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MICROCOMPUTER-CONTROLLED BUFFER GRADIENT GENERATOR FOR ION-EXCHANGE CHROMATOGRAPHY

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SUMMARY

A microcomputer-based system has been developed for switching between several buffer solutions to produce a mixture with independent elution gradients for two ions. The system has been used as a gradient programmer for the separation of amino acids by ion-exchange chromatography and the computer also supervises the automatic facilities of an amino acid analyser. In normal operation there is no monitoring of the concentrations produced but results obtained with the system demonstrate that the performance is reproducible and a good approximation to the specified gradient is obtained. Advantages of the system are that it gives the analyser a high separating power and it is versatile and easy to use.

INTRODUCTION

Separation of amino acids by ion-exchange chromatography requires variation in the composition of the buffer solution used for elution. This can be achieved by switching from one buffer to another in a stepwise manner or by using a continuous gradient produced by a gradient forming device. Buffer gradients were introduced for this purpose by Moore and Stein[1] to improve the separations obtained by their original method[2]. They used a simple single-chamber mixing system for producing a gradient. However, the first automatic amino acid analyser[3] used the stepwise system which was then adopted by the majority of instrument manufacturers. An exception was the Technicon NC-1 system, based on the work of Piez and Morris[4], which incorporated a nine-chamber gradient forming device developed by Peterson and Sober[5]. The NC-1 system has had a widespread application and many modifications of the original gradient specification have been proposed[6–8]. However, the increased speed of analysis, made possible by the use of small spherical bead resins and narrow bore columns, has made the automatic loading of samples desirable. The multi-chamber system became obsolete because it had to be manually filled with different buffers, though an automated two-chamber device has been described by Chilcote *et al.*[9].

The fully automatic gradient programmer, utilised in the Rank-Hilger Chromaspek, was originated by Thomas[10]. Two buffer solutions are mixed by switching a

pair of solenoid valves according to a graphic profile held on a rotating drum and scanned by a photo-conductive cell. This approach overcame the major problem of the multi-chamber device and it had an added advantage of being able to produce steeper gradients. Nonetheless, it was restricted to the independent control of one gradient because of the limitation of mixing only two solutions. In ion-exchange chromatography it is desirable to be able to control ionic strength and pH independently. High-performance liquid chromatography (HPLC) also requires gradient elution to resolve difficult mixtures. Separate pumps were originally used to mix two solvents at high pressure but single pump systems are being introduced[11–13] which mix two or more solvents at low pressure. The gradients produced by one of these systems[12] are controlled by microcomputer from a specification of the percentage of each solvent. We have utilised a microcomputer so that Thomas's approach can be extended to mix more than two buffers, thereby allowing the independent formation of two or more gradients. This paper describes the system (which has been named MICAWBER) and its attachment to a Technicon TSM amino acid analyser.

EQUIPMENT

Principle of operation

MICAWBER is designed to produce concentration gradients by rapid switching among a number of buffers. Typically, the time for which a buffer valve is open is between 1 sec and 1 min. The system is programmed to produce a mixture closely approximating to a profile of concentration gradients specified for each of two ions. The cumulative error between output and specification is used to determine which valve should be opened. The system consists of three modules built in these laboratories, *viz.* microcomputer, control console and valve unit as shown in the photograph (Fig. 1).

The microcomputer

This was mainly constructed from a kit (South West Technical Products Corp., Phoenix, Ariz., U.S.A.) based on the Motorola MC6800 microprocessor unit which responds to 72 instructions. A 16-bit address and 8-bit bi-directional data bus allows the MC6800 to communicate with 4K bytes of read/write memory, 1K byte of read-only memory, containing the resident operating program MIKBUR[14] and five universal Peripheral Interface Adapters (PIAs)[15]. When the "power up" or "reset" switch is operated, program control is passed to MIKBUG. An ASR33 teletype, attached through a serial control interface incorporating one of the PIAs, responds by indicating that the operating program is ready to receive commands. Punched paper tape data transfers and various diagnostic routines can then be run by MIKBUG. When the terminal is not required it can be disconnected without disturbing the system. Four input ports and four output ports are provided by the other PIAs; access to these is through their own specific memory addresses. Each port has eight peripheral data lines and two control lines available for interfacing to its part of the system.

The control console

Manual or automatic valve control is selected by a console switch. "Manual"

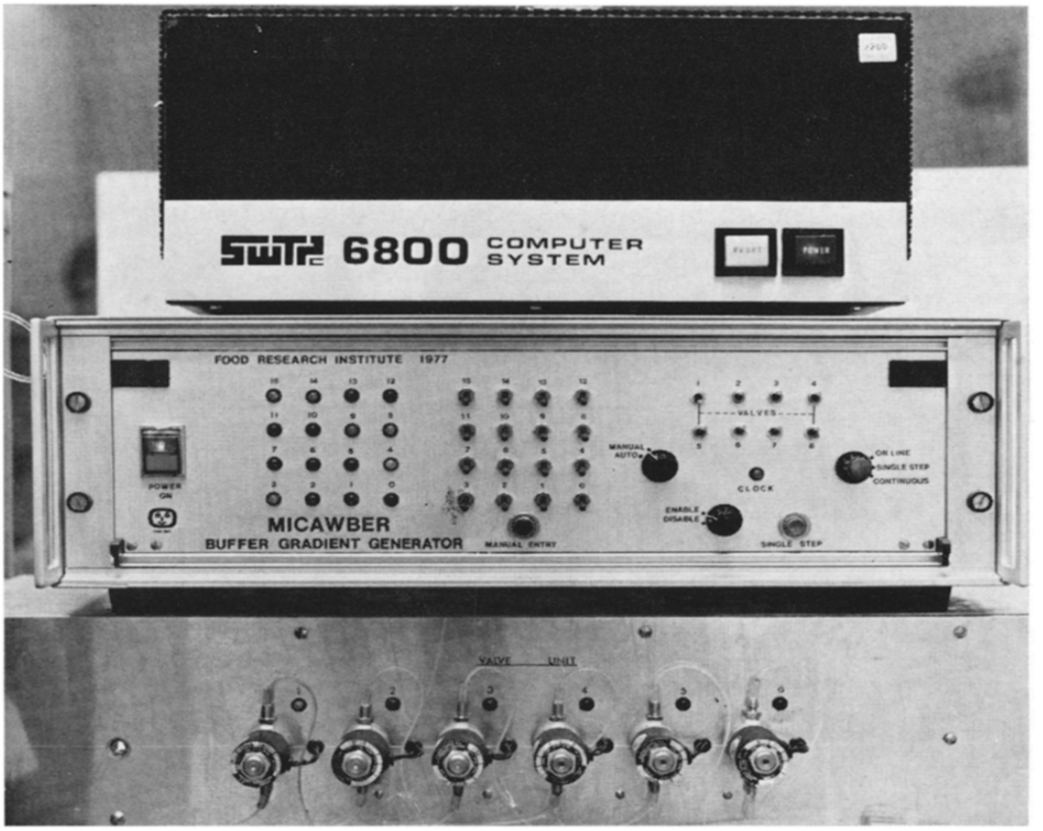

Fig. 1. The MICAWBER gradient programmer.

is used for testing purposes so that any valve can be energised by means of front panel switches. These are connected so that the lowest numbered has priority if more than one is selected. To permit independent operation of the microcomputer and the analyser, the system is effectively disengaged by the console's disable switch which sets all control output lines to a high impedance state. A 4 × 4 array of light emitting diodes provides a display of two bytes of data under program control. Two bytes of input data can be set up on a 4 × 4 array of toggle switches, and the associated interrupt request for computer attention is made by a "manual entry" push button. Acknowledgement by the microcomputer is necessary to reset the interrupt request line before further interrupts can be made. This form of "handshaking" is also required for non-maskable interrupts which are generated individually by push button or clocked at 10 Hz by a mains frequency step-down counter.

The valve unit

Six buffer reservoirs are connected in parallel via solenoid valves (type B2, 24 V d.c., normally closed, Skinner Precision Industries). The valves are mounted to give an upward buffer flow to remove air bubbles and may be switched either from the control console, or by the data by the on the output port designated for valve control. Optical coupling on the latter ensures electrical isolation of the valve

unit so that earth loops or inadvertent disconnections do not cause errors. Light emitting diodes are connected across each operating coil to indicate which valve is open.

Connection with the amino acid analyser and auxiliary units

The system is used in conjunction with a Technicon TSM amino acid analyser (TSM). The electrical connections are shown in Fig. 2 and fluid connections in Fig. 3.

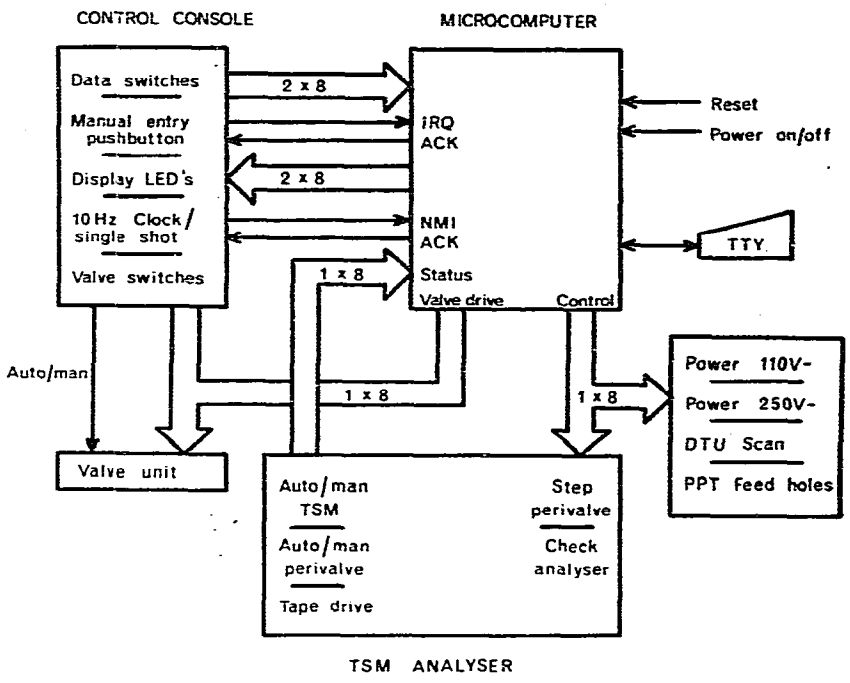

Fig. 2. System configuration (electrical).

In addition to regulating the buffer supply, the microcomputer controls the peristaltic valve (perivalve), which in turn governs the flow of the buffer mixture and reagents and operates the sampler. The unmodified TSM controls the perivalve with micro-switches activated by a control tape. In our system a blank control tape is loaded and MICAWBER operates by simulating the micro-switch closures with solid-state relays (D2410 International Rectifier) optically coupled to two output lines designated "step perivalve" and "check analyser". For the operation of auxiliary units, similarly connected relays provide mains power switching at 110 and 240 V and issue control signals to a Solartron Data Transfer Unit and Facit 4070 paper tape punch which are used to log the chart recorder output of the TSM. Three analyser operating conditions are monitored; these signals are derived from the "auto" indication lights of the TSM and perivalve, and the drive circuit of the tape motor. They are rectified, smoothed and optically coupled to three data lines of an input port. All connections have been made so that the TSM can be readily returned to its normal operating state. The output lines are connected in such a way that no commands are issued when (1) they

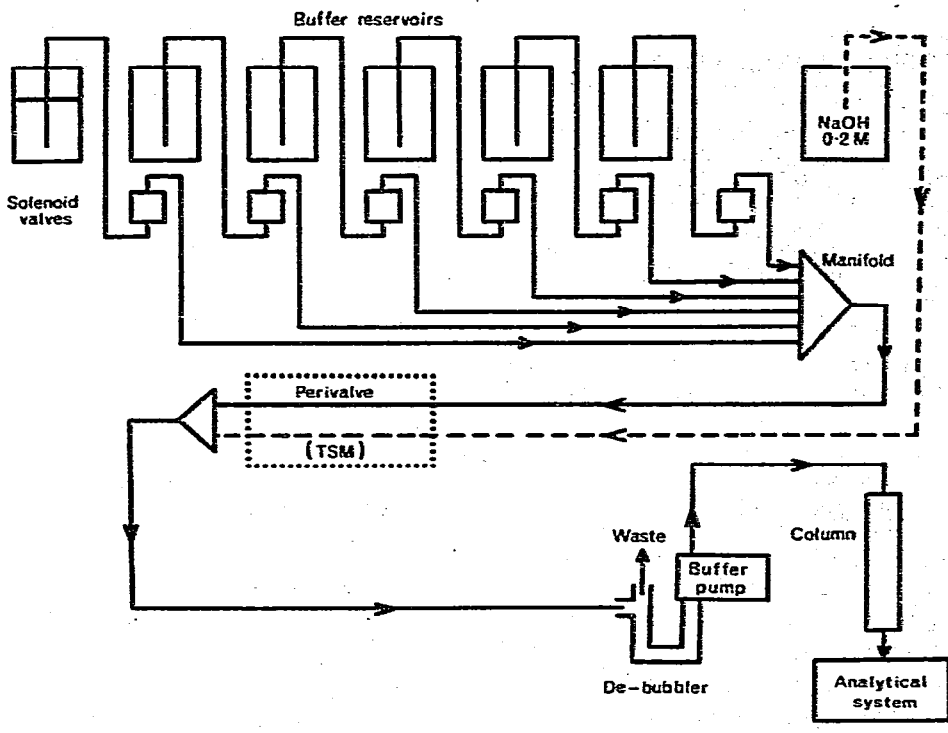

Fig. 3. System configuration (fluid).

are driven by a logical zero; (2) the console switch is at "disable"; or (3) the connectors are uncoupled.

Mainframe computer

Access to a mainframe computer was required for program development and for producing paper tapes. We used an IBM 1130 computer with 16 K words of memory and programs were written in FORTRAN.

METHODS

The valve switching algorithm

A method is required for opening and closing the valves controlling the buffers (Fig. 3) which will produce a mixture approximating in its ionic concentrations to a reference profile specified as a function of time. Only one buffer valve is open at any time and valve switching activity is minimized.

Table I is an example of a simple reference profile which consists of a set of concentration specifications. The specifications are, in general, independent of each other and each consists of a series of straight-line segments. Let the required concentration of ion i in the mixture be $r_{i,t}$ at time t. At the start of a segment these values are obtained directly from the profile, otherwise they are updated for each time interval:

$$r_{i,t} = r_{i,t-1} + g_i \qquad \text{for all } i$$

TABLE I

TYPICAL PROFILE SPECIFICATION

Buffer specification	*1*	*2*	*3*
pH	3.25	6.5	6.5
Sodium molarity	0.2	0.2	1.1
Scaling ratio 1.0			

Profile 1 Control data	*Accuracy tolerance*	*Elapsed time limit (sec)*	*Calculation interval (sec)*	*Data logging interval (sec)*
	2.26	10.0	0.2	4.0

Time (min, sec)	*Event*	*Concentration*		*Comments*
		Ion 1	*Ion 2*	
0,00		3.25	0.20	Initial conditions, *i.e.* Buffer 1
0,01	"step perivalve"			Perivalve from position 12 to 1
2,00	"step perivalve"			Perivalve position 2 (sample in place)
15,00	"scan logger"			Start data logging
20,00		3.25	0.20	
60,00		4.20	0.20	(pH gradient started at 20 min)
70,00		5.00	0.25	(Na^+ gradient started at 60 min)
90,00		5.80	0.60	
105,00		6.20	1.00	
105,00		6.50	1.10	Switch to Buffer 3
148,00	"check analyzer"			In perivalve position 2
143,20	End "check analyzer"			
150,00	"step perivalve"	6.50	1.10	Perivalve to position 3, NaOH to column
150,00		3.25	0.20	Switch to Buffer 1 to prepare for equilibration of column
160,00 to 160,35	"step perivalve"			Step perivalve from position 3 to 11 every 5 sec
164,00	End "scan logger"			Stop data logging and punch feed-holes
194,50	"step perivalve"			Perivalve to position 12
195,00	End of profile			End of profile number 1

where g_i is the concentration gradient of the ion calculated from the particular segment of the profile. A set of cumulative errors in concentration, e_i, is set to zero at the start of a run and is kept updated for each ion by the algorithm. Whenever there is a discontinuity in the profile the error term for that ion is reset to zero. For ion i and buffer solution v, let the concentration be $c_{i,v}$ in appropriate units.

The following procedure is implemented at each time step. Each buffer valve is considered in turn to calculate the set of cumulative errors, $a_{i,v}$, which would occur if the valve were opened for unit time:

$$a_{i,v} = e_i + r_{i,t} - c_{i,v} \qquad \text{for all } i,v$$

A score s_v is now calculated for each valve by summing the squares of these errors over all ions

$$s_v = \Sigma a_{i,v}^2 \qquad \text{over all } i, \text{ for all } v \tag{1}$$

Differential weighting between ions is achieved by the use of suitably scaled units of concentration for each ion. The "best" valve to open is the one with the lowest score but, to minimize switching, the valve currently open is left open, provided that its own score is less than a given tolerance. If, however, the time elapsed since the last valve change exceeds a specified limit, the "best" valve is nevertheless opened; this permits fine adjustments when they do not involve excessive activity. These switching rules do not imply that the score of the open valve is always within tolerance. The set of cumulative errors is updated to reflect the effect of the valve actually open:

$$e_i = a_{i,w} \qquad \text{for all } i$$

where w is the valve open. When a valve switch occurs, the previous valve is closed and the new one simultaneously opened.

Microcomputer programs

A computer program was written for running in the microcomputer to implement the valve switching algorithm and to provide signals for controlling the analyser. It is suitable for incorporation in read-only memory. Figs. 4–6 provide a simplified guide to the program logic. In particular, error handling and the setting up of information for the display lights have been omitted; these occupy about half of the program. The master program module (Fig. 4) initialises the microcomputer for subsequent operations and provides an output on the display lights of conditions detected by other sections of the program. Whenever the machine receives an "interrupt", lower priority work is deferred until the task demanded by the interrupt has been completed. The "manual entry" button supplies such an interrupt request and a second program module (Fig. 5) responds by reading the switches and setting indicators for interrogation during the next processing cycle. The interrupt control lines are prepared as "handshake-mode" outputs[15]. The largest section of the program controls the analyser and solenoid valves (Fig. 6). The clock issues the highest priority (*i.e.* non-maskable) interrupts to provide basic timing; the software selects a submultiple of this as the frequency for running the program. Since the program's execution time is not constant, valves are switched only at the start of each processing cycle, according to the results obtained during the previous cycle. This allows the time, for which a valve is open, to be controlled as accurately as possible. The program can switch up to eight analyser control signals on or off at times specified in the profile. The "step perivalve" command is automatically removed after 1 sec and "scan logger" commands are issued at a specified frequency. For simplicity, the program logic used to generate the signals has not been included in Fig. 6. Arithmetic is performed in fixed-point binary, the location of the binary point, word-length and sign convention depending on context. Since some words contain accumulating values, there is a possibility of arithmetic overflow. If this occurs, an indication is given to the operator but no recovery is attempted. The microcomputer has no hardware "multiply" instruction; the Motorola MULT16 subroutine[15], which implements Booth's algorithm, is used for 16-bit multiplication. Two further microcomputer programs were written, one to test the operation of the interface, operator controls and clock, the other to provide memory display–modify facilities whilst the main program is in operation.

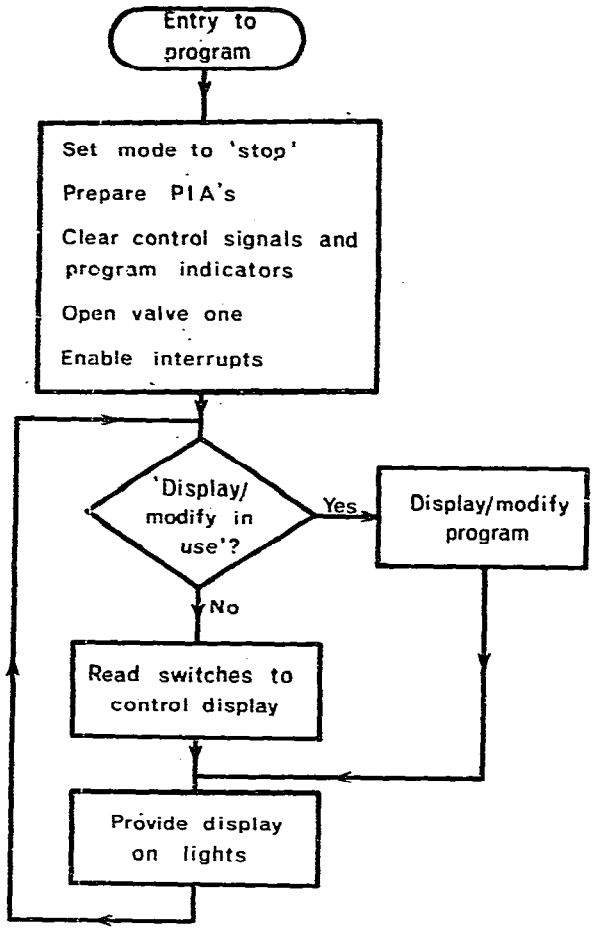

Fig. 4. Master program module.

Mainframe computer programs

Since the microcomputer programs comprise some 1500 statements and assemble to over 2 kilobytes, the programming could not reasonably have been performed in machine code. Program assembly facilities on the microcomputer considerably increase its cost so a cross-assembler program was written which reads statements punched on cards in Motorola assembly language[16], checks the syntax, converts to object (binary) code and punches a paper tape suitable for loading by the Motorola MIKBUG routine[14]. The cross-assembler is less than 1000 statements in length. Another program prepares the paper tape of profile information. It reads the specification cards in a convenient format, scales concentrations to accommodate to the capacity and precision of the relevant variables, calculates concentration gradients, and converts the results for output to tape. Valve switching algorithms were tested by programming them in the mainframe computer. The same specification cards were read and the consequent switching activities predicted. The resulting ionic concentrations were calculated using a mixing-chamber model[17,18] and a printed and graphical display of the performance was produced.

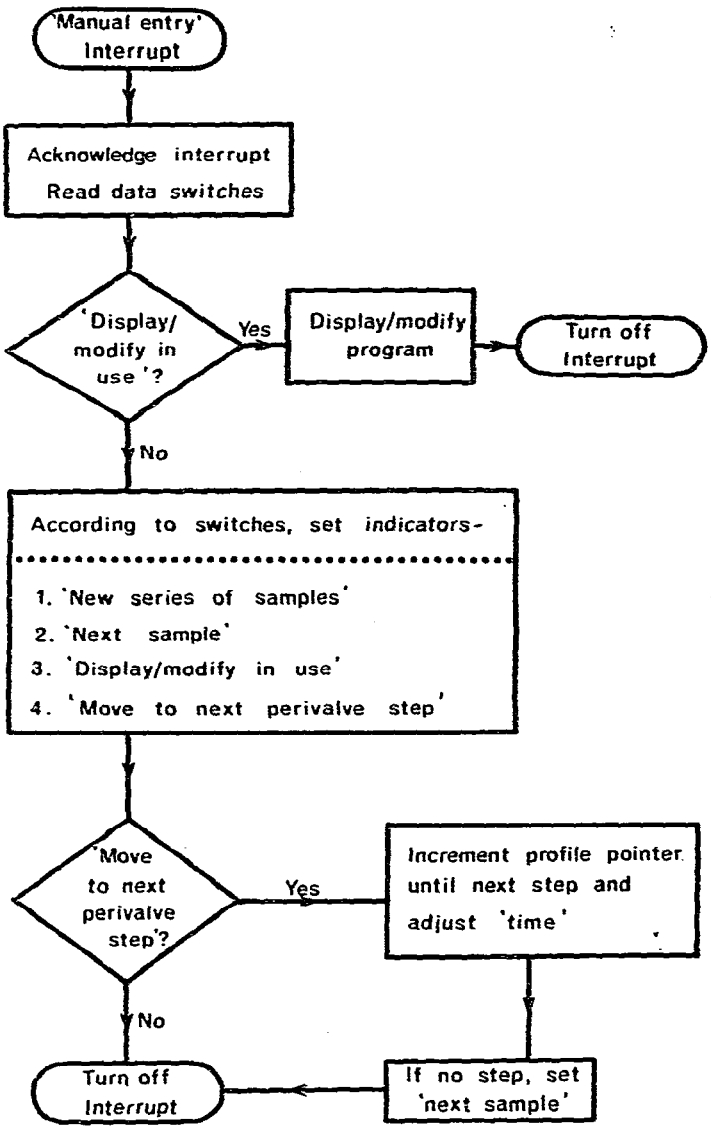

Fig. 5. Manual entry program module.

Operation of MICAWBER

The program permits the control of one or two ionic concentrations using up to eight buffers. Two binary programs, (1) analyser and valve control, (2) memory display–modify, are available on paper tape. These are loaded and prepared for running from the terminal, and are co-resident in memory. After the microcomputer has been powered down, reloading is necessary; this takes about 15 min. A paper tape defining the reference profiles must be loaded before the system can be used. This data file (Table I) contains the concentrations of the buffer solutions, up to five profile specifications, and a table of the profile numbers to be used with the various

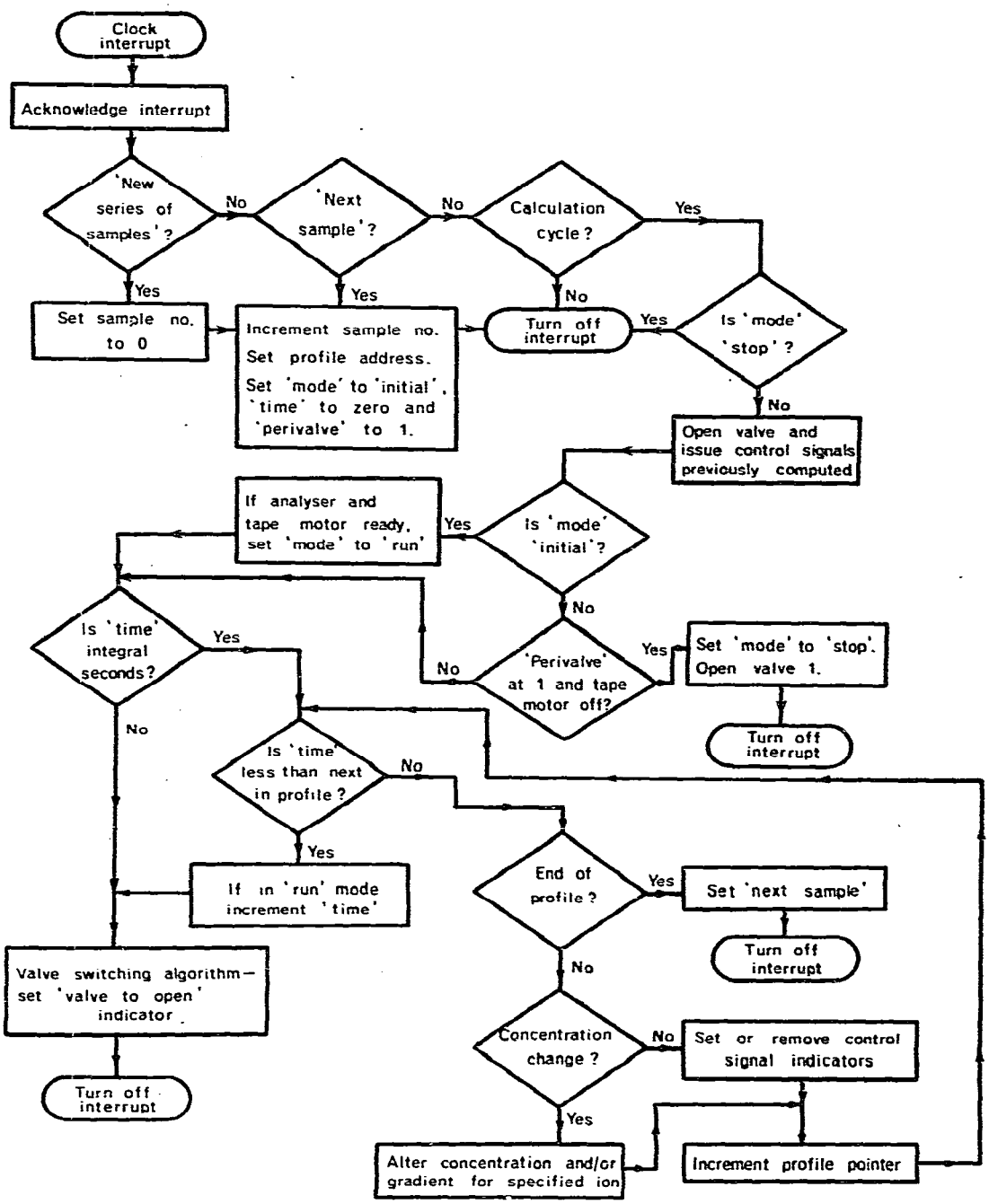

Fig. 6. Control program module.

samples. A profile is not practically limited in length and, as well as supplying the concentration–time relationships, it specifies the perivalve stepping regime, the time interval at which calculations are to be performed and the valve switching accuracy tolerance and time limit. When a new profile is required this information is put on

to computer cards for entry to the mainframe computer program which punches a paper tape suitable for loading. If the profile requires a step in concentration, the segment is entered as beginning and ending at the same time. Punching and loading take about 3 min each. The terminal is not required for any further operations until another tape has to be loaded. Usually this is only necessary when a profile is changed.

Operator commands from the data switches are indicated in Table II; the more crucial are only accepted when "manual entry" is pressed. The memory display–modify program is invoked by an operator command and it allows any memory location to be displayed on the lights and modified from the switches. It is used for monitoring various activities and for making minor changes such as altering the sequence of profile numbers to be followed. MICAWBER is put into operation by selecting the appropriate data switches and pressing the "manual entry" button. The valve switching algorithm is implemented for the concentration specification at zero time in the profile of the first sample. In this initial state, concentrations and elapsed time are held fixed until the analyser and perivalve have been prepared for operation. This is indicated to the microcomputer by the operator setting the TSM tape motor running. The program proceeds through the profile by switching valves and giving perivalve control signals, the first signal advancing the automatic sampler. The analyser status is monitored and abnormal conditions cause the display lights to flash. Severe errors terminate the program. Suitable commands in the profile check the status of the analyser and synchronise the data logging on paper tape. Feedholes are produced to separate the runs and the data are analysed off-line by computer[19]. At the end of a profile the next sample's profile is automatically addressed. When the TSM detects that the last sample has been analysed it turns the tape motor off. The program responds by progressively stepping the perivalve to its standby (twelfth) position and then entering a quiescent state.

TABLE II

OPERATOR CONTROL COMMANDS

Program Commands
(Obeyed when exact switch combination is set and "manual entry" pressed)

Switches	*Activity*
15 and 7	Go to initial state for first sample.
15 and 6	Skip in profile to next "step perivalve" (or end of profile).
15 and 5	Go to initial state for next sample.
8	Invoke memory display–modify program.

Indicator Display Commands
(Obeyed whenever relevant switch is on)

Switch	*Display*
0	Time of run in seconds (binary).
1	Analyser status (input); analyser control signals (output).
2	Perivalve position; valve, clock and interrupt status.
3	Sample number; profile number.
4	Time of run in minutes (binary-coded decimal).
Else	Error–warning display; program status.

EXPERIMENTAL

Tests on gradient production

The performance of the system was tested by experiments using solutions containing various concentrations of glycine and proline. These amino acids could be readily determined, without separation, by the analytical system of the TSM because of the difference in the colour produced by the reaction with ninhydrin. The ion-exchange column was not used. Each solution was calibrated by measuring the recorder response on the 440 nm and 570 nm channels when the solution was fed directly from the valve unit into the analytical system of the TSM. The results are shown in Table III. Tests were carried out by constructing two profiles specifying concentrations in recorder response units and checking the recorder output. The first profile (Fig. 7a) contained various combinations of gradients which were used to test the reproducibility of the system and its ability to control two independent gradients. The second profile specified constant "response units" at both wavelengths which were approximately mid-way between the response of any of the feed solutions. This profile was used to test the mixing ability of the system with various tolerance values. Further mixing tests were carried out in which the proline–glycine solutions were replaced with pH 6.5 buffer solutions containing appropriate amounts of methyl orange and bromo-phenol blue. The concentrations of the dyes were adjusted to give recorder responses similar to the proline–glycine solutions. In these tests the output from MICAWBER was fed directly into the TSM colorimeters.

TABLE III

SOLUTIONS USED FOR INITIAL TRIALS

Solution	*Proline (nmole/ml)*	*Glycine (nmole/ml)*	*Recorder response (%)*	
			440 nm	*570 nm*
1	50	0	9	2
2	500	0	77	11
3	0	125	17	86
4	395	110	68	75

Separation of amino acids

The system has been used to produce mixtures of buffers to separate the common protein amino acids by ion-exchange chromatography. Some of the basic amino acids are difficult to separate and various test profiles were tried before a satisfactory separation was achieved. Buffers used were: (1) pH 3.25, Na^+ 0.2 *M*; (2) pH 6.5, Na^+ 0.2 *M*; (3) pH 6.5, Na^+ 1.1 *M*. Buffer (1) contained thiodiglycol. All buffers were 0.1 *M* in citrate and contained 0.01% pentachlorophenol. The profile concentrations were specified in the units quoted.

RESULTS

The results of some mixing trials are shown in Fig. 7. Mixing tests using proline–glycine solutions are shown in Fig. 7b and c, while Fig. 7d shows a typical

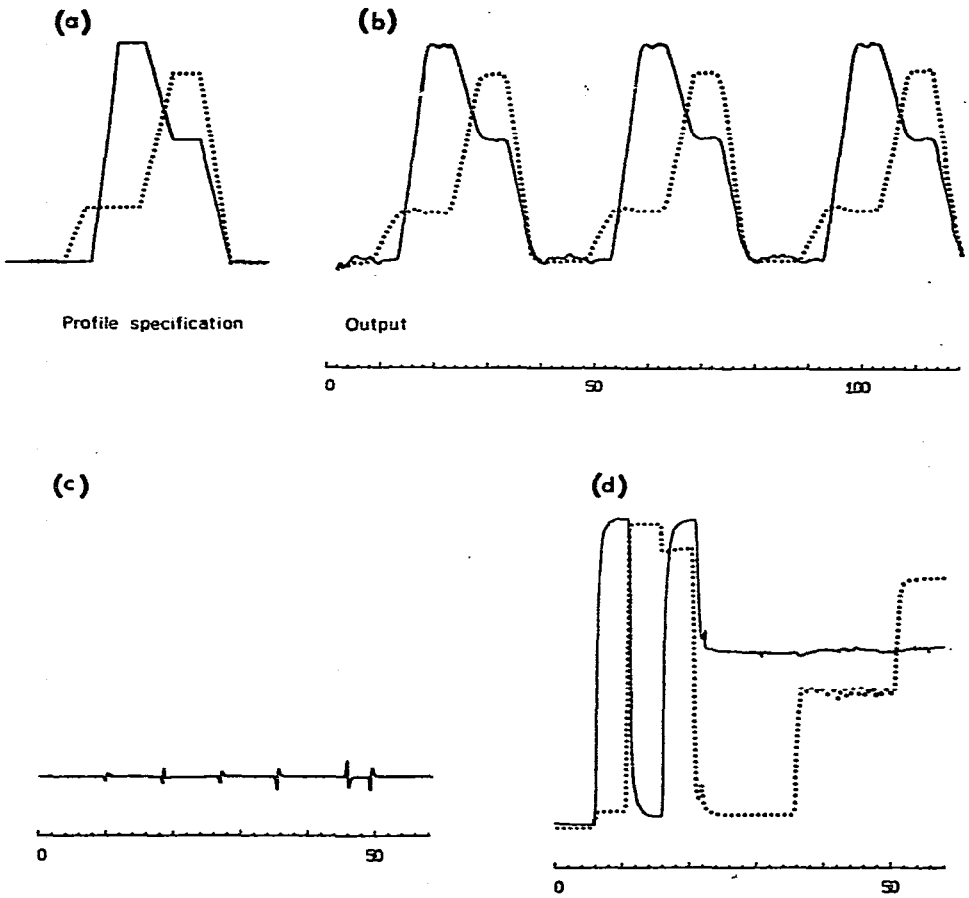

Fig. 7. Performance during initial trials. ——— = 570 nm colorimeter response, ····· = 440 nm colorimeter response. Horizontal scale, times in minutes.

result from the second series of tests using coloured solutions. In the latter case, the profile consisted of two parts. In the first, MICAWBER was instructed to open each valve for 5 min. The instructions in the second part were to maintain the colour response at 570 nm at 50%, while producing three intermediate levels on the 440 nm channel. An amino acid chromatogram produced by the complete system using the profile in Table I is shown in Fig. 8a. The variation in separation of basic amino acids produced by changes in the buffer gradients are indicated in Fig. 8b–e.

DISCUSSION

Development of method

The original idea was to use the mainframe computer to calculate the times at which each valve switching should occur and then transfer the results to the microcomputer by paper tape. A very simple microcomputer program would then issue the commands at the specified times. A model of this system was tested on the mainframe computer and the present design of the hardware was conceived following a

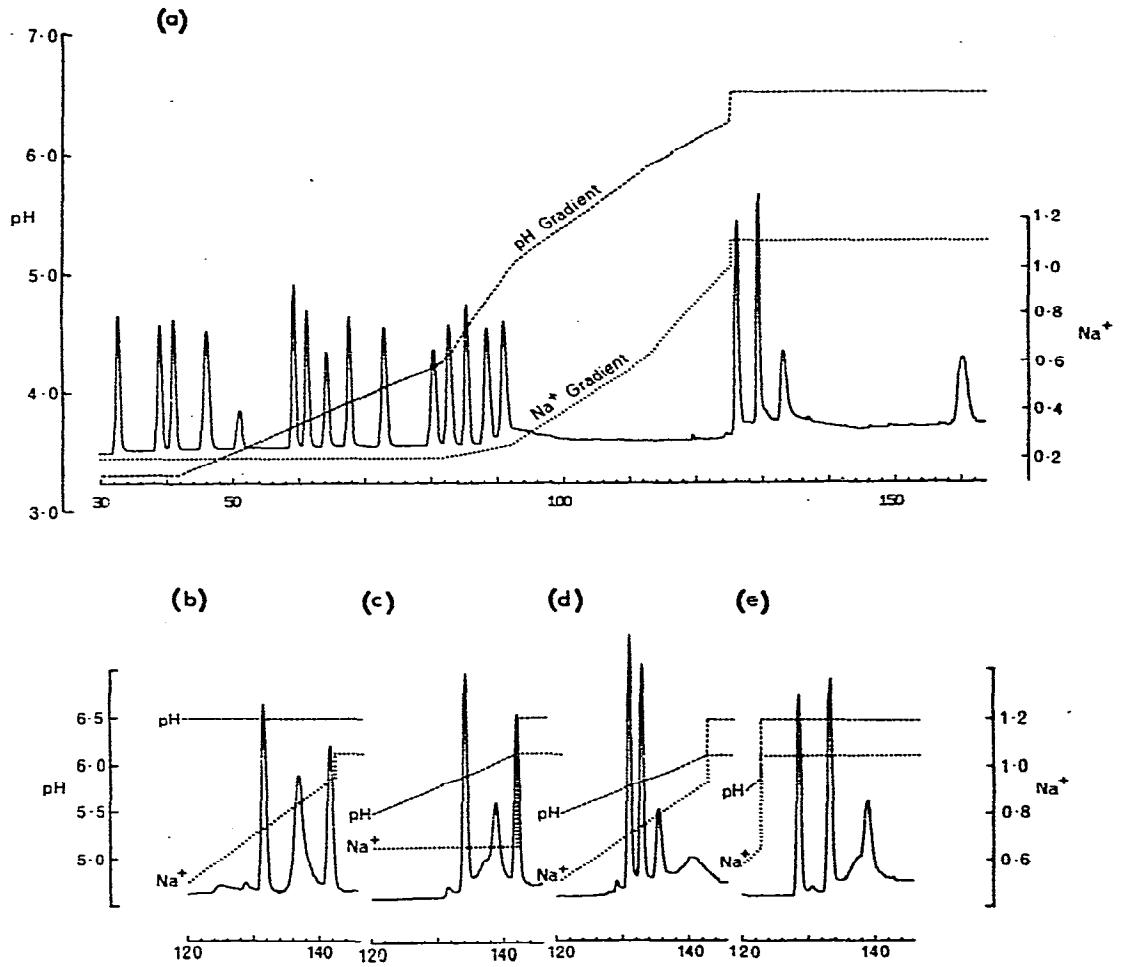

Fig. 8. Amino acid chromatograms using MICAWBER ——— = 410 nm colorimeter response – – – = pH gradient, ····· = Na^+ gradient. The times in the profile have been increased by 22 min to correct for the delay in the analytical system.

satisfactory series of tests. However, during the construction stage, further tests on the model revealed that an excessive number of commands might be needed to achieve the required accuracy. Attempts to overcome these difficulties led to the present system in which the microprocessor does most of the calculating and makes the valve switching decisions. It is an interesting demonstration of the flexibility of microcomputer-based systems that this radical change required no modification to the hardware. The possibility was considered of using ion-selective electrodes to provide a feedback of performance to the computer. The long-term stability of these appeared to limit them to titration systems[20,21]. However, Gaarenstroom *et al.*[22] have recently described a computer-controlled measuring system. The present state of ion-exchange theory is not sufficiently well developed to make practical use of accurately known concentrations. Pitt[23] and Inczédy[24] have produced mathematical models for predicting retention volumes of peaks from gradient elution ion-exchange chromato-

graphy, but it would be extremely time-consuming to perform an iterative process for optimising elution positions for twenty or more peaks. MICAWBER was partly designed to simplify the experimental work needed to determine the required profile and then to be able to reproduce it within a narrow tolerance. The long-term reproducibility of the system depends mainly on the precision of preparing the input buffers and thus it is an advantage that complex gradients can be formed from a small number of buffers.

Program testing was hindered by the lack of an EXORciser (MC6800 simulator); the MIKBUG testing facilities are not very powerful. Once the program was in a running state, however, its performance could be compared against the model run on the mainframe computer. The model was also useful in testing various valve switching algorithms since these are much more easily written in FORTRAN. In total, the systems analysis and programming took about 3 man-months and the electronic design and construction about 7 man-months.

The initial trials of the system (mixing proline–glycine solutions) gave encouraging results. Fig. 7b shows that under test conditions, opposing gradients could be formed which were reproducible and a good approximation to Fig. 7a, the specification. Fig. 7c shows that a fixed concentration can be maintained without any tendency to drift. This was produced with a tolerance specification which resulted in an 18-sec switching frequency and at this rate the output is well damped. The presence of the peaks caused some concern because they are of much longer duration and it was not clear why they occurred. They may be due to a beat frequency between MICAWBER and the analytical system of the TSM, an effect similar to that observed by Saunders[13]. In order to eliminate the analytical system, the second series of mixing tests was carried out using coloured solutions and passing the output from MICAWBER directly into the colorimeters. Under these conditions there was more random noise but no indication of any peaks. The mixing test shown in Fig. 7d is a stringent test of the ability of the system to control two ions independently. The difference in the noise levels of the two outputs when they are both near the mid-position is due to mixing in the colorimeters. In operation with standard buffers for separating amino acids, no sudden changes in baseline have been detected.

Performance of equipment

The system has been in use for nine months and, after some initial problems had been resolved, has been reliable. Two faults were found in the microcomputer: a single bit in read/write memory was permanently true and one of the memory circuit boards had an intermittent fault whereby, when data was stored at certain memory locations, one bit was lost some milliseconds later. This condition was not detected by the Motorola test program ROBIT. Both faults were due to individual chip failures during the first few hours of operation and are considered to be typical integrated circuit faults. No problems have been encountered regarding the corruption of programs or data stored in read/write memory for long periods, although initially it was thought that this might be a problem. Inaccuracies due to valve switching have not been detected indicating that the speed of response is adequate. Bristow[25] has reported the characteristics of a similar type of valve and we expect the valve life to be better than 3000 h at a 10-sec switching rate.

Performance of the method

The algorithm for valve switching seems to be adequate. For any particular ion, mixing is assumed to be linear in the units used (*e.g.* molarities) and independent of the other ionic concentrations. For the hydrogen ion the first assumption, at least, is untenable and so logarithmic units (*i.e.* pH) are used and treated as if they combine linearly. If this approximation is unacceptable the system must be regarded as acting as a proportional mixing system between buffers of different pH. In all the experimental work a scaling ratio of 1.0 between concentration units has been used. For amino acid chromatography an error of one unit in pH is thought to be of approximately equal importance to one unit in sodium molarity. This gives a relatively finer control of pH than of sodium molarity. An advantage of using cumulative errors as a basis for calculating scores, rather than approximating to a proportional system, is that small systematic deviations are eventually corrected. Since equal weight is given to all previous deviations, a disadvantage is that an uncorrected error occurring at some time in a run might be adjusted for at a much later time. This does not seem to occur in practice and the errors generally oscillate around zero with a period of a few seconds. Although the present program has been written for two ions there would be no problem in introducing a third or fourth. As can be seen from eqn. 1, the algorithm requires $i \times v$ multiplications, each taking 1.5 msec. The execution time of the rest of the algorithm is about 3 msec so the total time is approximately $3.0 + 1.5 \times i \times v$ msec. Since an interval of 200 msec between calculations seems to be satisfactory, the execution time is sufficiently short for the present purposes. For other applications, such as HPLC, requiring more frequent calculations, the time taken in calculating squares might be excessive. However, the use of absolute values rather than squares can result in valve switching producing an ever increasing error in one ion. It is necessary to square the errors before summation because the size of any change in error for an ion is then weighted according to the magnitude of the cumulative error of the ion.

It is very difficult to monitor the performance of the system in normal operation; reproducible chromatograms are produced and peak elution times are systematically affected by changes to the profile. Fig. 8a was obtained with only three buffers; a very similar chromatogram was originally obtained using the same specification of profile and an extra pH 4.2, 0.2 *M* buffer. The fact that this buffer can be removed indicates that the assumption of linear mixing in pH is reasonable. In Fig. 8b–e the separating power of gradient elution is demonstrated. These chromatograms were produced sequentially in an over-night run from five different profiles.

The advantages of MICAWBER are: (1) gradients are easily specified in working units; (2) a wide variety of profiles can be specified without the need to change the input buffer solutions; (3) five profiles can be stored at any time and successive samples can be run on different profiles; (4) it can be used as a step buffer system if required; (5) a relatively small number of buffer solutions are required.

Disadvantages are: (1) access to a mainframe computer is required for producing new profiles; (2) a terminal is required for program and profile loading; (3) gradients cannot be specified which end at an extreme buffer (in order to use an extreme buffer it must be introduced as a step).

CONCLUSIONS

It has been shown that a microcomputer can be used to generate multiple ion buffer gradients by switching between appropriate buffers. Further developments of the present system using lithium citrate buffers for the separation of amino acids from plant extracts[8] and using borate buffers for the separation of sugars[26] are envisaged. The availability of the microcomputer has already led to its use for providing a number of automatic functions and this aspect is also likely to be expanded. The system may also be useful in other areas requiring multiple gradients such as high-performance liquid chromatography.

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