure glass syringe draws sample through a connecting tube into the loop of a high-pressure sample valve, the Hewlett-Packard approach eliminates the dead volume of the connecting line (6 μl).

Improvements in the 1090 autosampler over the earlier 1080 include the following important changes:

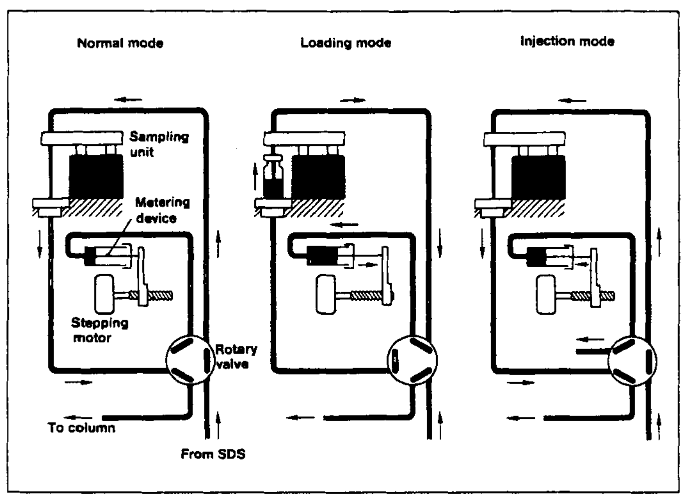

FIGURE 14. Operation of the Hewlett-Packard 1090 autosampler (see text for details). (With permission.)

1. The 1090 glass syringe is in the low-pressure portion of the system, so it is subjected to less stress, eliminating the need for the special high-pressure steel syringe used in the 1080.
2. The 1090 glass syringe is under stepper-motor control so sample volumes can be changed from injection to injection through programming, unlike the 1080 syringe, which could only be changed manually.
3. The 1090 glass syringe can be readily replaced by the user when it wears out or if other injection volumes are desired (e.g., 25- or 250-μl syringes are usable) vs. the 1080 steel syringe, which is fixed at 200-μl maximum volume.
4. The needle/column-top connection of steel to polymer in the 1090 gives a more-reproducible leakproof seal than the steel-to-steel seal used in the 1080.

In both the 1080 and 1090 injector designs, a six-port high-pressure valve is used to divert flow from going through the needle during the sampling portion of an injection. However, by eliminating the steel syringe in the 1090 design, Hewlett-Packard also eliminated the need for a second set of complex and costly high-pressure seals: the seals within the syringe.

The autosampler in the 1090 readily permits vial-to-vial transfer with precise timing of all steps. This feature is useful for such work as enzyme reactions, or chemical derivatizations such as the OPA derivatization of amino acids.[26] Greater software gives the 1090 the following capabilities:

1. The 1090 is fully programmable, providing random-sample sequencing, variable-injection number, variable-sample volumes, and vial-to-vial transfer with precision timing of events.
2. As many as 100 different gradient programs can be run, one for each sample vial.
3. The square array of 100 tubes is compact, and "magazines" of 10 tubes each can be added or removed.
4. Methods can be edited while samples are being run.
5. Precision is excellent (for areas): 0.1 to 0.3% RSD from 2 to 10 μl, and about 0.6% for a 1-μl sample.
6. To cover injection volumes from 0.2 to 250 μl, either a 25- or 250-μl syringe can be used.
7. A "wash" cycle can be programmed at any point to clean the outside (and inside) of the needle.
8. Variable-injection number from 1 to 99 is possible (provided sufficient sample is available).
9. Variable-sample volume from 0.1 to 25 μl (with the 25-μl syringe) can be set for any vial, much smaller than usual autosamplers.
10. Very little sample is required for an injection and very little is wasted (e.g., 1 μl can be injected from 5 μl).
11. A single computer is used for four functions:
    a. LC controller (gradients, temperature, flow, etc.)
    b. Autosampler control
    c. Diode array detector control
    d. Data gathering and manipulation
12. The auto*INJECTOR* (capable of making many injections from a single vial) can be upgraded in the field by the user to an autosampler, capable of making injections from many different vials.
13. A cooling option was later introduced so that biological samples could be preserved, or the entire instrument could be operated in a cooled room to 5°C.
14. Derivatizations are possible by taking material from more than one vial into a holding tube, such as primary and secondary amino acid samples by simultaneous OPA and FMOC derivatization. The reactions may also be done at elevated temperatures (to 100°C) for a programmable duration.

Concerning the last point above, the 1090 was used in a novel way to solve a problem that had baffled LC for many years: "How can both the primary and secondary amino acids be analyzed at the high speeds possible with reversed-phase LC?" Generally, amino acids are sufficiently small and polar that they show no retention by C18 reversed phase. Thus, direct chromatography generally uses the slower ion-exchange LC with postcolumn reaction with ninhydrin for detection. Precolumn derivatization of amino acids (1) adds a large pendant group to give amino acids sufficient retention for separation by reversed-phase LC and (2) adds a fluorescent (and UV) detectable group. Compared with ion exchange separations of amino acids (about 1 h), reversed-phase separations can be much faster than ion exchange, often permitting 21 amino acids to be quantified in less than 15 min. A problem is that the OPA-derivatization method does not react with secondary amino acids (proline and hydroxyproline). FMOC derivatization, on the other hand, does react with all amino acids, but gives a large peak of fluorescing side product that must be liquid-liquid extracted to prevent overlapping with some FMOC-amino acid derivatives. Hewlett-Packard scientists figured out how to take sample plus OPA- and FMOC-derivatizing agents into a holding tube, to move these reagents back and forth to perform mixing, and then to inject this mixture on the reversed-phase column. The larger size of the FMOC reagents gave them longer retentions, and thus the secondary amino acids remaining after reaction with OPA were derivatized so they eluted after the primary amino acids (as OPA derivatives).[27,28]

Disadvantages of the 1090 autosampler are that it can only be programmed through the computer used to control the 1090 (two are available) and this autosampler cannot be used with any other LC instrument. The 1090 does not have the "diluter" capability introduced by Gilson. Being pneumatically operated, the 1090 also requires a 60-psi gas supply; this is sometimes an inconvenience.

9. Shimadzu SIL-6A Autosampler

This Japanese injector was introduced for sales and service about 1986 in the U.S. (Figure 15).[29] This autosampler uses the valve-syringe approach to loading sample (like that described for the Perkin-Elmer ISS-100). One modification, like the Waters WISP injector, is a restrictor capillary connecting the eluant inlet and outlet line so that pressure is never fully cut off, even during valve actuation. Thus, this design minimizes the pressure pulse found when the valve is actuated, at the cost of some few percent reduction in peak height. The SIL-6A autosampler offers variable-injection number (1 to 30) and variable-sample volume (from 1 to 500 µl). At about $10,000, this autosampler offers many of the most recent features plus some unique data processor interactions:

1. A "priority sample" position permits interrupting the programmed sequence for running urgent samples.
2. The 100-vial circular rack is removable.
3. A single glass syringe loads samples and washes the inside and outside of the needle between injections. This wash time is programmable.
4. Programming is said to be very easy, with the possibility of programming ranges of vials at one time (e.g., vials 1 to 10) for the injection volume, number of repeat injections, file number, and run time.
5. All of the above data, as well as the current sample number, current repeat number, and run time are continuously visible on a video screen that is part of the autosampler.
6. Built-in diagnostic routines help with troubleshooting mechanical and electrical subassemblies, and can also turn off the autosampler, column pump, and column oven.
7. Injection time intervals can be set to 650 min.
8. Vials are 1.5 ml and 200 µl.
9. A contact closure can start an external data processor.
10. An RS232C port is available for external computer interaction.

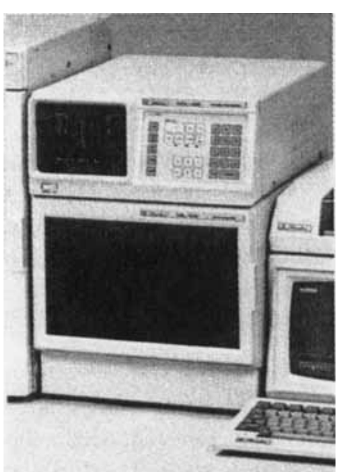

FIGURE 15. Shimadzu SIL-6A autosampler (bottom) and SCL-6A System Controller with video screen (top). (With permission.)

An advantage to the Shimadzu autosampler is that instrument parameters and sample numbers can be printed out on the Shimadzu C-R3A data processor. This autosampler is available, without an internal control unit, as the SIL-6A (for about $8000), and with the internal control unit as the SCL-6A (for about $10,000).

A limitation of the SCL-6A autosampler is that vial-to-vial transfer for dilution, derivatization, and internal standard addition is not possible. Also, random-sample sequencing is not possible.

10. Bio-Rad/Jasco Refrigerated AS-48 Autosampler

This Japanese injector was introduced for sales and service about 1983 in the U.S. by Bio-Rad (Figure 16).[30] The AS-48 was the first autosampler to have built-in cooling to preserve biological samples. A solid-state Peltier-effect thermomodule fixes temperature at about 8°C. At about $7000, this autosampler uses the valve-syringe approach to loading sample, like the Perkin-Elmer ISS-100 described above. However, since only full-loop injection is possible, partial-loop injection and, thus, variable-sample volumes cannot be used. Capabilities of this autosampler include:

1. Variable-injection number (0 to 9 injections) can be programmed in.
2. A "priority sample" position permits interrupting the programmed sequence for running urgent samples.
3. Cross-contamination from vial to vial is low, since the needle is immersed in a reservoir between runs and flushed with cleaning solvent.

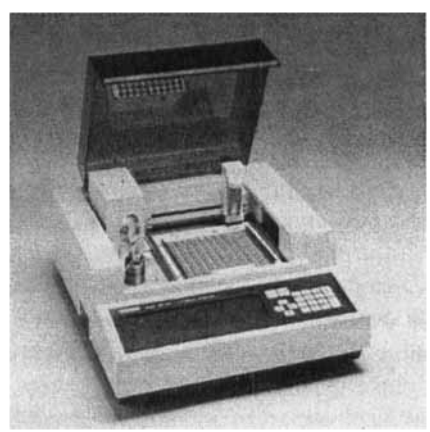

FIGURE 16. Bio-Rad/Jasco refrigerated AS-100 autosampler (a more recent version of the AS-48, which looks similar). (With permission.)

4. Reproducibilities are good, below 0.05% for the fixed-loop injection.
5. The AS-48 has two-way communication with an external integrator, i.e., the autosampler can start the integrator at the moment of injection, and the integrator can start the autosampler when the integrator is finished.
6. Vial size is 1.5 ml.
7. Run-time can be from 3 to 999 min.
8. After the last vial, the autosampler shuts off.
9. The system is completely electromechanical; no gas is required.

The impact of this autosampler around 1983 was the availability in a compact unit of internal cooling to preserve biological samples. Since this is an older instrument, it has many limitations compared with recent autosamplers. Vial capacity is low (48 vials), variable-sample volume, random-vial sequence, and vial-to-vial transfer is not possible. Computer interaction is very limited.

C. Unusual Specific Autosampler Configurations

Historically, the first years of high-performance liquid chromatography (HPLC) focused on LC plumbing, hardware, and column design. Later came microprocessor capabilities for doing clever automated quantification and report generation. This was followed by a number of clever methods for optimizing separations so as to quickly come to acceptable analyses. Today, the greatest effort is being made to address the current most time-consuming step in LC: preparation of samples. This effort will continue in the future. Several of the most recent autosamplers bring special talents to this labor-intensive sample preparation step.

1. The Chrompack/Spark Holland PROMIS Autosampler

This autosampler combines the capabilities of (1) a 96-well autosampler, and (2) dual high-pressure switching valves for sample cleanup (Figure 17). Costs are around $12,000 with dual-valve, and $9000 without the dual-valve capability. Innovative capabilities of this autosampler/dual-valve combination include:

1. Dual valves permit on-line sample cleanup in which sample is loaded onto a short column and impurities are eluted from this column. By "column switching" this short column onto the top of the analytical column, sample is later washed onto the main analytical column for separation. Strongly sorbed impurities can be eliminated by (a) subsequently washing of the short column with very strong eluant or (b) periodically discarding the short column. (This column-switching approach is an important new area, particularly with direct serum injection for analysis of drugs and metabolites.[13])
2. Dual valves permit on-line sample derivatization in which sample and derivatizing agents are loaded into a holding tube (or column), reacted for the proper time, and then flushed onto the analytical column.

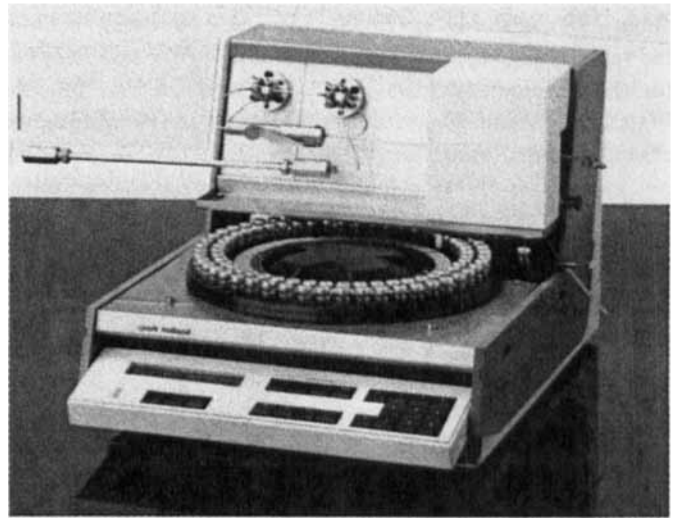

FIGURE 17. The Spark Holland PROMIS autosampler (once distributed by Chrompack) with dual high-pressure valves for column switching, sample clean-up, etc. (With permission.)

3. Dual valves permit other operations such as stream sampling with "heart-cut" of main components, column selection, preconcentration with alternating columns, etc.[31]
4. Up to 96 sample vials (1.5 ml) can be divided into eight segments, each using eight different methods.
5. Methods can include variable-injection volume (1 to 9), and variable-sample volume (1 to 999 μl).
6. Analysis can be chosen between 1 and 99 min.
7. After every "X" vial, a "reserved vial" can be injected and run by its own program; for example, a standard can be run periodically.
8. Eight marker signals can be assigned to any of the 96 vials.
9. For external events, signals can be time programmed "on" or "off".
10. Up to 16 methods can be stored internally.
11. A "priority sample" position can be used at any time without interrupting the main program.
12. Reproducibility by partial-loop injection is below 1%, and by full-loop injection below 0.5%.
13. Vial inserts permit sample to be taken from as little as 10 μl.

The system for transferring sample from vials involves inserting a coaxial sample/air-vent needle through the septum. Then, an internal syringe transfers a flush volume and proper sample volume to load either fully or partially a sample loop.

Limitations of this injector are that the round carousel makes the autosampler a bit large. Also, random-sample sequencing, variable-injection number, and variable-sample volumes are not possible except that these parameters may be changed for each of the eight possible segments, and the segments can be randomly programmed. Also, vial-to-vial transfer is not possible.

2. Varian AASP

The AASP (Advanced Automated Sample Processor) combines both the ability (1) to inject up to 100 samples with (2) the ability to clean up samples (Figure 18).[32] The original developer, Analytichem International (Los Angeles, CA), was acquired by Varian Associates which now manufactures and markets the product. The AASP can hold ten racks with ten miniature disposable cartridge precolumns (18 × 2.1 mm). Each precolumn holds 40 to 50 mg of any of 12 different silica-based bonded phases. The precolumns are intended to be used only once and discarded, giving this system some important distinctions over other cleanup systems. One-use precolumns potentially have a high tolerance and capacity for irreversibly sorbed components, such as proteins. In a properly designed separation, samples are loaded manually by the operator with a pipette onto the precolumns. Using a special wash station, an entire rack of ten precolumns can be quickly washed with solvents that elute salts and weakly retained interfering components. Then, up to ten racks (100 samples) are loaded onto the AASP. The AASP then sequentially conditions each precolumn (if desired) with an eluant, clamps the precolumn firmly onto the head of the analytical column, and then initiates the analytical run. Strongly retained components, such as proteins, not eluted during the run, are discarded when the precolumn is discarded.

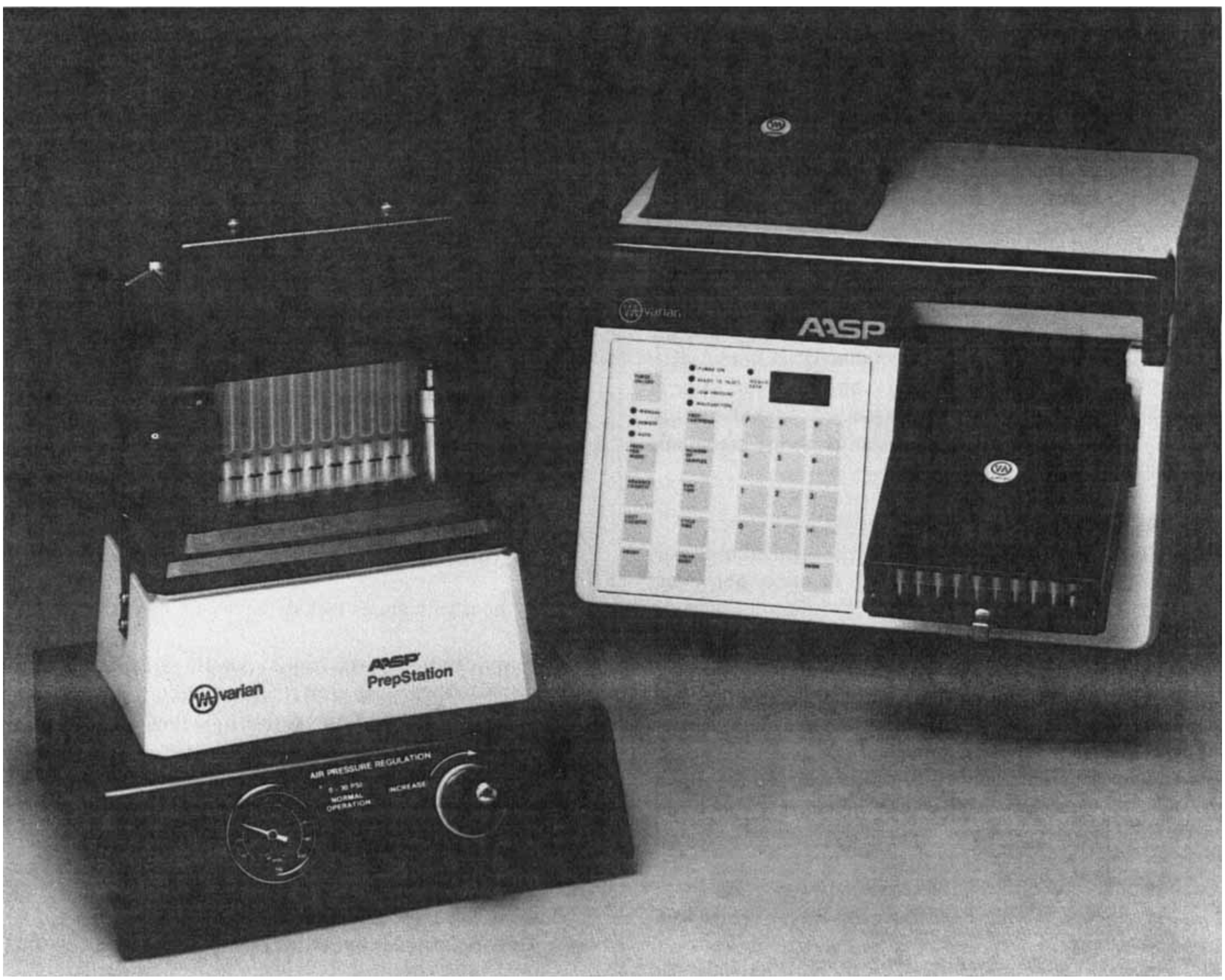

FIGURE 18. Varian "Advanced Automated Sample Processor" (AASP) (right) with sample "PrepStation" (left). (With permission.)

Because a variety of reversed-phase, ion-exchange, and normal-phase sorbents are available for the precolumn, it is possible to design some clever cleanup procedures. For example, by adjustment of pH to 7, ionized organic carboxylates might be retained on an anion-exchange precolumn, and, by changing the pH to 2, these are eluted later onto a reversed-phase analytical column for proper separation. The AASP has the potential for washing the precolumn with 25 to 250 μl of solution that can be wash solution, pH adjustment buffer, complexing agent, or even derivatizing agent.

Other advantages of the AASP are

1. Pressures to 6000 psi are possible, even though plastic precolumns are used.
2. Large sample volumes (50 to 500 μl) can be loaded initially, usually with quantitative transfer of the entire sample to the LC column.
3. Up to nine external event switches can be controlled by the AASP to initiate injection, start or stop integrators, etc.
4. The AASP can be initiated externally, for example, by a signal from a gradient controller at the end of a run.
5. For reliable operation, 22 internal tests can sound an audible alarm.

A disadvantage of the AASP is that disposable cassettes are a continuous cost (at about $20 per cassette), but this is often a small cost with some difficult biological samples. Additionally, the user is locked into the column sizes and packing materials offered by the manufacturer (although custom-packed precolumns can be obtained). Also, to obtain the full value of the AASP, it is necessary to develop methods carefully so that unwanted materials are not eluted when the AASP precolumn is connected to the LC column.

3. Varian 9090 Autosampler

The Varian 9090 appeared after the Gilson, Perkin-Elmer, and Hewlett-Packard 1090 autosamplers described above and has many of their features (Figure 19).[33-35] This includes the use of a microstepping glass syringe in a low-pressure side loop to draw sample into a high-pressure six-port rotary valve. A single keystroke "Auto Mix" permits the 9090 to be programmed to mix reagents by repeatedly drawing them in and expelling them out of the probe.

As in its earlier autosampler, Varian maintained the round carousel, but overcame many earlier deficiencies and made considerable improvements.

1. The entire top of the carousel is easily accessible.
2. The 105-tube rack has seven segments that can be removed individually.
3. Injections can be varied from 1 to 1000 μl (provided the loop is large enough to contain the sample by partial-loop filling).

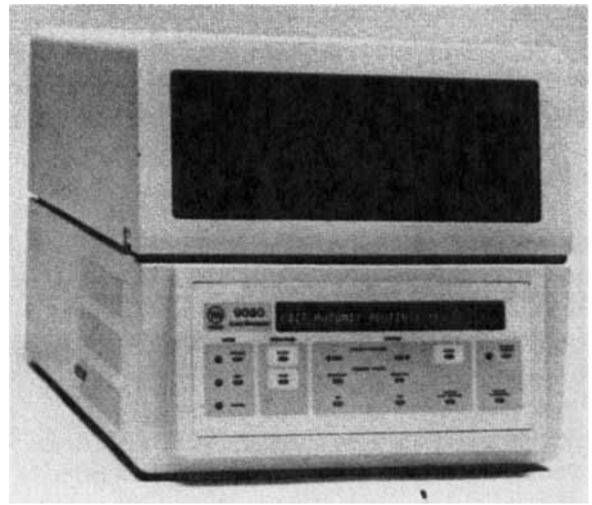

FIGURE 19. Varian 9090 autosampler. (With permission.)

4. The 9090 can be programmed internally or from an outside computer through an IEEE or RS-232 plug.
5. Methods can be edited while samples are being run.
6. Up to 99 methods of 10 steps are possible (such as transfer, mix, wait, or inject).
7. Up to 100 injections per vial can be made.
8. Microvials permit as little as 1 out of 10 μl to be injected.
9. Precision is very good, for example, with partial filling of a 10-μl loop, area precision is 0.2 to 0.4% from 2 to 10 μl, and only 0.7% at 1 μl. By full-loop injection of 10 μl, area precision is 0.2%.
10. Random-sample sequencing, variable-injection number (from 1 to 99), and variable-sample volume from (1 to 200 μl) are possible.
11. Derivatizations are possible by taking material from more than one vial and letting it react in a holding tube. This is used for derivatizing primary amino acid samples with OPA-derivatizing agents.[36]

More unusual features include:

1. Liquid-liquid extractions permit precise time of derivatizations such as PTC (phenylthiocarbamyl), giving the advantages of (a) derivatizing both primary and secondary amino acids and (b) producing more stable derivatives than OPA. After vial-to-vial transfer of components, an interfering side product from the PTC derivatization is eliminated by liquid-liquid extraction.[33-36]
2. The needle senses the vial bottom (for emptying microvials) and prevents Z-folded needles!
3. The next sample can be prepared for injection with runs as short as 1 min apart (a likely future need for fast LC).
4. A "priority position" permits urgent samples to be inserted at any time without interrupting the original

programming (a feature first introduced by Spectra-Physics).

Limitations of the 9090 are a 60-psi supply of gas is required to operate the pneumatics, and it is not possible to thermostat the racks, but it can be operated in a cool room to 10°C. Nor does the 9090 have a "diluter" pump to meter precisely in large volumes of diluents (a wash pump does clean the needle, but not with precise volumes).[36]

4. Spectra-Physics SP 8780 XR Autosampler

Introduced in 1985 as the SP 8775, this autosampler uses the valve-syringe approach to loading sample (like that described for the Perkin-Elmer ISS-100 (Figure 20). A digitally driven syringe withdraws sample for partial-loop injection, and helium displacement can be used for full-loop injection. Injection takes place when the six-port valve puts the loop in line. Between-run flushing cleans the line connecting the high-pressure valve to the needle used to pierce the septum. This autosampler offers random-sample sequencing, variable-injection number (1 to 10), and variable-sample volume (from 1 to 250 μl). At about $11,000, this autosampler has several features unique to LC sampling.[37]

1. An optional bar code-label reader gives positive sample identification, independent of how samples are loaded in the racks.
2. A "priority sample" position permits interrupting the sequence of samples for running urgent samples.
3. The four removable 20-position small circular racks, four on the instrument at one time, permit continuous operation of up to 20 racks (400 tubes) in series, i.e., first racks can be removed and replaced with racks of unanalyzed samples, and the system will continue to run these racks.

FIGURE 20. Spectra-Physics SP 8780 XR autosampler. (With permission.)

4. A 5-year warranty is provided with the instrument.
5. To operate with more viscous samples, the fill speed of the loop can be adjusted, as well as the delay before the valve is activated (making the injection) so viscous sample has time to fill the loop properly.
6. The system can communicate to outside computers using either LABNET or a RS-232C connector.
7. Programming is said to be very easy, and "dialog mode" and "help" keys permit easy programming.
8. Built-in diagnostic routines help with troubleshooting and locating mechanical and electrical subassemblies.
9. Reproducibilities are good; for fixed-loop injection (at 20 μl) precision is below 0.5%, and for partial-loop injection, reproducibility is below 1% at 10 μl.
10. Injection times can be set to 9999 min.
11. Several input/output relays can hold the injection, stop a pump, signal the start of injection, or start a gradient.
12. Four time-programmable event outputs are provided.

A limitation of the 8780 is that vial-to-vial transfer for dilution, derivatization, and internal standard addition is not possible. Also, cycle time between injections of 2 min may be long for fast-LC methods.

5. EM/Hitachi 655A-40 Autosampler

This Japanese injector was introduced for sales and service around 1986 in the U.S. by EM Science.[38] This autosampler uses the valve-syringe approach to loading sample (like that described for the Perkin-Elmer ISS-100). The 655A autosampler offers random-sample sequencing, variable-injection number (0 to 10), and variable-sample volume (from 1 to 100 μl or 5 to 500 μl, depending on the syringe used). At about $10,000, the 655A has many of the most recent features plus some very sophisticated (and well-documented) computer compatibility:

1. Position 107 is "priority sample" position that permits interrupting the programmed sequence for running urgent samples.
2. Position 108 is a "standard position" that can be sampled via the programming at any time during the sample sequence, at up to eight different volume levels. Plots of volume vs. area from 2 to 50 μl shows high linearity (correlation coefficient above 0.9998), potentially permitting calibration by injecting different volumes of the same sample.
3. The 108-vial removable rack sits in a water bath that can be either heated or cooled.
4. Cross-contamination from vial to vial is below 0.02% because the needle is only removed from the flow stream during the 1-min injection sequence, so the needle is constantly being flushed with eluant.
5. A second glass syringe washes the outside of the needle between injections.
6. A side port on the needle prevents the needle from clogging with septum material.

7. Operation can be continuous since a "sensing arm" checks for the presence of a vial, shutting down the system only if no vial is present. Hence, first vials can be removed and replaced with vials of unanalyzed samples, and the system will continue to run these vials.
8. Programming is said to be very easy, and in a "dialog mode" parameters can be copied from vial to vial by a simple "copy" command.
9. Data are displayed by a 40-character, two-line liquid crystal display.
10. Built-in diagnostic routines help with troubleshooting and locating mechanical and electrical subassemblies.
11. Diagnostic tests of mechanical and electrical systems can be activated externally when desired.
12. Reproducibilities are good; for fixed-loop injection and partial-loop injection are said to be below 0.6% for area.
13. Injection times can be set from 1 to 600 min.
14. Four time-programmable event outputs are provided.
15. When limited sample volume is available, as little as 1 μl can be injected from 13 μl in a microvial insert (usual vial size is 1.5 ml).
16. Injections as frequent as 1/min make the injector compatible with fast-LC, if the holdup volume can be tolerated.
17. At the moment of injection, four separate contact closures give "start" signals to an integrator, chart recorder, etc.
18. After the last vial (indicated by the sensing arm), a 1-s "stop" signal can be used to operate a flush valve, and 15 min later, a 1-s "all stop" signal is activated to shut down all components.
19. The autosampler can be activated by an external contact, such as an integrator.
20. An output permits transmission of digital data on vial number, injection volume, and other parameters.
21. A RS 232C port or PAN (private area network) port permits the unit to be programmed and controlled by an EM/Hitachi integrator (D-2000).

Another advantage to the EM/Hitachi autosampler is the powerful and well-documented[38] computer compatibility, permitting complete documentation and verification of sample processing using an outside computer (Figure 21). A 50-line BASIC program, published in Reference 38, for an IBM PC can be used to start the autosampler, read injection parameters for each vial, and store the information on disk. A second 25-line BASIC program, also published in Reference 38, permits the data to be read from the disk for printing a report.

A limitation of the 655A autosampler is that vial-to-vial transfer for dilution, derivatization, and internal standard addition is not possible.

6. Waters MilliLab Workstation

Introduced in 1986 at about $30,000, this robotic autosampler is one of the most sophisticated systems on the market (Figure 22).[39,40] The capabilities of the MilliLab fit between the most sophisticated autosamplers described above (about $10,000 to $12,000) and robotic-arm manipulators (at $40,000 to $60,000).[13] Compared with robotic arms, the system cannot perform many functions, for example, manipulating containers and objects, weighing samples, centrifuging solutions, etc.

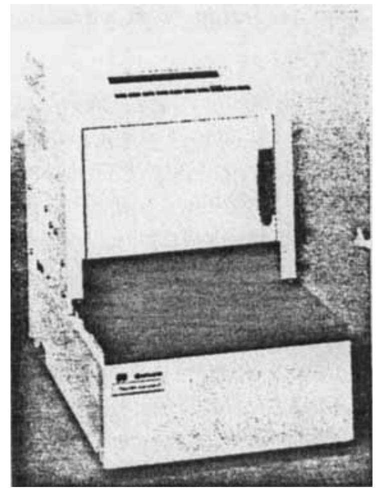

FIGURE 21. Hitachi 655A-40 autosampler (available with a water bath) and once distributed by EM Science. (With permission.)

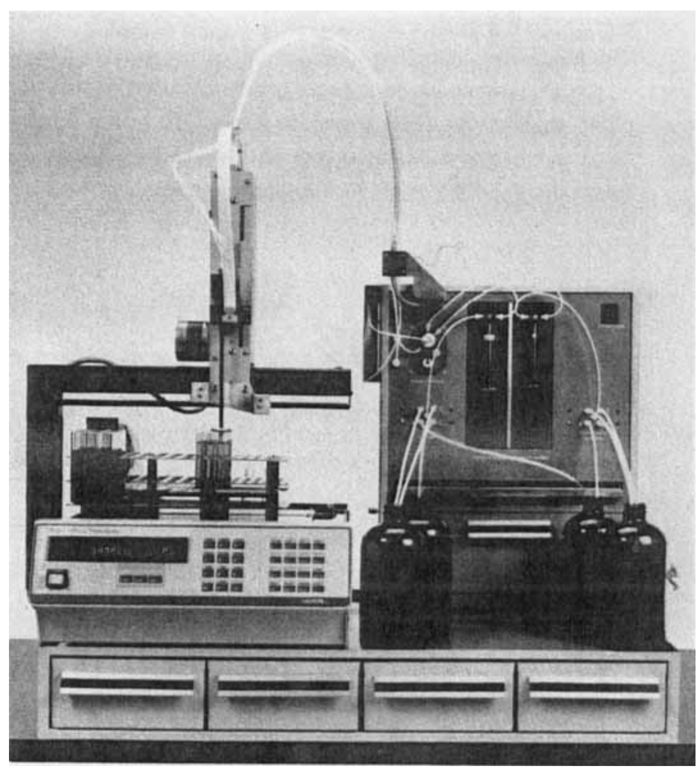

FIGURE 22. Waters-Millipore "MilliLab" robotic autosampler. (With permission.)

However, the MilliLab can perform some very complex manipulations beyond those possible with the most sophisticated robotic autosamplers available before.

The MilliLab consists of transport and fluidics modules. The transport module has a movable probe with an inflatable tip that can positively affix to various items (such as filters, injection valves, etc.). The probe can be programmed to take up specific X, Y, and Z positions above a test-tube rack, probe wash station, filter waste station, etc. The probe can pass sample through special filters and solid-phase extraction cartridges (functions usually not possible with other robotic autosamples, although Gilson has made this possible on recent autosamplers). The fluidics module, with two digitally stepped syringes (from 0.1 to 10 ml) being fed by three eluants each, can make vial-to-vial manipulations (to move samples) and can add any of six solutions, for making dilution, derivatizations, or standard additions. Other unusual functions of this new robotic autosampler include:

1. The MilliLab offers random-sample sequencing, variable-injection number (1 to unlimited), and variable-sample volume (from 1 to unlimited).
2. Liquid-liquid extractions are possible.
3. The system can communicate to outside computers using RS-232C connector.
4. Programming is said to be easy with many operations being preprogrammed.
5. Prepared sample can be loaded directly into a sample valve, loaded into vials for off-line injection, or loaded into an auxiliary autosampler rack.

With this new concept in robotic autosampler, many of the functions and capabilities are continuing to be developed. Limitations of the current version of the MilliLab may be the limited vial capacity (84 tubes of 13 ml or 120 tubes of 3 ml), the high price, the large size using much bench space and making portability difficult (34 in. wide × 28 in. deep, 130 lb).

III. PUMPING SYSTEMS AND GRADIENT CONTROLLERS

A. General Principles

Pumping systems and gradient controllers are described here, approximately in the chronological order in which they appeared, from about 1970 through 1987. To help in following the principles used in different types of pumps, they are grouped together into five categories (which is also the approximate chronological order of development):

1. *Parallel-flow pumps* typically have two pistons, and flow is supplied alternately from one and then the other. The inlet and outlet are connected in parallel. A set of inlet/outlet check valves are required on each piston.
2. *Membrane pumps* typically have one piston that operates at high frequency (500 cycles/min) while immersed in hydraulic oil. This piston chamber is separated from the fluid chamber by a metal membrane.
3. *Rapid-refill pumps* typically have one piston that refills sufficiently rapidly that only a small pulsation results. A pulse dampener is often used to minimize this pulsation.
4. *Series-flow pumps* typically have two pistons connected in series. For 1-ml/min flow, the main piston supplies 2 ml/min in its forward pumping stroke, and half of this flow is taken up by the second piston retracting. As the first piston refills, the second piston supplies flow at 1 ml/min. The key advantage is that only one set of inlet/outlet check valves is needed (on the main piston).
5. *Electronic-flow feedback pumps* are either parallel-flow or series-flow pumps. As the most recent innovation to minimize pulsation noise, during a "constant flow period" in the pumping cycle, the existing system pressure is stored in an electronic memory. The second piston then moves at a variable speed to deliver eluant at this constant pressure.

More details of these pumping principles are given in each section. Note that the most recent pumps are found at the *end* of the section, and often these have the most sophisticated capabilities and best performance/price ratio. Each succeeding generation of pumps shows technical improvements and more conveniences, often at lower costs.

Pumping systems have shown an enormous evolution in capabilities. Many pumping approaches have disappeared altogether for analytical LC such as the gas displacement pump from Varian, the gas amplifier pump from DuPont, the "flow amplifier" pump from Micromeritics and Durram, and the solenoid pump from Problematics. The piston displacement pump, used in early LC, is now seeing some renewed use for the specialized fields of supercritical fluid chromatography and microbore LC. These specialized pumps are not discussed here.

The specific examples of pumps discussed here generally represent important technological advancements that are still present in existing pumps, or the specific pumps themselves are still available. Most pumps have a reciprocating piston moving in a piston chamber. A special seal of filled fluoropolymer (often filled with carbon or other materials) around the piston prevents leakage of eluant (discussed below). Inlet and outlet ball valves (check valves) provide one-direction flow through the pump head. Pistons, balls, and ball seats are usually sapphire, and the head is usually stainless steel. Recently, titanium, "Hestelloy®" metal, Teflon®, and other polymers have been used for pump heads so the device can be operated with corrosive materials or iron-sensitive proteins.

Several factors have been acting simultaneously to make different demands on the pulsation noise that can be tolerated from pumps. As the size of particles has decreased, the permeability of columns has decreased, and column lengths have been made shorter to stay within the pressure limits of current pumps (usually 6000 psi). Less permeable columns tend to increase system "compliance" (see next section) and decrease the amount of pump pulsation noise that reaches the detector. However, simultaneously, detectors have become more sensitive, so pump noise has had to be further decreased.

This section shows that many very clever and innovative methods have appeared for precisely pumping LC eluants. As the size of particles continues to grow smaller, below 1-μ size, and columns move toward 1-mm lengths and micron-size diameters, so, too, will the technologies for "pumping" LC eluants change.

B. Basic Functions for All Pumps

For analytical LC with columns 1- to 5-mm id and 100 to 250 mm long, pumping systems generally supply pressures to at least 5000 psi with flows ranging from about 0.1 to 10 ml/min. Materials in the pump are relatively inert, with eluants usually contacting only stainless steel heads and ball housings; sapphire piston and check-valve balls and seats; ceramic ball stops; and certain polymers, such as carbon-filled high molecular weight polyethylene (Fluoroloy G) or similar Teflon®-based seals (Fluoroloy K).[41]

Pumps are often referred to as "solvent delivery systems" (but called "pumps" here), since they frequently are "systems" of various components and capabilities far more complex than simply pumping the eluant. These many additional features can greatly increase both the capabilities and costs of these pumps. Comparing pumps with pressures of at least 5000 psi and flows from 0.1 to 10 ml/min, costs can range from as little as $1200 for a one-piston sinusoidal-flow pump to over $9000 for more sophisticated pumping systems. More costly pumps have many of the features below plus more precise, accurate, and pulsation-free flow and flows over a greater range. The more sophisticated features found on recent pumps include:

1. High-pressure operation (at least 5000 psi, with 6000 psi being very common, and some 10,000 psi pumps were once marketed [e.g., Altex 100A pump])
2. Constant, pulsation-free flow
3. Built-in pulsation dampening and other pulsation control methods, especially important with refractive index and electrochemical detectors and low dispersion methods
4. High flow capability (to 10 ml/min, sometimes to 20 or 50 ml/min for scaling up to preparative work, flushing the system, or special techniques, such as fast LC)
5. Low flow capability to 0.010 or even 0.001 ml/min (so the pump can be used with microbore LC)
6. Digital or continuous flow settings to obtain a broad flow range and still keep precise and accurate flows; pumps often have the capability of changing the pump heads; for example, some pumps have 5-, 10-, 20-, or 50-ml/min pump heads, such as the Gilson pump
7. Solvent filters within the pump to ensure protection of ball valves
8. Built-in pressure meter and millivolt output for recording pressure
9. Overpressure shutdown protection if the system clogs
10. Underpressure shutdown protection if the system leaks
11. Constant pressure capability
12. External start switch that can synchronize the pump with an autosampler or low-pressure gradient generator
13. Solvent selection capability by a manual valve or an automated valve with no cross-contamination or leaks between solvents.
14. An internal timer to time program flow (i.e., change the flow at any given time or ramp flow up or down over a time period)
15. Built-in timer for events control so the pump can activate an injector, mark a recorder, or activate a gradient generator
16. Precisely resettable flows so reproducibilities are good
17. Precisely resettable isocratic compositions
18. Accurate flows so the "set flow" is the true flow
19. Accurate gradient generation so literature and experimental retentions can be reproduced
20. Compressibility compensation so flows are independent of back pressure, see "Compliance" below
21. Priming capability for fast solvent changes and for eliminating air bubbles
22. Low hold up volume for easy solvent changes
23. Recycle capability with little holdup volume to pass unresolved peaks repeatedly through the same column with little peak spreading within the pump
24. Tolerance to low-boiling "cavitating" solvents such as methylene chloride or ether
25. Tolerance to particles, such as dirt, bacteria, mold fibrils, etc.
26. Tolerance to micelles and surfactant solutions such as ion-pair reagents and sodium dodecyl sulfate solutions used in micellular chromatography
27. "Flushable" pistons so salts on the piston can be washed free, or sealed membrane pumps so no salts contact the piston
28. Tolerance to corroding solvents like halogen-containing methylene chloride and salt solutions (high-salt eluants can dry on the piston and lead to fast piston wear)
29. Inert chromatographically, for example, so proteins are not sorbed or denatured by such contaminants as iron in the eluant (e.g., "iron-free" for biopolymer separations)
30. Rugged for long service, without the need for seal change (a recent Beckman-Altex pump offers a 5-year warranty on the piston seal!)
31. Easily serviced when necessary, especially with easy seal change and without the possibility of breaking the sapphire plunger
32. Selectable voltage by switch, fuse, or software

33. Physically convenient (compact, lightweight, portable, and stackable)

This composite list of current pump capabilities shows that modern-day LC "pumping systems" are very sophisticated and have had to include many special features in their design.

C. Personal Computer (PC)-Controlled Pumping Systems

Each year the population becomes less "computer-hostile" (more computer literate) and simultaneously, PCs continue to improve importantly. Every year PCs:

1. Are more user friendly
2. Are less expensive
3. Are more standardized for interchange of programs
4. Are faster in processing
5. Have more internal memory
6. Are more dependable
7. Have more available programs (often word processing, data-base programs, spreadsheets, and BASIC programs are built in)
8. Are more flexible (e.g., multitasking permits word processing while controlling an instrument)
9. Provides better displays (screens that are flat, colored, and/or have higher resolution for graphics)
10. Are smaller, lighter, and more rugged (for portability)
11. Are lower in power requirements (for battery operation and thus more immune to power surges or power failure)

Innovations and improvements are certain to continue with developments such as faster (32-bit) computers and voice recognition, which will eliminate keyboards. Computers are now moving toward being thought of as a *capability* rather than a *device* sitting on a desk (like the transition of the calculator from large mechanical device to a wrist-worn capability). Freed of a power cord, the battery-powered lap-top computers of today can send and receive information between computers remotely by phone, even in a car via a mobile cellular phone.

More sophisticated LC systems contain up to five separate computers, doing the different functions of:

1. Controlling LC functions (flow, pressure, temperature, and gradient generation)
2. Running optimization routines to find the best separation
3. Controlling robotic autosamplers to inject, derivatize, and dilute samples
4. Manipulating data from detectors to identify and quantify compounds and determine chromatography performance (area, column efficiency, H vs. μ curves, etc.)
5. Manipulating diode array detector outputs to determine peak purity, optimize detection wavelength, deconvolute unresolved peaks, etc.

It should become more common that a single computer takes over several of these complex functions, as was done to some extent with the Hewlett-Packard 1080 and 1090 instruments and the Varian 5000.

An advantage of PCs for controlling some of these functions is that some capabilities potentially can be updated by software changes. However, realistically, even the most recent PCs become obsolete in about 2 years as new developments appear (such as faster 32-bit computers, larger hard memories, portability, better displays, etc.)

Several companies now have PC-based systems that combine the gradient-control and data-management functions. Advantages to such a system are[42]

1. Costs are less than integrators plus dedicated controllers.
2. Gradient control or data-management software can be updated when (and if) new programs become available.
3. Data can be permanently stored for further analyses, future comparison, remanipulation, and archival storage requirements.
4. More complex calculations, tailored calculations, and tailored reports can be user-programmed into the PC.
5. Other functions are possible (such as word processing; assistance from expert-systems, communication by phone, etc.).

It should be remembered that while the formal potential of the system is high compared with simple LC systems, there are some factors, often related to time, that can be problematic in everyday use with many PC-based systems. Manipulations that can take many minutes include retrieving chromatograms from the vast data bank of the hard disk (with a 16-bit computer), recalculating data from a chromatogram, finding the proper 5.25-in. disk with a chromatogram, and even calling up control conditions for a run.

D. Compliance, Pulsations, and Pulsation Dampening

"Sinusoidal" flow is produced by a simple pump if the piston is attached to a rotating wheel or circular cam. (Most modern pumps use a "shaped cam" to minimize pulsations, as described under the Waters 6000 pump.) Figure 23 shows the individual sinusoidal flow profiles for three separate heads, out of phase by 120°.[43,44] Following any one sinusoidal curve, flow output from the piston chamber is indicated by the portion of the sine curve above the horizontal line, and the piston chamber refilling is indicated by the portion of the sine curve below the horizontal line. If the flow is put through a resistance (such as a chromatography column), the pressure profile would follow this flow profile, and pulsations would result. Note that having three heads 120° out of phase is one technique to minimize pressure (flow) changes (see the low ripple in the "composite delivery" flow profile).

The ability of the LC system to "comply" and store energy

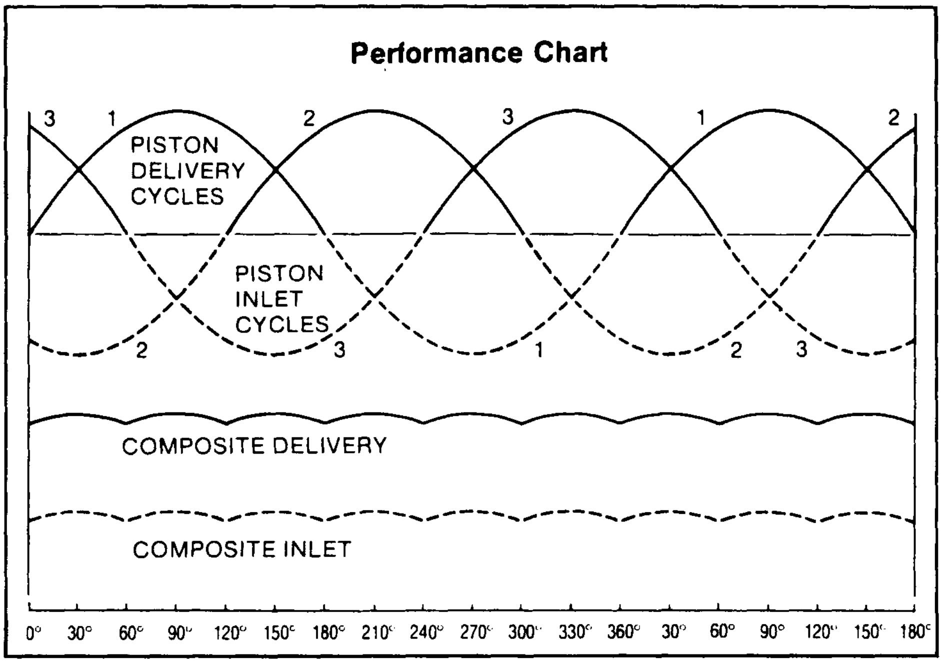

FIGURE 23. Sinusoidal output from each head of three heads 120 degrees out of phase gives the "composite delivery" flow profile in the middle. (From Anspec, Ann Arbor, MI. With permission.)

between pump strokes is termed "compliance". A LC system with no column can represent a system with zero compliance. Pressure and flow are zero on the "refill" stroke of a simple single-piston sinusoidal pump. No energy is stored between piston strokes. A system with good compliance may store energy (and subsequent flow) by compressing the eluant itself, by slight expansion of the tubing, and (intentionally) by pulsation dampeners. Thus, with a high-compliance system, energy is stored during the "pumping" portion of the piston cycle, and energy is released during the "refill" portion of the cycle.

Pulsation dampeners are devices with varying levels of compliance that are often used to smooth out the pulsations from a pump. Four types of pulsation dampeners have been described:

1. *Spring-loaded pistons or bellows,* explored in the early 1970s, are rather inefficient devices, generally too small to be of much use.[45] The spring acts to store the energy during the "pumping" part of the stroke and release the energy during the "refill" part of the stroke. To operate well, the piston must move easily, the bellows must be flexible, and the spring must not be completely compressed.
2. *Gas ballasts* are the most efficient devices for energy storage, and the "membrane" (gas-liquid interface) is infinitely flexible.[46] However, gas-saturated eluant feeding back into the flow stream could make bubbles in the detector or even disrupt column packing structure. Early fortuitous work with air-filled Bourdon-tube pressure gauges probably gave good pulsation dampening because the 10 ml of trapped air was compressed to a 0.1-ml bubble at 100 bar, and this acted as a gas ballast. Gas ballasts have been used in other applications since then to remove all detectable pump pulsations, even to a refractive index detector.[47] Such systems prevent gas-saturated eluant from feeding into the flow stream in various ways.
3. *Bourdon tubes* are created by partially flattening a round steel tube. At higher pressures, this flattened tube tries to achieve a more circular cross-section, and on the refill stroke the meter recovers its original oval shape and releases energy. If a Bourdon tube is then coiled, the tube will try to straighten during the pumping (pressurizing) stroke, and this movement is used mechanically to move a pointer on a pressure gauge to indicate pressure. The 10- to 20-ml volume in Bourdon tubes used in large pressure gauges can absorb some pulsations. When a Bourdon tube is made with 1/16-in. od spring steel and the fluid allowed to flow through it, it becomes the pressure meter used in several pumps (e.g., Waters, Autochrom, and Knauer pumps). Such small Bourdon tubes have little pulsation-dampening effect. However, some pulsation dampeners (e.g., Altex, Perkin-Elmer, and Waters) use a flow-through Bourdon-type tube made by flattening 10 to 20 in of $^{1}/_{4}$-in od stainless steel tubing. With some devices this is encased in a steel envelope or embedded in a semiplastic tar that improves the "springiness" of the device.

4. *Compressible fluids* are a key to a more recent pulsation dampener. These fluids have high energy storage capabilities and are available from several companies (e.g., Gilson, Rainin, and SSI). The eluant flows over one side of a thin, flexible, steel membrane 1 to 2 in. in diameter. A chamber on the other side is filled with a highly compressible fluid, like hexane or methanol. Pulsation dampening occurs as with the Bourdon tube, with energy storage by the compressible fluid during pressure surges. Often these stand-alone pulsation dampeners have other functions, such as a pressure-indicating meter, voltage output indicating pressure, and high- and low-pressure limit set points. This design for a pulsation dampener is similar to the device for determining flow that was used earlier in the Hewlett-Packard 1080 liquid chromatograph.

The recent inexpensive pumping system from Perkin-Elmer, the Trident, uses a coiled tube with a large volume of eluant (approximately 30 ml). The coiling will produce a slight flattening effect and create a spring-like action as the coil gets longer and shorter. With sufficient back pressure from the column, this effectively absorbs pulsations. Part of the pulsation dampening of this device is due to the compressibility of the fluid itself contained in the tube, and part is due to the elongation of the tube.

In the ensuing discussions of specific pumps, the use of these various pulsation dampeners will be noted. To minimize problems with pulsation dampeners (e.g., holdup volume, irreversible distortion, and leakage), the most recent generation of pumps is using electronic-flow feedback to make very fast changes in piston movement so as to match the pressure measured on the previous stroke. Pumps using this principle are described near the end of the section.

E. Pump Seals

A key development in LC was the introduction of the Bal C-cross-sectional seal around 1975. Sealing around the moving piston, the open part of the C cross-section faces into the high-pressure chamber of the pump so that higher pressures cause the "C" to open and the seal to become tighter. In addition, even poorly elastic materials, such as filled perfluorinated polymers, can have a spring built in to produce sealing at low pressures. Figure 24 shows a cross-section of a Bal-type seal around a piston showing the spring facing into the high-pressure chamber.[48] Other campanies now offer this type of seal.

Prior to the Bal-type seal, compression-type seals used an "O" ring or a packing surrounding the piston. Tightening a "packing nut" was required frequently to prevent leakage. Higher pressures usually led to greater leakage.

F. Gradient Generation

Another important concern with pumps is gradient generation. The earlier method is "high-pressure" gradient generation.

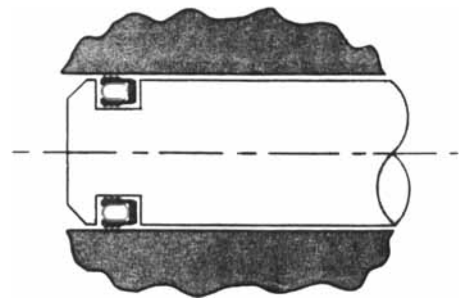

FIGURE 24. Cross-section of a Bal-type seal on a piston showing the open section facing the high-pressure zone (to the left) so increased pressure opens the seal and improves the tightness of the seal. (From Bal-Seal Engineering Company. With permission.)

This involves two or more high-pressure pumps supplying parallel streams of eluants to the injector and column. With "low-pressure" gradient generation, the valves leading to two or more eluants open with the proper timed ratios to produce the composition required. This puts different eluant segments, one after the other, in sequence into the low-pressure flow stream leading into the pump. A third "medium-pressure" gradient generation system is described later in detail for the Hewlett-Packard 1090 pump, in which 90-psi metering pumps supply the proper composition to a chamber preceding the high-pressure pump.

With either kind of gradient generator it may be necessary to use a mixer to mix either the parallel streams (from high-pressure systems) or the sequence of different eluant segments (from low-pressure systems). These mixers may be "active" or "passive". Active mixers are more common and consist of a small chamber with a Teflon®-coated magnetic mixing bar. Some companies supply different size mixing chambers compatible with microbore through preparative chromatography (e.g., Eldex and Gilson). Passive mixers consist of a column packed with nonporous glass beads and are used with some systems (e.g., Brownlee and Varian). Some systems do not seem to need mixers (e.g., Waters high-pressure gradient generation system, the Hewlett-Packard 1090 medium-pressure system, and the Spectra-Physics 8700 low-pressure gradient system).

The different gradient generation systems each have their advantages and disadvantages. With the older high-pressure gradient generation system, it is difficult to obtain very low and very high compositions, which require that one or the other of the pumps operate at very low flow output.[49] The higher costs of using multiple high-pressure pumps has led to many companies coming out with low-pressure gradient generators. A problem with low-pressure generation is the valve timing must usually be synchronized to the piston movement in order to obtain proper compositions. Often this synchronization is not easy to do at all flows from the pump. Besides the lower costs, an advantage to the low-pressure generation is that very low and very high compositions can be obtained with good reproducibility.

G. Specific Parallel-Flow Pumps and Gradient Controllers

These typically use two pistons with the two inlets connected together and the two outlets connected together (thus, the flow through the two heads is parallel). One head supplies flow, while the other head is refilling, and vice versa.

1. LDC/Milton Roy "miniPump" Sinusoidal Parallel-Flow One-Piston Pump

One of the first types of pumps used in LC in the early 1970s was the simple, sinusoidal-movement single-piston metering pump used in the chemical industry to add components to a process stream (Figure 25).[50] Understanding the advantages and disadvantages of these first miniPumps will make clearer the directions that later LC pumps took in the 1970s.

Pulsations in the flow for metering chemicals are generally not important, as long as the average flow per minute is constant and reliable. These miniPumps perform metering well in the 1000- to 2000-psi range using the then-available O-ring compression-type seal around the piston. Chief advantages of miniPumps are

1. Costs are low (about $1100 for a single piston, and $1700 for a dual-piston pump).
2. Pressure ratings are high (approximately 2000 psi later increased to 6000 psi).
3. High and low flow ranges are available from 0.03 to 15.3 ml/min (but this must be decided when purchased).
4. Heads of stainless steel ($530) can be interchanged with heads of inert Hastelloy C ($900), titanium ($1000) for iron-sensitive compounds (like some proteins) or corrosive reagents, or PEEK ($630) for biological applications.
5. Explosion-proof housings are available for process environments.
6. Preparative flows 2.25-fold higher than the rated flow can be obtained to 3000 psi with a preparative head (about $600) using the $^3/_{16}$-in. diameter glass piston vs. the standard $^1/_8$-in. sapphire piston.
7. Preparative flows fourfold higher than the rated flow can be obtained to 1500 psi with another preparative head (about $600) using the $^1/_4$-in. diameter piston for flows up to 60 ml/min in the duplex pump.

FIGURE 25. LDC/Milton Roy "miniPump" sinusoidal parallel-flow one-piston pump. (With permission.)

The presently available miniPumps are very similar to the early metering pumps, except the pumps of today use the Bal-type seal described in the introduction, so leakage around the piston is virtually absent to pressures of 6000 psi. Pressure is then often limited by the power of the motor; the motor stalling if pressure is too high.

miniPumps can be purchased in different flow ranges, depending on the number of pistons and strokes per minute (spm). A disadvantage is the flow range of the pumps is limited: about 10 to 100% of full stroke. Thus, single-head (Simplex) miniPumps have a flow range ratio of about 1:10, and double-head (Duplex) pumps have a flow range ratio of 1:20 (i.e., one head off and the other at 10% vs. both heads full on). Single-piston miniPumps have five flow ranges (in milliliters per minute): 0.032 to 0.32 ml/min (3.8 spm); 0.267 to 2.67 ml/min (31 spm); 0.483 to 4.83 ml/min (57 spm); and 0.767 to 7.67 ml/min (89 spm). Dual piston miniPumps have a double-piston head (each able to be set separately) running off a single motor, and three flow ranges are available, depending on the motor speed: 0.267 to 5.33 ml/min; 0.483 to 9.67 ml/min; and 0.817 to 15.333 ml/min. Flow is adjusted continuously with a vernier knob that adjusts the length of the stroke.

One limitation of the miniPump is the flow adjustment knob does not read in milliliters per minute, so the vernier must be calibrated or flow determined directly for each setting by catching and weighing solvent over a given time period.

Another important limitation of these miniPumps is that the flow output is not constant, but sinusoidal, even from the dual-piston pumps (180° out of phase). The piston is mechanically connected to a rotating circular cam, so a sinusoidal flow output from the pump is generated. With a pump at 31 spm, then, the piston spends about 1 s refilling, and 1 s pushing liquid out. The flow out of the pump head increases and then decreases in a sinusoidal pattern, following the piston movement (e.g., see Figure 23). With a single-head pump, outlet flow increases from 0 through 0.5 s and then falls to zero at 1 s and holds at zero flow from 1 to 2 s. With two heads 180° out of phase, the period from 1 to 2 s for the second piston mimics the flow from 0 to 1 s for the first head. This reduces somewhat the flow variation, but a strong sinusoidal pulsation still remains on the flow, which translates into pressure changes. These pressure pulsations can put considerable noise on most detector baselines and reduce the lifetime of packings. Pulsations are reduced by adding more heads, appropriately out of phase, or by increasing the system compliance.

A third limitation is that these miniPumps have no built-in overpressure safety device. Unless one is used after the pump, it is possible to damage seals or ball valves, break the sapphire piston, or destroy columns if a clog develops at the column outlet.

Waters marketed the LDC miniPumps as their C903 pump at about $1500 in the early to mid-1970s, plumbed for LC and modified with (1) the Bal seal for leakproof operation, (2) heavier motors for higher pressure operation (to 6000), (3) a pressure gauge, and (4) a built-in Bourdon-type pulsation dampener to increase the system compliance and reduce pulsations (Figure 26). These seals and motors are part of the currently available miniPumps from LDC, and an accessory pressure monitor and pulse dampener is also available (about $300).

Even today these miniPumps are a reliable and inexpensive approach to pumping not only LC liquids, but also corrosive derivatization reagents for postcolumn reactors (provided the pulses can be tolerated).

2. LDC/Milton Roy "VS" Sinusoidal Parallel-Flow One-Piston Pump

To overcome some of the disadvantages of the miniPumps described above, LDC recently introduced the "VS" pump as inexpensive as the miniPump, with other advantages (Figure 27):

1. Cost is low (about $1200).
2. Flow and pressure are in the common LC range (0.5 to 5.00 ml/min and 6000 psi).
3. Flow reproducibility is good +0.5%.
4. Flow can be directly set by a turn-counting dial calibrated in milliliters per minute, and flow can be set to the nearest 0.01 ml/min.
5. Heads of stainless steel ($200) can be interchanged with heads of inert Hastelloy-C ($750), titanium ($750), or (low-pressure rated) Kel-F for corrosive reagents (like reaction-detector reagents) or metal-sensitive compounds (like some proteins).

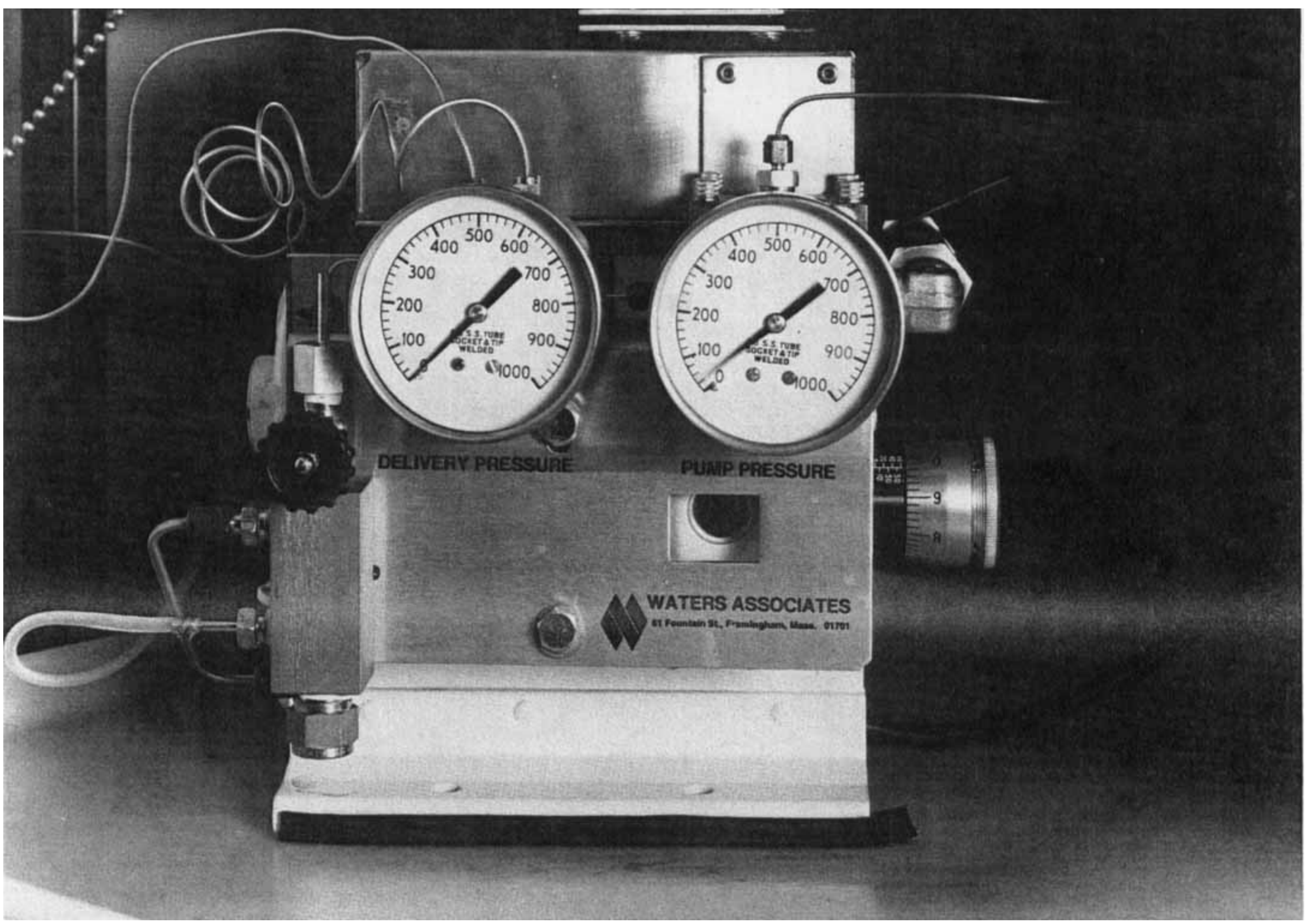

FIGURE 26. Waters-Millipore C903's early version of the LCD/Milton Roy miniPump modified for LC.

FIGURE 27. LDC/Milton Roy "VS" sinusoidal parallel-flow one-piston pump. (With permission.)

6. Components were once available with gold plating for increased chemical resistance (like the metal spring that creates the refill stroke).
7. Flow can be controlled externally by external electrical resistance.
8. Size is smaller (7 × 6 × 8 in., W H D, 10 lb) vs. the miniPump above (10 × 7 × 7, in., 16 lb), and heads are more compact (7 parts only) and more quickly disassembled for repair.
9. Check valves are used in an inexpensive ($27) disposable cartridge.

Disadvantages still remain with the VS pump, despite the many improvements over LDC's earlier miniPump. Flow remains sinusoidal, although flow rate is controlled not by the length of the stroke as in the miniPump, but rather by stroke frequency. Also, the VS pump has no built-in overpressure safety device.

3. Eldex Sinusoidal Parallel-Flow One-, Two-, and Three-Piston Pumps

Eldex sells small pumps similar to the one- and two-head pumps of LDC described above, except Eldex also markets a three-headed pump (Figure 28).[51] The pistons for the three-headed pump, 120° out of phase, are "driven by contoured cams optimized to give maximum drive motor efficiency and

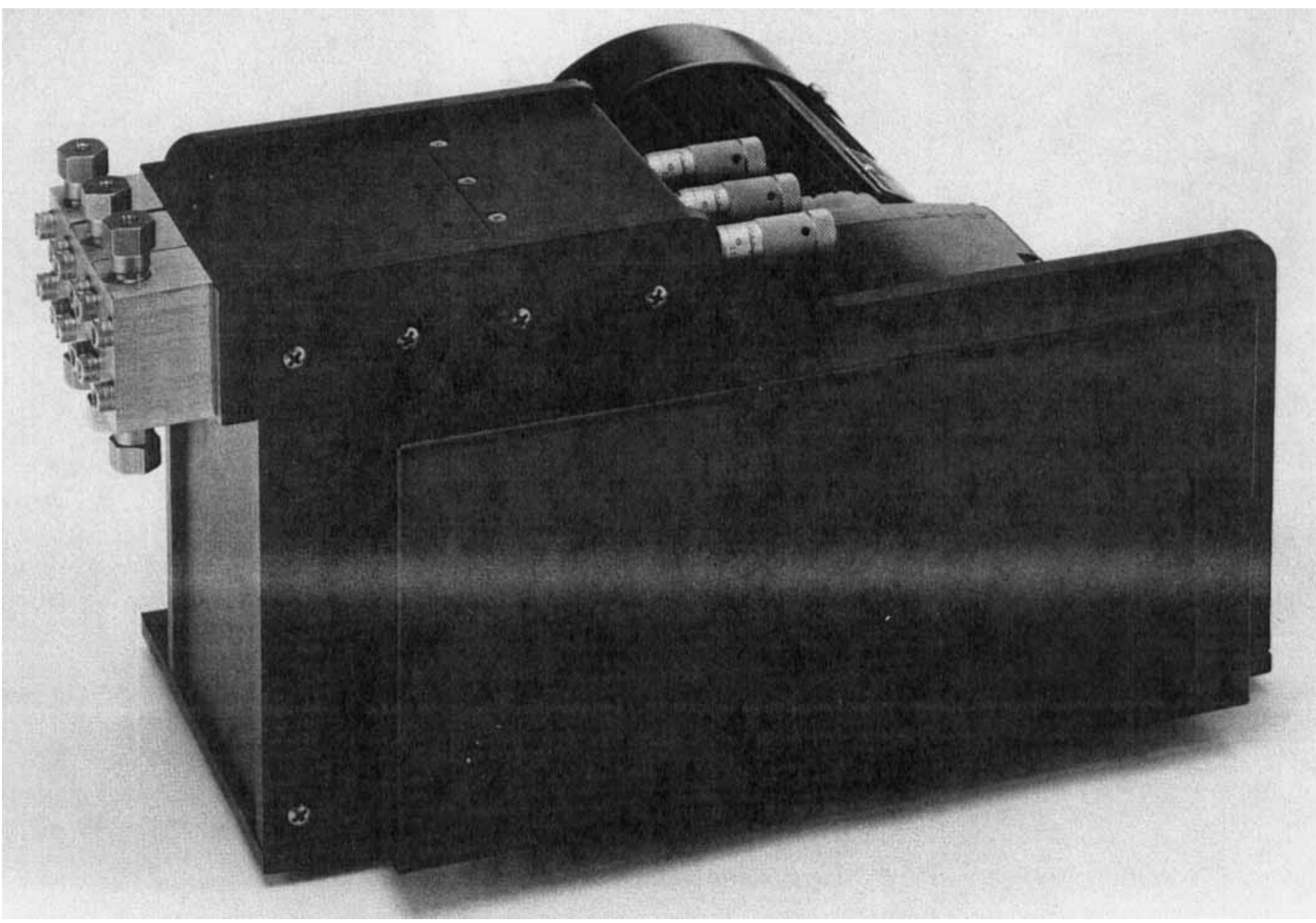

FIGURE 28. Eldex sinusoidal parallel-flow three-piston pump. (With permission.)

minimum pressure pulsations". However, the flow output for the one-headed pump is not of the "fast-refill" type (described below for the Altex 110A), and, hence, flow stops totally on the refill stroke if the system has no compliance. Eldex pumps use constant speed motors, and flow is controlled by a precision micrometer that fixes the piston stroke length and gives reproducible flows. The pumps offer a number of different options of pressure, head-number, or flow rate. For example, their high-pressure (5000 psi) pumps are available as follows:

One-head,	flows 0.2—8 ml/min, about $1300
Two-head,	flows 0.2—10 ml/min, about $2000
Three-head,	flows 1—100 ml/min, about $4800

Other models with pressure ratings to 1000 or 2000 psi have higher flows. Other unique features of these lower-cost pumps are

1. Special heads of KEL-F® polymer are available for pumping corrosive fluids (at low pressures) or metal-sensitive proteins.
2. Each head can pump different fluids since it can be adjusted independently (pulsations can be a problem).
3. Various Bal-type piston seals are available (Fluoroloy-G, a high molecular weight polyethylene; Fluoroloy-K, a fluorocarbon).
4. Springs are corrosion-resistant, gold-plated stainless steel.

Eldex also offers a pump monitor (at about $1000) that includes a liquid-backed low dead-volume (varying from 0.5 to 0.9, depending on pressure) pulsation dampener, pressure readout, and high- and low-pressure limits that will shut off the pumps.

As with the miniPumps from LDC, disadvantages are the flow adjustment knob is not calibrated in milliliters per minute, considerable pulsations exist, compressibility compensation is absent, and no overpressure protection is built in.

4. Eldex CMT-2 Low-Pressure Two-Eluant Gradient Controller

Introduced in the last few years, the "Chromat-A-Trol" (at about $2600) could be one of the most versatile (though simple) programmers on the market, having 24 external event channels (Figure 29). The system is easy to configure in many control possibilities since Eldex also supplies many peripheral devices. For example, capabilities include:

1. A low-pressure two-eluant gradient generator module and 0.5- or 3-ml mixer (at about $1000) can generate gradients from 1 to 99% (resolvable to 0.1%); from 0 to 0.9% or from 99.1 to 100%, resolvable to 0.05%. The mixer is glass for easy observation of air bubbles.
2. Up to 24 grounded outlets (110 V) can be time-programmed using their six-outlet modules (at about $500 per module).
3. Up to 24 switch closures (DC) can be time-programmed using their six-outlet modules (at about $500 per module).
4. Up to 24 multiple solvent selection valves (conneting a central low-pressure line to any of six lines) can be time-programmed (at about $1000 per valve, four required).
5. Two outlets are pulsed for activating an injector or fraction collector.
6. Time-programming can have dozens of "on" or "off" operations ranging up to 99 h.
7. An audible alarm can be time-programmed to alert the user.

FIGURE 29. Eldex low-pressure, low-cost Chromat-a-trol controller. This can time program (not shown): (1) rapid valves for low-pressure gradient generator, (2) slower valves for step gradients or solvent switching, (3) a six-contact closures (low current), and (4) six 110-V switched outlets. (With permission.)

5. Waters 6000A Parallel-Flow Two-Piston Pump

The first pumps taking into account some of the special needs of LC were the 6000 series of pumps, introduced in 1975 by Waters (Figure 30).[52] These became the most widely sold pump (approximately 10,000). This pump is described in detail because many of the design innovations were incorporated later in pumps from other companies (often with improvements), and many features remain in the current replacement pumps from Waters (the 501, 510, and 590 pumps, described below).

Introduced in October 1975, the M6000 pumps were much more expensive (about $4300 in 1975) compared with the very simple sinusoidal single-headed Milton Roy-type pumps that preceded them (about $800 to $1500 in 1975). However, the M6000 was properly named a "solvent delivery system" because it has many additional capabilities beyond simply providing flow, as did earlier pumps.

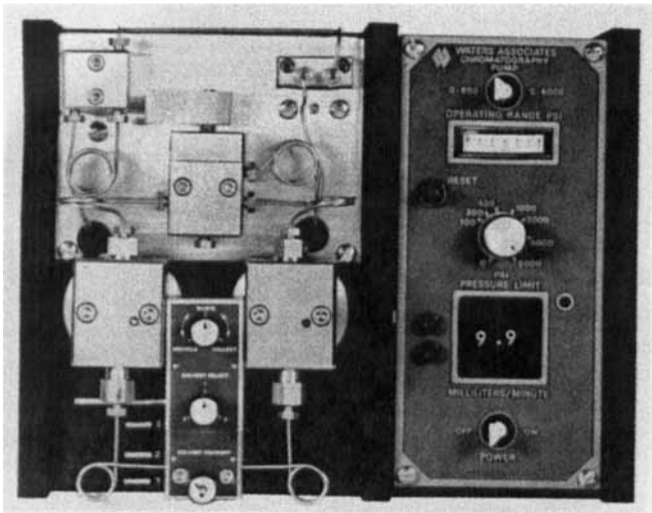

FIGURE 30. Waters-Millipore 6000A parallel-flow two-piston pump with inlet manifold. (With permission.)

The basic design of this pumping system introduced many major design innovations that became commonplace in later pumps. These innovations were

1. Two heads were used — while one head was filling, the other could be delivering fluid.
2. The cam design for moving the pump heads forward was shaped so the head speed was nearly constant, and, thus, flow was constant (i.e., nonsinusoidal) (Figure 31).
3. Sophisticated electrical motor control permitted flow control and eliminated fluctuating voltage problems, back pressure (load) effects, etc.
4. Internal microprocessor control permitted the pump to memorize flow settings and change flows according to back pressure (compressibility corrections).
5. A low-volume built-in pressure monitor prevented accidental damage to the column or pump and permitted convenient diagnosis or pump malfunctions, since the output could be displayed on a recorder and compared with malfunction patterns given in the instruction manual.
6. Up to four solvents could be selected by simply rotating a knob, with no cross-contamination of solvents.
7. Connections were provided for recycle chromatography, especially useful in steric exclusion chromatography to determine molecular weights. The partially separated band could be sent through the same column many times.
8. An output was provided for external control of the pump.
9. A "priming valve" was incorporated to help purge inlet line of old solvent and remove air bubbles from the lines and pump head.

The built-in pressure monitor eliminated a major source of artifacts found in early chromatographs: conventional Bourdon tube pressure gauges, usually connected by a "T" to the LC, often fed dirty eluant or gas bubbles into the flow line when pressure was allowed to change, such as during a gradient or at shutdown.

The shaped-cam approach had been marketed in the early 1970s in an all-Teflon® and glass (low pressure to 500 psi) metering pump from an early chromatography company in California called Cheminert, whose product line was acquired by Milton Roy in the mid-1970s.

The new design features used in the Waters M6000 pump were copied and improved upon by many instrument companies for several years in the 1970s.

Many of the principles used in the 6000 pump have been used by Waters (and other companies) in later versions of the pump, including the 510 (at about $6000), the 590 (at about $7400), and the 501 (at $4000).

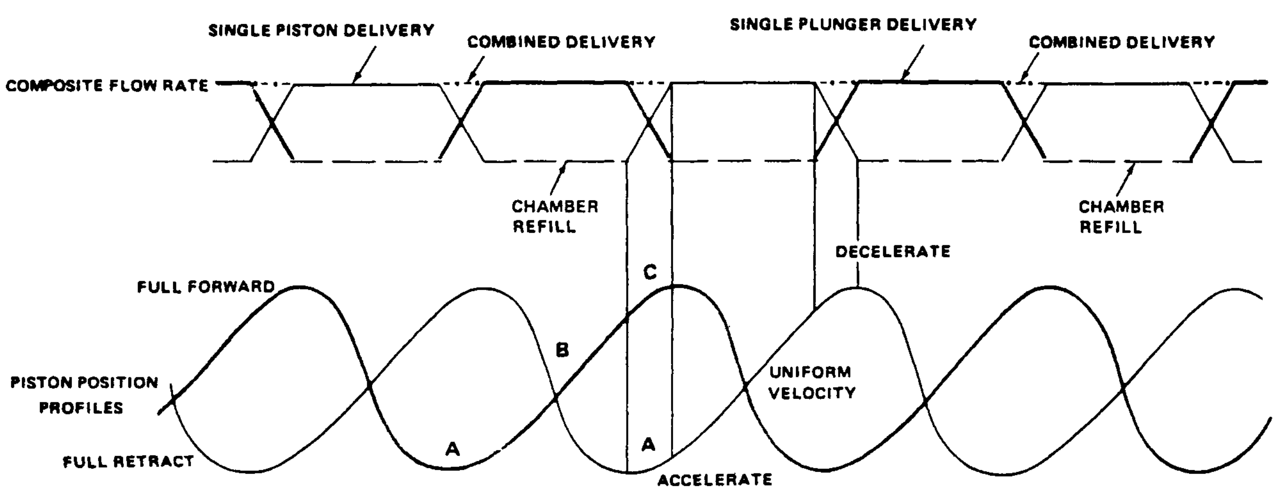

FIGURE 31. Piston position profiles (bottom) and resulting flow rate (top), showing how the shaped cam produces a constant flow output (dotted line, top) in the Waters-Millipore 6000A pump. (With permission.)

A deficiency in the Waters pump is the difficulty in removing air bubbles if they are sucked into the ball valves.

6. Beckman-Altex 100A Parallel-Flow Two-Piston 10,000 psi Pump

Introduced about 1978 for some $6000, the Beckman-Altex Model 100A pump was the first pump to compete importantly with the Waters Model 6000A pump (Figure 32).[53] Although it is no longer commercially available, the 100A was a success, and many were sold and are still in use. Altex may have significantly competed with itself and the 100A pump by later introducing the lower-priced single-piston 110A pump, described below. Using two heads driven from shaped cams, the 100A pump had a number of improved capabilities over the earlier Waters M6000 pump:

1. Flow range was broader (0.03 to 10 ml/min) with smaller flow increments (0.01 ml/min).
2. Pressures to 10,000 psi were possible (significantly above the 6000 psi of other pumps).
3. Flows to 28 ml/min and approximately 3000 psi were possible with optional heads.
4. Starting or stopping the pump could be performed remotely, e.g., with a time switch or pump controller.
5. The flow-through in-line pressure transducer could be set to read in pounds per square inch or atmospheres.
6. A digital display was used to set the upper pressure limit.
7. A simple switch permitted constant pressure operation (useful for column packing and microbore LC).
8. Flow reproducibility was very good (±0.3% or 0.003 ml/min, whichever is larger).
9. Flow accuracy (closeness of the set flow to the actual flow) was also very good (±1% or 0.005 ml/min).

The Altex 100A pump was one of the first pumps to state flow accuracy and reproducibility specifications. As with the Waters pump, an output was provided for recording the pressure so flow malfunctions could be easily diagnosed. Additionally, the pump used an electronic pulse correction similar to that described later for the Altex single-piston Model 110A pump. Thus, if cavitation of a low-boiling eluant or an air bubble

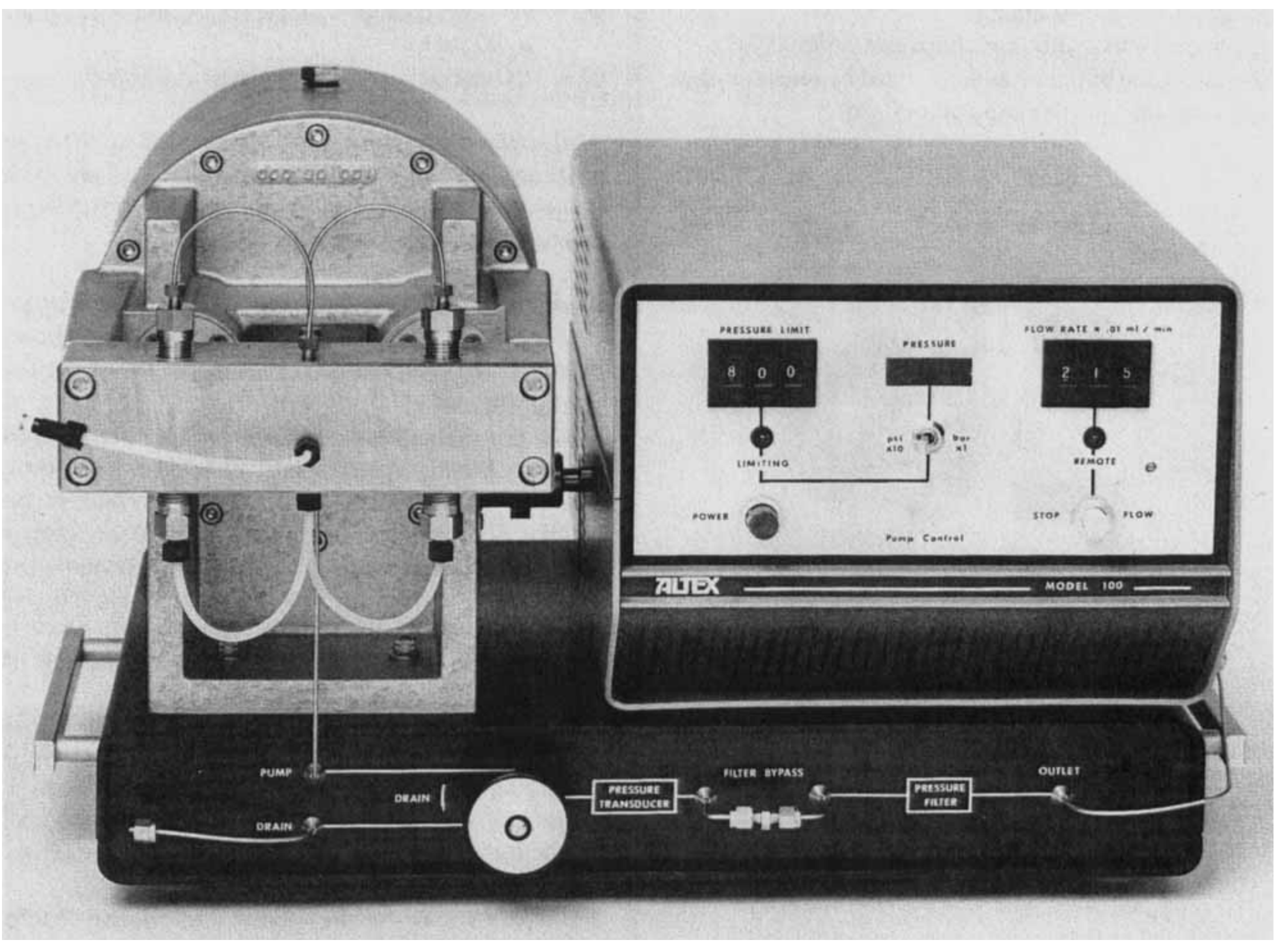

FIGURE 32. Beckman-Altex 100A parallel flow (10,000 psi) two-piston pump. (With permission.)

occurs in the pump head, rapid pumping (trying to raise the head pressure) will automatically clear the inlet. (The ease of priming this pump has a significant advantage over the Waters pumps.)

The Model 100A pump was built ruggedly and was very dependable. However, this ruggedness and dual heads proved to be costly and something of an "overkill" to LC pumping, so that the single-piston Altex Model 110A (described later) could offer important cost advantages.

7. Varian "Slim-Line" 2010 Parallel-Flow Two-Piston Pump

A Japanese pump designed about 1984, the 2010 (at about $3500) is sold and serviced by Varian (Figure 33, top).[54] The 2010 has direct flow settings from 0.01 to 9.9 ml/min. The 2010 is intended to be operated by the 2020 gradient controller (below). The usual features are present, including built-in pressure monitoring, overpressure shutdown, and automatic compressibility compensation. Special features of the 2010 include:

1. A switch-controllable microflow mode for 10 to 990 μl/min or 0.1 to 9.9 ml/min
2. Operation to unusually high pressures of 7500 psi
3. Flow precision of 0.3% (as determined by retention-time reproducibility at 1 ml/min and 600 psi)

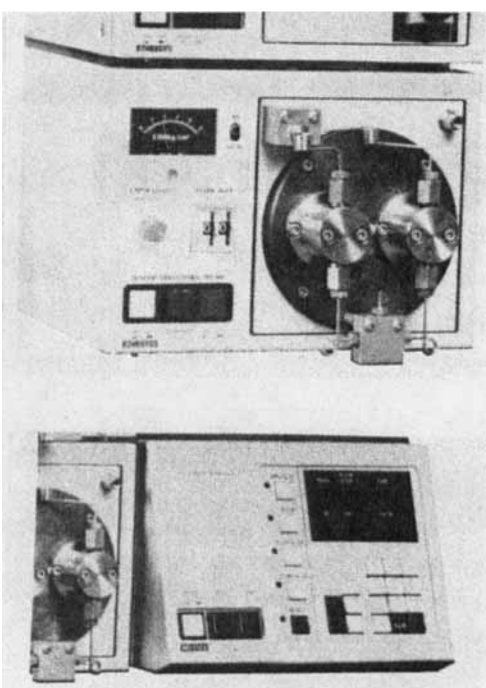

FIGURE 33. Varian 2010 parallel flow two-piston pump (top) and high-pressure two-eluant gradient controller (bottom).

A limitation of the 2010 is that no underpressure cutout is provided so eluant potentially can be lost in case of a leak.

8. Varian 2020 High-Pressure Two-Eluant Gradient Controller

At about $4000, the 2020 is able to operate two of the 2010 parallel-flow two-piston pumps described above for high-pressure gradient generation (Figure 33, bottom).[55] Features include:

1. Internal storage of ten programs of up to 20 steps per program is possible.
2. Program times are from 1 to 999.9 min in 0.1-min steps.
3. Both composition (in 1% increments) and flow can be simultaneously programmed.
4. Three external events contact closures can be time programmed.
5. An input permits external equipment to stop a program or the internal alarm signal to signal outside devices.
6. An audible alarm can be time programmed.
7. Battery backup permits moving the programmer without memory loss.
8. A "loop counter" permits repetition of program files up to 99 times.
9. "Override" keys permit 100% A or 100% B at any time.

Because of the low flows possible with the 2010 pumps (to 0.01 ml/min), this gradient controller has the potential of generating simultaneous composition and flow gradients compatible with narrow-bore columns.

9. Waters 510 Parallel-Flow Two-Piston Pump

The Model 510 (at about $6000) has direct flow settings from 0.1 to 9.9 ml/min and is available in a wider flow-range version (0.1 to 22.5 ml/min). (The 510 looks like the 6000 pump, but the solvent selection manifold between the pump heads is absent.) The standard version can be driven to 0.01 ml/min with the Model 610 gradient controller (described below). Both versions incorporate many of the features of the 6000, including pressures to 6000, built-in pressure monitoring, overpressure shutdown, flow delivery accuracy to 1.0% (set flow vs. actual flow), and flow reproducibility to 0.1% (flow variations vs. time). To reduce costs, the solvent inlet and outlet manifolds were eliminated.

Improvements in the 510 vs. the earlier M6000 include a new check valve and seal design that permit readily using low-boiling eluants such as methylene chloride (used in normal phase LC), and automatic compressibility compensation (instead of the manual compressibility setting in the earlier models).

10. Waters 590 Parallel-Flow Two-Piston Pump

The Model 590 from Waters is their most recent and sophisticated pump for common LC (Figure 34). Although

FIGURE 34. Waters-Millipore 590 parallel flow two-piston pump, programmable for events and flow. (With permission.)

more costly than most simple pumps ($7400 vs. $4000 to $6000), it has a wide range of capabilities.[56]

The more sophisticated programmable pump, the Model 590, has features of the Model 510, plus a number of other clever capabilities, chief of which are

1. A choice of models gives different flows and pressures:

Model	Piston chamber (μl)	Flow range (ml/min)	Pressure (psi)
590	100	0.001—10 (1 μl/min!)	6000
590 EF	225	0.003—45	2000

2. Constant pressure operation is useful for column packing, equilibrating a new system, or microbore LC.
3. Internal memory storage of nine methods with 40 flow changes is possible (but not gradient programming).
4. An internal clock permits automated shutoff and startup, permitting late-day runs and early-morning conditioning when the operator is absent).
5. A "help" key guides the user in programming (potentially eliminating instruction manuals).
6. Battery memory backup preserves memory when moving the pump or during power failure.
7. Pressure upper limits protect the pump and columns, and pressure lower limits conserve eluants should a leak develop.
8. Eight external event switches can be set to "open", "close", or "pulse" in order to choose solvents, change columns, make injections, etc.
9. Four control switches permit external devices (like an autosampler) to start a flow program after an autosampler injection.
10. A manual solvent-control manifold permits selecting any of three eluents into the pump, and a reference manifold permits, for example, sending the pumped solvent to wash detector cells.

Limitations of the 590 and 510 are similar, and these include the pressure limit of 2000 pounds for the "extended flow" model (vs. 6000 psi for the regular model) and the inability to readily interconvert between models (as is possible with models that permit exchange in heads). The 590 cannot drive another pump, so only flow programming and events control are possible. An external gradient controller is still necessary for either high- or low-pressure gradient generation.

11. Waters Delta Prep 3000 Preparative Parallel-Flow Two-Piston Pump

The Delta Prep is a pumping system with the flow range as wide as the 590 pump, but with a yet higher flow for preparative chromatography, from 1.0 to 180 ml/min (Figure 35).[57] This pump uses the same basic mechanical pump, but a piston volume of 400 μl/stroke (vs. 100 and 225 μl for the 590 and 590EP pumps, respectively). This makes the Delta Prep pump capable of giving a similar linear velocity for columns from 3.9 to 57 mm in diameter, i.e., from analytical to preparative diameters. As part of the Delta Preparative LC, the pumping system has built-in solvent selection valves that can choose up to four different eluants, for example, so step gradients can be changed during a run, or the column can be washed with strong eluant at the end of a run. One of these automated pump inlets can be used for "loading through the pump" large samples for preparative work.

Common inlet lines to the pump and outlet lines from the pump are used up to a manual "column switching valve", where different internal diameter lines are selected, either 0.04-in. id for preparative or 0.1-in. id for analytical work. The analytical side has a loop injector for partial-loop filling for injection up to 5 ml.

12. Anspec SM-90 Parallel-Flow Three-Piston Pump with Low-Pressure Four-Eluant Gradient Controller

The Anspec SM-90 pump with low-pressure four-solvent gradient programmer (introduced in 1986) is a relatively compact (42 lb, 7 in. H × 15 in. W × 21 in. D) and less expensive (about $9000) low-pressure four-solvent gradient generation system (Figure 36).[58] This system is a redesigned version of the pump discontinued several years ago by DuPont. Although the flow range is not exceptional (0.1 to 10 ml/min), other features of this pumping system are noteworthy:

1. The price is good (about $9000) considering that the pumping system provides four-solvent, gradient programming.

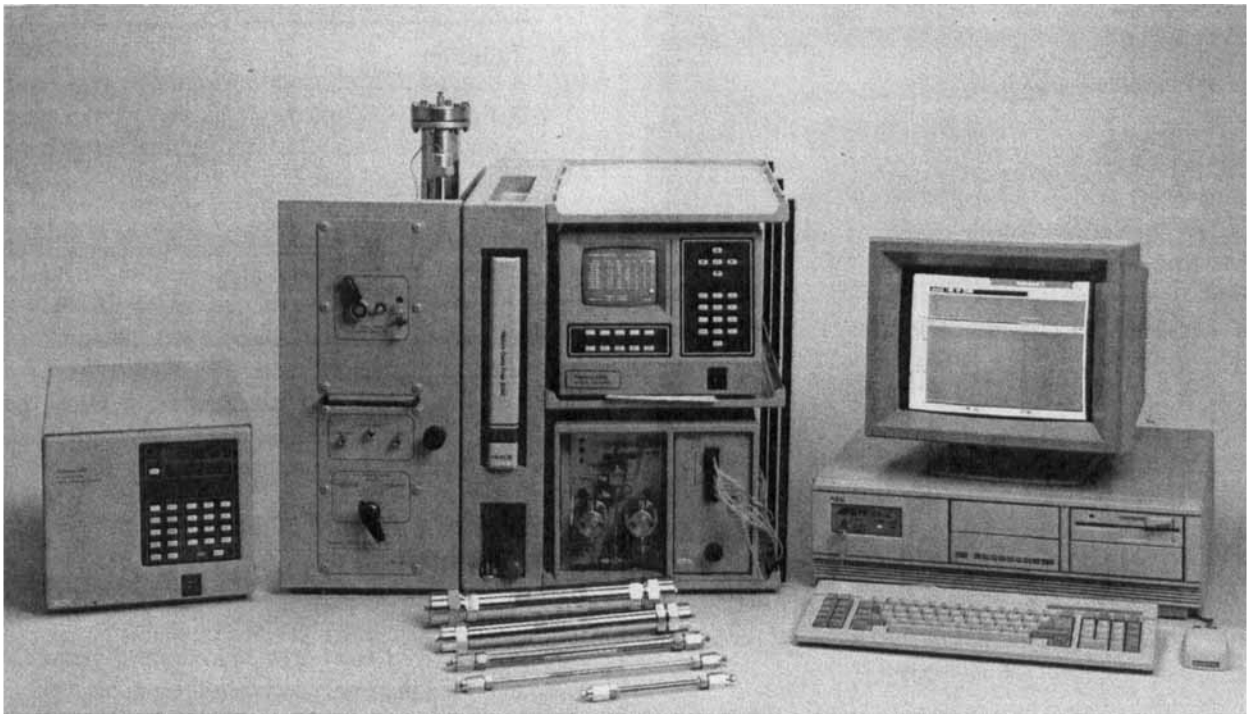

FIGURE 35. Waters-Millipore preparative parallel flow two-piston pump with remote four-eluant selection (center, bottom) and controller (above the pump) and other components for the Delta Prep 3000 preparative LC. (With permission.)

2. Pressures to 7000 psi are higher than for many pumps (usually 6000 psi).
3. Four external events can be controlled during a run.

Providing a 3-year warranty on parts and labor, this was the first LC instrument to offer a warranty longer than the 3- to 12-month warranties usually offered. Recently introduced instruments have since followed with 5- and even 6-year warranties. Service contracts often cost as much as 10% of the instrument costs per year, so this pumping system can be expected to show high reliability.

The SM-90 uses a single line of light-emitting diodes to display multilinear steps, numbered 1, 2, etc., with the time and percent A, B, C, and D for each step. A dynamic magnetic mixing chamber prevents synchronous operation of the mixing valve and the pump period. The three pump heads (63 μl each) move in sinusoidal motion, but 120° out of phase. A "solvent organizer" chamber with a helium degassing manifold and storage tray for four 1-l capped solvent bottles is available for $1000.

13. LDC/Milton Roy ConstaMetric I, II, and III Parallel-Flow Two-Piston Pumps

LDC/Milton Roy introduced the ConstaMetric series of pumps in 1975. They were one of the first pumps to use the parallel-flow two-piston design. The cam that drives the pistons was computer designed, allowing for constant velocity of the pistons. The discharge cycles of each piston overlap so that low pulsations are achieved, even without the use of pulse dampener or extra tubing to increase compliance.

These first pumps were called ConstaMetric II and ConstaMetric IIG. They had built-in flow-through pressure transducers, pressure indicators, high- and low-pressure limits (to shut down pumps if blockage or leaks occur), light-emitting diode (LED) indicators for warning of over- or underpressure, a reset button to restart the system, and an input for external flow control.

The ConstaMetric I was introduced in 1976 as the second pump for a gradient system. It did not have the overpressure safety features mentioned above.

The ConstaMetric III pumps (about $4000) (Figure 37)[59] were introduced as replacements for the ConstaMetric II and IIG. They were repackaged in a single, compact case and have the features listed above plus:

1. Flows from 0.1 to 9.99 ml/min from a $^1/_8$-in. piston can be continuously set from a front-panel turn-counting dial calibrated to read flow, with pressures to 6000 psi.
2. Two-solvent gradients can be generated under control of the "Gradient Master" or microprocessor controllers described below, using high-pressure mixing.
3. Microbore flows from 0.03 to 3.3 ml/min are possible if either model is purchased initially with the $^1/_3$ speed motor (about $300 more).

FIGURE 36. Anspec SM-90 parallel flow three-piston pump with low-pressure four-eluant gradient controller. (With permission.)

4. Preparative flows from 0.3 to 22.5 ml/min to 3000 psi can be obtained by the user substituting a $^{3}/_{16}$-in. piston liquid end (about $550).
5. Preparative flows from 0.4 to 40 ml/min to 1500 psi can be obtained by the user substituting a 1/4-in. piston liquid end (about $550).

14. LDC/Milton Roy ConstaMetric 3000 Parallel-Flow Two-Piston Pump

Introduced in about 1984, the LDC/Milton Roy ConstaMetric 3000 pump was changed from the horizontal configuration found with the ConstaMetric I and III pumps (11 in. wide × 21 in. deep × 6 in. high) to a vertical configuration more easily set one next to the other (6 in. wide × 18 in. deep × 13 in. high) (Figure 38). The price (about $4500) and specific performance specifications of precision, accuracy, etc. are the same as the ConstaMetric I and III pumps. Improvements of the 3000 over its earlier models include:[60]

1. Flows are set by thumbwheels to hundreths of a milliliter from 0.01 to 9.99 ml/min.
2. Pressure is indicated by LEDs.
3. A new piston guide reduces seal wear and extends seal life.

15. LDC/Milton Roy CM4000 Low-Pressure Three-Eluant Gradient Controller

The LDC/Milton Roy CM4000 pump and three-solvent gradient programmer combination provides a good example of how pumps are growing simultaneously more capable, less expensive (about $8500 for a pump and three-solvent controller), and more compact (Figure 39) (45 lb, 13 in. high × 6 in. wide × 19 in. deep).[61] The CM4000 has in one compact unit the following functions:

1. Flows from 0.010 to 10 ml/min in 0.010 increments can be set to pressures of 6000 psi.
2. Isocratic eluant mixtures of three-solvents can be set from the built-in controller and low-pressure mixing.
3. Gradients of three-solvents by low-pressure can be set from the controller using up to seven curve shapes or multilinear program steps.
4. A slave pump for four-solvent gradients is possible by combining low-pressure mixing (for three eluants) and high-pressure mixing (for the fourth eluant).
5. The pump can communicate to other instruments by either a RS232C or IEEE488 port.
6. Reversed phase isocratic separations can be optimized by connecting the pump through the IEEE488 port to an IBM PC using their "TAMED®" simplex optimization program for developing the best two-solvent mixture for isocratic separations.
7. Four external events can be controlled during a run.
8. The pump can be stopped or started remotely.
9. Analog voltage outputs permit plotting measured pressure and/or expected composition of the eluant.
10. Valves automatically close when the pump is shut down to prevent accidental siphoning of eluant through the pump.
11. High- and low-pressure limits and prime flow rates can be set from the face of the pump.
12. The pump can be set in the constant pressure mode.
13. A "help" button guides the user through programming (as discussed above for the Waters 590).

A limitation of the CM4000 is that on the pump the display is only a single line of characters to indicate run conditions and the gradient program. It is necessary to "scan" manually through the steps to determine conditions. However, when using the optimization program with the IBM PC, the screen displays all conditions.

For a non-PC-based instrument, the CM4000 has a large internal memory. With 27 segments per file, the CM4000 can store in memory 15 "control files" (each file setting initial flows, overpressure limits, and flow vs. time programs), 15

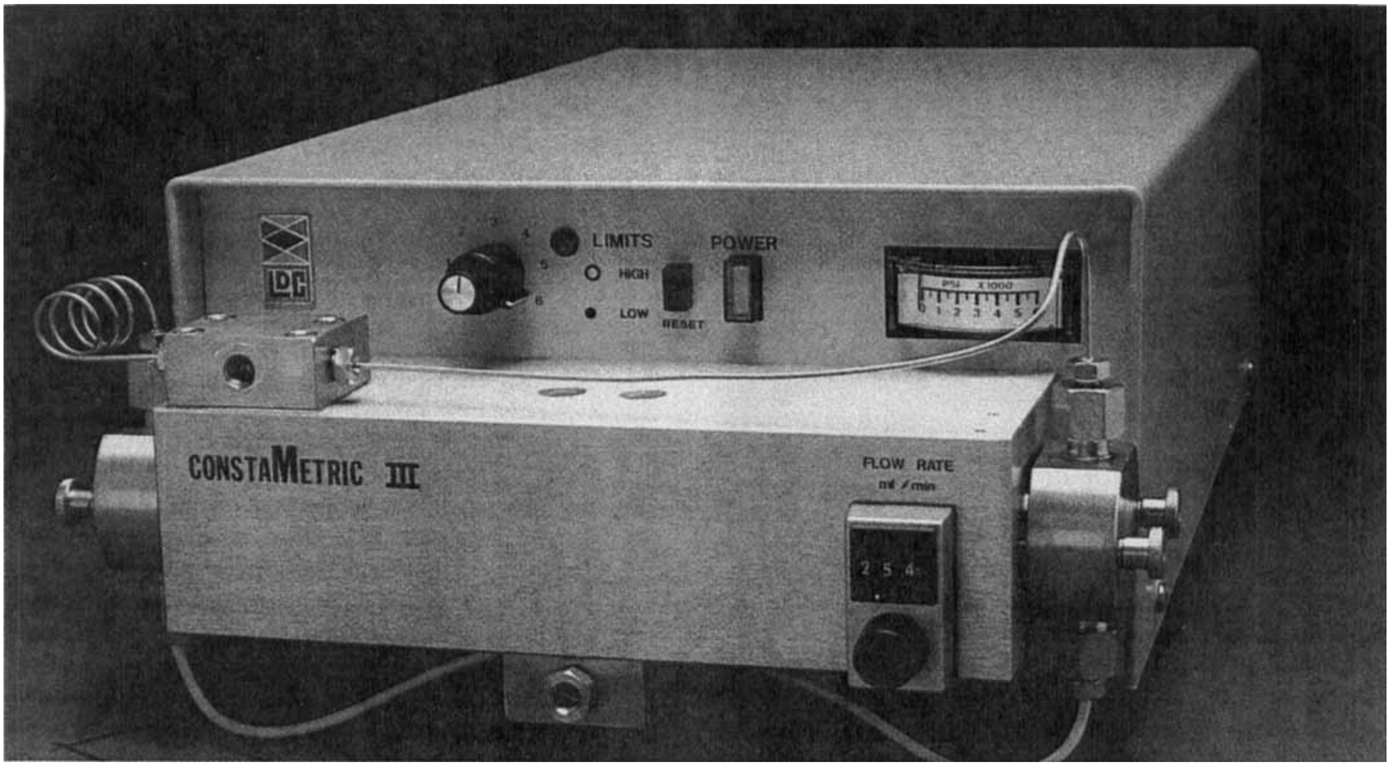

FIGURE 37. LDC/Milton Roy ConstaMetric III parallel flow two-piston pump. (With permission.)

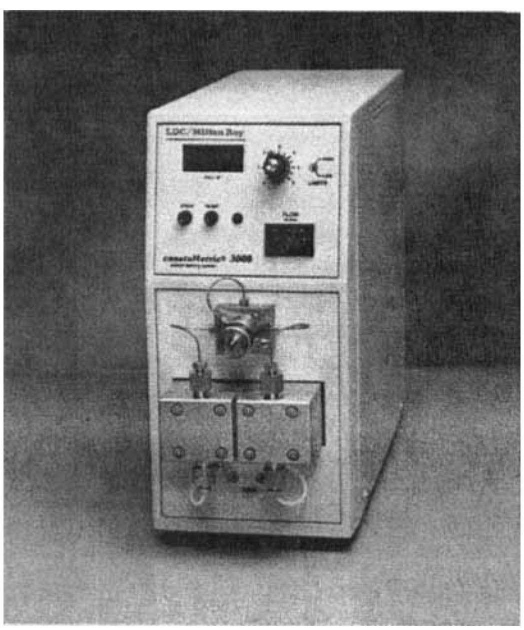

FIGURE 38. LDC/Milton Roy ConstaMetric 3000 "vertical design" parallel flow two-piston pump. (With permission.)

"gradient files" (for setting percent A, B, and C compositions vs. time), and 15 "time files" (for setting a time vs. the four external events contact setting of: "open", "close", or "24 V"). From the front panel, composition can be easily stopped during a gradient, and the "% key" can be used to adjust manually the solvent composition.

The small holdup volume of the pump (1.5 ml) makes response to new inputs of solvents rapid and gives the ability to respond quickly to small changes in composition (since low-pressure mixing is used). Composition can be adjusted to 0.1% setting.

This pump also illustrates another trend in more recent pumps: discussion of reproducibility and accuracy is growing less vague as more sophisticated approaches for measuring reliability are published. For example, this is one of the first pumps to discuss explicitly "compositional accuracy" (closeness to the true value); the company literature says this is within 1.0%. Flow rate precision (variability) is ±0.3% or 3 μl/min, whichever is smaller. Flow is generated by a frequency-controlled stepper motor, driving the pistons at 65 steps per microliter. The cam-following profile driving the heads is designed to apply constant suction on the solvent-proportioning valves, and one valve is open at all times to prevent cavitation and to ensure that a proper and reproducible mixture results, independent of the piston position. The conventional measure of gradient reproducibility is the average retention time reproducibility. For the CM4000, this is approximately 0.4 to 0.5% RSD for ten replicate runs of a six-peak standard mix (unlabeled)

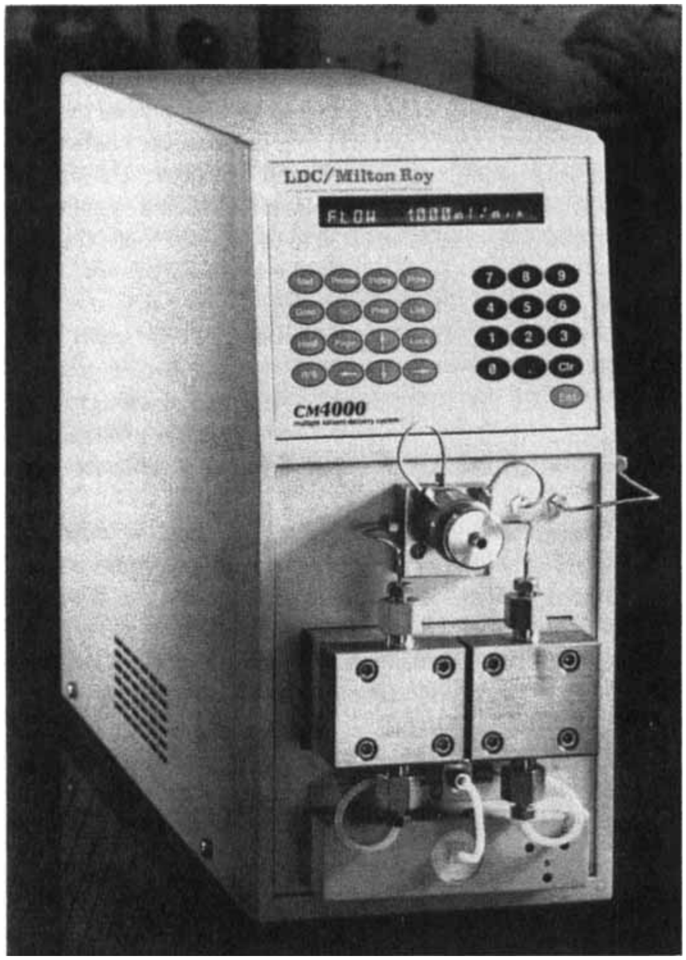

FIGURE 39. LDC/Milton Roy CM4000 parallel flow two-piston pump with built-in low-pressure three-eluant gradient controller.

separated on a reversed-phase column with a 10-min linear gradient from 0 to 100% eluant.

16. Waters 600 Parallel-Flow Two-Piston Pump with Low-Pressure Three-Eluant Gradient Controller

The Waters 600 pump and four-solvent low-pressure gradient programmer introduced in 1986 is Waters' first offering different from its usual high-pressure gradient generation system (Figure 40).[62] While low-pressure gradient formers are usually less expensive than high-pressure gradient generation (using multiple pumps), the Waters 600 at $13,500 does cost more than its least expensive two-solvent high-pressure system at about $12,000 (e.g., two 501 pumps at $4200 and a 680 controller at $4700). However, the low-pressure system has many more capabilities compared with the high-pressure gradient system, such as four-solvent gradient capability, and more reproducible gradient formation, especially at the gradient extremes or at very low flows.

Consisting of a separate controller with video display and a pump/column compartment unit, the two components can be

FIGURE 40. Waters-Millipore 600 parallel flow two-piston pump (bottom) with low-pressure three-eluant gradient controller (top). (With permission.)

stacked to take up only 12 in. of bench top. The pump section is a two-head, two-piston system similar to the fluid section of a Waters 510 pump (without the 510 control section). The Waters 600 has the following functions:

1. Possible flows are from 10 μl/min to 10 ml/min to 6000 psi and to 45 ml/min to 1000 psi in 0.010-ml/min increments.
2. Four-solvent gradients can be generated, using up to 11 curve shapes or multilinear program steps.
3. Four external events can be controlled during a run.
4. Methods can be linked, for example, to run different gradients for different samples or for flushing columns at the end of a set of automated runs.
5. The helium sparge can be time programmed (e.g., to turn off at night).
6. An optional column heater can have temperature programmed from the controller.

The Waters 600 uses a video screen to display tables of time vs. flow vs. percent A, B, C, D, or a gradient curve (five convex, one linear, five concave). The curves can be moved around on the screen to change or enter parameters, and five "soft" keys (whose function changes with the screen) permit selecting new tables, clearing lines, clearing the entire table, or saving a file. "Hard keys" prompt programming gradients, isocratic compositions, or events.

With 80 segments per file, the 600 can store in memory eight "gradient files" (for setting flow, percent A, B, C, and D vs. time) and eight "timed-events files" (for setting activation of the four external event contacts at specified times). A "manual override button" on the front panel permits setting isocratic conditions, even during a gradient run.

Flow is generated by a frequency-controlled stepper motor, as is true of all Waters pumps (vs. voltage control for pumps from most other companies). Mixing is achieved with a passive mixer. Composition can be adjusted at 1% increments, and compositional accuracy (closeness to the true value) is 0.5%. Compositional precision (variations from moment to moment in flow) is ±0.015% and flow precision (variations from moment to moment in composition) is ±0.1% for flows from 0.01 to 10 ml/min. The high precision of composition and flow is achieved by the Waters patented "RPS"® (Random Phase Synchronized) software.

Performance of the 600 is exemplified by the average retention time reproducibility of approximately 0.015% for ten replicate runs of 20 amino acids separated with a 12-min nonlinear gradient from 0 to approximately 30% B eluant.[63]

Performance of the 600 is also illustrated in company literature by a figure showing a step gradient (in 10% steps) from 0 to 100%, but at 150, 1000, 2000, and 4000 psi (determined by increasing flows from 1 to 5 ml/min).[64] Since a UV absorber was added to one eluant, step changes paralleled the gradient changes. The plots of a UV absorber in one component vs. volume delivered showed overlapping profiles, even over these wide flow and pressure ranges.

17. Waters 680 High-Pressure Two-Eluant Gradient Controller

Introduced in 1982 (now about $4700), the 680 gradient controller uses a small video screen to set up a tabular display of run conditions (see Figure 41). No graph of the gradient or its progress is given, only an elapsed run time. The 680 is capable of controlling any Waters pump for generating gradients or mixed isocratic eluants with the high-pressure method using two pumps. A two-eluant gradient system might cost about $18,000 with two 591 pumps (about $7000 each) down to about $11,000 with two 501 pumps (about $3000 each). The high-pressure gradient system gives less reproducible gradient formation than the low-pressure technique at the gradient extreme or at very low flows (i.e., when pumps must deliver very low flows). The 680 has the following capabilities:

1. As many as three pumps can be controlled for ternary or binary gradients or isocratic mixes of any composition.
2. Two pumps can be run as a gradient, and the third in a constant mode to pump, for example, a postcolumn reagent for derivatization, or a single pump for isocratic flow for a second column (although the pump can be operated on its own).
3. Reproducibility with a conventional system and the 510 pumps is excellent, for example, in a 0 to 10% gradient, area reproducibility is 0.3 to 0.5% RSD (retention time

FIGURE 41. Waters-Millipore 680 high-pressure two-eluant gradient controller. (With permission.)

reproducibility is 0.08 to 0.35% RSD) for nucleic acid bases on a conventional 30 × 0.46-cm column at 1 ml/min.

4. With a microbore column of 1-mm id (25 cm long) and a 45 to 80% gradient, precisions are from 1.6 to 2.6% RSD for areas and 0.03 to 1.3% RSD for retention times.[65]
5. Possible flows are from 10 μl/min to 10 ml/min to 6000 psi and to 45 ml/ min to 1000 psi in 10-μl increments.
6. Gradients can be generated using up to 11 curve shapes or multilinear program steps.
7. Four external events can be controlled during a run.
8. Methods can be linked, for example, to run different gradients for different samples or for flushing columns at the end of a set of automated runs.
9. The helium sparge can be time programmed (e.g., to turn off at night).
10. An optional column heater can have the temperature programmed.

H. Specific Membrane Pumps and Gradient Controllers

1. Orlita High-Speed Membrane Pump

One of the earliest approaches to eliminating the pulsation problems was used around 1970 with the German-made Orlita membrane pumps, used industrially to meter precisely chemicals into flowing streams, with very little possibility of leakage of the chemicals.[66] This design became that adopted by the very commercially important pump used in the Hewlett-Packard 1080 liquid chromatograph and adopted to the 1090 liquid chromatograph.

Membrane pumps minimize pulsation noise in two ways: the multiple heads provide overlapping strokes, and the stroking frequency is very high. For example, a three-headed pump might have the output strokes 120° out of phase to give the overlapping strokes shown in Figure 23. Displacement volumes per stroke are often very low (1 to 2 μl per stroke gives 1 to 2 ml/min) and the frequency of operation very high (500 cycles/min). Thus, modest compliance in the system would often give a very steady baseline.

The principle used for the high-speed pumping is illustrated in Figure 42. As the steel (not sapphire) piston moves forward (Figure 42 a and b), it traps a small portion of oil between the piston and the piston chamber, moving that oil forward to act on one side of a thin, stainless-steel membrane, then forces solvent out (Figure 42c).

Besides the high-frequency effect to eliminate pumping noise, the other major advantage is that seals around a piston are eliminated. This was especially important in the early days of LC (1965 to 1975) before the Bal seal was available, and compression packings were used to maintain piston seals.

A limitation of the Orlita pump is that there is no simple (electronic) control system for gradients, i.e., flow rate cannot be ramped down or up from two different pumps. Flow is approximately proportional to the depth the piston is allowed to move into the head. This depth is controlled by a difficult-to-use hand screw on the back of the pump. Also, more oil "slippage" between the steel piston and piston walls will take place at higher back pressure. Thus, there is expected to be some deviation in a plot of flow vs. back pressure (less flow at higher back pressures).

2. Hewlett-Packard 1080 One-Piston Membrane Pump and High-Pressure Gradient Controller

The Hewlett-Packard 1080 liquid chromatograph was a revolutionary integrated complete LC that startled the industry when it was introduced in 1977 (Figure 6). It was several years before any company could compete with the total performance of the 1080, although the price of the 1080 was very high ($40,000 to $60,000), and there were many problems with the early 1080s.

The pump and gradient controller were available only as part of a full LC system. The 1080 used modifications of the Orlita membrane pump described above, but replaced the hand screw used for setting the flow with a servomotor to move the head back and forth over the piston (along with other design changes).

The flow was actually measured and adjusted in a feedback loop that involved a complex system. A single-head pump at 500 cps was used for each of the two solvents. The output of each pump head was passed over a special pulsation-dampener/pressure-transducer, then through a resistor (several meters of 0.01-in. id tubing), and then to a dynamic mixing chamber and another pressure transducer. The pulsation-dampener/pressure-transducer was thermostated to 40°C to eliminate ambient temperature effects on the transducer (a technique now used frequently with high sensitivity refractive index and diode array detectors). This device used the flexible membrane technology incorporated in the pump head itself. Eluant flowed over a small, stainless-steel membrane (approximately 3 cm in diameter) in a chamber with a small holdup volume (1 to 2

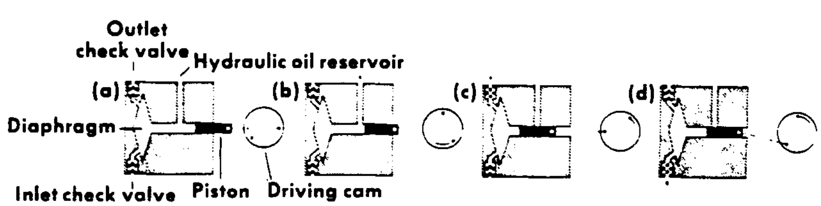

FIGURE 42. Operation of the Orlita and Hewlett-Packard 1080 high-speed membrane (diaphragm) pump (details in text). (With permission from Hewlett-Packard.)

ml). On the other side of the membrane was a "liquid ballast" of approximately 30 ml of fluid of high compressibility (isopropanol) that partially dampened pulsations. Flow control used a true feedback system in which the internal microprocessor calculated flow of each pump from the rate of pressure fall after each pump stroke.

Commands permitted either numerical printouts or timeplots of:

1. Measured flow from either pump head
2. Measured combined flow from both pumps
3. Measured pressure from either pump head
4. Measured combined pressure from the combined flow

The plots were useful for diagnosing problems in this, one of the most sophisticated pumping systems.[67]

3. Hewlett-Packard 1090 One-Piston Membrane Pump and Low-Pressure and Medium-Pressure Three-Eluant Gradient Controller

The Hewlett-Packard 1090 pumping system has many features different from other systems (Figure 13).[68,69] As part of a "family of integrated LC modules" it is possible to upgrade the system readily from isocratic single-solvent pumping (in two different ways) to two-solvent or three-solvent pumping. The 1090 uses a unique pump itself and a unique system for generating gradients not currently available in other instruments.

The PV5 is a *low-pressure* three-solvent gradient-generating system (about $9000) that precedes a single-pump module (about $7000). The PV5 uses a timed valve to select between three solvents that feed the pump.

The DR5 *medium pressure* gradient system is a second and more precise approach available in the 1090 for generating gradients and fits neither in the category of low-pressure nor high-pressure gradient generation (described in the introduction of the pump section). Rather, this more expensive gradient generation approach uses three low-pressure metering pumps (about $15,000 total) to feed the high-pressure membrane pump (about $7000). The DR5 medium-pressure gradient generation approach needs some more explanation since it is unique. Three medium-pressure (approximately 30 to 60 psi) metering pumps (one is shown in Figure 43, left), each with two 100-μl piston chambers, are under servo-drive control so that 7 nl is delivered with each step. As the volume delivered from one piston is nearly emptied, the second piston is connected by a rotary switching valve, thus avoiding problems from ball valves. Three of these dual-piston metering pumps feed into a "low-pressure compliance" chamber that can hold and mix the delivery from the three metering pumps. The pressure of the solvent in the low-pressure compliance chamber (at about 90 psi) is monitored for diagnostic purposes. This chamber has a variable volume depending on the flow chosen. This chamber uses similar flexible metal membrane approach as used in the high-pressure

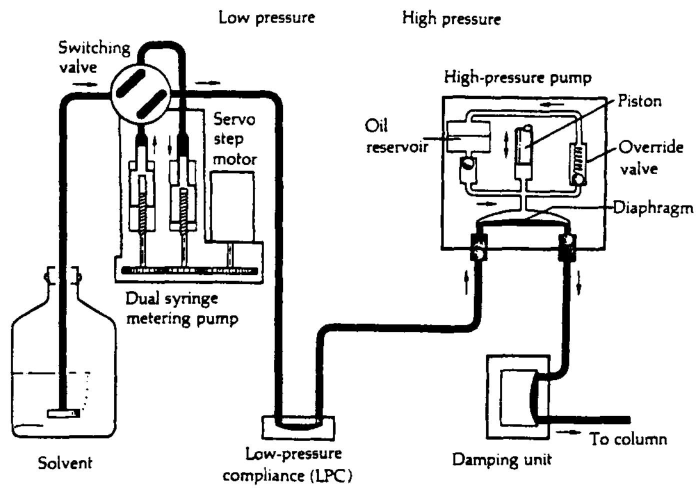

FIGURE 43. Operation of Hewlett-Packard 1090 "medium pressure" gradient generation method (details in text). (With permission.)

membrane pump and pulsation dampener. On the intake stroke of the high-pressure pump (Figure 43, right), the low-pressure compliance chamber is emptied. As the high-pressure pump is emptying onto the column, the three sets of dual-piston metering pumps feed the proper mixture of the three eluants into the compliance chamber, depending on the composition chosen, and they feed a volume necessary for the flow rate chosen. Figure 43 shows one set of dual metering pumps for one eluant feeding the low-pressure compliance chamber.

The high-pressure membrane pump operates on a unique principle, as follows (Figure 43). The membrane pump maintains flow precision, independent of flow rate or column back pressure, by constantly delivering all of the metered flow from the low-pressure compliance chamber. Six hundred times per minute a closed-loop oil pump pressurizes a flexible, stainless-steel diaphragm to 440 bar against a flat surface: the pump head, which holds the solvent inlet and outlet check valves. On the backward stroke, oil is drawn from a reservoir into the piston chamber, reducing the pressure on the diaphragm to less than the eluant pressure on the inlet check valve. The eluant in the low-pressure compliance chamber then flows into the high-pressure pump, raising the diaphragm. On the forward stroke, the oil must overcome a 440-bar (6600 psi) "override-valve" (Figure 43, upper right) before returning to the reservoir. The diaphragm is forced flat, pumping all of the eluant beneath it onto the column. Eluant delivery is thus independent of column back pressure, and no moving seals are in contact with the eluant at high pressure. The damping unit reduces the pressure ripples from the high-pressure pump, and it also contains a pressure transducer to monitor column back pressure.

Unique features of the 1090 include:

1. A choice of low- or medium-pressure gradient generation methods are possible at different prices, giving different possibilities for narrow-bore columns.

Gradient generation method	PV5 low-pressure	DR5 medium-pressure
Minimum gradient flow (μl/min)	500	100
Minimum column size (mm id)	2.1	1.0
Reproducibility retention time (% RSD)	<0.15	0.3—0.8
Reproducibility area (% sRSD)	0.2—0.8	0.7—1.1

2. The medium-pressure gradient generator gives gradients to a flow range lower and wider than most other systems (from 20 to 5000 μl/ml, 0.020 to 5 ml/min), and, thus, is compatible with microbore columns in the 1-mm diameter range.
3. The medium pressure gradient generator can be upgraded in the field from 1 to 2 to 3 eluants.

A limitation of the 1090 (like the 1080) is that the pump is part of an integrated LC unit, and it cannot be used alone outside of its large controller/solvent/detector housing. Also, while the capabilities are high, the price is higher than other instruments.

I. Specific Rapid-Refill One-Piston Pumps and Gradient Controllers

1. Beckman-Altex 110A and 110B Rapid Refill One-Piston Pumps with Electronic Flow Feedback Control

Altex (a Rainin Company at the time, acquired by Beckman in 1978) introduced in 1977 the first of what was to become an innovative and common pump in LC, the single-piston, rapid-refill pump (Figure 44).[70] Until this time, pumps had used two heads (Waters and Altex) or three heads (DuPont/Jasco) so that one head was supplying flow while the other was refilling. The rapid-refill, single-piston pump reduced mechanical complexity, reduced costs, and (possibly) reduced

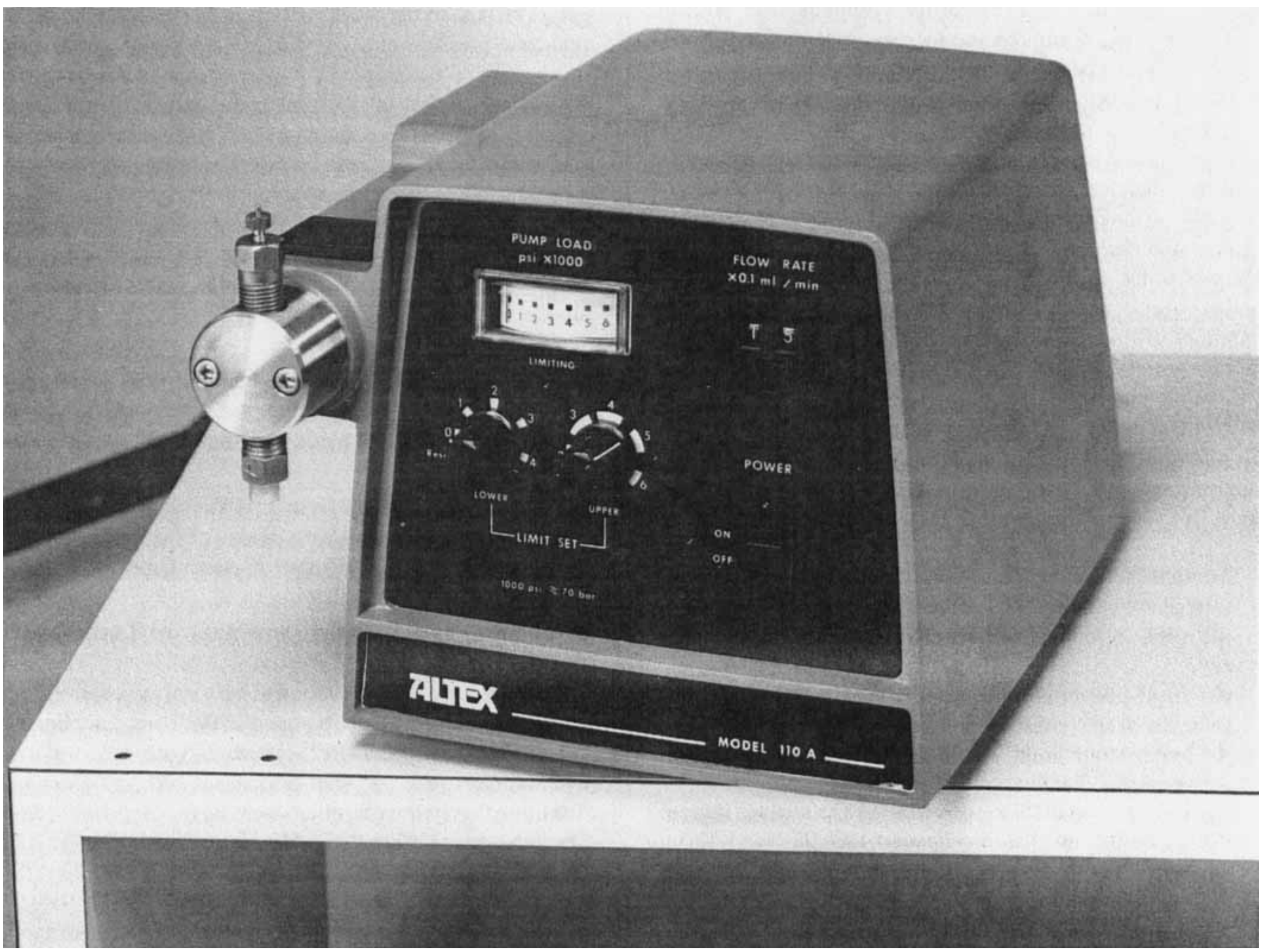

FIGURE 44. The first rapid-refill one-piston pump the 110A from Beckman-Altex (with electronic flow-feedback control). (With permission.)

potential areas for malfunctions. These factors became a strong impetus to the success of this type of pump. The cost of this pump was about $2000 when introduced in 1977 (now about $5000), considerably below the cost for two-head pumps (e.g., two-head pumps were about $6000).

This single-piston pumping approach uses some new technologies. The piston motion has three segments: (1) "normal pumping", in which the piston moves forward at a constant speed proportional to the set flow rate; (2) "rapid refill", in which the motor speeds up in order to retract the piston in less than 200 ms to refill the 140-μl pumping chamber; and (3) "pump-up" period, in which the motor continues at rapid speed but now moves the piston forward so the piston chamber is returned to the "memorized pressure level" in order to minimize the pressure dip between strokes. The memorized pressure level is taken during the normal "pumping stroke" portion of the stroke and is averaged electronically with previous values to calculate the piston displacement necessary to adjust for the system compliance. This piston displacement can be changed using the compressibility compensation control potentiometer, depending on the solvent, system compliance, seals, etc. (Note that with a 140-μl pumping chamber, and a flow of 140 μl/min, a cycle consists of about 60 s of pumping and then 0.2 s for refilling.)

The pressure level in the piston chamber is determined in an unusual manner. An electrical measurement of the torque load on the piston drive motor is proportional to piston chamber pressure, and this is generally equal to the pressure on the outlet side of the check valves unless the frit filter in the outlet chamber check valve is plugged. The flow and pressure capabilities of this pump were close to the usual range (0.1 to 8 ml/min to 6000 psi or 0.1 to 9.9 to 5000 psi). As with other pumps at the time, the 110A pump could be operated alone or through the external 420 system controller (described below).

Besides the reduced costs, other innovations were introduced to high-pressure LC with this first rapid-refill single-piston pump:

1. Compressibility compensation is automatic.
2. Low piston volume (140 μl) and total pump volume (500 μl) made solvent changeover and recycle chromatography easy.
3. A "high-pressure limit" that restarts automatically achieves nearly constant pressure operation.
4. A "low-pressure limit" would shut down the pump should a leak occur, thereby conserving eluant.
5. An inlet and outlet filter just before the ball valves reduces the possibility of piston-generated particles (and eluant particles) from affecting ball-valve operation, thus improving reliability.
6. A preparative head (at $1100) is available for flows from 0.28 to 27.9 ml/min to 2000 psi.

Limitations of the 110A are that the pressure indicator by pump load had limited accuracy (±20%) and that reducing the pressure pulsations on the refill stroke required adding an external "pressure filter" of the Bourdon-tube type.

The 110B is the current version of this pump, selling at about $3100; it differs only a little from the 110A pump (Figure 45).[71] The 110B has a square housing so they can be stacked one on top of the other, unlike the 110A. The 110B is also available with a preparative chromatography head (to 30 ml/min) at about $3300.

2. Beckman 114M "micro-Flow" Rapid-Refill One-Piston Pump with Electronic Flow Feedback Control

Introduced in about 1986, this pump, at $6300, is compatible with microbore and regular chromatography columns (Figure 46).[72] A simple switch permits the flow range to be changed from 0.001 to 1 ml/min (1 μl/min!) to 0.01 to 10 ml/min.

The 114B uses a similar electronic feedback control system as does the 110 series, except the torque load on the piston drive motor is not used to measure pressure. Rather, a strain gauge affixed to the outer wall of the piston chamber measures real-time pressure changes during each piston stroke. This is used to make electronic flow corrections and compressibility corrections in that the forward piston stroke is rapid until the piston chamber is brought up to the pressure memorized at the end of the previous stroke, where the piston was operating in a constant flow mode. In addition, a "floating piston" design reduces piston and seal wear to the point that Beckman-Altex guarantees the high-pressure piston seal for 5 years! Other performance parameters of the 114M and 110B pumps are similar. Additional conveniences are

1. A preparative head is available for flows to 30 ml/min.
2. Liquid crystal displays permit three-digit set points of flow, pressure upper/lower limits, and current pressure.

A disadvantage of the system is the higher price ($6300).

More sophisticated current versions of Beckman-Altex pumps are described later under their "System Gold".

3. Beckman-Altex 420 High-Pressure Two-Eluant Gradient Controller

Introduced in about 1980, the 420 system controller could be used to control Altex pumps for flow programming, high-pressure gradient generation between two solvents, and external event control (e.g., to start an autosampler and integrator).[73] Traditional system controllers were large, immovable devices. One innovative design to the 420 was a lightweight and compact calculator-size keyboard (approximately 9 × 11 × 3 in.) (Figure 47). This is connected by a ribbon cable to the larger and heavier electronics cabinet that can be placed out of the way. While the heavier keyboards have now become standard, when lap-top computers take over control functions, the compact portable control unit may return.

FIGURE 45. Recent version of the rapid-refill, one-piston pump, the 110B, from Beckman-Altex (with electronic flow feedback control). (With permission.)

The 420 system controller permitted two connected pumps to perform many functions for the first time, including:

1. Lower flows of both pumps can be set (from 0.01 to 10 ml/min) in lower flow increments (0.01 ml/min) (vs. 0.1 ml/min minimum flow and increment change on the pump face).
2. Eluant composition can be set, either isocratic or gradient from 0 to 100% in 0.1% increments at 10 ml/min and 0.01% increments at 1 ml/min.
3. The intended gradient composition can be plotted on a chart recorder with a 0- to 1-V output.
4. The chart speed can be set from the controller from 0 to 10 cm/min in 0.01-cm/min increments for recorders with chart speeds proportional to a TTL compatible pulse train, where 166.6 Hz gives 10 cm/min.
5. Time can be measured from 0 to 999 min, for example, to indicate elapsed time, to measure time duration, or for controlling either events, flow, or gradients.
6. Four electrical time-programmable relay contact closures can be opened or closed for marking recorders, initiating autosamplers, starting integrators, etc.
7. Four pneumatic time-programmable relay contact closures can be opened or closed for operating air-activated injectors, valves, etc.
8. A switch-selectable "remote input" permits the controller either: (a) to jump ahead to a last program, for example, to wash the system when a "last-vial" signal is received; or (b) to continue a program in a "hold" configuration, for example, to let a gradient run be started by an autosampler.
9. A time-programmable alarm (of adjustable loudness) can be set to activate after 0.01 to 999 min.
10. For an overpressure condition (signaled from 100A pump), a controller can active an alarm or initiate a specific gradient program (e.g., a low-flow flush cycle).
11. Memory can hold 19 programs with up to a total of 149 instructions and upgraded memory chips of 80-instruction increments each are available.
12. A battery backup prevents memory loss with power failure or moving of the instrument.

FIGURE 46. Beckman-Altex 114 M "micro-Flow" rapid-refill, one-piston pump with electronic flow feedback control. (With permission.)

Features like the expandable memory and electronic boards (that could be substituted for easy repair) predicted many of the features now commonly found in microprocessor-controlled instruments.

4. Varian 5000 Rapid Refill, One-Piston Pump and Low-Pressure Three-Eluant Gradient Controller

The very innovative Varian 5000 pump, a one-piston rapid-refill pump (Model 5560) with built-in ternary gradient capability, was introduced in 1978 (selling now for around $30,000, without detector) (Figure 48).[74-78] The high-speed valves that meter in any of three solvents are directly attached to the piston head of the pump. At this point, a mechanical inlet valve is also used to eliminate many of the problems with ball valves that often malfunction if air is trapped in the balls, if eluants are not sufficiently degassed, or if very volatile eluants are used (like those used in normal-phase LC). The mechanically operated valve is unique to the Varian system. Following the Hewlett-Packard 1080, this instrument was one of the first in which the pump and gradient system, pulsation dampening, mixing system, and computer control were one integral unit. Because of the use of low-pressure gradient generation and a single pump, the price was less than systems using high-pressure mixing (about $25,000 to $30,000 for three-eluants with autosampler).

Unique features of the series 5000 include the following:

1. It has wide flow range from 0.1 to 15 ml/min with good gradient generation possible with the low-pressure gradient system.

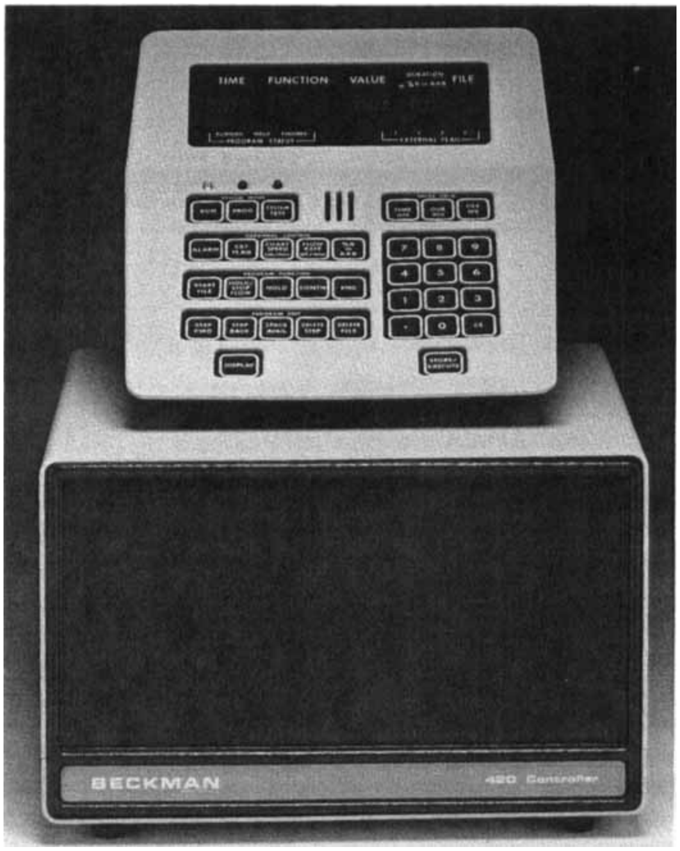

FIGURE 47. Beckman-Altex 421A high-pressure two-eluant gradient controller, a later version of the 420 that looked similar. (With permission.)

FIGURE 48. Varian 5000 rapid-refill, one-piston pump and low-pressure three-eluant gradient controller. (With permission.)

2. The video screen continuously displays all instrument conditions.
3. Microbore gradients compatible with columns down to 0.2-mm diameter have been shown possible in two ways:[76] (a) by splitting the flow from the pump and (b) by generating and containing the entire gradient in the hydraulic system of the pump.
4. Dedicated versions are available for amino acids and amine analysis using various kinds of separations, derivatization, and detection.
5. The system can be programmed from the Varian VISTA 54 microcomputer (at about $10,000), which can control up to four liquid or gas chromatographs, produce dual chromatogram plots (annotated with retention times, peak names, and drawn baselines), and perform many data calculations to quantify and identify peak.[77,78] This was one of the first small computer data systems introduced about 1979.

A limitation of the Varian 5000 pumping system is the pump is part of an integrated LC unit, and it cannot be used alone outside of its large controller/solvent bottle housing. Additionally, the pressure limit (5000 psi) is below other instruments (6000 psi). Since this is an earlier instrument, the memory capacity of nine methods is low by the standards of today.

5. Shimadzu LC-5A and LC-6A Rapid-Refill One-Piston Pumps and High-Pressure Three-Eluant Gradient Controller

The Shimadzu LC-6A single piston, rapid-refill pump (at about $4000) is a modification of the earlier LC-5A that had one of the widest flows available: push-button settings allowed selection of low flows (1 to 99 μl/min) and flows 100-fold higher (0.1 to 9.9 ml/min). Unfortunately, the LC-6A has a more conventional flow rate (0.1 to 9.9 ml/min) (Figure 49).[79] However, the LC-6A pump has some exceptional features:

1. Constant pressure operation at the push of a button is useful for column packing, equilibrating a new system, or microbore LC.
2. Pressure is higher than many pumps (to 7100 psi).
3. Upper and lower pressure limit switches preserve eluant (in case of a leak) and preserve the column (in case of a clog).
4. The pump can be run down to 0.01 ml/min using the pump controllers.
5. Three-pumps can be used for three-eluant gradients by high-pressure generation, using the SCL-6A controller (with appropriate interfaces) (Figure 50). This controller can operate also the column oven, two six-port high-pressure valves, two low-pressure six-solvent selection valves, an autoinjector, and the UV, electrochemcial, fluorescence, and refractive index detectors.

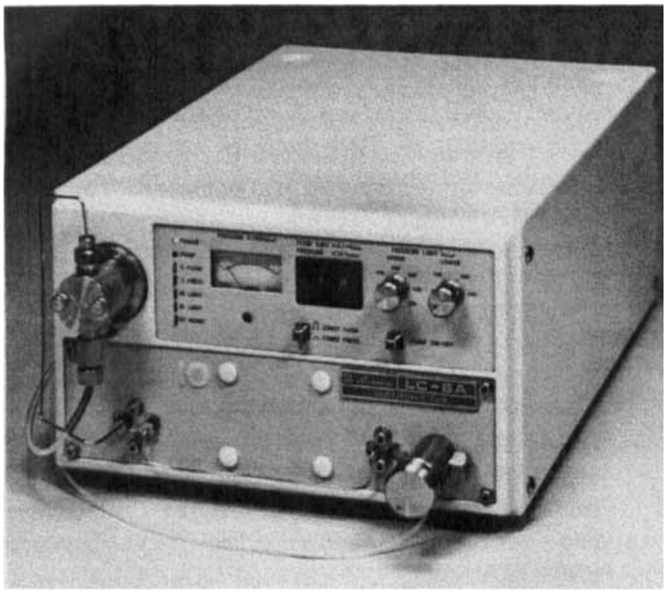

FIGURE 49. Shimadzu LC-6A rapid-refill, one-piston pump. (With permission.)

6. Perkin-Elmer 410 Rapid-Refill One-Piston Pump and Low-Pressure Four-Eluant Gradient Controller

Introduced in 1982, this pump and controller (at about $12,000) offers a new technology for generating gradients or isocartic mixtures from up to four eluants. Figure 51 shows control unit (right) and the pumping module[80-82] (left).

Perkin-Elmer has produced a complete LC system aimed at the biotechnology market with the "IsoPure LC System", by making this pumping system available in titanium (at about $14,000), the 410 Bio LC Pump. The biocompatible variable wavelength detector also is made of polymers and titanium, and (ion-exchange) columns are made of glass and Teflon.®

Figure 52 shows the unusual method for blending eluants used by this pump. The pump consists of two pistons that operate 180° out of phase. As the high-pressure piston delivers eluant (Figure 52, left) as a rapid-refill one-piston pump, the low-pressure piston (right) is drawing in the next increment of solvent blend. At the end of the high-pressure stroke (delivering 100 μl), the low-pressure pump delivers its entire volume in only 40 ms to the high-pressure head in preparation for the next stroke. A four-valve low-pressure system selects the proper mix of four eluants.

This pumping approach has three advantages. First, solvent blend for the next eluant increment is prepared in the low-pressure chamber over the same time interval as the delivery

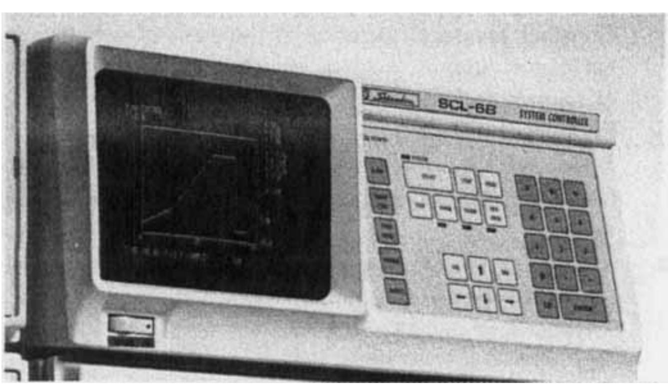

FIGURE 50. Shimadzu SCL-6A controller that can time control high-pressure three-eluant gradients, valves, detectors, data processing, etc. (With permission.)

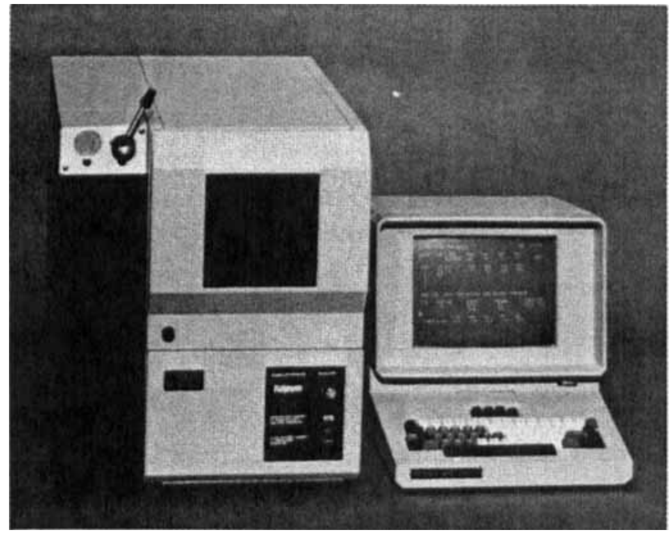

FIGURE 51. Perkin-Elmer Series 4 rapid-refill, one-piston pump and low-pressure four-eluant gradient controller. (With permission.)

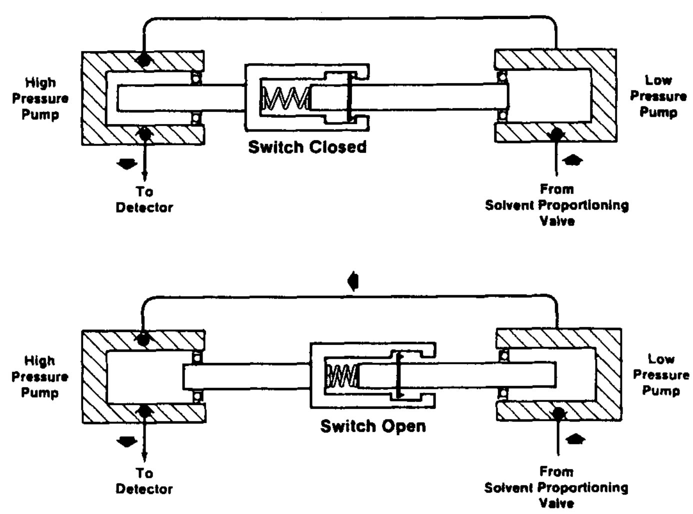

FIGURE 52. Operation of the Perkin-Elmer rapid-refill, one-piston high-pressure piston (left) and low-pressure piston (right) used to draw in and blend the four eluants. (With permission.)

stroke. The solvent-proportioning time for other pump systems may take as little as 0.2 s, but the 410 takes much longer (e.g., 6 s at 1 ml/min and 0.6 s at 10 ml/min). Since the actuation time of the solenoid valves used in low-pressure gradient generation limits precision and accuracy of the eluant blend, it is expected that the 410 give better performance. A second advantage is that mixing of the solvent components is expected to be better. A third advantage is the composition is expected to be independent of column backpressures (which can vary during a gradient), since the solvent is blended at a constant pressure and flow rate in the low-pressure side of the pump.

Since this is a one-piston (high-pressure) rapid-refill pump, a pressure pulse appears on the refill stroke, and a pulsation dampener is required. However, the resulting pressure dip was shown to be 1.4% of the back pressure (about 40 psi) with a 3000-psi back pressure.

In appearances, the pump head looks like a series-flow pump head (two adjacent piston chambers). While the series-flow pump head has a "make-up" piston without check valves *following* the main piston (with inlet/outlet check valves), this pump shows a low-pressure pump (with a single inlet check valve) *before* the main piston (with inlet/outlet check valves).

While the flow range (0.01 to 9.9 ml/min) and the upper pressure limit (6000 psi) are not exceptional, the 410 does offer many unique advantages:

1. Providing four-eluant mixing (to 0.1% compositions) is rare among pumps (others are the Anspec SM-90, Gilson 714, and LDC/Milton Roy 3000).
2. A low piston volume (100 μl each) and total pump volume (2000 μl) made solvent changeover easy.
3. Multitasking software permits editing methods while the system is running.
4. Linear, step, concave, and convex gradients are possible.
5. Battery backup protects memory for 5 years.
6. Up to 12 methods are available (9 in memory storage), 1 active, 1 inactive, and 1 default method.
7. Methods can have up to six time segments.
8. The display shows on a single page the gradient program, pressure, flow, current composition (of all four components), and temperature, eliminating the need to search through several screens for these data.
9. Methods can be "chained" together for optimization methods.
10. The keyboard can be locked.
11. Upper and lower pressure limits protect the pump and column.
12. Reproducibility is good: below 0.15% RSD for retention times with a linear gradient from 40 to 100% acetonitrile in water.

Besides the good retention-time reproducibility, the linearity between flow rate (F) and reciprocal of retention time (t_r) for a specific peak (F = Vr/tr) was noted to give a linear regression coefficient of 1.000, said to confirm that the correct solvent proportion is delivered to the column, regardless of flow rate or back pressure (Vr is retention volume). Area reproducibility was not reported.

Limitations of the 410 are that the flow, pressure, and computer memory for methods (nine methods) are not exceptional.

7. Gilson 303 Rapid-Refill (10,000 psi) One-Piston Pump

The flow, pressure, and flexibility of the Gilson pumps are currently broader than most other systems (Figure 53). Two pump drives are available. The standard drive of the 302 goes to 6000 psi (at about $2000), and the heavy-duty drive of the 303 with a more powerful motor goes to 10,000 psi (at about $2600). The pump drives are specified separately from the broad range of pump heads (six available). Pump heads are offered in the following sizes (pressures quoted for the high-pressure pump head costs at about $900 each):

1. Microbore LC, a head to 10,000 psi gives flows from 0.0005 to 5 ml/min (0.5 to 5000 μl/min).
2. Analytical LC an 80-μl head to 10,000 psi gives flows 0.001 to 10 ml/min.
3. Semipreparative LC, a 200-μl head to 4000 psi gives flows 0.0025 to 25 ml/min.
4. Semipreparative LC, a 400-μl head to 2000 psi gives flows 0.005 to 50 ml/min.
5. Preparative LC, an 800-μl head to 1000 psi gives flows 0.010 to 100 ml/min.
6. Preparative LC, a head to 580 psi gives flows from 1 to 200 ml/min.

The upper pressure limits of the pump heads are determined by the power of the motor driving the piston and the diameter of the piston. Wider diameter pistons have a lower pressure limit. If insufficient energy is provided by a motor against the back pressure of the piston, the motor will stall or a built-in

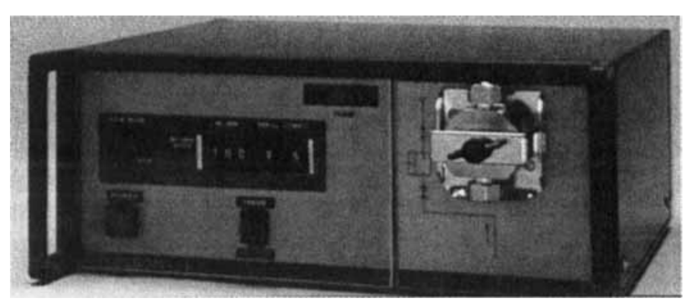

FIGURE 53. Gilson 303 rapid-refill (10,000 psi), one-piston pump. (With permission.)

saftey gear will slip in order to protect the motor. With the Bal seals, higher pressures make a tighter seal, so leakage is rarely the limiting factor.

This very flexible and modular pumping system has several special attributes:

1. Flow range (0.5 μl/min to 200 ml/min) and pressure range (to 10,000) are broader than most pumps.
2. Pump heads for repair or different flows or pressures can be inexpensively, easily, and quickly changed (by hand-screwing a holding bracket).
3. Certain pump heads (10 and 200 ml/min) are also available with a special washing chamber behind the seal for work with salt solutions that can dry and abrade pump seals.
4. Certain pump heads (10 and 200 ml/min) are also available in titanium for use with iron-sensitive biochemicals (and the special washing chamber behind the seal for work with salt solutions).
5. The pump can be used to "dispense" a fixed *volume* of eluant, when set in the "dispense" mode and activated.
6. For eluants that readily cavitate, the piston "refill" speed can be controlled over nine settings from 125 to 650 ms (either manually or from a system controller).
7. For eluant compressibility and system compliance, a "pulse compensation" setting can be controlled over nine settings (either manually or from a system controller). This controls the length of the first part of the piston forward stroke when eluant in the piston chamber is being brought to the system operating pressure.
8. Other computers can control the pumps (with a converter; however, in-house programming is required).
9. The 50- and 100-ml/min heads, once available with a special constant volume head, permit "recycle" chromatography for improved resolution on a fixed column length or for preventing degassing of solvents or cavitation of volatile solvents at high flows. A forechamber is filling when the main chamber is emptying to keep the volume constant within the closed system.

8. Gilson PC-Based 702/704 High-Pressure Two-Eluant Gradient Controller and Data System

The Gilson 702/704 System controller can make gradients and analyze data (Figure 54).[85] Introduced in 1984, Gilson is perhaps one of the first companies to offer a PC-based system for controlling the pumps *plus* analyzing data. The Gilson systems are also marketed and supported by Rainin Instruments (Woburn, MA). This very modular system typically consists of two pumps (at about $2900 or $3300 each); an Apple-II 64 kilobyte computer (about $2600); pump control computer software and external contact module ($1200); and miscellaneous components (mixers, pressure monitor, injector (about $2700). For data storage, it is possible to use either a 5.5-in. disk drive (about $400) (at approximately 16 average

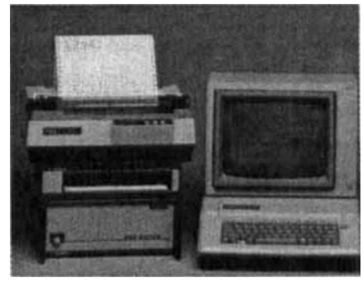

FIGURE 54. Gilson 704 high-pressure two-eluant personal computer (PC) bused gradient controller and data system. (With permission.)

chromatograms per disk) or a hard disk storage (10 megabytes) holding approximately 600 chromatograms (at about $3000). The data acquisition software and printer ($3600) can be used as a chart recorder with chart speed and two channels being controlled from the PC.

Gilson's philosophy of "eliminate needless duplication by planning for flexibility" reflects an extremely efficient approach to system modularity and the need to tailor a system precisely to the intended goals. For example, the pump head and mixing chambers can be selected for the type of chromatography.

The 702 offers some unusual benefits:

1. Manual changes in flow or percent B from the controller requires also selecting the time over which the change will be made, to avoid "shocking" the column. (For instantaneous change, this can be set to zero.)
2. Two-eluant and three-eluant gradients are made by high-pressure mixing using an unlimited number of linear programming steps displayed as a percent B vs. time plot (the gradient-profile) on the video.
3. The video continuously displays the gradient profile, progress of the gradient by markers on the gradient-profile, flow, percent B, and activation of external contacts.
4. Up to four external contacts can be time-programmed to turn "on", "off", or "pulse".
5. One external contact, as above, sounds an audible alarm.
6. "Looping" (repeating) the same program is possible up to 99 times.
7. "Linking" (one program to another) is possible for an unlimited number of programs (permitting automated method exploration or automated shutdown or startup).
8. Through any of four system controller "input events", a run can be stopped or started, for example, to use an autosampler to begin a run, or provide for an emergency stop, etc.
9. A single system controller can specifically address and individually operate up to 16 pumps (two at a time).
10. The computer can be used for other functions (word processing, BASIC programming, etc.) when not used to control the LC.

11. Obsolescence is delayed since PCs have the potential for being upgraded by software changes.

This last point, delaying equipment obsolescence, is exemplefied by three modified computer systems that came out for the Gilson hardware system: in 1986 one by Rainin uses an Apple MacIntosh computer, in 1986 one by Gilson that uses an IBM-AT, and in 1987 one by Gilson that uses an IBM PS/2 computer. Unfortunately, the two modified systems did not upgrade only software (around $1000 to $2000) to improve the system, but also upgraded the computer and interfaces, at a cost of several thousand dollars. Thus, it is probable PCs, even with their software upgrading capability, have a practical lifetime of only 2 to 4 years, since newer models generally are so much better (less expensive, larger memory, faster operation, smaller, etc.).

A software improvement to the 702 is the "Data Master" system. The 704 software plus printer can produce printouts of real-time chromatograms, store whole chromatograms, do postrun calculations for area percent, perform internal or external standard calibration, calculate with any of five baseline correction routines, and draw in the baseline actually used for integration. In addition, all chromatography conditions can be printed on the chromatogram, and printouts can include the gradient conditions, pressure, or temperature profiles, or overlay of a second chromatogram.

A limitation with this, as with many PC-based systems, is it can be very time consuming to find a particular chromatogram and manipulate it. Additionally, this older system cannot be reprogrammed while it is running (i.e., it is not multitasking).

9. Rainin PC-Based "MacRabbit" High-Pressure Three-Eluant Gradient Controller

Using a MacIntosh 512-kilobyte "Memory Enhanced Computer" ($1700) or the 1-megabyte "MacIntosh Plus Computer: ($2200), Rainin made the following improvements over the Gilson 702/704 controller (at about $1500 for the software):[86]

1. Gradients from two or three solvents are made by high-pressure mixing.
2. Gradients can be constructed by use of the "mouse" or direct number entry.
3. Six output contact closure relays are provided (vs. four relays in the 702/704 above).
4. An analog signal output from 1.9 to 6 V DC can be time-programmed to control the detection wavelength between 190 and 600 nm of the Knauer 87 variable wavelength UV/visible detector (available also from Autolabs).
5. The screen display with the MacIntosh computer is more flexible, permitting the flow, the gradient profile, and the progress of the gradient to be displayed.
6. Method storage is higher on the 800K double-sided 3.5-in. disk vs. the 5.5-in. disk with the Apple II.

A limitation of the Rainin MacRabbit controller is that it is not compatible with computer-controlled quantification (as was the Gilson 702/704 controller above). A Shimadzu C-R3A Chromatopak is suggested as a data processor. Also, this system cannot print out directly from the MacIntosh computer or store raw chromatogram data or computed chromatograms.

10. Gilson PC-Based 714 High-Pressure Four-Eluant Gradient Controller and Data System.

As with the Rainin MacRabbit system above, this software program (at about $1500) also requires a computer different from the first Gilson controller. The new system uses the IBM-AT or IBM-PS-2 PC using MS DOS and Microsoft Windows. The 714 can control up to four Gilson pumps for high-pressure mixing and perform all the functions of the 704.

As a data processor, the 714 can save, analyze, and plot up to four detector channels, applying different parameters to each channel for optimal integration. The system can also reanalyze automatically many files of raw data. Also chromatograms can be displayed in real time or with postrun display of chromatogram on the screen or as printouts. Portions of the chromatograms can be expanded, and data presentation can be custom designed.

A limitation to this approach to four-eluant gradient generation is the high cost of four high-pressure pumps (at $2900 to $13,500 each) to generate gradients. Also, the probability of pump failure is possibly fourfold as great with four pumps vs. a single pump with low-pressure gradient mixing. The accuracy (closeness to a true value) of four pumps at produced gradients also may be questionable.

11. Eldex 9600 Parallel-Flow Two-Piston Pump with Low-Pressure Three-Eluant Gradient Controller

Similar in formal appearance to the Spectra-Physics SP8000 (with electronic flow feedback serial flow pump), the 9600 is its most recent and sophisticated pump for common LC (Figure 55). At $7000 with four-eluant reservoirs and plumbing for sparging built in, the 9600 is proposed as the best "price-to-performance ratio".[87,88] While flows (0.01 to 10 ml/min) and pressure capability (to 6000 psi) are not exceptional, the 9600 does have some of the most advanced capabilities:

1. Gradients can be generated down to 0.05 ml/min.
2. Total holdup volume of the pump is only 1000 μl, making it very compatible with low-pressure eluant mixing.
3. Flow precision is ±0.3% RSD, and composition repeatability is better than 1%.
4. "Floating piston design" provides long seal life.
5. Internal memory is preserved with a battery backup for 6 months.
6. A 1-year warranty on parts and service is provided.
7. Outputs permit not only pressure, but also percent A, B, and C to be plotted on a recorder.

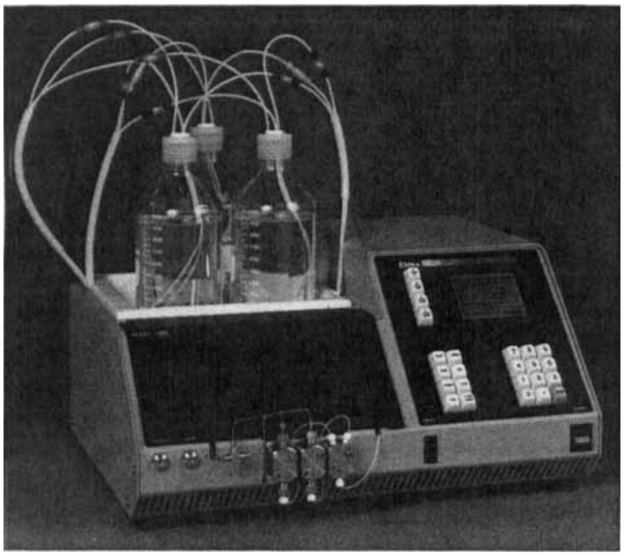

FIGURE 55. Eldex 9600 parallel flow two-piston pump with low-pressure three-eluant gradient controller. (With permission.)

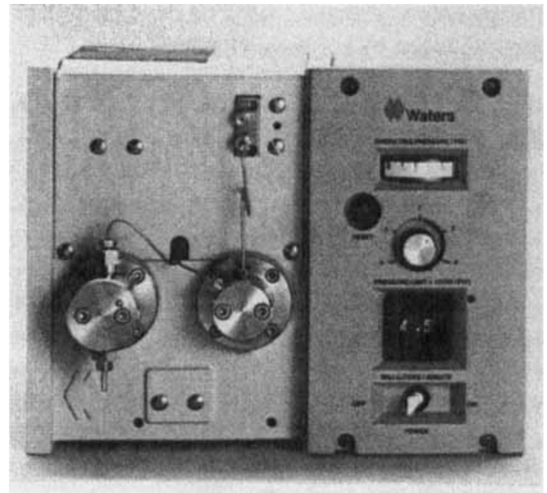

FIGURE 56. Waters-Millipore M45 low-price series flow two-piston pump that preceded the 501 pumps. (With permission.)

8. Four external event contact closures and four TTL (transistor-transistor logic) contacts are provided for synchronizing the pump with autosamplers, injectors, integrators, etc.
9. Remote "stop", "start", or "hold" inputs are provided.
10. Programming is easy with this menu driven system.

This is such a new system, limitations are not yet clear.

J. Specific Series-Flow Two-Piston Pumps and Gradient Controllers

One of the least expensive and more versatile designs to enter the LC pump market is the "series flow" two-piston system with only one set of check valves (ball valves). Differences can be seen by comparing the series flow two-piston pump using only two sets of check valves (Figure 56) to the older parallel-flow two-piston system using four sets of check valves (Figure 30). As will be seen in this section, many newer pumps use this principle.

The two pistons may be housed in a single head (e.g., the Autolab/Knauer pump) or in two separate heads (e.g., Spectra-Physics and Waters). The "pumping piston" has inlet and outlet ball valves, and the outlet of this passes into a second "damper piston" with no ball valves. If a flow of 1 ml/min is desired, the pumping piston actually puts out 2 ml/min during its forward stroke, but the damper piston is retracting at the rate of 1 ml/min, thus taking up half the flow. As the pumping piston is refilling, the damper piston now moves forward at the rate of 1 ml/min.

This type of pump has been given various names. "Serial flow" may be most descriptive since other two-piston pumps (with two check valves on each piston) produce a "parallel flow". Another name is the "take-up piston design" or "accumulator piston design", since the second piston, without check valves, stores and "takes up" or "accumulates" the flow during the refill portion of the first piston cycle.

This clever design of having the damper piston operate out of phase at half the output of the pumping piston has advantages:

1. More-constant flow of two out-of-phase pistons is obtained.
2. Only two ball valves are used (vs. four ball valves are used in the conventional parallel-flow two-headed systems).
3. Repair, cleaning, and failure of the two ball valves may be half as frequent as a one-piston rapid-refill pump at the same final flow (but identical to one set of check valves in a parallel flow pump).

Specific pumps and pump controllers using the two-piston serial-flow approach are now discussed.

1. Waters 501 Series Flow Two-Piston Pump

A recent version of the M-45 pump introduced about 1982 (but with lower pressure level of approximately 5000 psi), the Waters 501 goes to 6000 psi and can be set between 0.1 and 9.9 ml/min when operated alone (Figure 56).[89] Under control of a Waters gradient controller, flow can be set from 0.01 to 10 ml/min in 0.01-ml/min increments. Gradient reproducibilities based on retention time are 0.6% RSD, and areas are 1% RSD for a 0 to 10% gradient. Other features include:

1. Price is low (about $3000) compared with other Waters pumps (with prices ranging from about $6000 to $8000).
2. Compressibility compensation is automatic with changes in operating pressure in order to maintain a constant flow.

3. An internal pressure meter can also be set for overpressure protection.

Several limitations of this pump are apparent compared with the more sophisticated versions of Waters pumps. For example, no inlet manifold is provided for eluant selection, and the low-pressure monitor selection option (0 to 600 psi) is absent. Additionally, pulsations are greater and a Bourdon-tube-type pulsation dampener is inserted between the two piston chambers to reduce pump noise, and this increases pump dead volume.

2. Autochrom 500 and Knauer 64.00 Series Flow Two-Piston Pump

The Knauer pumps were introduced in Germany about 1982. These are distributed and serviced in the U.S. as the Autochrom 500 pump (Autochrom, Milford, MA). Figure 57 shows that the pumping piston and damping piston are contained in a single head. The 500 offers some unusual features:[90]

1. Price is modest (around $3500).
2. Upper and lower pressure limits protect the pump and column and prevent loss of eluant with leaks.
3. Interchangeable heads are available (at about $700) for microbore LC (0.02 to 2 ml/min to approximately 6000 psi), analytical LC (0.01 to 9.9 ml/min to approximately 6000 psi), or preparative LC (0.5 to 50 ml/min to approximately 3000 psi; 50 ml/min is nearly twice the usual preparative flow of this type of pump).
4. Pump heads are easy to repair by removing four screws without the chance of snapping off the sapphire piston.
5. Piston volume is very low (20 μl from the pumping piston and 10 μl from the secondary damping piston).
6. Pump holdup volume is low (250 μl for microbore, and 300 μl for analytical pumps) for rapid solvent changeover and low gradient delay with low-pressure gradient generation.
7. Flow can be controlled by an analog input from external flow controllers such as the Autochrom OPG/S Gradient Controller or M320 Gradient Workstation.
8. Pressure is continuously displayed.
9. The pump can be externally started or stopped by a switch closure.
10. Rapid repair is provided by next-day parts (or unit) delivery.
11. Compressibility compensation corrects for changes in operating pressure to maintain a constant flow.

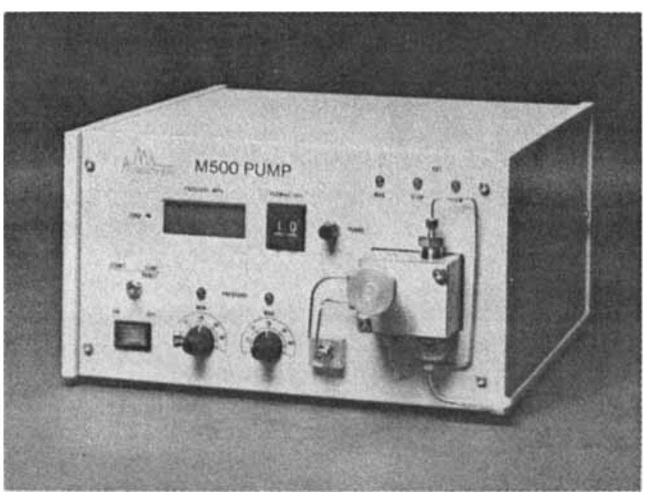

FIGURE 57. Autochrom M500 series flow two-piston pump (the Knauer 64.00 pump). (With permission.)

3. Autochrom OPG/S Low-Pressure Three-Eluant Gradient Generator

The OPG/S is available in a number of configurations, depending on whether it has two- or three-eluant gradient capability (Figure 58).[91] The OPG/S uses an inert high-speed valve at the pump inlet to switch between two or three eluants during the inlet stroke of the pump. Unusual features of the controller are

1. The price is low (ranging from about $2500 to $3500).
2. Flow ranges are broad. Models are available for low flow (0.02 to 5 ml/min), medium flow (0.05 to 50 ml/min); high flow 2 to 400 ml/min, or wide flow range (0.05 to 1000 ml/min).
3. Versatility is broad. The OPG/S has been interfaced with both two-piston parallel-flow, two piston series flow, and

FIGURE 58. Autochrom OPG/S low-pressure three-eluant gradient controller that can be used with most pumps. (With permission.)

one-piston rapid-refill pumps, including pumps from Beckman-Altex, Autochrom, Eldex, LDC, Perkin-Elmer, Gilson, Waters, and others.

4. Autochrom PC-Based M320/CIM High- or Low-Pressure Two-Eluant or Three-Eluant Gradient Controller

This "M320/CIM" is one of the first sytems to use a PC as a LC controller (Figure 59).[92] The CIM (abbrevation for "Control Interface Module") uses Autochrom software (M320, about $2000 with interface and cable) with an IBM PC (or IBM compatible computer, or DEC microVax-based computer) and any of three different CIMs selected initially to generate gradients by three means:

1. Two pumps (analog, frequency, or serial controlled) for high-pressure gradients (about $2100)
2. One pump for two-eluant low-pressure gradient (about $2500 with valve)
3. One pump for three-eluant low-pressure gradients (about $2800 with valve)

The CIM can control a wide variety of components and pumps (e.g., the frequency-controlled pumps from Waters or Kratos, or the analog-controlled 0 to 5- or 0 to 10-V Beckman-Altex, Autochrom, LDC, etc.).

The CIM intelligent module is a microcomputer that stores methods that are downloaded from the PC, where the CIM controls the local pumps (the later Beckman "System Gold" controller uses a similar system). The M320/CIM uses the IEEE-488 multiinstrument control bus and RS232 communications, so Autolabs anticipates future modifications of the M320 software for future automation. Other exceptional features of the M320/CIM system are

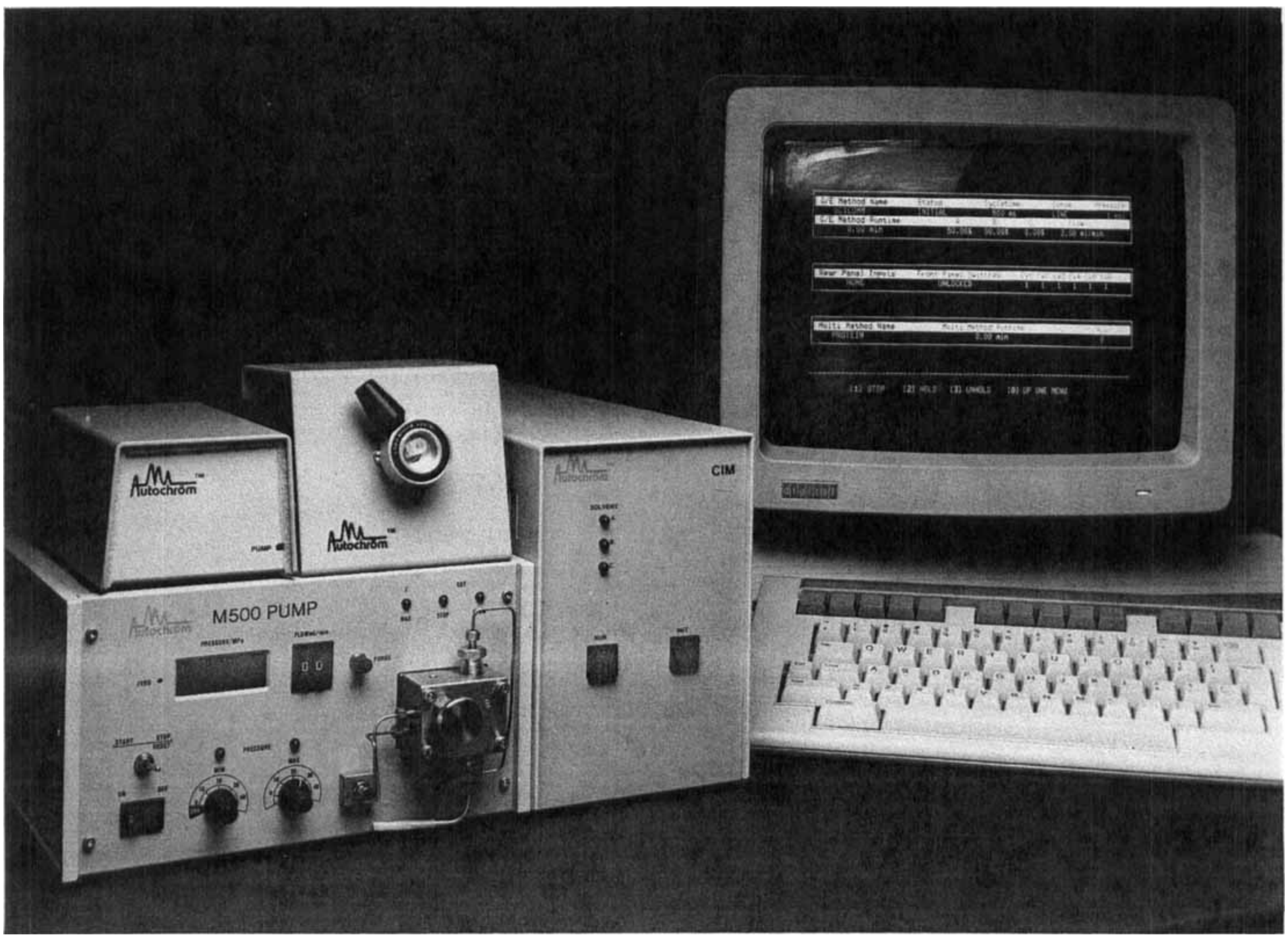

FIGURE 59. Autochrom PC controller (left), the CIM (center), and low-pressure two- or three-eluant gradient mixer (left, top). The CIM can be down-loaded from the PC or also used locally (see text). This is one of the first PC-based systems, a forerunner of today's common PC-based approach. (With permission.)

1. The PC is free during runs for inputting methods, processing data, word processing, etc.
2. Low-pressure gradients can be generated over a wide flow range from 0.05 to 400 ml/min (with three tubing kits).
3. Solvent proportioning is coordinated with the intake stroke of single-piston rapid-refill pumps (such as the Beckman-Altex 110A and 114 pumps).
4. Up to 15 CIMs can be attached to one PC, and each can use a different means of gradient generation and pumps from different manufacturers.
5. Each CIM can program external devices; for example, the CIM can program the autosampler and detector parameters via the computer.
6. Each CIM can control up to six events that can be time-programmed for "on", "off", or "pulse".
7. Each CIM can store ten methods in local memory for unattended method scouting, and these can run linear, convex, concave, or step gradients.
8. Each CIM can use a different data analysis system, if it is compatible with a RS232 port, such as the Spectra-Physics integrator (4290/4270), Nelson Analytical data system (3000), or Autochrom M625 data workstation.

Capabilities from the PC are

1. Several parameters can be programmed to change with time (time programmed), including flow, events, or eluant composition for gradients (convex, concave, linear, or step), as well as autosamplers and detectors.
2. Fifteen CIMs can be monitored simultaneously in an abbreviated format.
3. Each CIM can be monitored individually in a detailed format.

Main advantages of the Autochrom system are the wide versatility and the possibility of incorporating equipment from many manufacturers into a single control system, at the cost of only about $3000 each. Since the PC is free during runs, it can be used as a word processor, spreadsheet, etc. (The "unloading" to a local controller is thus a method to go around having the computer be multitasking.) Both the 320 controller (described here) and the OPG/S controller (described in the section above) can activate a number of other less-expensive free-standing devices, including a three-eluant selection valve (about $850), a ten-port high-pressure valve (about $1250), a high-pressure six-column selection valve (about $1550), a high-pressure two-column selection valve (about $800) and a solenoid interface converting an external event signal to four 12-V signals (about $460).

K. Recent Electronic Flow Feedback-Controlled Pumps and Gradient Controllers

The following most recent pumps typically use some system of sophisticated flow feedback to eliminate pulsations. This approach actually began with the Beckman-Altex rapid-refill one-piston Model 110A pump over a decade ago (1977). With the 110A pump (described above), the torque on the piston drive motor was used to measure the pressure in the piston chamber at the end of the pump stroke. This pressure was memorized electronically and controlled the rapid forward movement of the piston to bring the piston chamber back up to the same pressure before the "constant flow" motion of the piston was again in control. Later Altex pumps were improved by using a strain gauge on the head itself to determine the pressure better (e.g., the 114M pump, described above). As will be seen below, many pumps available in the last 2 years use two pistons (either in parallel flow or series flow), some fast-response system to measure pressure, and sophisticated internal electronics to use the pressure reading to reestablish constant flow when flow switches between the two heads. Specific methods used by some different companies are described below.

1. Micromeritics 760 Series Flow Two-Piston Pump with Electronic Flow Feedback Control

The Micromeritics 760 (Figure 60) is nearly identical to the Anspec SM-909.[93] The 760 is an upgrade of the company's earlier 750 pump. The 760 (at about $3500) is available with three different heads (at about $1000 each) covering the flow range from microbore LC (0.002 to 1 ml/min), to conventional LC (0.1 to 5 ml/min), to semipreparative LC (0.5 to 20 ml/min). The 760 is one of the first to use a unique method for "flow multiplexing" to produce pulseless flow without pulse dampeners. This approach is beginning to be used in many new pumps (such as the other pumps in this section). Micromeritics' flow multiplexing divides the complete cycle of the pump into two delivery phases: one of constant pressure and one of constant flow. During the constant flow portion, the existing system pressure (determined by a fast piezoresistive pressure strain gauge) is stored in an electronic memory. As this piston completes its delivery stroke, the unit is switched to the constant pressure mode. Both pistons deliver briefly in this constant pressure mode to ensure pulseless changeover. The second piston then continues to pump at constant speed until empty, then the first piston takes over, and the constant flow mode is switched back. Micromeritics says this provides "absolutely pulseless flow, without pulse dampeners".[94,95] The low pump volume (440 μl total) is important to the operation of the low-pressure gradient former, the Model 753.

Other unique features of the 760 are

1. Pressures to 7200 psi are higher than usual (3000 psi for the semipreparative head).
2. The pump may be ordered with an optional RS-232C interface (about $400) and analog interface (at about $400) so it can be controlled from a PC.

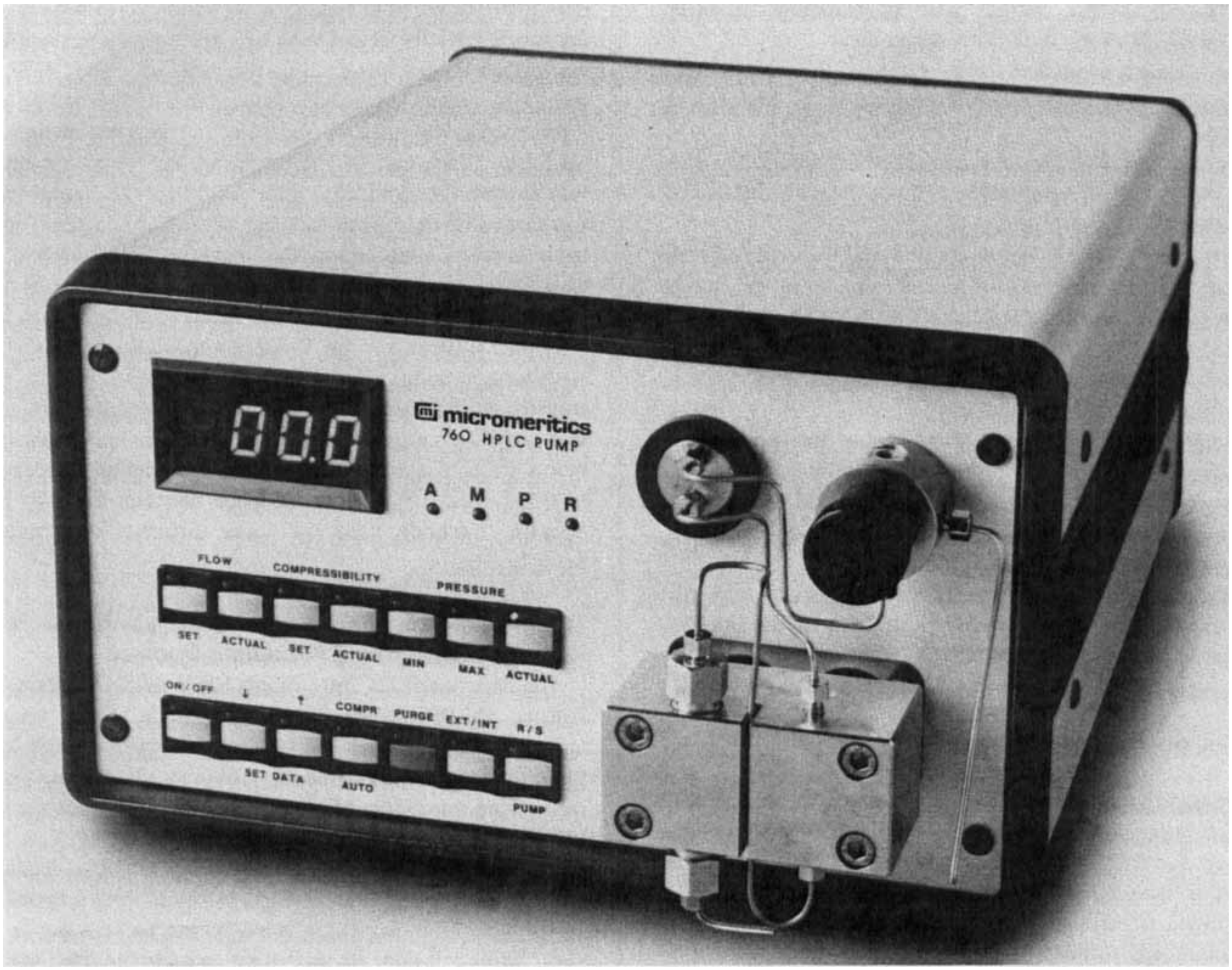

FIGURE 60. Micromeritics 760 series flow two-piston pump with electronic flow feedback control. (With permission.)

3. A piston-rinsing option is available (at about $600) so salt solutions will not destroy the piston seals.
4. A battery protects the memory.
5. Flow accuracy is better than 0.5%, and flow reproducibility is better than ±0.1%.
6. Compressibility can be automatic or can be set manually.

2. Micromeritics 752 Two-Eluant Low-Pressure Gradient Controller

Although the 753 gradient controller (below) can generate any gradient or isocratic mixture among three eluants, the earlier 752 gradient programmer has some unusual advantages:

1. Two-eluant gradients have many possible shapes (nine concave, nine convex, and one linear).
2. Two-eluant gradients can have a third eluant added at constant composition.
3. Isocratic mixtures of three different eluants can be generated to 0.1% composition and flows with 0.5% precision.
4. Gradients can be from 2 to 999 min in length.

3. Micromeritics 753 Three-Eluant Low-Pressure Gradient Controller

Micromeritics (along with Pharmacia) was one of the first companies to offer low-pressure gradient generation for LC in the mid- to late 1970s. The 753 gradient controller builds on that technology (Figure 61). The 753 model is intended to be used with the 750 pump having a low holdup volume. Chief features of the 753 gradient generator are

1. Low-pressure mixing of either two or three eluants is provided in a low-volume chamber (1.5 ml).
2. The cycling of the two three-way solenoid valves is tied to the flow rate (as the time of the intake stroke decreases, so does the time of the proportioning cycle).

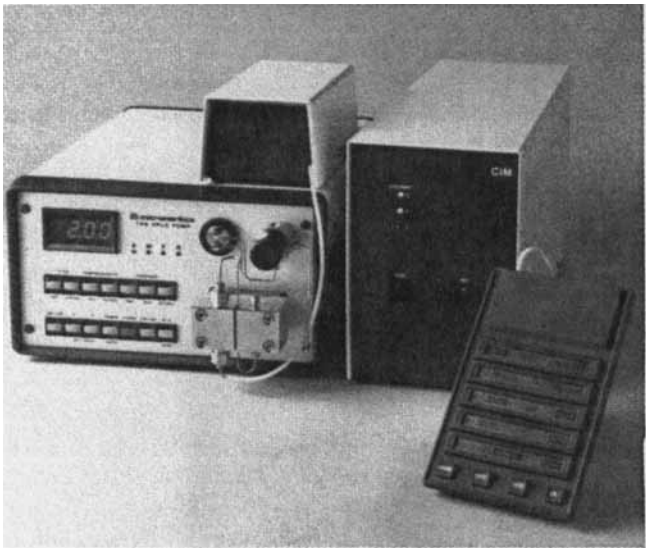

FIGURE 61. Micromeritics 7610 two- or three-eluant low-pressure gradient system with the mixing valve on top of the pump (see Figures 57 to 59 above), and a 760 series flow two-head pump with electronic flow feedback control. (With permission.)

3. The gradient generator can be operated from the front panel, remotely with a PC-based control module, or by an externally applied voltage of 0 to 10 V.
4. Continuous solvent profile from e $^1/_5$ to e 5 can be generated for three different eluants simultaneously (and this includes any isocratic combinations).

4. Isco 2350 Rapid-Refill One-Piston Pump with Electronic Flow Feedback Control

In 1986, the Model 2350 was introduced as Isco's fourth-generation pump (at about $3400).[96,97] While this pump *looks like* a series flow two-piston pump in that the output of the left-hand piston (with ball valves) (Figure 62, bottom) enters a second similarly placed chamber (with no ball valves), this second chamber is a diaphragm damper and *not* a series flow pump head. Thus, there is only one piston, one set of piston seals, and one set of check valves.

To minimize pulsations, this pump uses an electronic compensation mechanism to adjust each stroke to give the same flow as measured from the last stroke. The motor-driven servo uniformly accelerates to pump motor as needed, within each stroke, to bring the piston chamber up to the pressure of previous strokes.

The 2350 model has several features available that make it desirable as a simple but high-quality workhorse pump. The unit is only 12.5 in. wide, and it provides a side column compartment and place for a built-in manual injection valve ($600 to $700 more) "to conserve space and eliminate bench clutter . . . while protecting the column against mishaps and temperature fluctuations". (A heating accessory that attaches to the column is also available.) The 2350 fits snugly on top of the UV/visible variable wavelength detector (one has a built-in chart recorder, making a complete isocratic LC instrument for about $8400). Although many specifications are usual (flow is 0.01 to 10 ml/min with the 80-μl head and pressure goes to 6000 psi, push-button electronic programming permits many unusual features:

1. Feedback electronic control varies motor speed, as needed, within each cycle, and then corrects pump rate after each piston stroke to give a flow-rate constancy of ±0.5%.
2. Internal pump software can keep track of the *volume* of solvent passed through a pump, and flow changes can be programmed after a specific volume has been pumped (instead of after a specific time interval, the usual "time-programming" process).
3. Flow changes can also be time-programmed.
4. An audible alarm indicates overpressure, underpressure, when a preset volume is delivered, or when a certain time has elapsed.
5. Memory is backed up by battery for 7 d, for moving the pump or in case of power failure.
6. Accumulated time or volume is easily reset.
7. The pump can control the chart drive of an Isco recorder to keep the X axis proportional to volume; for example, 1 cm of chart paper can be set to represent always 1 ml of volume through the column, independent of flow.
8. The pump automatically restarts after pressure drops below an upper pressure limit, giving an approximate constant pressure mode.
9. Bright digital displays show continuous data, including current pressure in psi or MPa, and upper and lower pressure limit, initial, current, and final flow rates can be called up.
10. External control of the pump is by digital control through an RS-232C port, or by analog control with a 0- to 10-V input from a gradient controller. Also, flow can be externally stopped or started by contact closure or a TTL (transistor-transistor-logic) signal.
11. Pressure sensing is not approximated by pump motor current, but rather by a separate strain-gauge pressure transducer that contributes nothing to the system dead volume.
12. Pulsations were found to be comparable to a dual-piston pump (approximately 15 psi, 0.66%, at 2280-psi back pressure with methanol at 2 ml/min flow; this is one of the few pumps specifically listing pulsation levels).
13. The head assembly can be removed in minutes for easy replacement, and the sapphire piston can be snapped out for cleaning, with little possibility of accidental damage.
14. A preparative chromatography pump head (240 μl) giving flows from 1 to 30 ml/min can go to 2500 psi and may be added later (at about $600).
15. For corrosive eluants or iron-sensitive proteins, "inert" components of Hastelloy® C-276 (pump head, damper

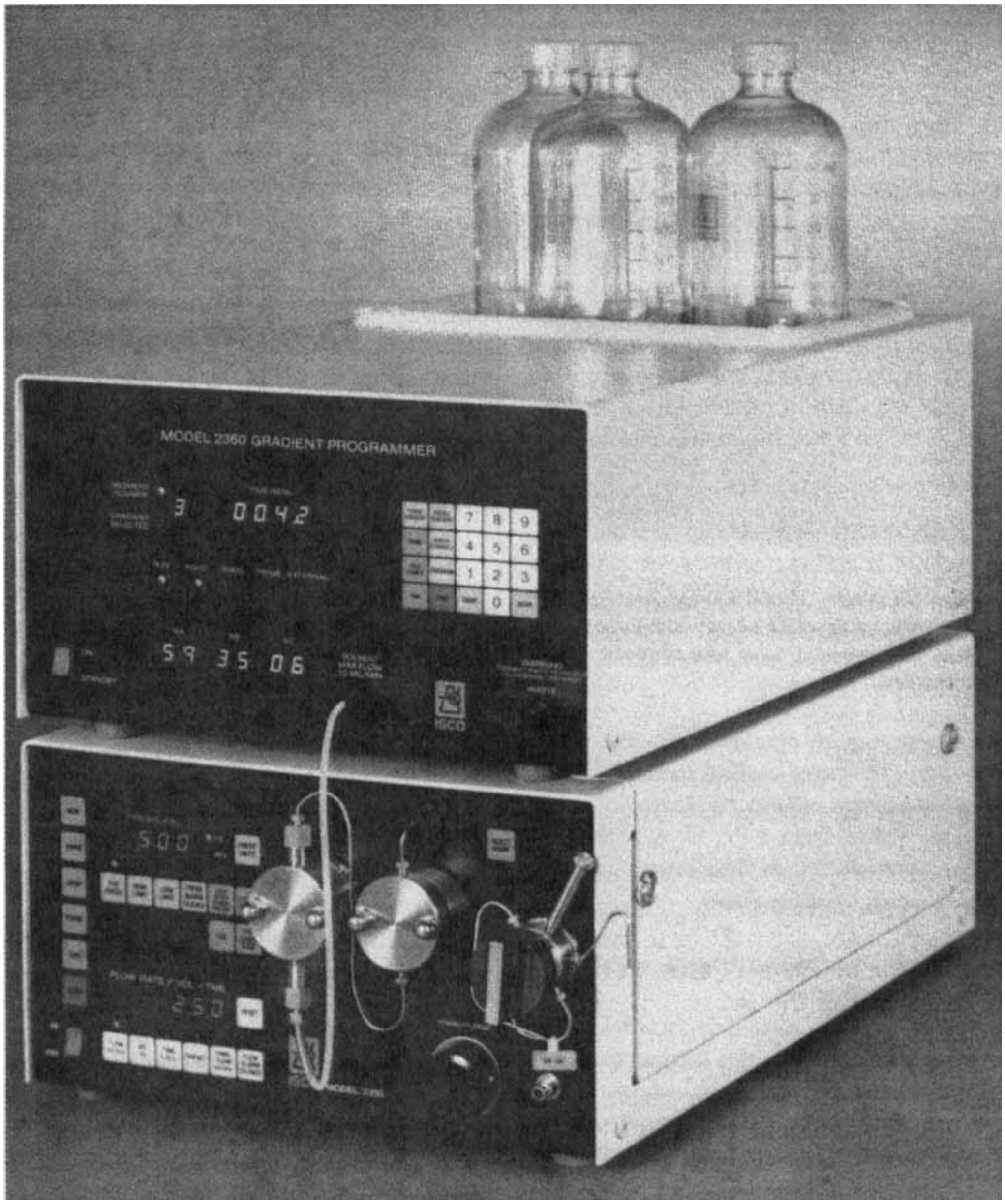

FIGURE 62. Isco 2360 low-pressure three-eluant gradient controller (above) and Isco 2350 rapid-refill, one-piston pump with electronic flow feedback control (bottom). The left "head" is actually a diaphragm damper-chamber and this is not a series flow pump. The pump also houses a six-port manual injection valve (lower right) and column chamber (lower right). (With permission.)

head, tubing, and injection valve assembly) may be ordered (about $800 more for the inert or preparative head). They say that this is equal to or superior to titanium for resistance to corrosion by acidic halide buffers, and the metal parts have much less tendency to cold-weld together.

16. For use with salt solutions, the piston can be washed behind the seals to eliminate salt crystals that can damage the seals.
17. Upper and lower pressure limits protect the pump and column from overpressures and prevent eluant loss by leaks.
18. Pressure can be recorded externally (0.1 V per 1000 psi).
19. "Floating piston" design minimizes seal wear.
20. A 3-year warranty on parts and labor is provided.

5. Isco 2360 Low-Pressure Three-Eluant Gradient Controller

In 1986, Isco offered low-pressure gradient generation for about $2800 for the flow range of 0.5 to 10 ml/min, and about

$3200 for the preparative flow range of 2 to 30 ml/min (Figure 62, top). (An inert version of Hastelloy® costs about $3000 for the 0.5- to 10-ml/min flow range.) An internal reciprocating pump draws eluant through proportioning valves to replenish a low-volume mixing reservoir that supplies the pump on demand. Gas produced by mixing solvents at low pressure is nucleated and leaves the reservoir at this point. This design accommodates rapid-refill one-piston pumps as well as more common two-piston pumps. Multisegmented gradients in 1% increments from 0 to 100% are possible with the 2360. Besides having the possibility of being used with almost any LC pump, no sparging of the eluant is required under most circumstances. Additional features of the gradient programmer are

1. Low-pressure mixing of either two or three eluants is provided in a low-volume chamber.
2. The gradient generator can be operated from the front panel or remotely with an Isco PC-based control module.
3. Multilinear segments of varying length (to 16 h total of up to nine segments each are possible.
4. Ten gradients can be stored in memory.
5. Internal memory is protected up to 7 d by battery.
6. The display shows the current gradient segment, running time, and a percent of each eluant, and this can be "frozen" by a single button.
7. A delay timer with audible signal can be set to compensate for the calculated lag time it takes for the gradient to go from the programmer to the column, allowing manual or automatic injection just when the gradient reaches the column.
8. The 2360 internal piston may be flushed manually, when necessary, or continuously to prevent salt from affecting the seals.
9. Rear-panel connections facilitate interfacing with most autosamplers, pumps, and fraction collectors.
10. The gradient programmer can be controlled and programmed by an external computer, and outgoing commands can be used to start external autosamplers, integrators, etc. (The IscoChem Research software package accomplishes this along with data management.)
11. A 3-year parts and labor warranty is included.
12. The interface card and control software is usable with almost all brands of pumps and many computers (e.g., the IBM model PC and model PS-2 as well as the Apple II series).

A difference between this low-pressure "on demand" generator and the Micromeritics generator above is that Isco supplies gradients made up of multiple line segments instead of a continuous curve. The proportioning cycle of the solenoids in the 2360 is based on the flow of an internal low-pressure pump, and not the high-pressure solvent delivery pump. Thus, gradient accuracy is said to be independent of the high-pressure pump used.

6. Isco ChemResearch® PC-Based High-Pressure Two-Eluant and Three-Eluant Gradient Controller and Data System

This system from Isco (hardware) and ChemResearch® (software) includes a single-pump ternary or dual-pump gradient control system, external events signals, and a data management system.[98] This system is similar to the Gilson system (Figure 54). The graphic presentations are different, and the Isco system can use linear segments or eight concave or convex curve shapes for two-eluant gradient construction. As with the Gilson system, a full data acquisition system can be used simultaneously with the PC. The Isco ChemResearch® interface module permits control of up to 16 external devices. (The Gilson system can control four external devices.) The data management system offers the following:

1. High-resolution chromatograms can be displayed on a video unit and printed (in real time, as peaks elute), as well as gradient shape and some run parameters (flow, composition, time, etc.).
2. Peaks can be integrated immediately after the run to quantify components.
3. Peaks can be quantified based on internal, external, or addition standards.
4. Unlimited raw data can be stored offline on disks.
5. Retention time, widths, skew, sharpness, height, percent height, area, percent area, and effective plates are calculated.
6. Peaks can be reintegrated and peak parameters recalculated after a run.
7. Chromatograms may be subtracted, added, or ratioed.
8. The X and Y axes can be manipulated to expand chromatogram display.
9. Chromatography conditions such as flow, pressure, gradient shape, date, etc. can be listed on the video unit and printed on the chromatograms.
10. Two detector inputs can be processed simultaneously.

With the Isco controller, even two dissimilar pumps can be controlled, provided they use voltage, frequency, or a RS-232-C input. The cost is about $2500 for the software alone, which rises to about $4700 with an Apple IIe computer and printer or $5000 with an IBM Personal System/2 and printer.

Isco invented and patented the two-pump high-pressure method for gradient formation and licensed it to Waters, Varian, and Tracor.[98] Isco notes some advantages to high-pressure gradient generation vs. low-pressure gradient generation:[99]

1. Seals and valves are equilibrated with one eluant and component and do not experience varying eluant compositions.
2. Gradients are delivered more quickly.
3. The option exists to convert the system instantly to two isocratic systems, and an operational system remains in place if a problem develops with one pump.

7. ABI Analytical (Kratos) Spectroflow 400 Series-Flow Two-Piston Pump with Electronic Flow Feedback Control

The ABI 400 has many features similar to the Micomeritics 760 pump (above), including the electronic flow control (Figure 63). Although the flows of the 400 are lower than usual (0.01 to 4.99 ml/min), this pump offers some special features.[100]

1. Pressure of 7000 psi is higher than usual (most pumps go to 6000 psi).
2. High- and low-pressure thumbwheel settings protect the system, and the settings can be seen at any time.
3. Both flow and pressure are continuously displayed.
4. The pump can be controlled by frequency-generating devices such as the Kratos detector with gradient option or the Kratos DS650 Data System.
5. An optional continuously irrigated pump head is available to wash away salt deposits on the piston to give long piston life.
6. A preparative chromatography head is available for flows up to 30 ml/min.

8. ABI Analytical (Kratos) Spectroflow 430 Low-Pressure Three-Eluant Gradient Controller

The 430 gradient generator (Figure 64) is very similar to the Micromeritics 753 gradient controller in performance.[101] The 430 is intended to be used with the Kratos 400 pump having a low holdup volume. Chief features of this gradient generator are

1. Low-pressure mixing of either two or three eluants is provided in a low-volume chamber, at atmospheric pressure, that permits excess gases from the mixing to bubble out.
2. The gradient generator can store and maintain up to ten gradient programs with up to 55 steps each.
3. Six switch closures can be used to turn "on" or "off" a Spectroflow 400 pump, autosampler, or detector.

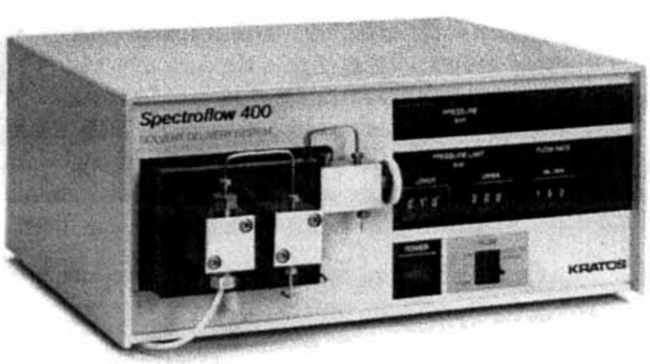

FIGURE 63. ABI Analytical (Kratos) Spectroflow 400 series flow two-piston pump with electronic flow feedback control. (With permission.)

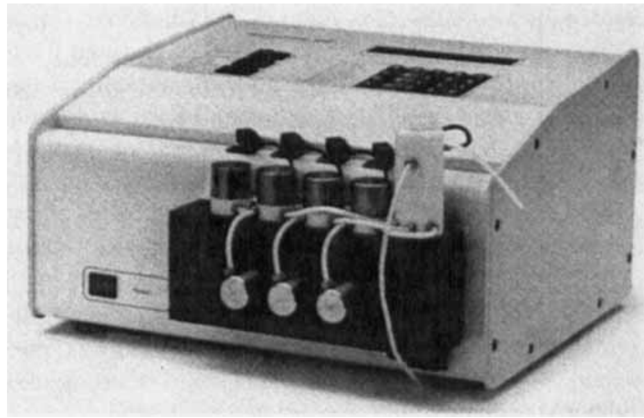

FIGURE 64. ABI Analytical (Kratos) Spectroflow 430 low-pressure three-eluant gradient program. (With permission.)

9. Spectra-Physics SP8000 Series-Flow Two-Piston Pump with Low-Pressure Gradient Controller and Electronic Flow Feedback Control

The SP8000, introduced in 1987, is one of the most recent and advanced pumping systems on the market (Figure 65).[102] At about $9200 for the pump and a controller for three-eluant low-pressure gradient generation, this pump uses a "flow-multiplexing" system similar to the Micromeritics 760 pump (described above), except series flow pump heads are used. Being one of the most recent pumps, it has some of the most advanced capabilities:

1. Besides the usual flow of 0.01 to 10 ml/min to 6000 psi settable in 0.01-ml/min increments, preparative heads are available for flows to 30 ml/min to 2500 psi (about $1100).
2. Gradients can be generated down to 0.05 ml/min.
3. For use with corrosive materials or metal-sensitive proteins, the system is available in titanium for about $13,000.
4. An isocratic version is available for $3400.

FIGURE 65. Spectra-Physics SP8800 series flow two-piston pump with low-pressure gradient controller and electronic flow feedback control. (With permission.)

5. A helium degassing manifold and four plumbed reservoirs are part of the system.
6. Pump heads are "bayonet mounted" for easy removal and repair.
7. Total holdup volume of the pump is only 800 μl, making it very compatible with the built-in low-pressure eluant mixing.
8. Flow precision is 0.2% RSD and composition repeatability is 0.2% RSD, based on chromatographic retention time of peaks.
9. Area reproducibilities are about 0.65 to 0.85% RSD.
10. Compressibility compensation is automatic.
11. "Floating piston design", described recently by Bal seal company, is used to provide long seal life. (Most previous designs fixed the piston rigidly in place.)
12. Internal memory is preserved with a battery backup for 100 h.
13. Compared with most other systems, especially PC-based systems, the unit is very compact and relatively portable.
14. The pump is expected to be reliable, since a 5-year warranty is provided.

Built-in diagnostics indicate three things:

1. "Flow unstable" is indicated with a numerical value.
2. Repairs are suggested, such as "outlet check valve defective".
3. A guide takes the user through repair with a "help" key, providing 400 lines of text.

In addition, an internal "maintenance log" can be set to remind the operator when to perform any of several maintenance operations based on the liters of solvent pumped. Extra dead volume due to a gradient mixer is eliminated since the two series-flow pump heads mix the sequences of eluant put into the flow stream by the low-pressure mixer.

Limitations of the SP8000 are not very important. However, because this is one of the most recent and advanced pumping systems, one expects it to have the best features of all pumps that preceded it. Two lines of available text make monitoring all parameters difficult. A "screen" can show the elapsed time, percents of the three eluants, and pressure, but other screens must be called up to monitor flow, maximum pressure settings, gradient program number, or flow stability. Since the trend has been toward four-eluant gradient generation, it is surprising this pump used only three eluants. Other limitations are the internal memory is limited to only ten files, and no low-pressure cutout is provided.

The price is low ($9200) compared with some three-eluant low-pressure gradient systems (e.g., Hewlett-Packard and Waters at about $13,000 to $16,000). However, there are low-pressure three-eluant gradient systems costing much less (e.g., Autochrome, Isco, and LDC at about $5000 to $7000). It would have been welcome if a greater part of "being most recent" would have included being least costly.

10. Bio-Rad 1350 Soft-Start Parallel Flow Two-Piston Pump with Electronic Flow Feedback Control

At around $3500, the 1350 provides unusual pressures (to 6000 psi) and flows (0.01 to 9.9 ml/min, in 0.02-ml/min increments) (Figure 66).[103] Additional features include:

1. Electronic adjustment of motor speed minimizes pump pulsations.
2. Their "Soft Start" slowly ramps the flow to the set flow and is said to prevent "column shock" that potentially can take place with pumps that start up abruptly.
3. A titanium version (at about $4000) is available for work with corrosive salts or iron-sensitive protein work.
4. A preparative head is available for flows to 40 ml/min.
5. Upper and lower pressure limits protect the pump.
6. Solvent compressibility can be selected from three preset values.

11. Bio-Rad 400 HRLC High-Pressure Two-Eluant and Three-Eluant Gradient Controller and Data System

The 400 gradient controller uses an IBM-XT and IBM-AT (or compatible) PCs (such as in Figure 54). The 400 gradient software is intended to be used with two of Bio-Rad's 503 "Soft Start" pumps, gradient mixer, manual injector, floppy disk, hard memory, and two-channel data collection and manipulation software (total cost around $16,000). With multitasking, the computer can run three separate two-pump LC systems (or two separate three-pump LC systems) and accept data. (Detectors and autosamplers are not included in this price.)

Other advantages of this system are

FIGURE 66. Bio-Rad 1350 "soft-start" parallel flow two-piston pump with electronic flow feedback. (With permission.)

1. The system is multitasking, so any of the three two-pump systems can be independently viewed and data from two detectors on each manipulated at any time.
2. Color graphics display chromatograms and edit baselines.
3. Ternary gradients with high-pressure mixing are possible.
4. Dedicated systems for specific applications are available, many of them in food anaylsis (e.g., fermentation monitor; fruit quality analysis, preservative analysis, fish spoilage analysis, corn sweetener analysis, nonnutritive sweetener analysis, etc.)
5. Sixteen contact outputs and nine BCD digits for sample number input are provided.
6. Integration permits baseline manipulation overlay, addition, subtraction, ratioing, and normalizing of chromatograms and time integration parameters to optimize autointegration.
7. Reports are generated using areas or peak heights, internal or external standards, linear or nonlinear calibrations, all with printout of chromatograms on either a color or black-and-white printer.
8. Sophisticated data analysis parameters from a series of reports can be combined to give summaries, data bases, and trends.
9. Screen manipulation is easy by filling in blanks, answering prompts, or default values can be used.
10. Only four-screen displays show all operations:

    (a) *Methods screen* has a timetable, controls solvent percents, external events, etc.
    (b) *Graph screen* displays chromatograms and gradient profiles, and these can be rescaled, offset, marked with codes, etc. Data can be manipulated from screen to screen to add, ratio, or overlay chromatograms.
    (c) *Integration screen* gives the automatic integration parameters, calibration values, and data storage information using different calibration approaches, calculation methods, and (linear/nonlinear) data regression systems. Postrun printout and name files of each input are also controlled.
    (d) *Sequence screen* is used for automatic operation to indicate the sample and gradient method to be used, number of injections, calculation method, as well as sequence to reprocess stored data in the operator's absence.

12. Beckman-Altex PC-Based "System Gold" Rapid-Refill One-Piston Pump with Electronic Flow Feedback Control and High-Pressure Two-Eluant Gradient Controller

Beckman-Altex pioneered the development of the rapid-refill one-piston pumps in 1977 (see the 110 and 114 pumps above). In 1987, the company combined its best pump technology with new detector technology to form the basis for the "System Gold" range of application-oriented LC systems. System Gold "modules", at each LC, have an internal microprocessor that communicates by an all-digital bidirectional link to the PC used as the system controller, either a NEC lap-top (about $1100) or IBM model AT, model PS, or model 2 (about $3000 to $5000). A single NEC lap-top PC can control up to eight 406 analog interface modules (at $2300 each) and provide on the lap-top a real-time display of the status of all systems, for example, flows, pressures, gradient profiles, etc. (but no data collection). For data collection with the System Gold, a single IBM PC using IBM software (software costs about $7000) can control two complete LC systems, including full detector data collection, data analysis, data display, as well as postrun data processing. Two channels of data can be collected on each system. There are several advantages to having a single controller/data system communicating with the modules:

1. Only one keyboard/operator interface needs to be learned and used.
2. Instrument status and display can be monitored on-screen, using parameters that users select as important to their applications.
3. Chromatograms are displayed with instrument parameters and one or two channels of data displayed separately or superimposed.
4. A single "analytical method" for *all* of the modules (e.g., pumps, detectors, data acquisition, etc.) can be created and stored in memory.

The two computer-control systems can be used with any of the four Beckman-Altex pumps:

1. 110B ($3500) one head, (isocratic) operable alone or by computer (see above)
2. 114M ($6500) one head, (isocratic) operable alone or by computer (see above)
3. 116 ($6800) one head, (isocratic) operable only by computer (described below)
4. 126 ($13,300) two heads, (gradient) operable only by computer (described below)

Either the 116 or 126 pumps (with the computer controller) can program a valve for letting the pump select remotely among four eluants. The computer-controlled valves, one for each pump head, *looks like* a low-pressure gradient-generating system; however, it is not. These valves are not of the high-speed type required for gradient generation. However, the valves do provide a unique capability currently found only with the Beckman-Altex system, in that the computer can select any of the four eluants to go to either of two pumps, and the two pumps can then generate a two-eluant gradient by high-pressure mixing.

The two-way communication between the PC system controller and the local modules gives on-screen or printed diagnostic information (flows, pressures, detector wavelengths, etc.). Additionally, automation is simplified by including all system parameters such as flows, pressures, detector settings, etc. in each method, which can be stored on hard or floppy disk for transfer to other systems.

Various combinations of PCs and interface modules, plus detectors and pumps, provide nearly a dozen application-oriented configurations for specific markets, for example, for nucleic acids, peptides, proteins, amino acids, preparative separations, quality control, and method development. Three representative systems show some of the many possible configurations of pumps and controllers:

1. Simple, low-cost system: "Protein I System" (Figure 67) (about $16,000). The 116 one-eluant pump module that can be time-programmed to select among four-eluants to the pump head (about $7000) is controlled by a NEC lap-top computer ($1100) that can control a 506 Autosampler ($10,000) and program the detector wavelength, attenuation, autozero, etc. on the 166 programmable UV/visible detector ($5600). (A printer and data system cost extra.)
2. Simple, medium-priced system: the "338 Gradient LC System" (Figure 68) (about $19,000). Two 110B pumps ($3600) are controlled by a 406 Analog Interface Module (about $2300) and programmed by a NEC lap-top computer ($1100). The Analog Interface permits the lap-top computer to control the pumps for high-pressure two-eluant gradients, as well as program the wavelength,

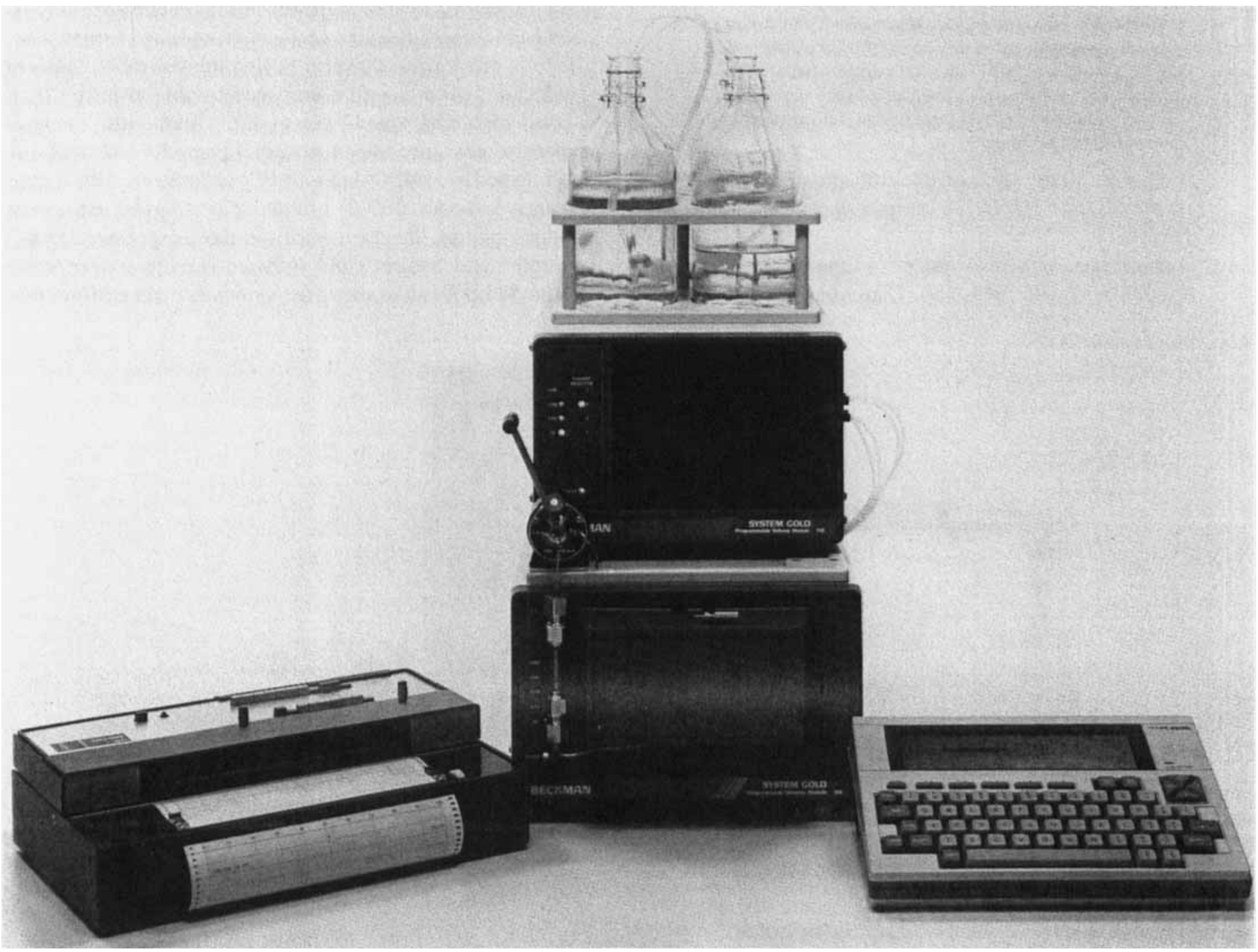

FIGURE 67. Beckman-Altex System-Gold "Protein I System", showing two 110B rapid-refill, one-piston pumps (left), controlled by a NEC lap-top computer (right) controlling a 116 one-eluant pump module (with one rapid-refill, one-piston pump) (middle top) sitting on a 116 Programmable UV/vis detector (middle bottom) feeding data to a chart recorder (left). (With permission.)

FIGURE 68. Beckman-Altex System-Gold "338 Gradient LC System", showing two 110B rapid-refill, one-piston pumps (left), controlled by a NEC lap-top computer (right, top), interfaced with a 406 Analog Interface Module (right, middle) and both sitting on a 116 Programmable UV/vis detector (right bottom). (With permission.)

attenuation, zero, etc. on the 166 programmable UV/visible detector ($5600). (A printer and data system cost extra.)

3. Sophisticated, expensive system: "Amino Acid System" (Figure 69) (about $49,000). Using the 126 two-eluant pump ($13,300) that can be time-programmed to select among four-eluants to either pump head, the system is controlled by with IBM PS/2 Model 50 PC with hard disk, floppy disk drive(s), and color graphics (about $5000) and System Gold Chromatography Software ($7000). The PC controls the pumps for high-pressure two-eluant gradients, sets the wavelength, attenuation, zero, etc. on the 166 programmable UV/visible detector ($5600), controls the 80-position 506 Autosampler (described in the previous section), and processes data on the video screen. A 235 Column Heater and 231 Post Column Reactor are also included. (A printer costs extra.)

A limitation of the System Gold may be the high cost of software ($7000), compared with about $1500 for Gilson-compatible systems and about $2000 for Autochrom-compatible systems, described above. However, the System Gold software may be more sophisticated and may provide more capabilities. There is often a resistance to buying the services of software production, even though a user may be able to justify hiring a consultant, which may be less useful. Additionally, the least expensive new (one-eluant isocratic) pump, the 116, with the least expensive NEC lap-top PC controller is still rather expensive (about $8000). In the other format (two-eluant gradient pump), the 126, with the most expensive IBM-PC controller and System Gold software is extremely expensive (about $25,000). However, the computers can perform other

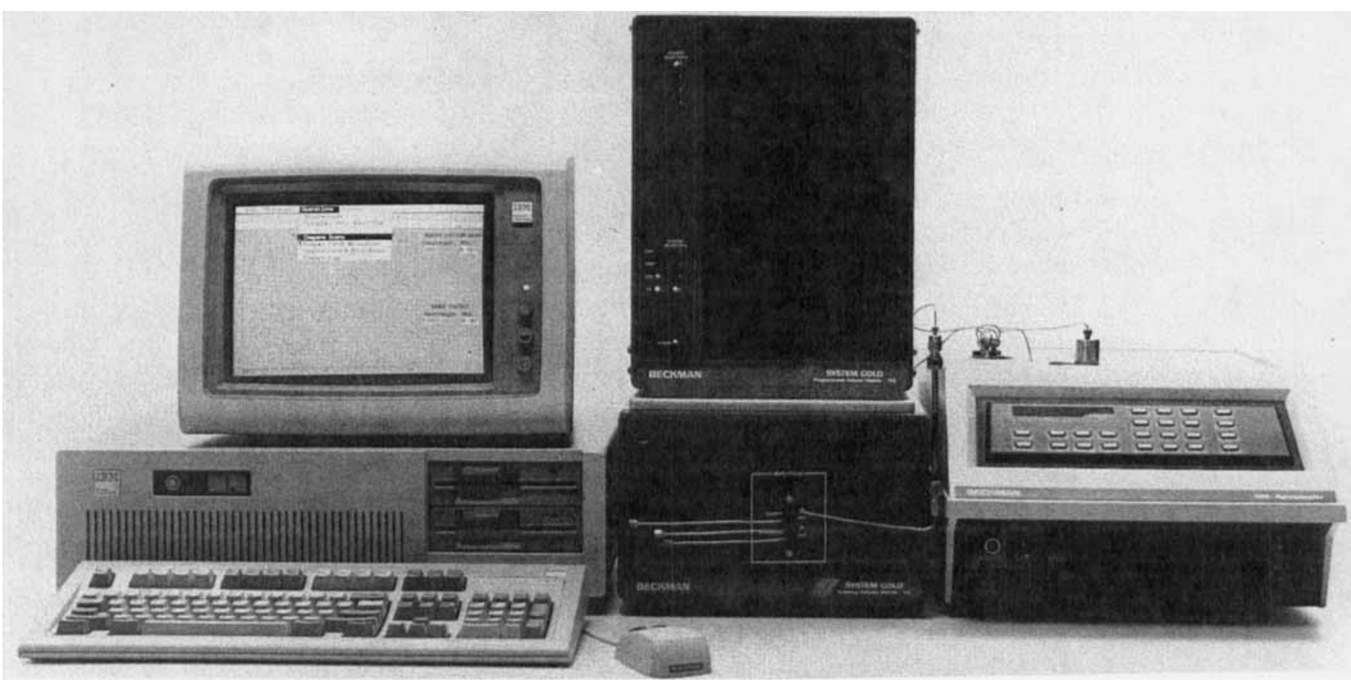

FIGURE 69. Beckman-Altex System-Gold "Amino Acid System", showing a 506 autosampler (right), 135 column Heater and 231 Post-Column Reactor (not shown), a 126 two-eluant pump module (with two rapid-refill one-piston pumps) (center) on top of a 116 Programmable visible detector, next to an IBM PS/2 Model 50 PC (left). (With permission.)

functions, such as controlling multiple modules, two complete LC instruments, analyzing data, and selecting eluants remotely or by time program.

IV. DIODE ARRAY DETECTORS (DADs)

A. General Principles

The detector to receive perhaps the greatest interest has been the DAD. It has the ability to record complete spectra at many times during a gradient run. It differs from "multiwavelength detectors", which typically record chromatograms at several wavelengths, but complete spectra are not taken at any time.

Perhaps a more general term is "rapid scan detector", since some such instruments do not use arrays of diodes; for example, the Barspec instrument uses only a single diode and moves the light source over the diode.

Today, most of the major U.S. and European LC companies offer DADs, as do several Japanese firms.[103] DADs typically pass the entire spectrum of light through the sample cell, then onto a dispersing element (grating), and finally to a diode array for detection. Diode arrays typically have from 35 to over 500 elements on a tiny chip less than about 0.5 cm wide and 8 cm long. A DAD typically takes the spectrum from 190 or 200 nm up to approximately 350 to 600 nm of a 3- to 10-μl volume of eluant from the column as often as 1 to 100 times a second. This can lead to a vast amount of information (see Section V).

A key component of the DAD is a microcomputer not only to take the information at high speed from the diode array, but also to process and display the vast amount of information in a useful way. Making that information useful quickly is a source of widely varying capabilities of different instruments. The ability to process the information, and technical support to make that instrument useful to the needs and applications of the user, should be given the utmost consideration when buying a DAD.

Appearing about the same time as the DAD was a multiwavelength detector of intermediate capability and performance. Typically, these are able to monitor a maximum of two to eight wavelengths at the same time and construct chromatograms at each of these wavelengths. Wavelength ranges and data sampling rates are similar to the DAD, however, computer and memory capacity are not so great, therefore costs are lower. Often multiwavelength detectors can perform several of the functions of the DAD, including wavelength ratioing, postrun chromatogram reconstruction, and baseline subtraction. These multiwavelength detectors are not covered here.

The capabilities of all of the DADs are in rapid flux today. Most of the innovations are coming from the ability to do new and clever things with the data and in presenting that data faster. Already the 16-bit computer used with current instruments is being replaced by 32-bit computers.

Capabilities of DADs are continuing to evolve. Currently, the types of plots include:

1. *Single wavelength plot* (absorbance plot, or simply a "chromatogram") shows the usual chromatogram of absorbance vs. elution time at a specific wavelength. Figure 70 (bottom) and Figure 71 (bottom) show chromatograms at different wavelengths on the Millipore-Waters 990 DAD.[104]
2. *Dual-wavelength plot* or *multiwavelength plots* show two or more chromatograms (absorbance vs. time) on the same video screen (or printout). The chromatograms may be overlapping (see Figure 70, top) or one above the other (Figure 72).
3. *Spectra* (absorbance vs. wavelength) are shown on individual peaks.

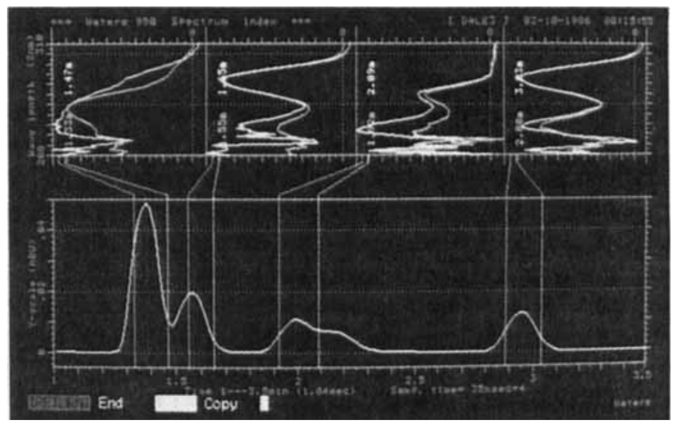

FIGURE 70. Dual (color screen) presentation of chromatograms (bottom) on the Waters-Millipore 990 DAD. The automatic overlay of spectra (top) acquired from the upslope (in blue), crest (in red), and downslope (in white) of each of four peaks is then automatically normalized. (With permission.)

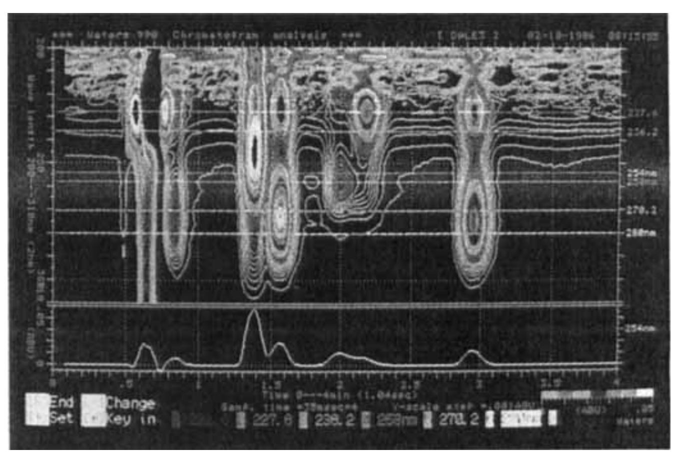

FIGURE 71. Dual (seven color screen) presentation of chromatogram (bottom) chosen at the desired wavelength by driving a cursor over the contour plot (top) on the Millipore-Waters 990 DAD. (With permission.)

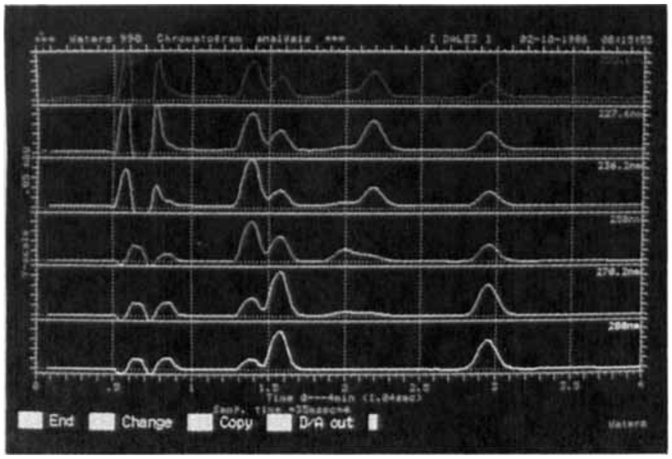

FIGURE 72. Seven-wavelength plot (each in a different color) on the Waters-Millipore 990 DAD. (With permission.)

4. *Spectral overlays* (stacked spectra) show two or more spectra that are overlaid for comparison. These may also be normalized so they have the same height. Figure 70 (with lines in different colors) shows the automatic overlay of spectra (top) acquired from the upslope, crest, and downslope of each of four peaks on the Waters Millipore 990 DAD. Figure 73 shows an overlay presentation used in a spectral library search on the LKB DAD, and Figure 74 shows that up to six spectra can be overlaid by the Millipore-Waters 990 DAD.
5. *Three-dimensional perspective (3-D) plots* ("topograms" of LKB) are those of wavelength vs. time vs. absorbance with the possibility of rotating the point of view around the absorbance axis. 3-D plots are shown from LKB (Figure 75) and Millipore Waters (Figures 76 and 77).
6. *Contour plots* show wavelength vs. time vs. absorbance with a "bird's-eye view" down on the wavelength vs. time axis with contours connecting common absorbancy zones (the middle of Figures 78 and 79).

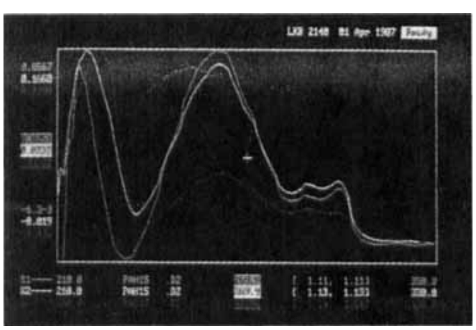

FIGURE 73. Spectral overlay presentation used in a spectrallibrary search in which the known and unknown peaks are normalized to the same height, showing a poor match at low wavelength on the LKB DAD. (With permission.)

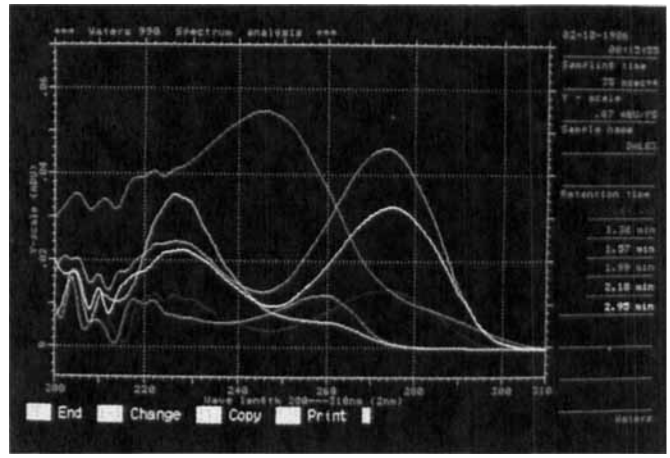

FIGURE 74. Spectral overlay of six spectra on the Waters-Millipore 990 DAD. (With permission.)

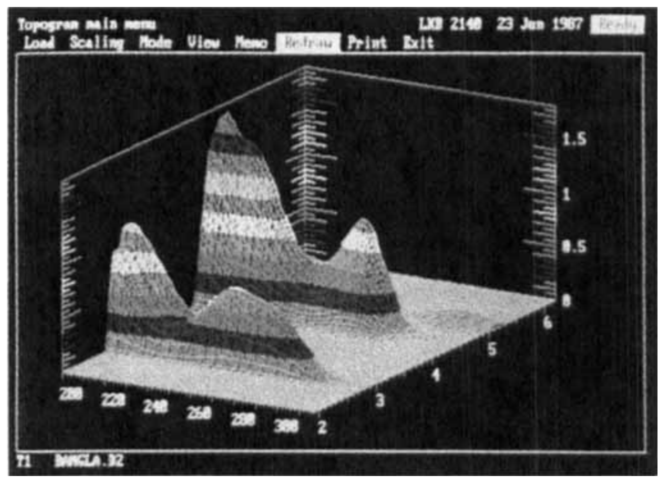

FIGURE 75. Three-dimensional perspective plot ("Topogram") of a nucleoside separation, showing wavelength (left to right), elution time (center to upper right), and absorbance (bottom to top) using "Wavescan EG" software and LKB "Rapid Spectral Detector". The view can be rotated around the vertical (absorbance) axis and tilted on the wavelength-time plane. Twenty different color-coded absorbance levels are possible in the time-wavelength planes. (With permission.)

7. *Ratio-plot* (or "ratiograms") is the ratio of absorbances at any two wavelengths vs. time for qualitatively assessing purity of peaks if the two wavelengths are chosen properly (not at an isosbestic point). Impure peaks will show a sloping ratio vs. time as can be seen in Figure 80 (upper line, right) and Figure 81 for ACTH peptides. In Figure 80 the first peak appears pure because the ratio of absorbance at wavelengths 280 and 254 is flat.
8. *"Peak-max"* plots are those in which a composite chromatogram is shown of time vs. absorbance, but each peak is displayed at the wavelength of maximum absorbance.

FIGURE 76. Waters 990 DAD showing the computer (left) and optical unit (right rear), and printer (right front). The three-dimensional perspective plot on the color screens shows wavelength (top to bottom), elution time (left to right), and absorbance (the visual vertical rise of the absorbance). The view can be rotated around the vertical (absorbance) axis and tilted on the wavelength-time plane.

9. *Corrected plots* are those in which the background absorbance of the eluant (gradient or isocratic) is subtracted from the sample chromatogram.
10. *Difference plots* are those of absorbance vs. time in which the absorbance is the difference in absorbance of any two preselected wavelengths.
11. *Percent transmittance plot* gives both small and large peaks on scale, small peaks are exaggerated, and large peaks reduced.
12. *Log absorbance plot*, like the percent transmittance, has the advantage of keeping both small and large peaks on the same scale; for comparing two chromatograms, for example, one at twice the sample size as the other, both large and small peaks will be displaced by the same distance (e.g., $\log 2A = \log 2 + \log A = 0.3 + \log A$). This permits visually assessing if two spectra are from the same compound if lines of different height always show the same separation (not possible by comparing simple absorbance plots).

The total capabilities of DADs are in rapid change today. DAD hardware changes are slower, but each manufacturer now makes frequent changes in:

1. New, faster, and less expensive computers
2. Faster presentation of data
3. New and more clever manipulations of data

B. Early Diode Array and Multiwavelength Detectors

The first multiwavelength detector known to this author was described at a European chromatography meeting in Montreux, Switzerland, in 1973. To present the vast amount of information,

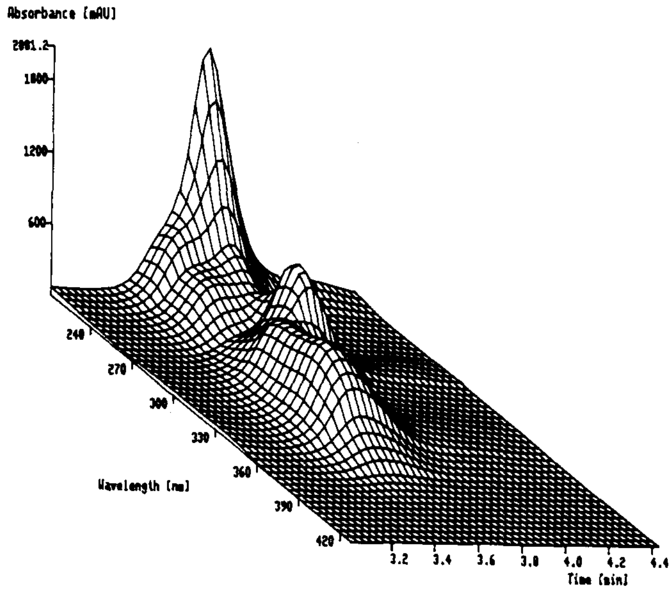

FIGURE 77. Three-dimensional perspective plot as a black-and-white printout showing wavelength (upper left toward lower right), elution time (left to right), and absorbance (the vertical hills, bottom to top) using the HP 1040A DAD system. The view can be rotated around the vertical (absorbance) axis and tilted on the wavelength-time plane.

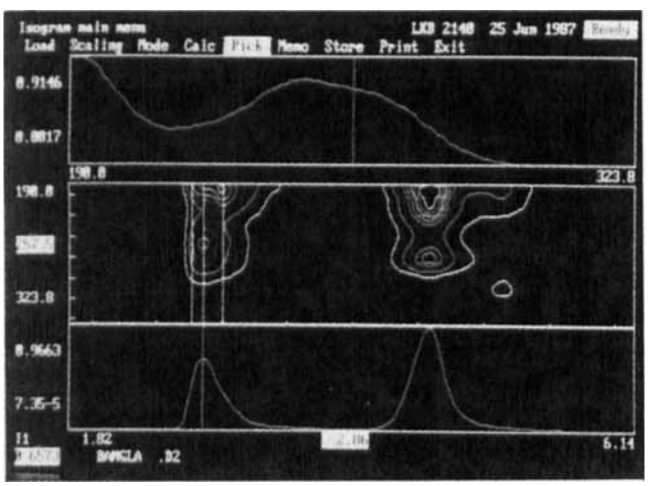

FIGURE 78. Split-screen capability (in color) of the LKB DAD showing: (1) a contour plot (center with wavelength running top to bottom) and time (left to right); (2) a spectrum (top) at the time of the contour plot cursor (the cross near the center); and (3) a chromatogram (bottom) at the wavelength of the contour plot cursor. (With permission.)

the authors used a movie, with the forward motion of the film being time, the Y-axis was intensity, and the X-axis was wavelength (as found in a usual spectrum). This was a most unusual presentation as a blank baseline, the spectra, changed as absorption spectra rose and fell as each peak eluted. This portended the first commercial DAD.

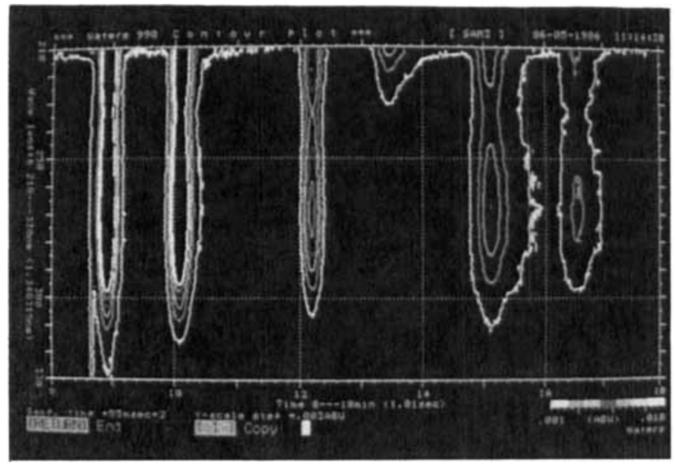

FIGURE 79. A contour plot (wavelength running top to bottom) and time (left to right) in seven colors on the Waters-Millipore 990 DAD. (With permission.)

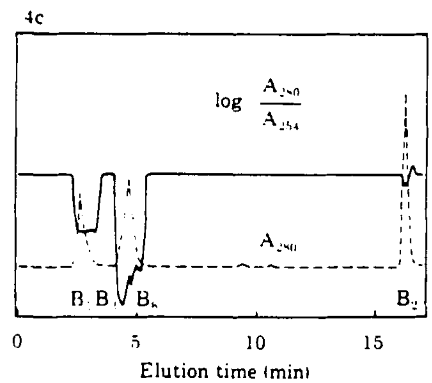

FIGURE 80. Ratio-plot of various B vitamins on the LKB DAD. (With permission.)

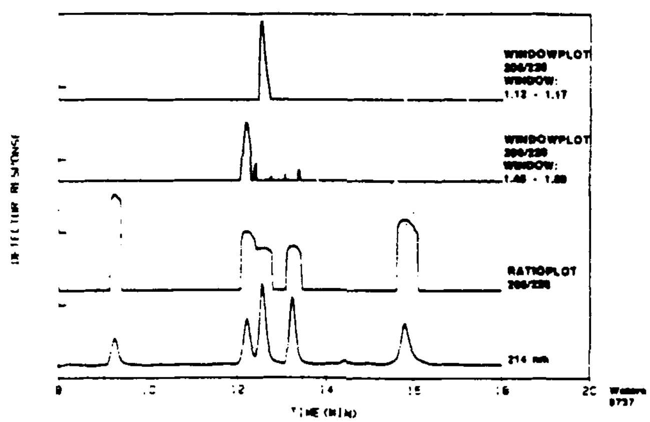

FIGURE 81. "Peak-max" plot on the Waters 990 DAD (see text for details). (With permission.)

An early effort to show the usefulness of obtaining spectra was demonstrated by Varian in 1972 in Frankfurt, Germany, with a Varian syringe pump instrument set up for stopping eluted peaks inside the cell of a scanning spectrophotometer. Also, this was one of the first occasions when a LC instrument was actually operating at a trade show.

One of the first wavelength scanning detectors was from Spectra-Physics in about 1975. It used a vibrating mirror to scan the dispersed spectra over a single-element light-sensitive diode. Since a large and costly computer was required to handle the data, costs were very high (about $50,000).

Micromeritics came out with a DAD with similar specifications to the Spectra-Physics instrument at a lower price that did not use the mechanically vibrating mirror, but probably was the first company to use an array of diodes. Again, speed was slow and costs were high.

In 1977 Hewlett-Packard came out with its full microprocessor-based liquid chromatograph.[105,106] The following year they made available their first dual-diode scanning-type detector. This could stop flow and scan through a spectrum from 190 to 600 nm in approximately 2 min by driving the photodiodes along the dispersed spectra. In about 1982, Hewlett-Packard introduced the first of the modern rapid scan DADs. This had many new capabilities. A chromatogram at the desired wavelength was generated on-line as the chromatogram eluted. A postrun "perspective plot" of time (X axis) vs. absorbance (Y axis) vs. wavelength (Z axis) could be plotted on a chart recorder in some tens of minutes after the run. The video screen (or printer plotter) could display a chromatogram at any desired wavelength with the six or eight individual spectra of each peak plotted above.

In 1984, the Hewlett-Packard 1090 was introduced with much faster software. Software updates have been made since. The latest versions are described below.

In late 1985, LKB came out with a DAD using colored graphics (both on the video screen and printer) and some novel methods for computer display and manipulations. They introduced the "bird's-eye" contour plot view of the chromatogram (their "topogram") with the advantage that absorbances or wavelengths are not obscured by other sections of the spectra, as happens sometimes with the perspective plot. In the contour plot, time runs from left to right, and wavelength runs from bottom to top. Contour zones (not lines) were colored solid to represent isoabsorbance zones, and these zones could be displayed either on the video screen or on a colored printout. (These zones were replaced with simpler colored lines on their most recent version, described below.) A monitor line could be run along the time axis to display separately spectra (absorbance vs. wavelength) on the video screen or printout anytime in the run, or a similar line could be run along the wavelength axis to display the chromatogram at any wavelength.

C. Hewlett-Packard 1090 Diode Array Detector

In 1984, Hewlett-Packard introduced their 1090 liquid chromatograph with an updated version of the DAD (Figure 82) that they introduced in 1982 in the 1080 liquid chromatograph.[107] The 1090 DAD uses a 211-element diode array. Unusual features of this DAD include:

1. The video screen can display a chromatogram at any wavelength from 190 to 600 nm, as well as the "current spectra" while the peaks are eluting.
2. Spectra from 190 to 600 nm can be memorized at approximately 25 spectra per second, therefore very fast-eluting peaks can be distinguished without stopping flow.
3. Detection can be performed in parallel for up to eight wavelengths, with separate integration and calibration on all of the wavelengths.
4. Integration parameters can be changed off-line after the run, to optimize the integration without the need for sample reinjection (baselines can be drawn in).
5. Raw data (area count at each time and wavelength) can be stored automatically on a disk drive (the HP 9121D).
6. Perspective plots can be drawn after the run, at any rotation about the Y axis (absorbance axis).
7. Multipoint calibration can be used for accurate quantification over a broad sample range.
8. Known and unknown chromatograms can be superimposed to aid in peak identification.
9. The detection wavelength can be programmed to change at any time; the "autozero" function eliminates baseline shifts.
10. The signal-to-noise ratio for each peak can be optimized by selecting both detection wavelength and peak width.
11. The signal-to-noise ratio can be optimized by averaging data points in the time axis.
12. The detector cell is usable with low-dispersion methods since the path length is moderately long (6 mm) and volume low (4.5 μl).
13. Data presentations typically include a chromatogram at any wavelength with up to six spectra across the top of the page as well as full run conditions.
14. A single computer, the HP-85, can be used to control the detector, manipulate data, and program in BASIC for special data manipulations (provided the user knows BASIC programming).
15. Numerical and graphic data (e.g., as several overlapping chromatograms or spectra) can be displayed on the video screen of the HP-85 PC.
16. Numerical or graphic data as low-resolution chromatograms or spectra can be printed out on the roll paper of the HP-85 PC (a narrow 10-cm paper).
17. Numerical or graphic data as high-resolution chromatograms or spectra can be drawn on the high-resolution, multicolored printer plotter (HP-7470A).
18. Data can be transferred by two analog signal outputs to integrators (such as the HP3390A), or chart recorders,

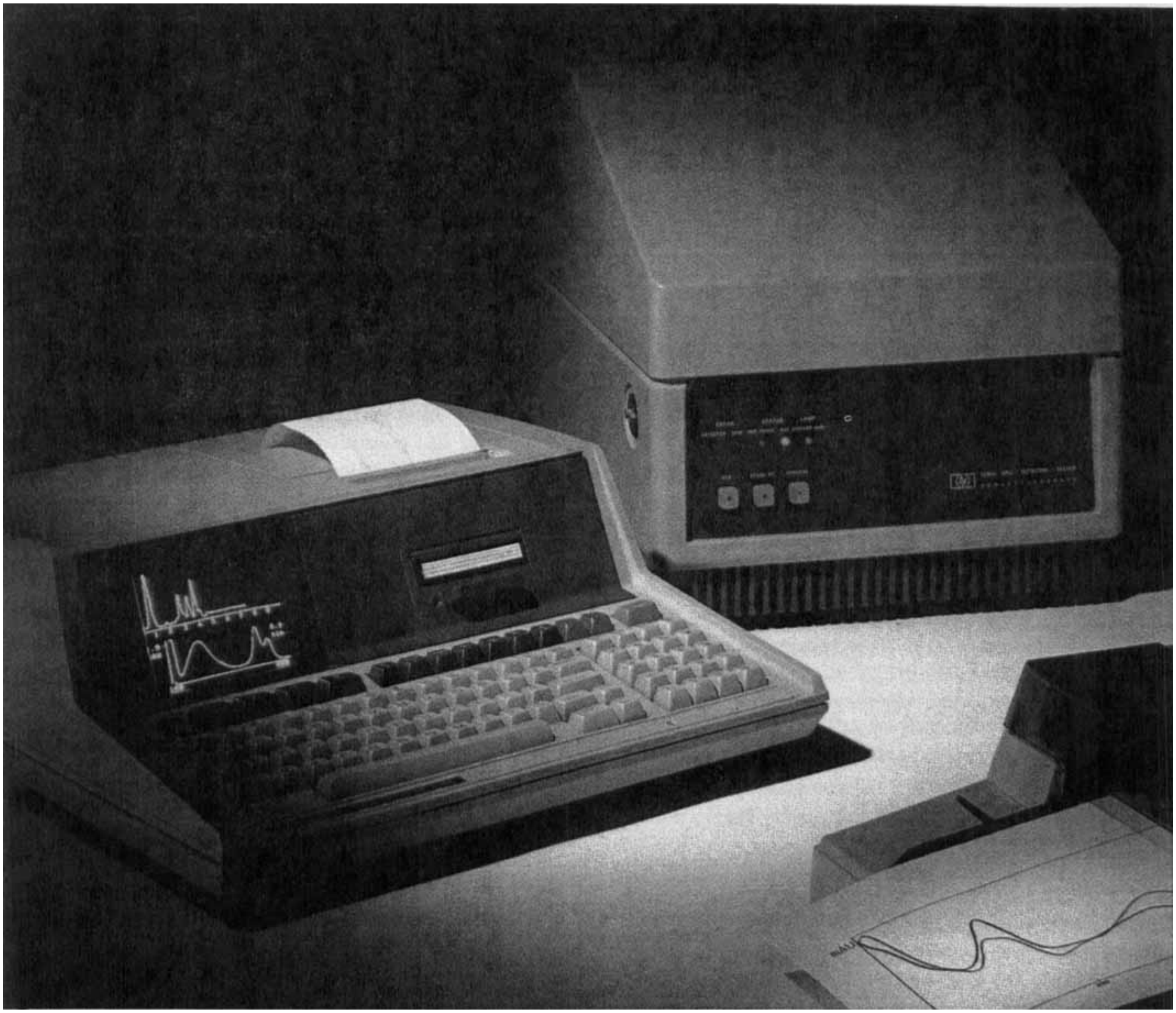

FIGURE 82. The Hewlett-Packard 1040 DAD showing the optical unit (rear, right) and computer (left). The memory unit is not shown. In the current 1090M system, the small computer is replaced by an IBM-compatible computer for faster data computation and color presentations. (With permission.)

or dual flexible disk drives (such as the HP-82901M) for high-speed data recording.

An easily overlooked innovation with the Hewlett-Packard systems has been the HP-85, a PC with a built-in video screen and *printer*. Few other PCs have built-in printing and graphics capabilities. However, the video screen is very small (approximately 3 in.), and the printer paper is very narrow (approximately 10 cm).

A problem with the Hewlett-Packard systems, in general, are that they are a completely integrated package without the possibility of using individual modules separately. Hence, the LC control function, autosampler control, detector, and integration systems are intimately tied together, and this cannot be purchased or used separately with other LC instruments. Because modules are not readily substituted one for another, this is often not as convenient for troubleshooting components, for example, by substituting one DAD for another. As consequence of being an early introduction into the DAD market, the high speed of data collection (25 spectra per second), and the wide wavelength range (190 to 600 nm) plus data from 211 diode elements, the 1090 produces very large amounts of data and requires sophisticated memory storage. Consequently, the price for a system is high (about $25,000 to $35,000). Later DAD detectors reduced costs by more limited, but more clever, use of software.

D. Varian Polychrom 9060 DAD

Introduced in 1984 (Figure 83), the Varian Polychrom 9060 has the following features:[108,109]

1. A video screen PC-based screen is used to present data and chromatograms (vs. a plotter-based system).
2. The wavelength range is from 190 to 367 nm.
3. The bandwidth can be set from 4 to 152 nm.
4. Scan rates can be set at 5, 11, or 16 scans per second.
5. Linearity is good: 1% up to 1.5 AU at 263 nm with acetone in water.
6. Noise is low: 60 μAU (0.00006 AU); conditions are 4-nm bandwidth and 11 scans per second and a time constant of 0.5 s with methanol at 239-nm detection.
7. Drift is below 1000 μAU/h (conditions are 239-nm detection and thermostating to 1°C).
8. Flow sensitivity is below 1000 μAU/ml/min (conditions are 1- to 2-ml/min flow range and 239-nm detection).
9. Spectra are taken automatically at peak maxima and at inflection points.
10. The detector cell can be used to 2000 psi.
11. The detector cell is in an easily accessed interchangeable cassette.
12. The detector cell is compatible with low-dispersion chromatography by using a 6-mm-long cell of 4.7-μl volume.
13. A RS232 port permits sending data to an external computer.
14. Time constants can be selected from 50, 250, 450, or 2000 ms.
15. The lamp may be programmed to shut off after the last sample and turn on for warmup in the morning of the next day.
16. The lamp is prealigned for easy change.

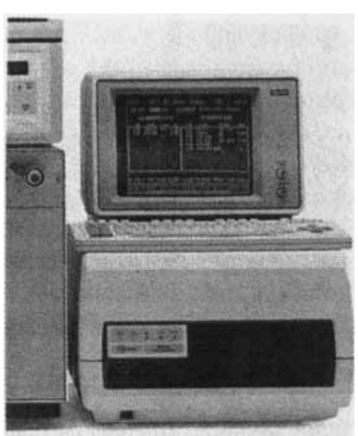

FIGURE 83. Varian 9060 DAD showing the optical unit/processor (bottom) and video screen/keyboard (top). (With permission.)

17. Self-diagnostic software routinely tests the system with every startup.
18. Software permits 80 spectra to be stored per run.
19. A single deuterium lamp provides spectra from 190 to 367 nm.
20. The video screen can be programmed to display chromatograms at any wavelength, stored spectra, peak ratios, or to run data.
21. Analog outputs provide chart recorder chromatograms at any wavelength, ratio plot, etc. (These outputs can be used for printer plotters or auxiliary computer data storage.)
22. The video display is very versatile, giving the possibility of displaying chromatograms at any two wavelengths, or a peak-max plot in which a composite chromatogram is shown with each peak displayed at its wavelength of maximum absorbance.
23. Two analog channels permit output of chromatograms, ratio plots, or purity parameter (at 10 or 1000 mV).

The last item, the Varian "purity parameter", gives a more quantifiable measure of whether or not a peak is pure.[110] Additionally, an identical purity parameter as a known peak eluting at the same retention time provides very strong evidence that the known and unknown peaks are the same. The Varian purity parameter is an algorithm for determining a wavelength, and this wavelength is weighted for the level of absorbance. A symmetric absorbance vs. wavelength plot would give a purity parameter corresponding to the wavelength of maximum absorbance. However, most common molecules absorb more at lower wavelengths, and, hence, the purity-parameter wavelength is generally at a lower wavelength than the usual absorption maxima.

E. Pharmacia LKB Diode Array Detector

In 1985, LKB (recently merged with Pharmacia) introduced its DAD detector with color monitor, color graphics, and color printer (Figure 84).[111] Wavelength selection is based on passing the UV light from a deuterium lamp through the detector cell and onto a diode array. The spectrum from 190 to 370 nm places 180 nm of light across the 265-element diode array, giving better than 1-nm resolution per element.

The full product consists of a number of components:

1. The "Rapid Spectral Detector" itself consists of an "optical unit" and a separate "data unit", a 16-bit dedicated computer for holding up to 400 spectra (about $15,000).
2. A 640-K RAM IBM-compatible PC such as the IBM-PC, IBJ P:C-XT, or AT&T-XT for acquisition, storage, and postrun evaluation of the spectra data (about $3000).
3. A high-resolution color monitor (e.g., CH8460B) (about $1000).

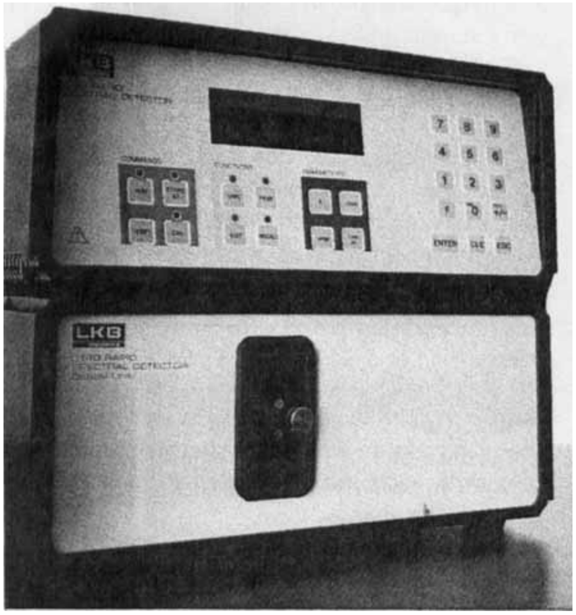

FIGURE 84. LKB DAD optical unit (bottom) and control unit (top). The data manipulation computer and memory system and printer are not shown. (With permission.)

4. WAVESCAN® software for the proper computer ($2000).
5. "Chromatography Software" for integration of chromatograms on the IBM computer (about $1000).
6. "Spectral Search" software provides two programs: (1) a "Library Editor" program for the user to create, edit, and store his own library of UV spectra, and a "Library Search" program to compare rapidly unknown to known spectra[112] (about $2000).
7. Hard disk memory for storage of raw data (about $2000).
8. A color printer such as the IBM Colorjet Printer.
9. Miscellaneous interface cables, cards, and coprocessors.[113]

A new feature of this DAD is that many display capabilities can be chosen to feed through four analog outputs. For quantification or displays, these signals must be fed to a four-pen recorder or a four-channel data logging system with a video. The LKB DAD can store in local memory 4000 spectra (e.g., at 4 spectra per second = 1000 second = about a 20-min run). The detector makes a digital record at each point in time of the absorbance at each wavelength. These data can be unloaded onto hard disk memory, if desired. This DAD can be programmed to take spectra either at fixed time intervals or as a peak elutes (on the upslope, crest, and downslope of the peak).

The power of the LKB DAD is that postrun manipulations can produce very complex displays. Slowing individual chromatograms, spectra, contour plots, 3-D perspective plots, ratio plots, or "total wavelength chromatography plots", by bunching wavelengths from 4 to 180 nm wide, permits a composite chromatogram to show the total complexity of the sample.

Split-screen formats permit very useful combinations of the above plots. These are

1. *Contour plots* of two samples can be displayed.
2. *Two dimensional graphic windows* can be used to show, for example, two spectra or two chromatograms. Data in one window can be moved or stretched in either axis for comparison.
3. *Three-window plots* show the contour plot with a spectrum (absorbance vs. wavelength plot) above, determined by the location of the time-axis cursor, and a chromatogram (absorbance vs. time plot) below determined by the location of the wavelength-axis cursor.
4. *Spectral overlay* of up to four spectra from the same or different chromatogram runs is possible, and each can be normalized to fit within the display window (thus, identical spectra should exactly overlay). High-sensitivity spectra can be filtered to eliminate noise.
5. *Chromatogram overlay* of up to four chromatograms from the same or different runs is possible, and each can be normalized to fit within the display window. High-sensitivity chromatograms can be filtered to eliminate noise. Chromatograms can be moved and the X or Y axes increased or decreased (scaled) to compare runs better.
6. *Chromatogram overlay with mathematical operations,* for example, subtracts the signal at one wavelength from that at another, and small peaks can be made visible, if the wavelengths are properly chosen. Figure 81 shows that ratioing the signal at 220 nm over the absorbance at 280 nm gives a "ratiogram" that can be used to assess the purity of the peaks.

The software in this system permits a "match factor" that is a measure of spectral similarity. The match factor is calculated by a point-by-point comparison of the unknown spectrum vs. a reference spectrum. Since both spectra are normalized to the same height, so concentration differences are unimportant. Absorbance differences are accumulated and subtracted from 1.000, a perfect match. A match factor of 0.970, for example, indicates a 3% difference between the unknown and known spectra. Their data suggest that match factors below 0.970 indicate possible discrepancy in identity.[114]

Other unusual capabilities of the Pharmacia LKB detector include:

1. Four recorder outputs to a four-pen chart recorder can display (1) chromatograms with variable peak width from 4 to 180 nm; (2) spectra; (3) spectra suppression plots; and (4) logarithmic absorbance ratios.
2. The absorbance can be measured of fast-eluting peaks,

because spectra are taken in 12 ms and time constants can be set down to 200 ms (and up to 10 s).
3. The detector cell is 5 μl with a 5-mm path length.
4. Noise is below 300 μ-AU (at 235 nm, 1-s integration time).
5. Drift is below 300 AU/h (at 235 nm).
6. Attenuation is settable from 0.001 to 1 AUFS.
7. Menu-driven programs permit fast manipulations of spectra.

Limitations may be the LKB is not a dual-beam instrument, so lamp voltage fluctuations or lamp aging could affect the signal. A complete system is costly (about $40,000), and visible wavelength above 370 nm is not available. Much of the power of the LKB DAD comes from postrun manipulations. The on-line run output is the contour plot. Other DADs (such as the Waters 990 and Hewlett-Packard 1090) can display in real time (as the chromatogram is developing) many other displays, such as spectra, wavelength ratios, etc.

F. Millipore-Waters 990 DAD

In about 1986, Waters-Millipore introduced its 990 detector, now upgraded to the 990+ ("990 plus") DAD detector with color monitor and color graphics (Figure 76).[115] Wavelength selection from 190 through 800 nm is based on passing the UV light from a deuterium lamp through the detector cell and onto a 512-element diode array.

The full product consists of the optical unit itself, and a separate NEC Powermate II computer, software, and printer plotter (all for about $23,000). Additional software permits searching of spectral libraries.

The power of the 990+ is that postrun manipulations can produce very complex displays, showing individual chromatograms, spectra, contour plots, perspective plots, ratio plots. A special "spectrum index plot" bunches wavelengths to permit a composite chromatogram to show the total complexity of the sample. Split-screen formats permit very useful combinations of the above plots. One of these, the "window plot", bunches the signal from all wavelengths and then sharply reduces (attenuates) the displayed signal on either side of the signal maximum. This plot can be very useful for controlling fraction collectors to isolate sample in preparative chromatography (Figure 81, top two lines). Software permits a "goodness of fit", which is a measure of spectral similarity.

Other capabilities of the 990+ DAD detector include:

1. The absorbance can be measured of fast-eluting peaks, because spectra are taken in 12 ms and time constant can be set down to 200 ms.
2. The detector cell is 8 μl with a 10-mm path length.
3. Noise is below 100 μAU (at 254 nm).
4. Drift is below 500 μAU/h (at 254 nm).
5. Wavelength accuracy is ±1 nm.
6. Resolution is 1.4 nm.
7. Linearity is from 0.1 to 1.6 AU.

G. Barspec Chrom-A-Scope

Introduced in 1986, the Barspec Ltd. (Rehovot, Israel) produced a rapid scan detector that is less costly than many other systems (Figure 85).[116] This instrument uses an IBM PC for data manipulation. Not a DAD, but still a rapid scan detector, a single detection element is used after the detector cell and after a grating driven by a special mechanism that permits 10 spectra per second to be recorded. This gives them "straight optics" in which sample is irradiated only by a narrow wavelength of light. Barspec suggests that this is better than "reverse optics", which irradiate the flowing sample continuously with white light, which potentially can damage some samples by UV irradiation or heating. These optics along with an inlet and exit slits give a well-defined bandwidth with lower stray light and higher sensitivity (baseline noise is better than 100 μAU). The scanning mechanism also allows continuous dark current correction giving drift said to be "virtually zero". The Chrom-A-Scope has these additional exceptional features:

1. Cost is low (about $15,000) compared with many earlier systems (about $25,000).
2. The unit is compact (13 × 9 × 10 in.).
3. Two wavelength ranges are available, a 190 to 370-nm model and a 190- to 700-nm model.
4. Display of data is versatile, with the possibility of 3-D (perspective) graphs, contour plots, and three simultaneous chromatograms (with different parameters).
5. A high-speed 14-bit A/D converter and "direct memory access" to input data to the computer, instantaneous spectral acquisition is achieved with instantaneous 3-D plots as the chromatogram is developing.
6. It is possible to replace the silicon-diode detector with a photomultiplier tube when dealing with low-intensity light.
7. At startup (or when requested) the computer performs diagnostic tests of all detector power circuits and control system logic circuits.
8. Extensive troubleshooting information is provided by the software.
9. Ventilation slots and the lamp location in the back plane of the instrument aid in thermal stability and low inside temperatures.
10. Wavelength repeatability is better than 1 nm and wavelength accuracy ±1 nm.
11. Spectral bandwidth is adjustable from 5 to 180 nm in 1-nm increments to produce summed spectra.
12. The IBM-PC and software uses MS-DOS® so the computer can be used with many other software systems for word processing, spreadsheets, data bases, etc.
13. The detector is potentially compatible with low dispersion and fast methods because response time is fast (software

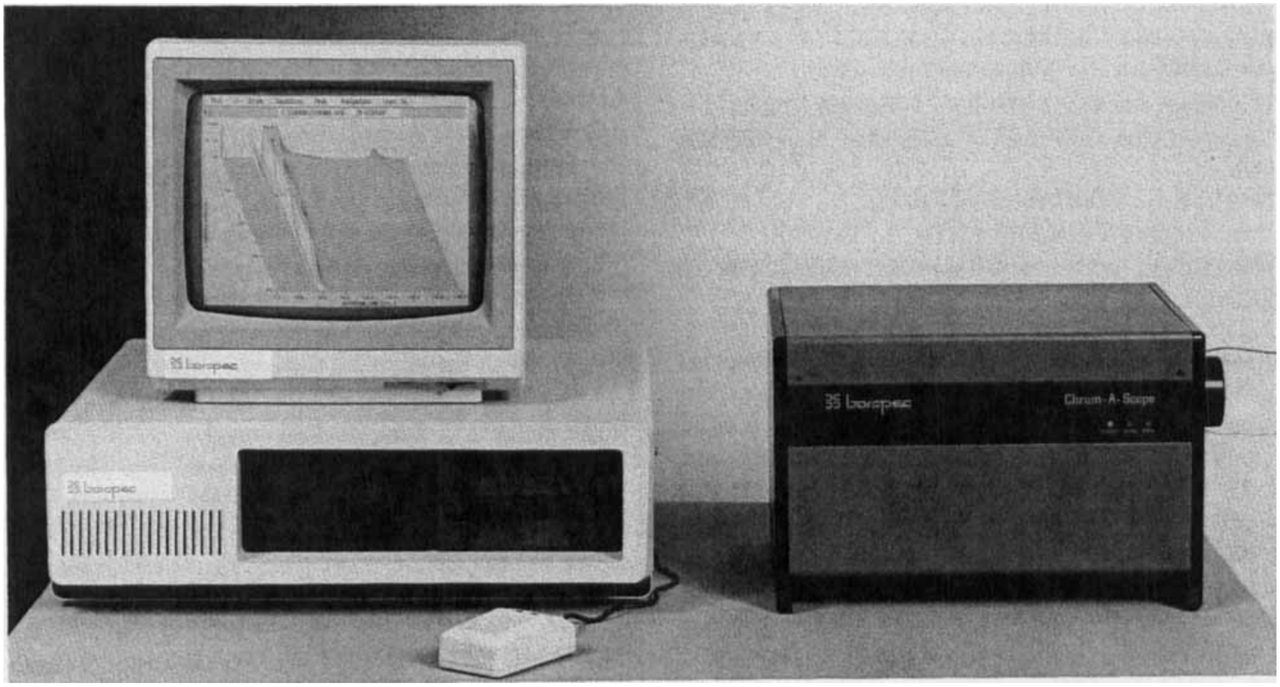

FIGURE 85. Barspec Chrom-A-Scop DAD optical unit (right) and the IBM control computer and memory system (left). (With permission.)

programmable from 100 ms to 15 s), spectra scanning is fast (100 ms), cell volume is low (5 μl), and path length is good (0.5 cm).

14. Integration capability is built into the software.
15. A seven-line liquid crystal screen provides three self-prompting screens that make use simple.

Interaction is said to be very user-friendly, and mastery of the operation of the Chrom-A-Scope is said to take only about 1 h. The user interaction is like that pioneered by Apple Computer Company, with the concepts of "desktop-mouse-icon-window" and the capability of free intermixing of text and graphics. The "mouse" controls an on-screen pointer. The pointer controls drop-down menus and icons representing functions (e.g., moving a file to a small picture or icon of a trashcan permits it to be discarded). Windows permit different functions to be seen on the screen at one time. Color or black-and-white graphics are possible. Graphic data of chromatograms can be transferred into the text of a report, giving publication-quality manuscripts. Direct output to a laser printer enables a one-step production of papers and reports.

The software package consists of three modules:

1. *Real-time control module:* permits the user to create, define, and save methods and run time parameters, to specify run conditions, and perform experiments. Data can be saved in the temporary or permanent memory, or viewed on the video screen.
2. *Analysis module:* permits raw data, spectra, chromatograms, instrument settings, and analysis results to be stored on the hard memory or disks. The user may perform smoothing, differentiation, integration, ratioing, report preparation, calibration, least squares analysis, and other methods. Spectra and chromatograms may be viewed simultaneously.
3. *Report module:* permits data (either graphic or alpha-numeric) to be used in the Chrom-A-Scope PC or other IBM-type computers and these data are compatible with word processors, spreadsheets, other computers, and lab networking.

H. Perkin-Elmer LC 235 DAD

Introduced in 1987 (Figure 86), the LC 235 has the following innovative features:[117]

1. Cost is low for DADs (about $13,000).
2. Display of data is versatile, with dual wavelength plots, ratio plots, or a mode in which a plot of any chromatogram (at fixed wavelength) has the wavelength at which the peak shows maximum absorbance printed above the peak.
3. A seven-line liquid crystal screen display provides three self-prompting screens that make use simple.
4. The optical case is dried with dessicant, sealed, and then heated and thermostated to eliminate ambient temperature variations to minimize drift.
5. The detector cell can be used to high pressures (2200 psi).
6. The detector is compatible with low dispersion, fast methods because response time is fast (100 ms) cell

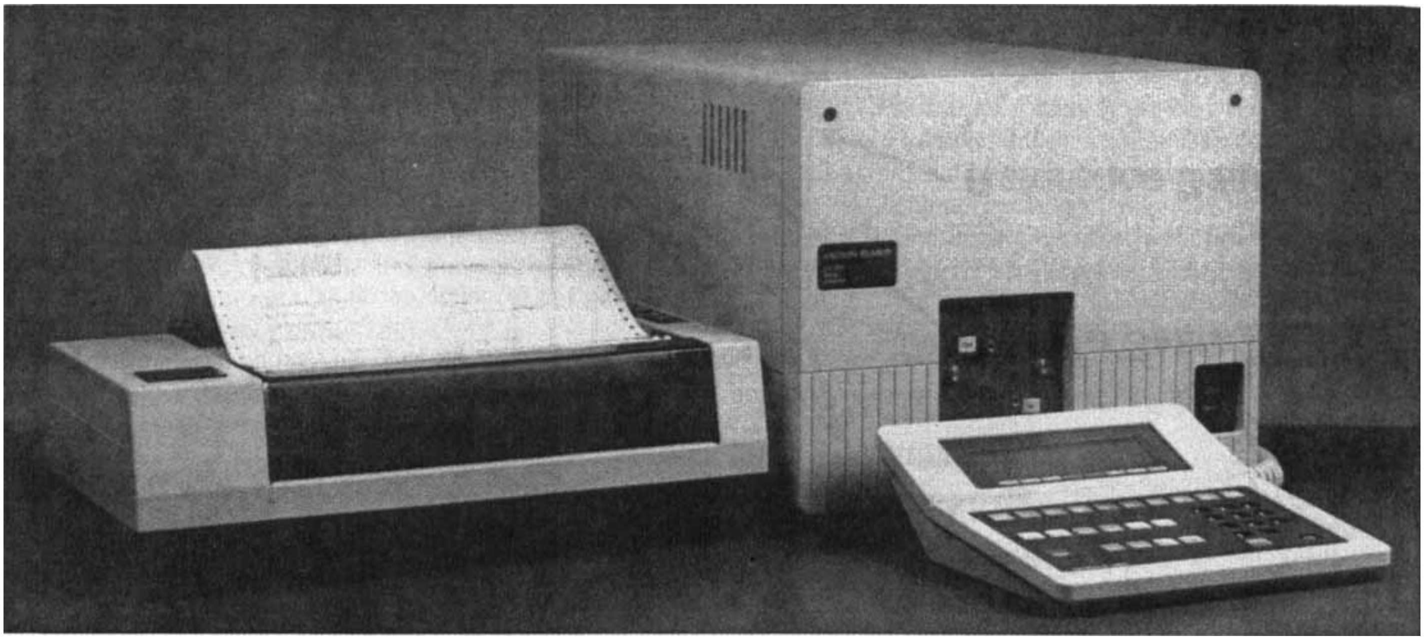

FIGURE 86. Perkin-Elmer LC-235 low-cost DAD with optical unit (right rear) and control unit (right front). (With permission.)

volume is low (8.5 μl), path length is long (1 cm), and peak spreading is low (16 μl at 4 sigma).

The detector cell design is innovative. The cell is made of a polyamide polymer that has low thermal conductivity. Thus, momentary changes in eluant temperature will have minimal effect on the cell dimensions, and, thus, flow noise is said to be reduced over metal cells. This low thermal effect means that thermostating the inlet line is unnecessary, so the peak broadening from the cell can be very low. Because the cell is a polymer and the inlet and outlet tubes are titanium, the cell is biocompatible, i.e., no ferrous metals are present to affect some proteins. Also, corrosive salts and acids have no affect on the cell. The end windows are shaped lenses and the aperture adjusted so that the light is focused through the center of the cells. Refractive index effects from samples and eluant gradients are thus minimal.

The discretely addressable diode elements are different from diode arrays in which the reading of each diode element precipitates the reading of the next diode element. The LC 235 DAD, to minimize noise from signal switching, uses a separate amplifier with each array element. The result is signal-to-noise performance that rivals their conventional variable wavelength detector, the LC-95 (one of the highest sensitivity-variable wavelength detectors). The keyboard is detachable with a 34-key membrane-type keypad. Most controls are under single keystroke command (such as run, scan, print, etc.) though eight self-prompting "soft keys" change their label and function, as different liquid crystal displays are called up. The backlighted, seven-line, liquid crystal display gives real-time absorbance and some detector parameters.

The keyboard permits very versatile display techniques that can help assess peak purity. For example, standards and sample runs can be overlaid, upslope can be compared to downslope spectra for showing that they are identical (or different); spectra of known and unknown peaks can be overlaid (either normalized or scaled to any height); ratio plots at any two wavelengths can be plotted, and retention times, plus wavelengths of maximum absorbance, can also help determine peak purity.

The "output-2" screen permits the choice of the second display to be either wavelength A, wavelength B, A + B, A − B, or A/B (this last display is the ratio plot).

The "detector" screen prompts the user for selection of (1) the A and B wavelengths (from 190 to 600 nm); (2) the sensitivity of the A and B channels (from 0.001 to 1 AUFS); and the bandwidth of the A and B channels (from 5 to 175 nm).

Wider bandwidth scans increase the signal from a trace peak, provided no contaminants are present. On this screen, the above six parameters may be changed up to nine times during a run.

In the "spectra" screen, the modes for taking data can be chosen:

1. At the peak apex (the "manual" soft key)
2. At 20% up the peak (the "upslope" spectra), at the apex, and at 20% down the peak (the "downslope" spectra) repeatedly between a "start" and "end" time (the "auto" soft key)
3. At any specified programmed times
4. At any time manually (the "scan" key on the console)

A two-pen recorder is used for data. On one channel, the "auto" mode for data presentation, the recorded chromatogram gives perhaps the most powerful capability of this detector. At each peak apex on the bottom of the recorder is listed the spectra number, retention time, maximum absorbance wavelength, and the "peak-purity index".

The Perkin Elmer peak-purity index is a specialized algorithm using a numerical value to quantify the purity of a peak. It is an automated data manipulation in which the ratios of absorbances at different wavelengths are summed. An index of 1 should be found for a perfectly pure peak; 0.91 to 1.5 is acceptably pure, and a number larger than 1.5 strongly indicates coelution of two or more peaks.[118] A ratio plot or "ratiogram" is shown on a second channel. If the two wavelengths are chosen properly (not at an isosbestic point), two overlapping peaks will show a sloping ratio vs. time, as can be seen in Figures 80 and 81.

During a run, up to nine changes can be made in any of the parameters, such as detection wavelength or full-scale sensitivity.

A limitation of this detector is the small number of diode elements, only 35, compared with the 512 elements used in some DAD detectors. Thus, with a 10-nm bandwidth, a wavelength range from 200 to 550 nm might be covered.

V. CONCLUSIONS

This review has described the various components important in modern automated LC. The most recent autosamplers, pumps plus gradient controllers, and DADs, show some trends that most likely will extend into the future and will also feed into adjacent fields (such as capillary electrophoresis and flow injection analysis). One very important trend is the increasing use of PCs to control LC components and manipulate data. With the increased use of PCs, there are some needs that are apparent.

Programming of PCs for LC components should grow increasingly more "friendly", and, thus, be simpler, faster, more difficult to make errors, and require less operator training. One current programming device is to use icons: small pictures representing the function, such as a waste can for the "delete" function. "Menu-guided" programming is also important today, since the user is guided through choices of the various parameters. This helps the novice and minimizes forgetting to enter parameters. Future menus should permit the in-place "default" values to be easily changed so users can fit in their own "most used" values, menus should have the provision of "requiring" that critical values be entered before moving to a new field, and menus should permit only "acceptable ranges" of data be entered (e.g., flow might be limited to 0.1 through 10 ml/min). When choices are required, future menus should list the choices, e.g., Pump A, Pump B, etc., instead of requiring the operator to type in the letters, which takes time and typing errors stop the program. These kinds of programming options should be very easy to request. "Shallow" menus are also preferred, with much data on a single screen, without the need to search through layer upon layer of screens to do the programming. Color screens will make use of these menus easier and more accurate. "Mouse" and "joy stick" movement of the cursor is often preferred by the novice to simplify programming, but single-key commands are more often desired by the expert "power users" who want programming speed. Both the novice and advanced user will want "soft keys" that can be user-programmed to perform a complex sequence of key-strokes at the touch of a single key. Future computer innovations in LC might use "touch screens" (recently used on a preparative LC),[119] voice control (recently used with robots),[40] drawing pads, and yet-to-be-discovered methods. New systems should provide all of these conveniences for both the sophisticated and novice user to make programming faster, simpler, and more accurate.

With faster and more powerful computers, "multitasking" computers will become common, so that the computer can be used for one function while it is performing a second function; for example, word processing, report generation, or programming a second instrument can be done while data are being taken and manipulated from a first instrument. Additionally, a single PC will often be able to control many LC instruments.

There is a trend toward a single PC and software that can (1) control the LC, (2) control an autosampler, (3) control a DAD, and (4) accept and manipulate data from several detectors. The first company with a commercial capillary electrophoresis instrument, Microphoresis Systems, has recently introduced its own software innovations[120] to make more secure and more useful the vast amounts of data that their instrument can produce. The data loads possible in instruments are seen in this capillary electrophoresis system. For example, if three replicates are made of a 20-min run (i.e., 60 min or 3600 s per sample) using a 96-position autosampler taking data at ten times per second simultaneously from four UV or fluorescent detector channels (possible with the Microphoresis system), this produces 1.4 million data points, or 1.4 megabytes of data from one unattended 4-d sequence of runs (3600 s/sample × 96 samples × 10 data points/sec × 4 detectors). A similar run using a single rapid-scan detector with a 500-element diode array would take 172 million data points, 172 megabytes. For PCs, since double-sided high-density floppy disks hold 0.36 to 1.4 megabytes and hard disk memories typically hold 20 to 120 megabytes, there is clearly an imminent future need for better data management. Better data management may take the form of (1) better screening of data (possibly by algorithms to discard uninteresting baseline points); (2) automatically backing up data to store and secure it from accidental loss; and (3) improved and faster on-line interpretation of data for quicker use. Toward the last two needs, the Microphoresis approach automatically backs up the hard disk on a built-in "streamer tape" at regular intervals (each evening, and/or when a certain amount of memory is filled). It also prioritizes data by several levels of importance or quality so the best data are more easily accessible. These kinds of software innovations are likely to continue to feed into the LC field. The techniques we have today in LC for programming and computer manipulations are still far from the optimal. There is an ongoing need for innovative programming methods as well as a need for faster movement of computer and software introductions into the analytical instrument arena.

Future automation in LC will include built-in diagnostics and easy repair by the operator by interchangeable electronic and mechanical components. Safety sensors will not only prevent samples from being lost, but will also have the capability of signaling the user, even remotely, by phone should any components malfunction. Such remote pagers are now used for process analyzers and clinical analyzers.

The business-oriented laboratory manager of today knows the value of time and productivity. Now that automated LC instruments are available, these managers are beginning to require that LC and other instruments be productive continuously, 24 hours per day, 365 days a year. The laboratory robotics field is beginning to make instrument design engineers aware of the need to reduce failures by using more durable parts such as valves or solenoids rated for millions of operation cycles.[121] For a 5-year instrument lifetime, older instruments making 10 runs per day at 200 work days per year need only operate 10,000 cycles in their lifetime. Modern instruments might make 20 runs per hour, 24 hours per day for 365 days per year to provide nearly a million cycles in a 5-year lifetime, a 100-fold increase in instrument load. Instrument design is beginning to take on this increased expectation, often at only a little increase in cost.

The value of 24-hour operation and the onset of faster methods (e.g., fast-LC) can mean that a failure can be extremely costly in terms of lost productivity. Thus, a need is to have safety sensors that can signal the user even remotely by phone should the instrument fail to operate properly. Such remote pagers are now used for process analyzers and clinical analyzers.

Automation with LC components will continue to pay for itself in ever faster and more productive problem solving, all the way from the method development stage through sample preparation and sample analysis to data analysis and report generation, and (in the future) to intelligent use of that information for automatic feedback and control and effective decision making.

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