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COMPUTER-CONTROLLED PREPARATIVE LIQUID CHROMATOGRAPH

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SUMMARY

Preparative liquid chromatography promises to be (and is) more widely used than preparative gas chromatography, but there are still problems and limitations to be overcome before the very high resolution of analytical liquid chromatography can be scaled up effectively in weight throughput terms.

A preparative liquid chromatograph is described in detail together with a computer control system with an easily modified programming system for developing strategies and algorithms for intelligent eluent collection control.

INTRODUCTION

The impact of analytical liquid chromatography (LC) can be described, without exaggeration, as dramatic. When it was first introduced, gas chromatography (GC) had a similar impact and some bold predictions were made for its preparative potential. Despite some successes, the results have never quite lived up to the promises. Equally bold predictions are easy to make for LC: are there any reasons to suppose they are more likely to be justified? In some respects advantages over GC are clear, but there are also a number of difficulties and limitations which emerge from our experience, and from that of others. The strategy of preparative LC method development has been well described recently by DeStefano and Kirkland[1].

Some advantages of LC are: (1) The range of applicability of LC is much higher than that of GC. Solubility rather than volatility is required. (2) Prior experience of low-speed, low-resolution LC and TLC is considerable. (3) Sample degradation in solution at ambient temperature is lower than in gas at high temperature. (4) Solute recovery is easier from a liquid, except for highly volatile compounds, for which preparative GC is probably more suitable. (5) Ambient temperature operation is cheaper, safer and more convenient. (6) The smaller geometry natural to LC because of a lower diffusion coefficient is easier to engineer. (7) Convenient detectors are mostly non-destructive. (8) Stop–start flow control without resolution loss is convenient. (9) The fact that identification techniques like nuclear magnetic resonance and mass spectrometry are now much more sensitive increases usefulness.

But some problems are: (1) Solubility in a suitable eluant to give separation is required. (2) Solvent purity, particularly with regard to non-volatile residues, is critical. (3) The capacity of the column packing is limited and marked increases seem unlikely. (4) It becomes increasingly difficult to engineer larger columns for high pressures. These high pressures present no problem for analytical-size columns. (5) High current cost of packing; this may ease with wider use. (6) The optimum injection slug distribution and how to engineer it. Should one try to use the infinite diameter effect[2]? (7) Fire hazards from large volumes of solvent under pressure. (8) How, and if, to use changes in the eluant composition. (9) Intelligent collection control, and overall instrument automation.

Application of preparative LC and research into the solutions of some of these problems are the subject of work in our laboratories. Besides describing the chromatograph in detail, some progress in solving the last problem, that of intelligent collection control, is the subject of the rest of this paper.

CHROMATOGRAPH

The chromatograph is constructed from components from several sources. The pump is an LC version from Haskel (Burbank, Calif., U.S.A.), Type No. 27500-3, with a 21:1 pneumatic-to-liquid pressure amplification factor, and a 70-ml stroke. The maximum pressure rating is 3450 p.s.i., but the available compressed air supply rarely exceeds 90 p.s.i., giving an effective limit of about 2000 p.s.i., which has been ample for our needs thus far. A 321 grade stainless-steel reservoir of about 20 l capacity, which can be evacuated to degas, or nitrogen purged, but not pressurised, is filled with solvent (minimum 1 l) via a 100 mm diameter glass sinter, porosity G3, filter funnel. Magnetic stirring, pneumatically powered, is useful to ensure a homogeneous solvent system.

All pipework uses Crawford Fitting (Solon, Ohio, U.S.A.) Swagelok 316 grade stainless-steel compression fittings and Whitey ball valves, obtained from their Great Britain agents (Techmation, Edgeware). Two valves controlling eluant and sample flow are pneumatically operated. Both homemade actuators—Kinetrol (Farnham, Great Britain) Model A, 90° rotary actuators—and, more recently, completely assembled ball valve and actuator combinations which can be purchased completely assembled (part No. SS-41S2-131DA, for example) have been used.

Pneumatic actuation avoids having electrical equipment in sparking range of large volumes of inflammable solvent, and conveniently provides a high torque required by some valves. Actuators that are spring-to-close and air-to-open provide safety shut-off in case of air pressure failure. All equipment is placed on stainless-steel trays whose volume can contain any leak up to the reservoir volume as an additional safety precaution.

Sample is dissolved in the eluent and can be injected from a syringe using a three-way Type SS-41XS2 ball valve, or when separation conditions are established, the solution can be placed in a home-made coil pump. Similar commercial models of pumps are made, for example, by Cecil Instruments (Cambridge, Great Britain), *i.e.*, Type CE 210. Direct nitrogen pressure drive allows sample to be injected either with solvent flow off or on provided the gas drive pressure is higher than the pressure generated by the main solvent delivery Haskel pump. An interlock system, as provided

on all commercial models, prevents connection of the gas pressure in erroneous and potentially very dangerous directions and is essential for safe operation.

Both glass and metal columns have been used (though glass columns cannot safely be used with the coil pump sample introduction system because it is possible by wrong operation to pressurise the glass column with gas, which would be highly dangerous if the glass fractured for any reason). Glass columns, either commercial types from Jobling (Stone, Great Britain) or heavy-wall glass tubes with ends ground to fit Swagelok 1-in. compression fittings, have restrictively low pressure limits of a few hundred p.s.i., but are a help in development of separations or instrumentation in some cases because one can sometimes see the sample and its distribution. Currently all 316 grade stainless-steel systems are in use based on machined and vacuum-braced combinations of Swagelok compression fittings. Vacuum brazing to British Standard 1845 (1966) is carried out by Torvac (Cambridge, Great Britain). These components are now available from HETP (Macclesfield, Great Britain). The column ends are formed from an SS-1610-C end cap with an SS-100-R-2 or SS-200-R-2 reducer brazed in. The inside is machined out to the tube outside diameter, plus appropriate tolerance specified by ASTM A269 (1971) to allow for tube O.D. variation, to give a square bottom. The tube thus penetrates deeper than the standard Swagelok fitting depth. At the column bottom a disc of double weave 304 grade stainless-steel mesh, 2300 × 325 wires/in., from Sankey Wire Weaving (Warrington, Great Britain) holds the packing material (down to about 1-μm particle diameter). To avoid impeding the liquid flow below this mesh disc, a second disc of single warp and weft coarser mesh, for example No. 85 mesh, 180-μm aperture to British Standard 410 (1962) in 304 grade stainless steel, is placed as a support. Both discs are held around their periphery by the column tube itself by first swaging the ferrules in place on the tube without the discs in place, ensuring that the tube is firmly seated into the fitting. When the fitting is reassembled with the discs in place the position of the ferrules, now gripping the tube tightly, forces the tube down onto the meshes, crimping it in place and preventing any packing from escaping round the edge. This system has very low volume, negligible dead volume, and much lower (tenfold) flow impedance than metal sinters. When dismantled, the meshes can be removed very easily and replaced or cleaned by reverse flow if necessary.

An identical system at the top can produce an injection slug into the eluant stream which, when introduced, spreads fairly evenly over the whole column area. Central on-column injection can also be achieved by using a larger reducer (1/8 in. or 1/4 in.) brazed into the top fitting and feeding sample down a central 1/16-in. tube and eluant around the outside of this tube using a teepiece, Swagelok part No. SS-200-3, and reducing PTFE ferrules, part No. PS100/200 from Phase Separations (Clwyd, Great Britain) which allow adjustment of the depth of the 1/16-in. central sample tube penetration. To avoid disturbing the packing during injection a tightly fitting disc of 5-mm thickness PTFE sinter (40-μm nominal porosity) from Vycrapor (Irvine Industrial Estate, Ayrshire, Great Britain) loaded by a 321 grade stainless-steel size M10 crinkle washer from Everbright Fasteners (Twickenham, Great Britain) has been found especially satisfactory. This spring also reduces or accommodates any settling of the packing, which can be a problem with spherical slurry packed particles. An undersize hole drilled almost through the PTFE sinter prevents back diffusion into the eluant stream. Filtration of the sample solution is desirable to prevent filtered

particles from blocking this PTFE sinter after many injections. Filtration of the eluant stream is also wise and a short pre-column 3/8 in. × 50 mm, part No. 341 from HETP, is suitably packed with coarse silica, 100–200 μm particle size range.

DETECTION

Several detectors have been used. A Cecil Instruments Type CE 212 single-beam variable ultraviolet (UV) detector and a Type CE 515 scanning double-beam UV–visible detector, which can also give good spectra at high sensitivity, have been used with success but a Perkin-Elmer (Beaconsfield, Great Britain) Type PE 124 double-beam spectrometer modified to accept Cecil Instruments type flow cells has been used most often for economic reasons. The flow cell is bored out from 1 to 3 mm diameter but retains a 10-mm pathlength to increase the light transmission. (Unlike spectrometers designed with flow cells in mind, the beam in a PE 124 is about 3 × 12 mm and thus too little light would pass even a 2-mm-diameter aperture cell.) Inserts to give a 1-mm pathlength are under construction, since a 10-mm pathlength is often found to give excessive sensitivity for preparative applications.

The variable-wavelength facility has proved invaluable, giving variable sensitivity and sometimes variable selectivity, although occasional attempts to use wavelength variation have been thwarted by selectivity in the opposite sense to that wanted. Indeed UV has sometimes proved extremely misleading in that some large peaks have produced minute weights on recovery and other minute or invisible peaks may represent considerable amounts. Refractive index detectors may not prove quite so misleading but have other disadvantages. On-balance variable-wavelength photometers provide satisfactory detectors and the ability to vary wavelength is even more useful than in analytical applications, where it has proved so powerful.

COLLECTION

Two eluant collection systems are in use. One is based on a conventional fraction collector which has many tubes of small volume, and is only suited for the collection of the results of one injection. The other type of device routes liquid to fewer but larger bottles, each dedicated to containing a particular component collected from a number of injections. The fraction collector approach is initially attractive in separation development but soon becomes tedious and inefficient for repeated injections. However, collection control must be intelligent and reliable to use a limited number of collection containers efficiently.

Several types of fraction collector have been used though none seem to have been designed with chromatography in mind, for they either operate on a fixed time or on a fixed volume, whereas the increase in peak volume as capacity factor k' increases from 0 to 10, say, suggests a tenfold increase in the fraction volume collected. A sophisticated collection algorithm can further reduce the number of tubes considerably and also increase the accuracy of collector changeover.

A rotary nine-way collection valve has been designed and built to route the column output to collection bottle for multiple injection systems. Connections are made using 1/16-in.-O.D. PTFE tubing and Cheminert connections from Laboratory Data Control (Riviera Beach, Fla., U.S.A.). A PTFE stator is pressed by stacked disc

springs to DIN 2093 from Industrial Trading (Worcester, Great Britain) against a Kel-F rotor, a slot in which connects to nine ports in turn. A pneumatic cylinder, part No. 01 0050 01 020, Enots (Birmingham, Great Britain), drives a pawl and ratchet mechanism which indexes the rotor one station per actuation.

COLLECTION CONTROL

A number of devices have been produced by instrument manufacturers to control collection of eluant from gas chromatographs. Some early devices were too easily tripped out of synchronisation by spurious peaks or spikes; others controlled on rigid time cycles which could not tolerate changes in retention time. More recent devices that combine several trip levels with time windows in which peaks must fall are much more satisfactory but still lack the intelligence of a human operator. It is this ability to avoid automatically placing peaks in the wrong collection bottles that computer control can now provide—combined with enough sense to know when human intervention is needed. Fortunately, in LC flow can be stopped, can await operator intervention, and can be restarted without detriment to the separation, which is an advantage over GC. Since the algorithm for collection control is in many ways less demanding than that for chromatogram integration, computer control should be feasible at similar cost, and even a falling cost.

COMPUTER SYSTEM

The computer used is a Computer Automation (Watford, Great Britain) LSI 2/10G with 16k of memory. Two teletype-like serial asynchronous data transmission channels meeting the C.C.I.T.T. recommendation V24 are provided, one via the system communication board, which also provides a real-time clock, and another via a distributed input/output system (DIOS) board. Programs are written in "BASIC" in the manufacturers' interactive version with additional routines in assembler code. These provide real time like a stopwatch as a BASIC variable and numeric and string variable transfer to and from the second (DIOS) teletype port from calls in BASIC. All data are transmitted and received in serial asynchronous ISO 7-bit code conforming with BS 4505, EIA RS-232C, C.C.I.T.T. V24, ISO/R2110, and ASCII. The advantage of using this data transmission format is that all devices can be tested independently with a teletype or similar peripheral, or even via a modem a remote timesharing computer. Plugs and sockets, pin connections, and signal type and format are well defined by national and international standards. This makes implementation by non-experts in computing and electronics much easier.

INTERFACE BETWEEN COMPUTER AND CHROMATOGRAPH

The interface between computer and chromatograph consists of Serdex modules from Analog Devices (East Molesey, Great Britain), a STX 1003 transmitter, and SCL 1006 clock package[3], to convert serial commands, single characters sent by the computer from the DIOS teletype port, such as *, !, £, @ etc. to open and close switches, or ? to command an analog-to-digital conversion of the spectrometer reading, whose result is transmitted back as a serial digit stream with sign and termi-

nated by a carriage return and line feed characters. (A detailed description of a similar system has been given by Overstreet *et al.*[4].) Electric switches actuate solenoid valves which allow pneumatic operation of valves and other equipment close to the chromatograph. The analog-to-digital conversion is carried out by an Analog Devices ADC 1100 dual-slope $3\frac{1}{2}$ BCD digit device giving 0.05% accuracy and a 42-msec maximum conversion time.

The BASIC compiler is core resident and interactive, including immediate execution of the commands LET, PRINT and GOTO. Errors are reported as each line is typed in, or during execution, and can be corrected immediately and program execution continued from the point at which the error occurred. This makes program development very easy and on-line activation of the various valves or reading a value from the photometer detector from the teletype is quick and easy. While this type of system is very inefficient in computing terms, it can still gather data, think and act fast enough for control of this system. Particularly during an algorithm development phase it is preferable to tolerate this computing inefficiency in exchange for efficient use of the programmer and chromatograph operator.

Using BASIC it is most convenient to let the program "free run" and not to attempt multi-tasking, as might be more natural with a more powerful and efficient multi-tasking real-time language such as RTL/2 (ref. 5). Thus an analog detector value is converted and read, some computation and decision takes place, and then the next value is read. Since the computation time varies, the time interval between values can vary widely. This is in contrast to the fixed (but usually steadily increasing to take account of increasing peak width) intervals used by most integration algorithms. Most minicomputer versions of BASIC take between 10 and 100 msec to evaluate a simple expression and decide which program line to jump to. Thus, taking conversion and transmission times into account, the time to respond might vary from 100 msec if the reading has not changed to 1 sec when a complex decision has to be taken. Since each line is recompiled, or more precisely interpreted, each time it is executed, and the computer is idle during all transmission and analog-to-digital conversion, this is highly inefficient. Speed increases of ten- to fiftyfold could probably be achieved by using a compiled multi-tasking RTL/2 or assembler program, but at the price of a commensurate increase in program development time. Since chromatographers are an order of magnitude more expensive to employ than computers, this seems a sensible trade-off.

Liberal use of core store, now much cheaper, allows quite a large amount of program and also retention of far more past data than is feasible with most integrators or multi-instrument integration computer systems. BASIC with additions occupies about 8k words, leaving at least 1k words, or 500 digital values, and the remaining 7k words for program.

CONTROL ALGORITHMS

The basic philosophy of the algorithms is to simulate the action of an intelligent human operator and to control sample introduction, collection or shutdown, as appropriate. Two modes of operation that require a different approach are the survey run with little or no prior information, and the repetitive injection mode using the same sample to accumulate a larger weight of products, when detailed information

about previous runs is available and must be used to ensure that the same peaks are found and placed in the correct container. As noted in the section on collection, in the former case a multi-vessel collector is most suitable, whereas in the latter case a smaller number of larger-volume containers which can be totally enclosed and nitrogen purged for longer-term storage is more suitable. Two distinct but interlinked programs are seen as necessary from experience so far: information collected on peak time, size and width during the survey run needs to be stored for use during repetitive operation.

Several factors suggest that the algorithm must be considerably simpler than those found necessary for GC, *viz.*

(1) Dynamic range of UV (and all other detectors) is much lower than for the flame ionisation detector most common in GC.

(2) Large solvent peaks are rare, and would not suggest a useful separation for preparative LC. It is from shoulders on these steep slopes that some difficult integration problems are found.

(3) Delays in decision about peak start, maximum and finish lead to quite small losses in the collected peak, whereas the error in calculated area can be very large.

(4) Drifting baselines should not be encountered unless gradient elution is being used, and even then the amount of drift it is practical to tolerate is much less because of the low dynamic detector range. (Gradient pumping systems currently available have insufficient capacity and speed for preparative columns: a typical separation on a 1-in. column would require 1 l of solvent and take about 10 min).

(5) Because samples become excessively diluted, peaks retained much beyond capacity factors $k' = 10$ will be used rarely. Thus the range of slopes and peak heights will vary only about tenfold, much less than is common in GC applications.

During development of algorithms and programs, a phase that is still continuing with experiments to assess the best methods, the following points of interest, difficulty or solution have been noted:

(1) Noise, if a problem, can be smoothed out with a number of functions. In view of the slow operation of BASIC, a simple weighted running average of the past two or three points combined with optimal analog smoothing should be adequate. Providing thresholds are correctly chosen, some noise can be tolerated without additional smoothing.

(2) Spike rejection can be achieved by peak width comparison and as having insufficient area, techniques familiar from GC integration. Because the system first diverts to waste, and delays for a short time while a positive decision is made to divert further to collection, spikes are not a serious problem.

(3) Baseline checking, and noise level measurement (say from the r.m.s. of 20 values) are essential before injection. The initial baseline value and noise level is stored for reference during and at the end of a chromatogram. Only after a satisfactory baseline has been recognised, are the flow and pressure, and perhaps the column geometry and the type, input from operator measurements. The computer can now calculate the time at which the solvent front is expected. Column efficiency in terms of plate height might also be input, either from test mixture data, or —more hazardously— from a peak in the chromatogram which might be anomalously broad.

(4) Recycle of liquid from the column to conserve solvent which is much more expensive than nitrogen or helium is convenient, especially during method development. It is important to be sure that contaminated solvent is not returned to the reservoir. If the detector shows a value even slightly above the established baseline, the computer prevents recycle by diverting to waste. At all times when there is any doubt about whether a peak is correctly identified, the column output is routed to waste. This degree of intelligence gives a high degree of security against contaminating either the reservoir or the collected samples. If the detector is set to a long wavelength, especially in the visible region, this can be unsatisfactory, since UV-absorbing impurities will not be detected at all, and it may be safer not to recycle. At very short wavelength too, say 220 nm, where solvent purity is critical, it may also be prudent to avoid recycle.

(5) Injection is best made after the eluant flow has been stopped and the real-time clock reset to zero. Injection can be made either by hand, or for a time interval from the coil pump containing sample to give a known volume for the pressure applied. The clock is again reset and flow re-started. Any unexpected peaks emerging before the solvent front (or the calculated time for excluded molecules) can trigger an alarm, for example, by sounding the bell on the teletype and perhaps also shutting down until manual control takes over.

(6) Peak start can be detected either by a simple trip set at a level above the baseline, by slope, or by rate of increase of slope (zero, first and second differentials). The threshold value, or values, are best related to capacity factor k' or time.

(7) Peak maximum can usually be sensed easily except that often some peak will go off scale on a UV recorder, or at least into a non-linear region above 2 absorbance units. Location of the peak maximum time may be complicated by this, and area calculations will be wrong.

(8) Peak heights are only of interest in giving retention volumes to calculate areas, perhaps approximate by integration standards.

(9) Valleys between peaks are readily detected but a changeover from one collection vessel to another at this point will result in maximum yield but less complete separation than would be achieved by heartcutting.

(10) Heartcutting is more difficult to define in general terms and it seems most convenient to program each situation individually. Changeover to collect a middle fraction, or fractions, at some part of the height of the first peak seems reasonable, but may be less satisfactory if different samples, with different composition, are used. More difficult is deciding the point at which to start collecting the second "pure" fraction; perhaps a height double the valley level above the baseline is sensible for peaks of roughly equal size. Purity must of course be traded for yield. Middle mixed fractions from the valley regions can also be recovered and recycled.

(11) Incomplete resolution seems best collected with a multi-vessel fraction collector. If the fraction size is set (calculated and timed) to correspond to between 1/5 and 1/20 of the peak volume at that retention volume, then decisions about heartcutting are postponed until the whole chromatogram is revealed. The computer can reduce the number of fractions to the minimum needed to avoid resolution loss; during periods where no peaks are detected, eluant may be routed to waste or recycle, or collected if detector insensitivity is suspected.

(12) The computer can also conveniently provide a printout of peak times,

volumes, collection points, peak efficiencies, resolution, and other parameters. Combined with the analog record, this provides a comprehensive record of the separation.

FUTURE DEVELOPMENTS

The main hardware addition planned is to duplicate (or even triplicate) the entire pumping system. This will allow a rapid change of eluant to elute strongly retained components quicker and in a smaller volume and at higher concentration. Simple gradient elution could be produced by interposing a stirred chamber between the two pumps and the column. Eluant change could be computer controlled without difficulty either at a fixed time or when no more peaks were eluted with the first lower-polarity liquid.

Pressure, and thus flow programming, of pneumatic amplifier pumps is relatively simple to engineer; two or more flow-rates could be computer controlled to speed elution of later peaks when time becomes more important than resolution.

If a detector that can scan is used (for example, the CE 515 detector made by Cecil Instruments), the computer could stop flow and obtain a UV spectrum of each peak. With the addition of an autoranging accessory, our "research chromatograph" based on this detector is already automated almost to stop and scan on activation of a single pushbutton, which could be "pressed" by the computer. Alas, the automation of tedious tasks like transferring solutions for recovery on a rotary evaporator is likely to prove more difficult.

As the algorithms for controlling the chromatograph become reliable and accepted, the way is open to replace an expensive processor system with a compiled multi-tasking version of higher speed and efficiency "hard programmed" in a microprocessor, just as has happened with GC integrators. This development is only likely to be feasible for commercial products. In the meantime the cost of an integrated processor/interface/high-level software package for laboratory research applications will also fall to a level where it is a small part of the total cost of a complete chromatograph.

REFERENCES

1 J. J. DeStefano and J. J. Kirkland, *Anal. Chem.*, 47 (1975) 1103A, 1193A
2 J. H. Knox and J. F. Parcher, *Anal. Chem.*, 41 (1969) 1599.
3 *Serdex Users' Guide*, Analog Devices Inc., East Molesey, Great Britain, 1973.
4 A. D. Overstreet, D. G. Larsen and P. R. Rony, *Int. Lab.*, March/April (1974) 42.
5 *RTL/2 Language Specification*, ICI (Mond Division), Runcorn, Great Britain, 1974; available from SPL International, London, Great Britain.