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HIGH-CAPACITY MULTI-CHANNEL COMPUTERIZED READ-OUT SYSTEM FOR MULTI-COLUMN CHROMATOGRAPHY

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SUMMARY

A computerized multi-channel read-out system for multi-column chromatography is described. It has a capacity of a hundred chromatograms per day when three channels are used and this can be expanded to four hundred chromatograms per day by the use of all twelve channels. The system is compared with other high-capacity systems for automated chromatography.

INTRODUCTION

We have previously described an automatic read-out system for multiple column chromatography connected via data-logger and paper tape output to a computer[1].

The development of a magazine-fed fraction collector for multi-column liquid chromatography[2] together with capillary column techniques for multi-column chromatography[3,4] has greatly expanded the potential capacity of multi-column systems for liquid chromatography. It has therefore been necessary to develop multi-channel automated read-out systems capable of handling the large numbers of chromatograms that can be produced by the new multi-column capillary systems.

GENERAL DESCRIPTION OF THE SYSTEM

The multi-channel read-out system is built around the recently described new type multi-collector[2]. The test tubes with the colored reaction mixture on which a transmittance reading is desired pass from the magazine to the left in the collector (Fig. 1) to the magazine on the right past a central latch that moves the tubes row by row past a set of suction tubes that dip in and out of the test tubes on signal from the central programmer. The reaction mixture is passed tube by tube through the colorimeters visible in the foreground and the transmittance is recorded on the multi-pen recorders to the right in the picture. These recorders are equipped with retransmitting slide-wires that send signals simultaneously to a data-logger and via the proper interphase systems directly on-line to a computer for data-handling.

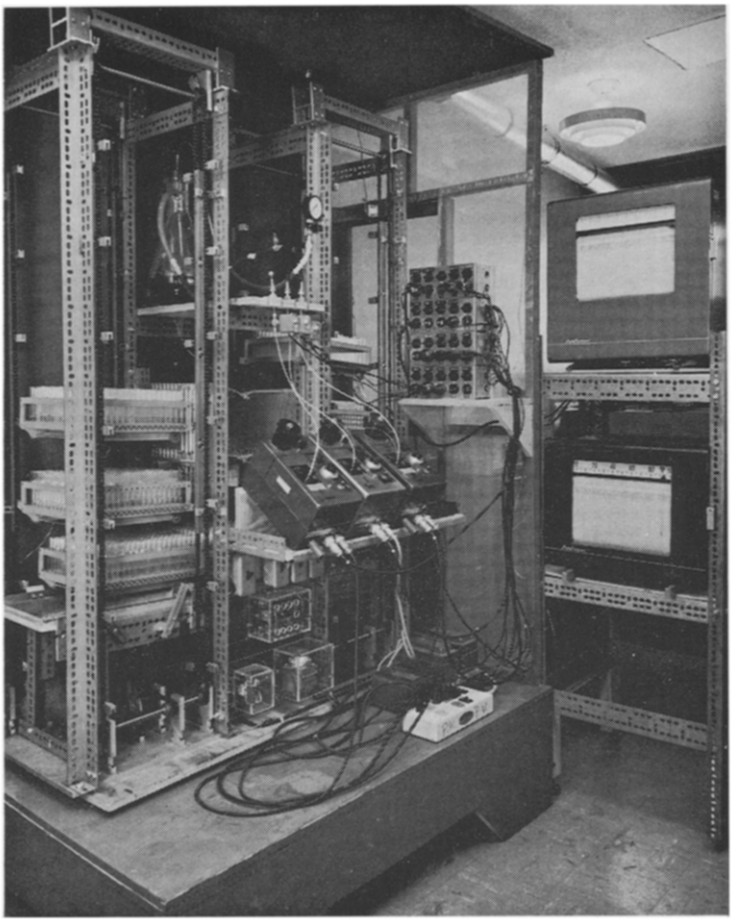

Fig. 1. The multi-channel read-out system set up as a 3-channel system. In the background the magazine-fed multi-column collector[2]. In the foreground the three colorimeters. The control panel (Fig. 3) is to the right above the colorimeters. A three-pen and a two-pen recorder are located on the rack at the extreme right. The suction "dipper" mechanism (Fig. 4) is behind the colorimeters and the controlled vacuum system (Fig. 6) is on the shelf above in the center of the multi-collector. The data-processing equipment (Fig. 7) is hidden behind the plastic sheet wall located in back of the control panel.

DETAILS OF CONSTRUCTION

Programming of events

The sequential steps in the automated read-out procedure are programmed through a punched tape programmer (Industrial Timer, Parsippany, N.J. No. 242) shown in Fig. 2. The program repeats sequentially twenty cycles, each consisting of the following steps: suction tube dips into test tube, valve opens to central vacuum to draw liquid through flow cell, valve closes to central vacuum, recorder drive is activated, switch to data-logger and computer system is closed, suction tube is lifted out of test tube, latch is activated to move a new row of test tube into position and so

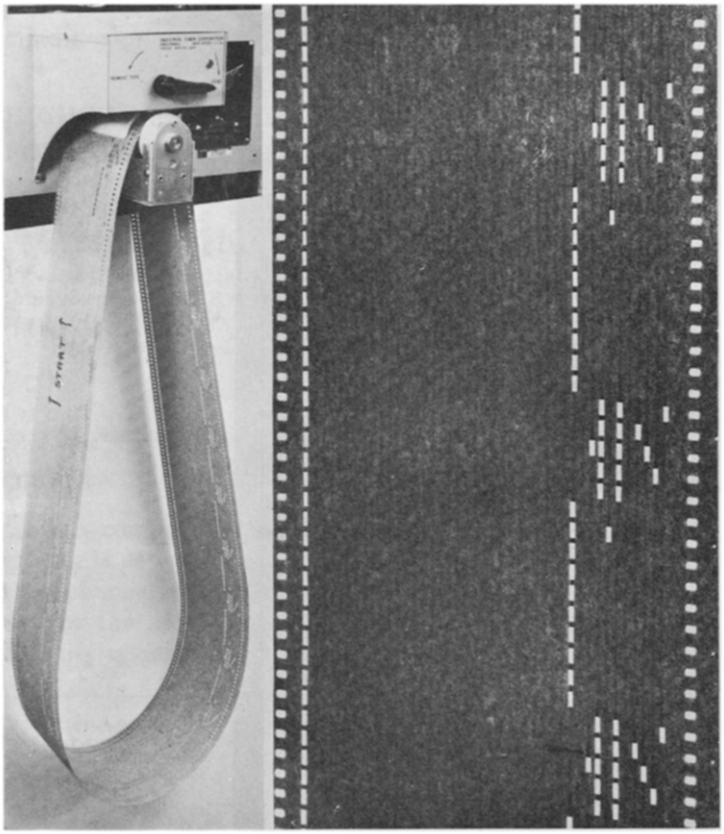

Fig. 2. The event programmer. To the left the timer with tape; to the right the programmed tape. A synchronous motor mechanism moves the tape from hole to hole each 1/2 sec and events are programmed by perforating the required number of holes in each channel. A contact closure is effected as electrical brushes pass over the perforations.

on. At the end of the twentieth cycle a longer delay is introduced after the latch has been activated to allow a new test tube carrier to move into position at the latch. The program is punched channel by channel by a special accessory to the tape programmer. The finished tape is shown to the right in Fig. 2.

The contacts made in the tape programmer when brushes travel over the holes punched in the tape can carry only a very moderate amount of current. We have therefore in all cases let this current activate a coil in heavy duty mercury relays mounted behind the plates in the central control panel shown in Fig. 3. We have also mounted on and off switches for each operation so that the impulse from the programmer can be passed on to or be withheld from the peripheral equipment as needed allowing single steps in the procedure to be isolated and tested separately.

Drawing the samples through the flow cells

We decided in the construction of the read-out machinery to use the colorimeters from the Technicon Autoanalyzer system primarily because these colorimeters have proven themselves over the years in day by day automated analyses in many labora-

tories the world over. The flow cells have been the tubular flow cells used in the Autoanalyzer N system. We have found that these flow cells can be used in our application without a debubbler if they are arranged at an angle of 45° to the horizontal as illustrated in Fig. 1. This is an important feature in the system since it has been found that air bubbles that inevitably occur now and then in the early part of the suction phase do not get trapped in the flow cell if it is arranged in this position.

We used in our earlier system[1] pumps to pump the liquid through the cells. This system has, however, over the years proven somewhat cumbersome needing a great deal of maintenance to function well. We have therefore switched to a simplified system in which a central vacuum is used. The arrangement is illustrated in Fig. 6. Vacuum from house vacuum or a small vacuum pump is adjusted through a Cartesian vacuum regulator (Manostat No. 6A, Greiner Scientific, New York, N.Y.) and the use of a bleed valve to the proper experimentally determined setting (approximately 15 in. mercury pressure). The regulated vacuum is connected to a large suction flask

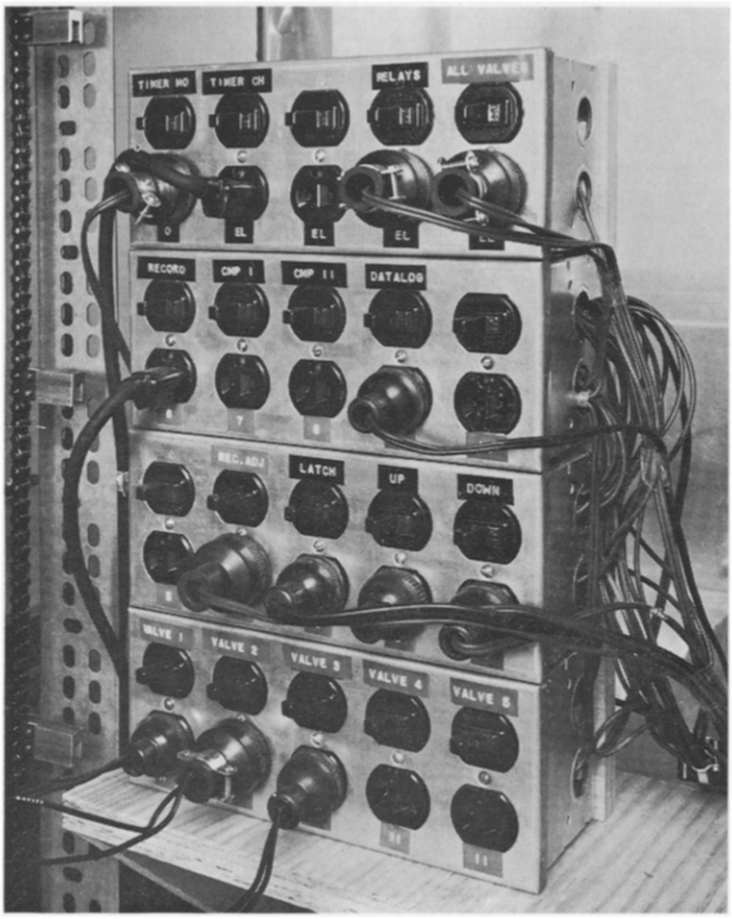

Fig. 3. The control panel. Wires from the event programmer are led to the coils in mercury relays located behind the switches. The loads are connected via switches to the load contacts on the mercury relays. All operations can be controlled from the switches on this panel.

from which it is distributed to individual flow cells through needle valves that regulate the flow and through on/off solenoid valves that open and close at command from the event programmer.

Teflon tubing and stainless steel connectors are used throughout in the suction line. Heavier (1/8 in. diameter) teflon tubing is used on the vacuum side of the flow cell. Thin teflon tubing (AWG No. 22) leads from the flow cell to the suction tube that dips into the liquid in the test tubes. The teflon tubing is carried through the inside of this piece of protective stainless steel tubing to the tip (Fig. 4).

The "dipper" mechanism

The suction tubes and the method of dipping into the test tubes are illustrated in Figs. 4 and 5. Fig. 5 shows the air cylinders used to push the teflon tube carrying stainless steel tubes into the test tubes and back up. They are double acting air cylinders (Clippard Inc., Cincinnati, Ohio No. 3BDS-6) that work on the principle that compressed air applied to the top side of the cylinder will drive a stainless steel rod downwards 6 in. Air applied to the bottom port of the cylinder will send the rod back up. A piece of stainless steel tubing is attached to this rod and the teflon tubing coming from the flow cell is passed through a hole in a short adapter connecting rod and stainless steel tubing and pushed through the inside of the stainless steel tubing

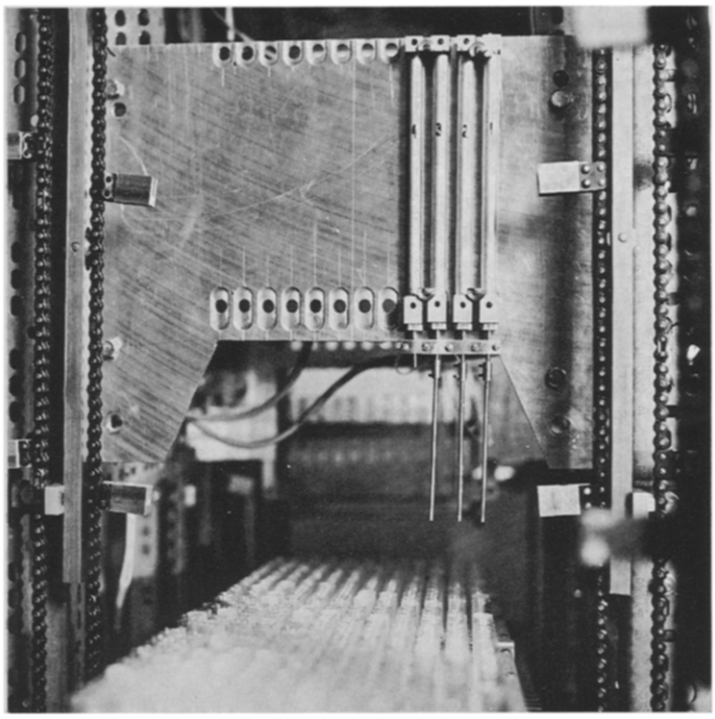

Fig. 4. The "dipper" mechanism. When compressed air is introduced at the top of the air cylinders (Fig. 5) the stainless steel tubes attached to the cylinders carry thin teflon tubing into the test tubes. When compressed air is introduced at the bottom of the cylinders the tubing moves out of the test tubes.

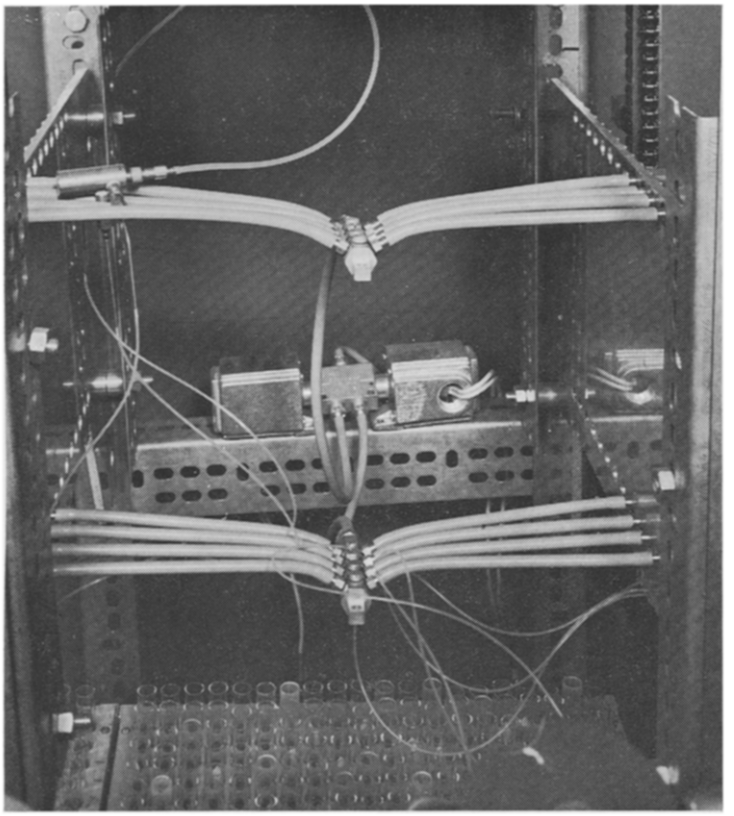

Fig. 5. The compressed air system. A solenoid valve in the background directs the air either to the top of the air cylinders (for dipping down into the liquid) or to the bottom of the cylinders (for retraction from the test tubes).

to the tip of this tubing. Through the valve and distributing system shown in Fig. 5 compressed air is sent first through the top port of the air cylinder by activating the right side of the solenoid valve (Clippard No. AVSC-115) shown in the background of Fig. 5. It is later returned to its "up" position by activating the left side of the solenoid valve opening the lower set of plastic distributing tubes to the compressed air.

The system as shown in the pictures has two sets of "dipper" mechanisms. One is used for bubbling carbon dioxide through the reagent mixture in the special reaction for 17-ketosteroids described earlier[5]; the second "dipper" mechanism is the mechanism used for sucking sample into the flow cells.

DATA-PROCESSING

The high capacity of the multi-channel automatic colorimeter unit cannot be fully utilized unless the calculation of the data is handled by computer. We have estimated that it will take five full-time clerks using ordinary electric calculators to calculate the 8-hour output from the machinery. Clearly much of the advantage of the high-capacity system would be lost without high-speed data handling.

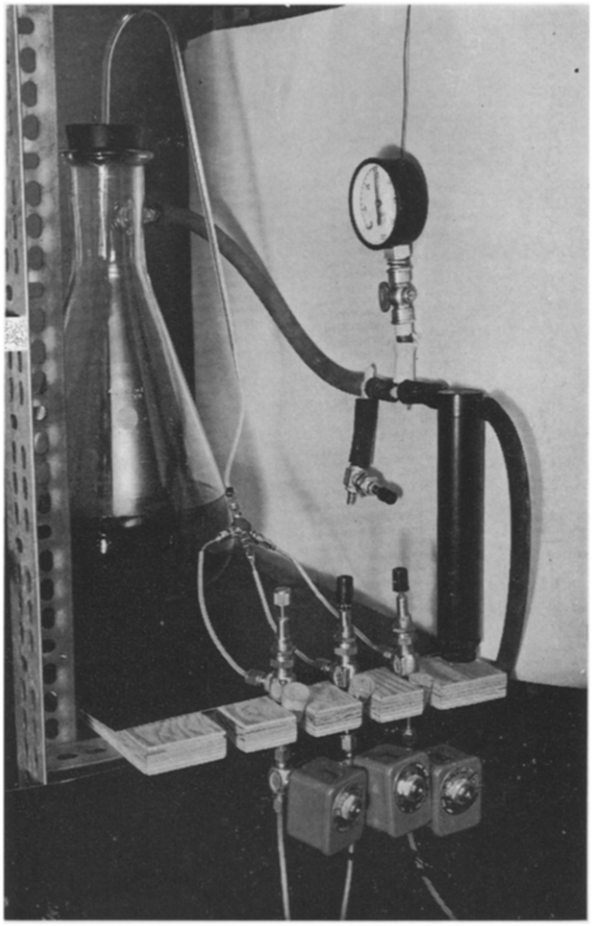

Fig. 6. The regulated vacuum system. The vacuum regulator (the black cylinder to the right) maintains in conjunction with the bleed valve located under the vacuum gauge the proper vacuum. A suction flask collects the reagent. Suction is applied to the flow cells in the colorimeters through the needle valves and solenoid valves shown at the bottom of the illustration.

We use for the data processing simultaneously two computer systems: a data-logger and a direct on-line system.

The data-logger (Fig. 7, top)

This is a commercial unit (Technilogger, Technicon Corporation, Chauncey, New York) that can receive data from up to eight colorimeters simultaneously and transform them to paper punch output at the same time labelling the input sequentially.

The on-line system (Fig. 7, bottom)

The signals from the retransmitting slide-wires in the multi-pen recorders are besides being passed to the data-logger also sent via a proper interphasing unit (Model AD-10, Technical Electronics, Bayside, New York) to an IBM model 1827 analog/

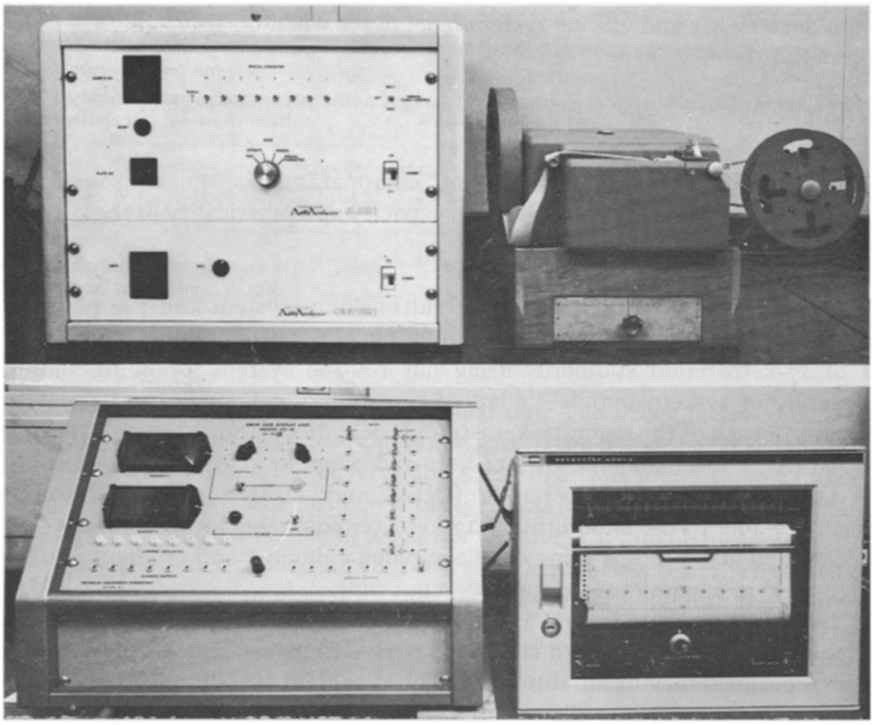

Fig. 7. The data handling systems. At the top the data-logger with its paper punch. At the bottom the on-line interphaser with a recorder for feedback of calculated data to the laboratory.

digital interphase for digitizing and from there to an IBM 360/30 computer for calculation. Calculated data can be transmitted back immediately to the laboratory and displayed on recorders in the laboratory.

Programming

The programs for the calculation of the chromatograms are currently under development and only the first relatively simple steps have been programmed. Peak values are summed and areas compared with similar areas for a set of simultaneously run standards to give amount of unknown present. The results are available as printed outputs and there are also several graphic outputs including plots of optical density values and micrograms compound per fraction cut sequentially registered to give a visual chromatogram to supplement the printed output.

The advantage of two independent computer systems

It may appear redundant to employ simultaneously two different computer systems. Experience has shown, however, that it is almost a necessity in high-capacity systems not to have to depend on a single system. We have not infrequently had computer breakdown lasting for a full working day. The data-logger paper tape has then been available for calculation once the computer was repaired and back in operation. Many man-hours of calculation time are saved this way. Running a double system also makes it possible to check one system against the other. Obviously

results from data-logger and on-line system must agree when both systems are functioning properly.

PERFORMANCE

We have in the performance testing of the apparatus concentrated upon the following aspects: reproducibility, carry-over, capacity and practicality of the system.

Reproducibility

The reproducibility of the combined multi-column system and the read-out machinery has been experimentally evaluated by chromatographing aliquots of mixtures of 17-ketosteroid standards using our routine system for multi-column chromatography of 17-ketosteroids[1,6] adapted to capillary columns. Approximately 50 μg amounts of each of the seven 17-ketosteroids dehydroepiandrosterone (DHEA), androsterone (A), etiocholanolone (E), 11-keto-androsterone (OA), 11-keto-etiocholanolone (OE), 11-hydroxy-androsterone (OHA) and 11-hydroxyetiocholanolone (OHE) corresponding to the main urinary 17-ketosteroids were chromatographed on twelve 6-foot capillary columns using capillary teflon columns[3] and gradient elution chromatography[6] in a simultaneous multi-column run.

We have for comparison on a commercial automated gas chromatograph (Barber-Colman) run a similar set of standards twelve times sequentially to compare the relative reproducibility of an automated liquid column system with that of the automated gas chromatograph. The conditions for the gas chromatographic assay were: column substrate, neopentyl glycol succinate 2% on Gas-Chrom P, mesh 100/120 (from Applied Science Lab., State College, Pa.) coated using the technique described by HORNING *et al.*[7]; hydrogen flow, 40 ml/min; air flow, 350 ml/min; column temperature, 220°; injector temperature, 250°; detector temperature, 250°; column length, 6 ft., I.D. 3 mm. Pregnane-3,20-dione was used as an internal standard. The steroids in the gas chromatographic assay were run as trimethylsilyl ethers prepared as suggested by KIRSCHNER AND LIPSETT[8]. The gas chromatograms were calculated in three different ways: by peak height multiplied by the peak width at half height, by the use of peak height alone and by multiplying peak height by retention time for a given compound, in all cases comparing with the internal standard.

The last of these methods gave the highest accuracy in the calculations and has been used for the comparison with the liquid system.

It can be seen from Table I that the reproducibility is clearly better for the automated liquid chromatography. The automated gas chromatography although considerably less reproducible must still be considered quite adequate for most biochemical work.

Carry-over

Since teflon tubing dips down into the reaction mixture in the test tubes and moves from tube to tube it was considered necessary to experimentally evaluate the possible carry-over from tube to tube. The carry-over consists of two factors. One is the mixing with the previous sample as new sample is drawn into the flow cell. The other is caused by sample sticking to the outside of the teflon tubing. The first factor can obviously be eliminated by drawing sample long enough through the flow cell until

TABLE I

COMPARISON OF THE REPRODUCIBILITY OF AUTOMATED LIQUID CHROMATOGRAPHY AND AUTOMATED GAS CHROMATOGRAPHY

In both cases twelve chromatograms of seven 17-ketosteroids were run and means and coefficient of variations (standard deviation in % of the mean) calculated.

	Coefficient of variation (%)							
	DHEA[a]	*A*	*E*	*OA*	*OE*	*OHA*	*OHE*	
Liquid chromatography	2.5	2.0	1.2	3.0	2.6	1.2	1.3	av. 2.0
Gas chromatography	2.6	3.1	3.1	2.9	3.1	2.5	7.2	av. 3.5

[a] For abbreviations for individual steroids see text.

all traces of old sample have been washed out. The other factor was found to be negligible because of the surface characteristics of the teflon. The relationship between volume sucked through the cuvette and carry-over was found to be as follows: at a suction volume of 0.8 ml a carry-over of 8.7 % was found, at a suction volume of 1.2 ml the carry-over was 0.8 %, and at a suction volume of 1.7 ml no measurable carry-over could be determined indicating that it was at least below 0.2 %. This then would be maximum carry-over produced by the teflon tubing dipping into the solutions in the test tubes.

Capacity

The aim in the development of the new system was to construct a high-capacity system. This has been obtained through the use of multiple channels. Some increase in capacity has also been gained through the use of vacuum suction instead of the pumps previously used to get the samples to the flow cells.

The current 3-channel system has a capacity of approximately 100 17-ketosteroid chromatograms per working day when 60 test tubes per chromatogram are read and estimating the seven main urinary 17-ketosteroids.

It is expandable to a 12-channel system by installing a total of twelve colorimeters with the necessary extra multi-pen recorders. The potential capacity of the system is therefore 400 chromatograms per working day when applied to a biological 17-ketosteroid mixture.

Practicality

The apparatus has been in routine use over the last twelve months in our laboratory. It has after initial "debugging" been found quite reliable with little maintenance needed. No specially trained personnel is needed for the operation of the instrumentation. Female laboratory technicians without special background in handling of instruments have been running the routine chromatograms on the machinery through these months.

DISCUSSION

Recent years have seen chromatography dominated by an explosive growth of gas chromatographic and thin-layer methods. Other chromatographic methods have been pushed somewhat into the background during this development.

It is our contention that the potential of column chromatography with proper upgrading and automation of procedures and a switch towards high resolution, relatively fast, multi-column capillary column techniques is fully as great as that of the newer techniques although no one technique can be expected to be the method of choice for all applications. Liquid–liquid and liquid–solid column chromatography will, however, we believe still be found in many cases to be the preferable chromatographic method.

The multiple capillary column techniques have been developed to overcome two of the very real shortcomings of column chromatography in practical work: low capacity and slow speed. With the new automatic read-out apparatus described here a factual capacity of 100 chromatograms and a potential capacity of 400 chromatograms per day has been reached. We have currently in our laboratory the capacity to do 100 capillary column separations in a day using our special multi-column fraction collectors. We can do this by starting one set of 50 columns in the morning and setting up another set in the afternoon. The investment in chromatographic pumps to achieve this capacity has, however, been high and for that reason new greatly simplified systems that eliminate the chromatographic pumps are currently under development. We believe that it will be possible in some routine chromatographic separations to expand from a hundred to several hundred capillary column chromatograms per day with these new systems without excessive demands on space and facilities.

To our knowledge the only other chromatographic system that can produce chromatograms and calculated values for 100 chromatograms per day or more is the "Cassandra" system for automated paper chromatography described by BUSH[9]. It is, we believe, of interest to compare the two systems on a number of points of importance for the performance of the total systems to give the chromatographer in search of high capacity automated systems a guide to the system best suited for a given application.

Comparison of the multi-column system with Bush's "Cassandra" system for high capacity chromatography

Resolution and speed. Techniques for liquid chromatography have traditionally been by far the slowest of all chromatographic techniques. New developments over the last decade have, however, changed the situation radically.

The first to systematically explore experimentally and theoretically the possibilities for fast liquid chromatography was HAMILTON[10,11], who showed that the separation of amino acids on ion-exchange columns could be significantly accelerated by the use of high pressure and together with his collaborators[12] studied a number of the variables affecting the chromatographic resolution. The theoretical potential of capillary columns in liquid chromatography was pointed out by GIDDINGS[13] who showed that theoretically the separating ability is higher in liquid chromatography than in gas chromatography since the theoretical limit to the number of plates is roughly 1000 times larger in liquid than in gas chromatography. Recently SNYDER[14,15] has evaluated the problem of maximum resolution per unit time in liquid–solid adsorption chromatography experimentally and theoretically and evolved equations and charts for maximum bed efficiencies in this type of chromatography as a function of column pressure, column length, separation time and particle size. The highest theoretically possible bed efficiencies cannot currently be realized in liquid–solid

chromatography because of technical limitations in pressures that can practically be obtained but as pointed out by SNYDER[14,15] a maximum number of 60,000 theoretical plates can conveniently be obtained today without exceeding known technology. Long runs (72 h) are, however, necessary for this. Resolutions equal to that obtained with packed columns in gas chromatography and far better than resolutions obtainable in thin-layer and paper chromatography can, however, be realized in high pressure, fast speed chromatography. SNYDER[15] in his experimental work reached bed efficiencies in 40 min of more than 2000 theoretical plates in the separation of hydrocarbons and he reports plate numbers of over 4000 in 2 1/2-hour runs working with a synthetic mixture of hydrocarbons separated on 32 ft. long columns at 10 atm. pressure. We have (unpublished) with the capillary technique described earlier[3,4] in routine work realized theoretical plate numbers of about 4000 in 4-hour runs separating corticosteroids on 6 ft. long columns at 30 atm. of pressure. Fast high-pressure capillary column chromatography of nucleotides was performed by HORVATH *et al.*[16] on 6 ft. columns and at 75 atm. pressure using pellicular column materials with separation of complex mixtures possible in from 75 to 90 min. These authors draw the overall conclusion that this liquid chromatographic technique is comparable in speed, resolution and quantitative range to gas chromatography.

It is clear from these examples of high-pressure long-column chromatography that practical techniques now exist that make fast column runs possible and with much better resolution than is possible in thin-layer and paper chromatography as pointed out by SNYDER[14,15]. Liquid chromatography is getting close to packed column gas chromatography in speed for similar resolution as demonstrated by HORVATH *et al.*[16]. The very interesting work by PIEL[17] with high-pressure small-particle liquid chromatography in which high plate numbers were realized in very fast runs, would indicate that we are only at the threshold of further developments in this area and that with future improvements in technique liquid chromatography may overtake packed column gas chromatography in speed for similar resolution.

The better resolution in the multi-column capillary column system will, we feel, for many applications be found to be its most important advantage when compared with BUSH's automated paper chromatography. A calculation of theoretical plate numbers for the paper chromatographic estimation of the corticosteroids cortisol, tetrahydrocortisone and tetrahydrocortisol in BUSH's system was performed by TAIT AND TAIT[18]. Theoretical plates under optimal conditions were found to be 750 for levels below 40 μg and 390 for levels between 40 and 100 μg. We found running 50 μg samples of the same corticosteroids using the multi-column system with capillary columns[4] and using gradient elution chromatography that we in 4-hour runs could reach theoretical plate numbers of 3800–4200 for these same substances. The very much higher resolution in the multi-column system is clear. That the higher resolution is an important feature in practical chromatographic work is shown by the fact that urinary 17-ketosteroids can be separated quite well by a single column run on alumina in the multi-column system, but it takes two separate paper chromatograms and a Girard separation to effect the same separation in BUSH's system[19].

Choice of detectors. Another important advantage multi-column systems have when compared with automated paper chromatography is the wide choice of detectors available. If stream-splitting is used in the column chromatography a number of detectors can be applied to portions of the same fraction, for example, colorimetry,

fluorescence and liquid scintillation counting. The flame ionization detector used in gas chromatography has become available as a detector in liquid chromatography[20] as has a very sensitive U.V. detector[21]. It is obviously also possible to use the BUSH[9] system as a detector in column chromatography letting the effluent from a chromatographic column form spots on paper that then can be treated and quantitated in the "Cassandra" apparatus. In situations where the precision of the paper chromatographic system is adequate this could become a powerful combination utilizing the potentially higher resolution of the liquid column systems and the convenience of the paper system. The processing speed in the Cassandra apparatus great as it is would, however, have to be further accelerated before this would become a practical proposition for high capacity work.

The Cassandra system is in comparison more limited in choice of detectors. Besides some reagents are incompatible with the system and it is sensitive to changes in temperature and humidity.

Load. It has long been recognized that column chromatography has a decisive advantage here compared with other types of chromatography and this is, for example, shown in experimental studies like the one by TAIT AND TAIT[18] comparing paper and column chromatography of steroids. Much higher loads were possible on the columns without resolution being affected and impurities had less of an effect on the chromatography.

Reproducibility. Although great strides have been made in improving the precision of the method for automated paper chromatography values for coefficient of variation for urinary steroids estimated with the Cassandra system would seem clearly higher than similar values obtained in the multi-column system. BUSH[19] gives values of 4.2–8.2 % for the coefficient of variation in batch operation of biological samples for some common urinary steroids against the 3–5 % we have obtained routinely in similar experiments with the multi-column system. It must, however, be admitted that the increase in precision obtained by BUSH with the many refinements in technique over the years makes his automated paper chromatography system adequate in reproducibility for most practical applications.

Analytical capacity and practicality. Although as indicated we should shortly in our laboratory have the capacity to run several hundred capillary column chromatograms in a day it must be conceded that this with current techniques will require considerably more space than a similar-sized operation involving paper chromatography since a medium-sized room would have to be dedicated to column chromatography to make the higher capacity possible. A minimum of four large vacuum ovens will be necessary for evaporation of solvents and relatively large amounts of solvents will be needed per day, for example, with current techniques an estimated 24 l of benzene per 100 capillary column chromatograms of urinary 17-ketosteroids. Clearly paper chromatography is less demanding in space and solvents at this time although high-capacity work with this technique is rather space consuming also. It is also possible to recover much of the solvents used by trapping the solvents on evaporation.

It should be emphasized that the further development of microparticulate techniques for column work[17] may make a drastic miniaturizing of procedures possible when the aim is to obtain as practically as possible as high capacities as possible in liquid chromatography with maintenance of high resolution. Space and solvent demands may with such techniques drop to a fraction of what is currently needed.

Apart from requiring more space and more of an expenditure in solvents and reagents we believe that the multi-column technique otherwise in convenience and speed in preparing the chromatograms is fully as practical and fast as the paper chromatographic technique. The transfer step is, we believe, easier to automate in a column system than in paper chromatographic techniques. Even with manual syringe transfer techniques, however, the 20–25 chromatograms per hour given as the effective production rate of a good technician in the paper chromatographic system[19] can without strain be duplicated by a technician working with the multi-column system if machine filling[4] is used for the columns. We believe that the time currently spent taking tubes in and out of the vacuum ovens and adding reagent automatically with an automatic syringe pipette roughly equals the time spent running the BUSH system's machinery for the automatic preparation of paper chromatograms and that running the scanner is about equal in complexity to running our read-out machinery.

Choice of system. The chromatographer deciding between the multi-column system and the Cassandra system for high capacity chromatography would as major considerations for a given application have to weigh the better resolution, higher precision and wider choice of detectors in the multi-column system against the lower cost per analysis and smaller space requirements of the automated paper chromatography system. Obviously a great number of other factors would also enter into a decision about what system to choose. Skill of personnel, familiarity with particular procedures, availability of system components, system complexity, maintenance problems, computer access and economic factors are but some of the factors to consider when selecting a system. There is no obvious best choice for all applications.

The multi-column system compared with other chromatographic systems

Although no other high-capacity chromatographic system than the "Cassandra" system has been built it is obviously possible by investing enough in the necessary equipment to get high capacity in other chromatographic systems.

The commercially available automated gas chromatograph (Barber-Colman) can theoretically give a capacity of about twenty-two 1-hour chromatograms within a 24-hour period although we have found it not quite reliable enough to leave running unattended overnight. By combining four such chromatographs one can reach a capacity of close to 100 chromatograms per day.

The relative advantages of a 100 chromatogram multi-column system compared with such a system would be: no need for derivative formation, wider choice of detectors with higher specificity possible through the use, for example, of colorimetric and fluorescence reactions, higher load, cheaper apparatus and higher reproducibility with about equal resolution in the two systems as long as ordinary packed columns are used in the gas chromatograph. The advantages of the gas chromatograph would be: faster analysis time for the first of a batch of analyses, higher sensitivity in some applications, lower cost per analysis, convenience of being able to use a commercially available unit.

Systems could be set up using commercially available thin-layer scanners. Compared with such systems the multi-column system would have the same relative advantages and disadvantages as in the comparison with the automated paper chromatographic techniques.

Further developments

We have as mentioned newer simplified systems for multi-column chromatography under development in an attempt to economically reach the high capacity necessary in the chromatographic step to keep up with the expanded read-out capability. Also under development are simplified colorimetric channels using an inexpensive colorimeter/spectrophotometer instead of the colorimeter used in the current system. This will be cheaper per channel and will eliminate the expense of filters. In all this work as in the current apparatus commercially available apparatus is modified for inclusion in the combined system.

We hope later on to construct a special multicuvette spectrophotometer module that would use a 25 cuvette rotating cuvette arrangement and a single spectrophotometer and recorder to give 25-channel read-out capacity. This would be much cheaper per channel than the system described here since the cost of such a unit would be basically that of a spectrophotometer and a recorder plus the special construction cost involved. We hope also to construct a read-out system for high-specificity colorimetry which will include fast scanning of a pertinent part of the absorption curve, data recording on magnetic tape and computer analysis of the absorption curve.

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