Journal of Chromatography, 280 (1983) 370–375
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 16,169

Note

Improvements to a rotating disc multiwavelength ultraviolet high-performance liquid chromatographic detector

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(Received July 22nd, 1983)

During the last year the versatility of UV absorbance monitoring in high-performance liquid chromatography (HPLC) has been greatly enhanced by the development of detectors which permit simultaneous, or rapid sequential, monitoring at several wavelengths. These approaches to HPLC monitoring provide spectral information about each eluting peak thus aiding in characterisation and the determination of peak purity. Detection systems of the first type incorporating linear diode arrays are now commercially available[1] but they are expensive and a relatively low cost alternative exploiting a rotating disc holding four narrow band-pass filters was developed at this laboratory[2]. Common to both types of detector is the use of a microcomputer to store and handle the spectral data, and the cost of this item constitutes a substantial portion of the total. This paper describes modifications to the earlier system[2] permitting the use of a lower cost computer, and the results achieved with it are reported separately[3].

The microcomputer used previously was a Zilog 1/20A which required only minimal adaptation to accept the output signal from the photomultiplier and the pulses from the rotating disc, and was programmable in Fortran. In the current adaptation a Commodore PET microcomputer was used in conjunction with an analog interface board. The programs for this system were encoded in (1) Machine Code to access the signals coming from the detector; (2) Basic to handle the data stored.

This dual approach provide the speed required for receipt of signals whilst retaining the convenience of the simpler language where speed was less essential.

THE DETECTION SYSTEM

Multiwavelength unit

The multiwavelength detector unit has been described in a previous report[2]. Some further details of its components are given in Table I. The rotating disc which supports four narrow bandpass interference filters was positioned directly in front of the HPLC flow cell. As each filter came into position, one of four aluminium flags attached to the edge of the disc, activated a slotted optical light sensor. A fifth aluminium flag with its own independent light sensor was used as a "start" reference signal to ensure that the rotational sampling sequence was maintained.

A constant speed of rotation of the disc assembly was required to ensure re-

0021-9673/83/$03.00

TABLE I

TECHNICAL DETAILS OF THE DETECTOR

Speed of rotation of the filter disc assembly	120 rpm
Photomultiplier	R372 (Brandenburg power supply Model 475R)
Dynode resistors	100 kΩ metal oxide
Pre-amplifier	LM11 Sensitivity 1 μA/V
Logarithmic amplifier	Burr Brown 4127 (set to cover 0–1 AU)
Slotted optical light sensors	RS Type 304–560
Analog to digital converter	Datel ADC-149
Sample/hold amplifier	Datel SHM-1
Multiplexer	LF13331 Quad bi-fet switches
Differential amplifier	LM11 Unity gain
Digital to analog converters	Analog devices AD7542KN
Input/output chips	6522
Control logic	TTL gates

producible measurements of absorbance values from an identified portion of each filter. The performance of the stepping motor used in this work was found to be superior to that of the synchronous motors tested.

A photomultiplier was used to monitor the light transmitted through the flow cell. To minimise electrical noise a pre-amplifier was built into the base of the detector (see Fig. 1 for the circuit diagram). The signal from this amplifier was in turn connected to a logarithmic amplifier which gave an output signal directly proportional to absorbance.

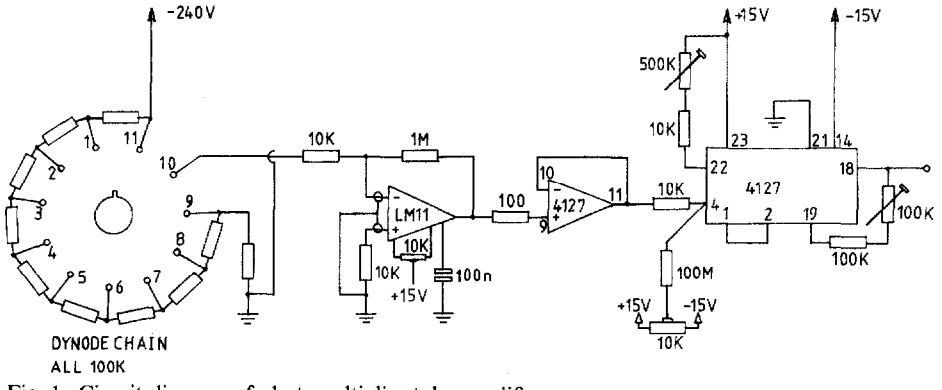

Fig. 1. Circuit diagram of photomultiplier tube amplifier.

Analog interface board

The analog signal from the logarithmic amplifier was connected via a multiplexer, differential amplifier and a "sample and hold" amplifier to a 14 bit digital to analog converter (see Fig. 2). The signals from the slotted optical light sensors were connected to the interface board via two inputs on the multiplexer. Finally the

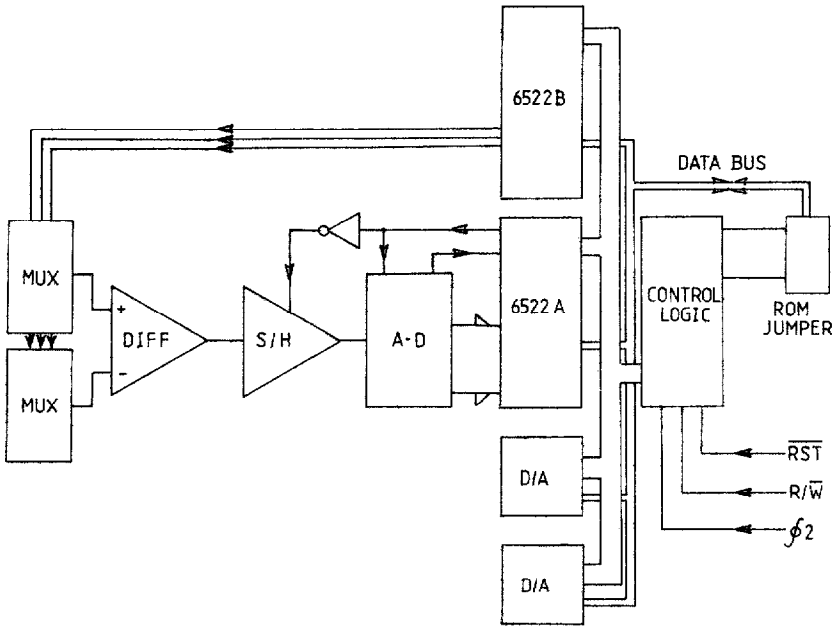

Fig. 2. Block schematic analog interface board.

output signal from the pre-amplifier (which represents transmission) was connected to a fourth input on the multiplexer. This signal was only monitored for diagnostic purposes.

The control logic which handles the transfer of data from the interface board to the microcomputer was configured so that the appropriate signals to and from the device were directly compatible a spare socket in the PET. (This is one of the two spare "Read only Memory" sockets.) This compatibility was achieved by using two input/output chips (6522A & B in Fig. 2) which are identical to the input/output components used in the standard PET microcomputer. With this arrangement the computer supplied many of the necessary control signals for implementing the circuitry on the interface board.

The machine code program written for utilising the interface system generated the necessary pulse to activate the acquisition of each 14 bit value from the analog to digital converter. On completing this task the analog to digital converter generated a pulse of its own which indicated to the program that the conversion had been completed and the value was ready for storage. The total conversion and storage time for each 14 bit value was approximately 300 μsec.

The control logic of the interface board also enables two 12 bit digital to analog converters to be used to provide chart recorder outputs, so that the digitised chromatograms can be represented in the usual manner as a chart trace.

Data acquisition

With this detection system it was possible to scan variation in absorbance reading, as each filter passes across the optical light path aligned with the HPLC

flow cell. These profiles of each filter could be inspected in the form of a discrete series of numbers, which have been stored in the computer. Alternatively they can be presented as a trace on the chart recorder (see Fig. 3). The event marker "spikes" seen on the traces coinside with every twentieth value. The system was programmed to automatically take in 250 values across each filter, after receiving the pulse generated by the appropriate aluminium flag. Inspection of the above data enabled a flat plateau region to be chosen for each filter, and this process was crucial in minimising baseline fluctuations during rotation of the disc. In practice each filter was represented by a minimum of four adjacent values (selected from the 250) which were then summed to represent the final absorbance value to be stored in the computer's memory.

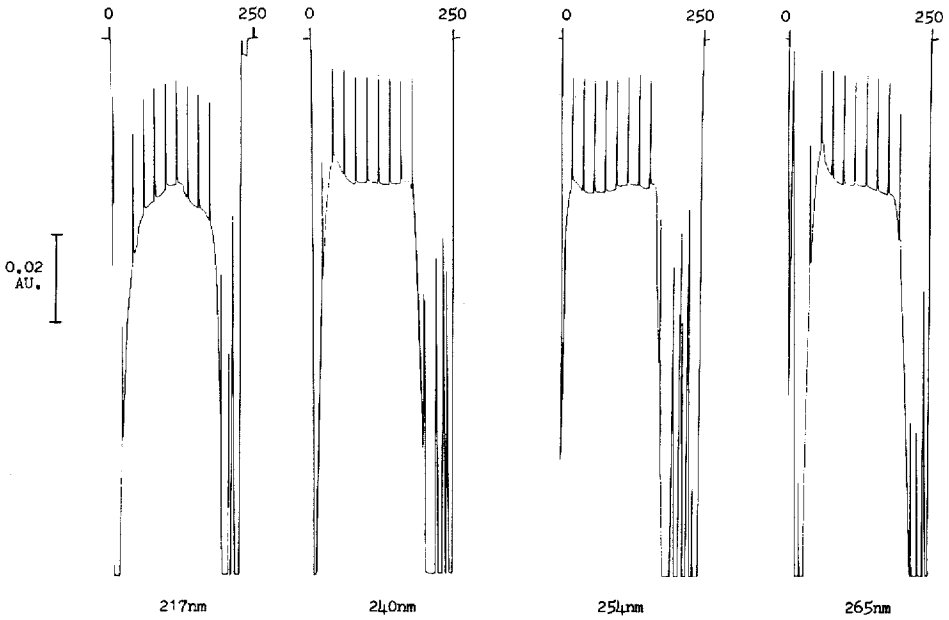

Fig. 3. Transmission profiles of UV filters. The event marker spikes coincide with every twentieth value recorded.

PROGRAMMING THE MICROCOMPUTER AND INTERFACE BOARD

The various programming tasks and their order of execution are shown schematically in a flow diagram in Fig. 4. The programming requirements of the system can be separated into three main sections: (1) Pre-selection of settings required for each particular analysis. (2) High speed acquisition and reduction of 1000 (*i.e.* 250 × 4) 14 bit values to represent four absorbance readings for each revolution of the disc (*i.e.* every 500 msec). (3) Processing the stored analytical data on the completion of each chromatographic run.

Pre-selected analytical settings

When selecting the various analytical conditions the computer's speed is not

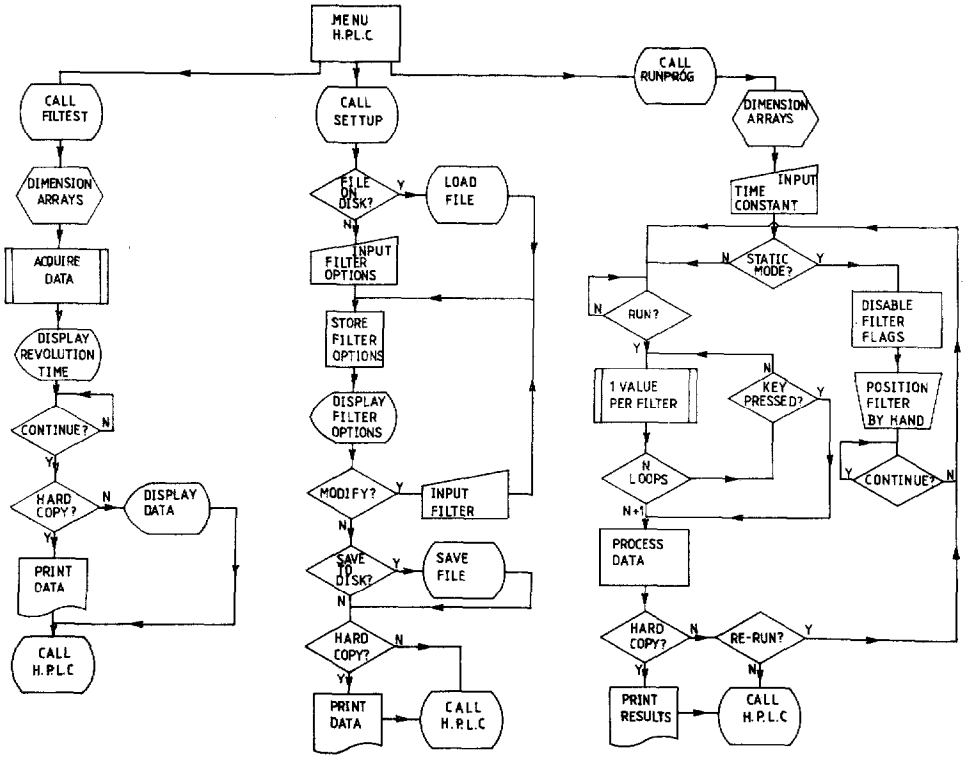

Fig. 4. Flow diagram of system software.

important. This means the programming can be done in Basic, a relatively slow computer language when run on the PET. This program enabled the following parameters to be selected, by prompting the analyst (with messages on the computers screen) to enter the necessary information at the computer's keyboard: (a) which filters to monitor (up to a maximum of 4); (b) the selected plateau region for each filter; (c) chart output sensitivities: 0.008–1.00 AU full scale.

This information was then stored in the computer for later use by the data acquisition routines. A permanent record of the conditions could be stored on the floppy disc unit to be recalled for use on other occasions.

Programming functions during the analysis

The number of tasks that have to be dealt with during each revolution of the disc assembly (*i.e.* every 500 msec) necessitated the use of the high speed achieved when programming in Machine Code. The following sequence of events underlines the magnitude of the tasks completed by the Machine Code program: (a) Sense the START pulse for each rotation. (b) Sense the pulse from the second light sensor, indicating that a filter is coming into position. (c) Take in 250 14-bit values across the filter: extract the values from the selected plateau region, which then represent the final absorbance readings. (d) Store the absorbance readings in the correct part of the PET's memory: check to see if this value should be output to a chart recorder:

if so implement the correct sensitivity setting before transmitting the reading. (e) Repeat (b) to (d) above for the other three filters. (f) Repeat (a) to (e) above 2400 times for a 20-min chromatogram.

In addition to producing two chromatograms on a chart recorder during the course of the analytical run, the system also stores the data for all four wavelengths; the latter may be used to provide digitised chromatograms if required.

Processing the digitised chromatograms

After acquiring the analytical data from a chromatographic run, the processing requirements were conveniently executed in Basic. This part of the program reduced the stored values to a series of peak height measurements based on the absorbance readings for each of the four filters. In addition the retention times associated with each set of peaks was calculated, and the equivalent absorbance ratios determined from the peak height values. These processed values could be listed on the printer and used as a basis for identifying the components in the original sample.

CONCLUSION

The system described above has been in routine operation for several months and the results have been reported in a separate publication[3]. It has been shown that the detection system provides multiwavelength data with signal-to-noise characteristics which compare favourably with those of the more conventional single-wavelength detector. (The overall noise level of the system is approximately 10^{-4} AU. As the interface system provides a digital resolution of $1.5 \cdot 10^{-5}$ AU, it is clear that the digitising process is not the limiting factor in the detector's performance.) The cost of the described development is at least a factor of 5 less than alternative commercial systems based on photodiode arrays[1].

NOTE

Listings of the programs and further details of the electronic circuit are available on request.

ACKNOWLEDGEMENTS

I would like to thank T. Catterick, B. B. Wheals and P. C. White for their helpful advice and S. S. Matharu for constructing the detector housing.

REFERENCES

1 S. A. George and A. Maute, *Chromatographia*, 5 (1982) 419.
2 T. Catterick, *J. Chromatogr.*, 259 (1983) 59.
3 P. C. White and T. Catterick, *J. Chromatogr.*, 280 (1983) 376.