Journal of Chromatography, 292 (1984) 412 417
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 16,630

Note

A personal computer-based gradient system for high-performance liquid chromatography with low-pressure mixing

RUURD VAN DER ZEE* and GJALT W. WELLING
Laboratory for Medical Microbiology R.U., Oostersingel 59, 9713 EZ Groningen (The Netherlands)
(First received July 1st, 1983; revised manuscript received December 19th, 1983)

An essential aspect of high-performance gradient elution is a gradient control system. Reliable commercial equipment for generating reproducible gradients is available, but is rather expensive. During the past few years personal computers, primarily designed for home entertainment, have become more and more popular, resulting in a marked decrease in the price of these machines. Although their computing power and memory capacity may not always be enough, such devices have been successfully applied in the laboratory[1–5].

We now describe the use of an inexpensive personal computer interfaced with a three-way solenoid valve for gradient elution. This low-pressure gradient system produces reproducible binary gradients and can be used in conjunction with any pump. Since the microcomputer is provided with a BASIC interpreter (8K) and a 6502 mnemonic assembler, the system is not only cost-effective, but is also easily maintained and can be adjusted to particular circumstances.

MATERIALS AND METHODS

An Acorn Atom computer (Acorn Computers, Cambridge, U.K.) was purchased in the 12K RAM + 12K ROM assembled configuration, including the optional VIA (the 6522 Versatile Interface Adapter). The microcomputer, featuring a 6502 microprocessor, was fitted with a utility Rom, *i.e.*, AXR1 (Electronisch Centrum Delft, Delft, The Netherlands), to allow for text in the graphics mode. The system also includes a commercial cassette recorder to store machine language and BASIC programs, a portable television set, *e.g.*, Philips Model TX, and a small interface circuit as illustrated in Fig. 1, to drive a three-way solenoid valve. The PTFE-coated valve used was a 12-V LFYA valve (Lee Company, Westbrook, CT, U.S.A.). It has a response-time of 55 msec, an internal volume of 7.4 μl and a pressure range to 1000 psi.

The chromatographic system further consisted of a Waters M 6000 A pump, a Rheodyne 7125 injector, a C_{18} column (40 × 4.6 mm) packed with Supelcosil LC-318 (Supelco) and a Pye Unicam LC-UV detector. Solvents were continuously degassed with helium.

To study the performance of the whole system, stepwise gradients of methanol and 0.5% acetone in methanol were employed, and the absorbance at 254 nm was

0021-9673/84/$03.00

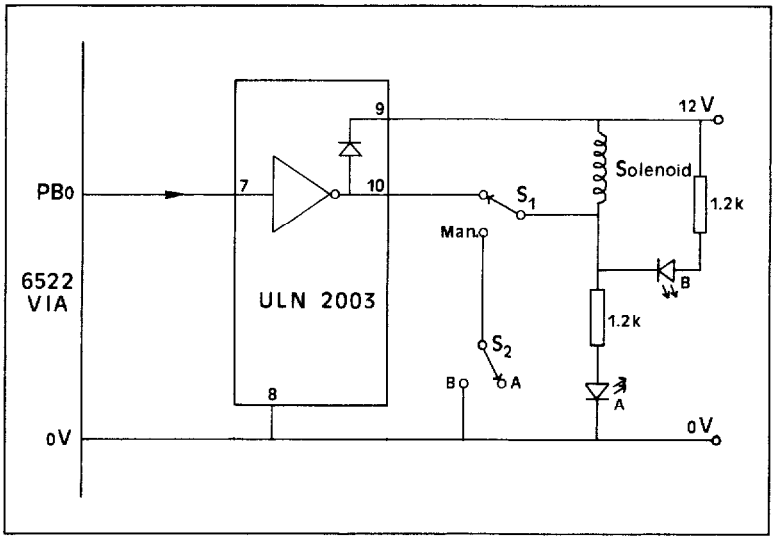

Fig. 1. Circuit diagram for the interface between the Atom microcomputer and the three-way solenoid valve. One of the output signals (PB0) of the 6522 VIA was taken from the internal connector, and connected to one of the Darlington inputs of the ULN 2003 Darlington driver (Sprague). This seven-stage Darlington driver integrated circuit was fitted in the Atom. All other components, including the solenoid valve, were mounted in a separate box. Two switches, S1 and S2, were included to allow for manual operation (man.). Selection of either solvent is made visible with two corresponding indicator hight emitting diodes (LEDs). The numbers 7–10 refer to the pinnumbers of the ULN 2003.

monitored. Standard proteins were eluted with a gradient of *n*-butanol–ethanol (20:80, v/v) in 12 m*M* HCl as described previously[6].

The main part of the operating program (available from the authors) was written in BASIC, and accounts for the input of data, equilibration of the column with the initial solvent composition and of the actual performance during elution. A small machinelanguage subroutine was used to obtain the correct timing. The high-resolution graphics of the Atom computer, with a resolution of 256 × 192 pixels, was used to plot the desired gradient after the input of the appropriate data (Fig. 2).

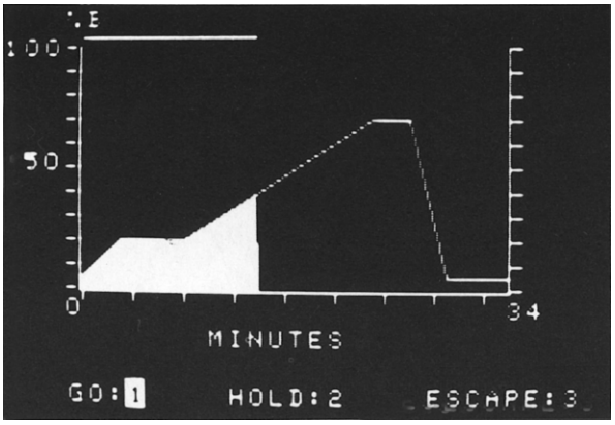

Fig. 2. Display showing the plotted gradient during operation.

Because most readers will not be familiar with the Atom-BASIC dialect, the essence of the gradient-operating routine is shown in Applesoft-BASIC (Fig. 3), to facilitate implementation on other systems. Although the program is rather self-explanatory, a few aspects will be discussed here.

The gradient is built up from linear segments. Data needed for gradient operation are the initial concentration of solvent B (in %, v/v) and for each subsequent gradient segment, the duration (in min) and final concentration for that segment, T(S) and B(S) (see lines 8–12 in Fig. 3).

A time-scaling factor, F, is used to keep the cumulative width of the segments below a maximum of 200 pixels, when the gradient shape is subsequently plotted on the display (see Fig. 2). The width, W(S), in pixels of each segment is calculated as shown in line 15.

For each element per segment the relevant solvent concentration is calculated with the linear function in line 23. With the use of an extra parameter, the latter could be replaced by a non-linear function if non-linear gradients are needed. Each element consists of F cycles, during which each time a TTL-compatible output port (in our configuration PB0 of the VIA) is set to an "on" or "off" state (lines 26 and

```
 1 ...
 2 ...
 3 REM input data
 4 INPUT N: REM number of segments
 5 DIM B(N),T(N)
 6 T(0)=0: D=0
 7 INPUT B(0): REM initial %B
 8 FOR S=1 TO N
 9 INPUT B(S): REM final %B of segm. S
10 INPUT T(S): REM time (min) of segm. S
11 D=D+T(S): REM duration of gradient
12 NEXT S
13 F=1+D/10: REM scaling factor
14 FOR S=1 TO N: REM scale segment widths
15 W(S)=20*T(S)/F: NEXT S
16 ...
17 ...:REM plot gradient
18 ...
19 REM operate gradient
20 K=ASC("HOLD")
21 FOR S=1 TO N: REM for each segment
22 FOR E=1 TO W(S): REM for each element in segm. S
23 C=B(S-1)+(B(S)-B(S-1))*E/W: REM %B for each element
24 FOR R=1 TO F: REM repeat F cycles
25 FOR P=1 TO 99: REM for each %
26 IF P<C THEN POKE -16295,0: REM output port="ON", sel.solv.B
27 IF P>=C THEN POKE -16296,1: REM output port="OFF",sel.solv.A
28 IF P<C POKE ...: REM plot point to display
29 CALL DELAY
30 K=(PEEK(-16384))-128: REM ASCII-code of pressed key in K
31 IF K=ASC("QUIT") THEN P=99:R=F:E=W:S=N
32 NEXT P
33 IF K=ASC("HOLD") THEN R=R-1: REM stay tuned
34 NEXT R
35 NEXT E
36 NEXT S
37 GOTO 17: REM next gradient
```

Fig. 3. The gradient operating program, rewritten in Applesoft-BASIC.

27). This results, by means of the Darlington driven solenoid valve, in the alternate selection of solvent B and solvent A during a certain amount of time. Combination of the amounts of both solvents gives the desired solvent concentration (v/v) for that cycle.

Starting (or continuing), halting and cancelling a gradient is achieved by a keyboard-scan routine in line 30. Starting or continuing a gradient can be performed by pressing key G (go) or any other key except key H (hold) and key Q (quit). In most other BASIC programs this can be replaced by a KEY or INKEY statement.

A simple machine language routine (DELAY) was used to adjust one cycle period to 3.0 sec, to obtain a proper timing for the gradient system. Therefore, the minimum duty time in one valve position is 30 msec, corresponding to 0.5 μl at a flow-rate of 1 ml/min.

The processing of the gradient is made visible by a POKE instruction to the appropriate display-memory location, yielding direct information with regard to solvent composition and elapsed time (see Fig. 2). After each gradient analysis the system returns to the initial solvent composition in a stand-by mode.

Listings and/or copies of the original Acorn program on cassette tape, together with an instruction sheet, are available on request.

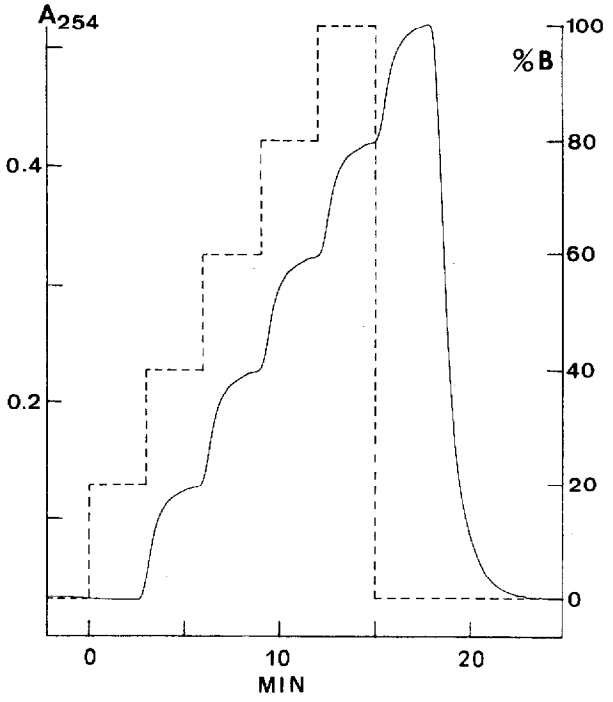

Fig. 4. Stepwise gradient of solvent B (0.5% acetone in methanol) in solvent A (methanol) at 1 ml/min. The concentration of solvent B was increased in steps of 20%. The absorbance at 254 nm (———) of the solvent resulting from the applied gradient (---------) was monitored with the outlet of the injector directly connected to the detector.

RESULTS AND DISCUSSION

One of the most important aspects of the gradient system is proper functioning of the solenoid valve. Three different types have been in use, two of which, Ultrograd 11300 (LKB, Bromma, Sweden) and MMD-3-D2 Micro Delta (Fluorocarbon Company, Anaheim, CA, U.S.A.), were less suitable for application in this high-performance liquid chromatographic (HPLC) gradient control system because of leakage, or a slow or irregular reponse-time. Only the LFYA valve could be used without modification and could accurately deliver the required solvent compositions. This enabled a satisfactory functioning of the whole system when tested with stepwise gradients of 0.5% acetone in methanol, while monitoring the absorbance at 254 nm (Figs. 4 and 5). Even for 1% steps in solvent composition, corresponding to a difference of 30 msec in the switching time of the solenoid valve, correct solvent compositions were obtained (Fig. 5). In this way the increase for each step of 0.5 μl of solvent B in solvent A per cycle period (3 sec) could easily be achieved, which is important for small volume gradients at lower flow-rates. As a more general test for normal operating conditions, protein standards were eluted with a linear gradient from a large-pore size C_{18} column. The separation obtained was indistinguishable from the one obtained with a commercial system (Waters), which needs an additional pump for high-pressure mixing (data not shown).

Although the system can essentially be used with any pump having a nearly continuous inlet flow, a mixing chamber might be necessary when a single-piston

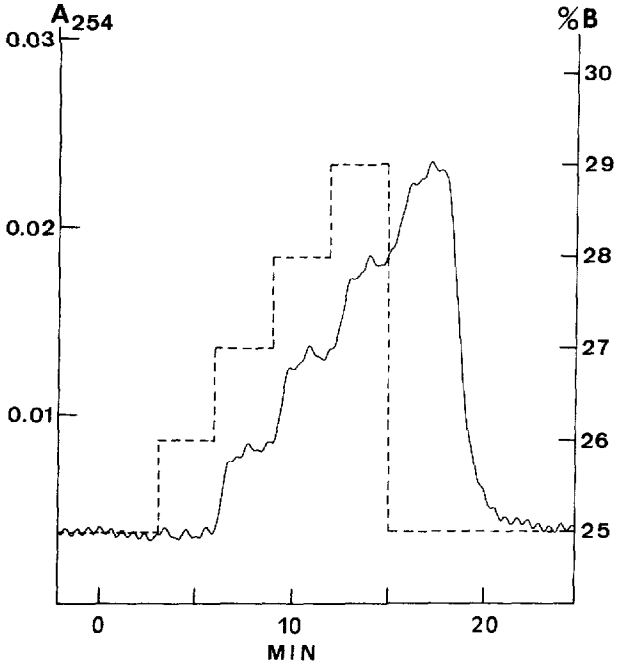

Fig. 5. Stepwise gradient as in Fig. 4, but solvent B was increased in steps of 1%.

pump is used, to compensate for small fluctuations in the solvent composition due to interference between switching of the valve and the frequency of the piston-stroke. In the system described here, with a dual-piston pump (Waters M 6000 A), interference occurred also, but only when the duration of one piston-stroke (stroke volume: 100 μl) coincided with the period of 3 sec, used by the computer program for one valve-switching cycle, *i.e.*, at a flow-rate of 2.0 ml/min. In the rare situations that this flow-rate was needed, a mixing chamber of about 0.3 ml reduced any fluctuations in the solvent composition. A mixing chamber could also reduce the small fluctuations visible in Fig. 5. However, this would have an adverse effect on the step-response, and was therefore not employed at other flow-rates.

This low-pressure mixing gradient system, in conjunction with the LFYA valve, has been extensively used in our laboratory for more than a year. The gradient system was applied successfully to the separation of Sendai virus proteins by reversed-phase and anion-exchange HPLC[7].

This system, consisting of a micro-computer, interface, valve, television set and cassette recorder, provides an economical way ($500–600) to transform an existing isocratic HPLC system into a programmable gradient HPLC system.

NOTE ADDED IN PROOF

Recently a less expensive 60/15 PSI valve (LFYX) with all other specifications equal to those of the LFYA valve has been introduced by the manufacturer.

ACKNOWLEDGEMENTS

We thank Mr. H. Boerema for his advice on the general applicability of the system. This research was supported by Grant GUKC IVh 81-8 from the Koningin Wilhelmina Fonds.

REFERENCES

1 B. Durham and C. R. Geren, *Anal. Biochem.*, 116 (1981) 331.
2 G. S. Begg and F. J. Morgan, *Anal. Biochem.*, 119 (1982) 12.
3 R. Ambrosetti, N. Ceccanti and C. Festa, *J. Phys. E.*, 16 (1983) 265.
4 B. Chornik, *Rev. Sci. Instrum.*, 54 (1983) 80.
5 M. S. Caceci and G. R. Choppin, *Compt. Chem.*, 6 (1982) 161.
6 R. van der Zee, S. Welling-Wester and G. W. Welling, *J. Chromatogr.*, 266 (1983) 577.
7 G. W. Welling, J. R. J. Nijmeijer, R. van der Zee, G. Groen, J. B. Wilterdink and S. W. Welling-Wester, *J. Chromatogr.*, (1984) in press.