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VERSATILE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC COMPUTER SYSTEM FOR SOLVENT DELIVERY, GRADIENT CONTROL AND DATA ACQUISITION USING THE COMMODORE 64 PERSONAL COMPUTER

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SUMMARY

An interface for a high-performance liquid chromatographic system based on the Commodore 64 personal computer has been designed as an alternative to the expensive and somewhat inflexible solvent programmers and data acquisition systems offered by commercial manufacturers. The system consists of the Commodore 64 with a single disk drive and monitor, and an interface that directly controls the flow-rates of two Waters pumps for solvent delivery in either isocratic or gradient mode, as well as analog-to-digital conversion of chromatographic data and either graphic data output to a dot-matrix printer or digital-to-analog conversion for output to a chart recorder. The interface permits computer-controlled flow-rates of up to 10.0 ml/min, with an accuracy of 0.0047 ml/min at flow-rates of 4.1 ml/min and an accuracy of 0.03 ml/min at flow-rates of 10 ml/min. The software for data analysis permits tabulation of elution times and areas for the peaks in a chromatogram with an option for baseline correction. The data are stored on floppy disks together with information pertinent to the chromatogram (flow-rate, solvent composition, etc.).

INTRODUCTION

The separation of complex chemical and biochemical mixtures by high-performance liquid chromatography (HPLC) is a laboratory method of increasing popularity. However, the cost of the equipment required for many HPLC applications (gradient control, integration of chromatographic peaks, etc.) is not trivial, and is to some extent out of proportion to the technology involved. As an inexpensive alternative to the solvent programmers and data acquisition/analysis systems offered by commercial manufacturers of HPLC equipment, we have designed an interface for a Waters HPLC system based on the Commodore 64 personal computer. Our goals in this design were: (1) to permit computer control of the solvent pumps, with the

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capacity to permit user design of desired gradient profiles, pre- and post-gradient isocratic flow, and recycling capabilities; (2) to store on disk the chromatographic data, and also in each disk file to list the parameters relevant to the run (flow-rate, length of run, solvent composition, etc.); (3) to develop interactive data analysis procedures, enabling tabulation and printing of data summaries, including elution times and integrated areas of chromatographic peaks, etc. A previous report shows that the Commodore Vic 20 microcomputer can be used to supply time events for interfaces to pneumatically operated HPLC valves[1].

We have designed a simple computer–HPLC interface consisting of driver circuits for two solvent pumps, analog-to-digital concersion of column detector signals, and digital-to-analog conversion of digital data for output to a chart recorder. Emphasis has been placed on simplicity and economy, but the system is nontheless sufficiently user-friendly that laboratory personnel unfamiliar with computers can use the system rapidly and efficiently. The cost of components for the HPLC interface is less than 300 dollars. Considering the low cost of a Commodore 64 with disk drive and monochrome monitor, the present design thus offers a very attractive alternative to the gradient controllers and data modules offered by commercial manufacturers.

APPARATUS

Hardware

A block diagram showing the HPLC pumps, the UV detector, the recorder, and the Commodore 64 computer system is shown in Fig. 1. The Commodore 64 is built around the MOS 6510 microprocessor and uses two MOS 6526 Complex In-
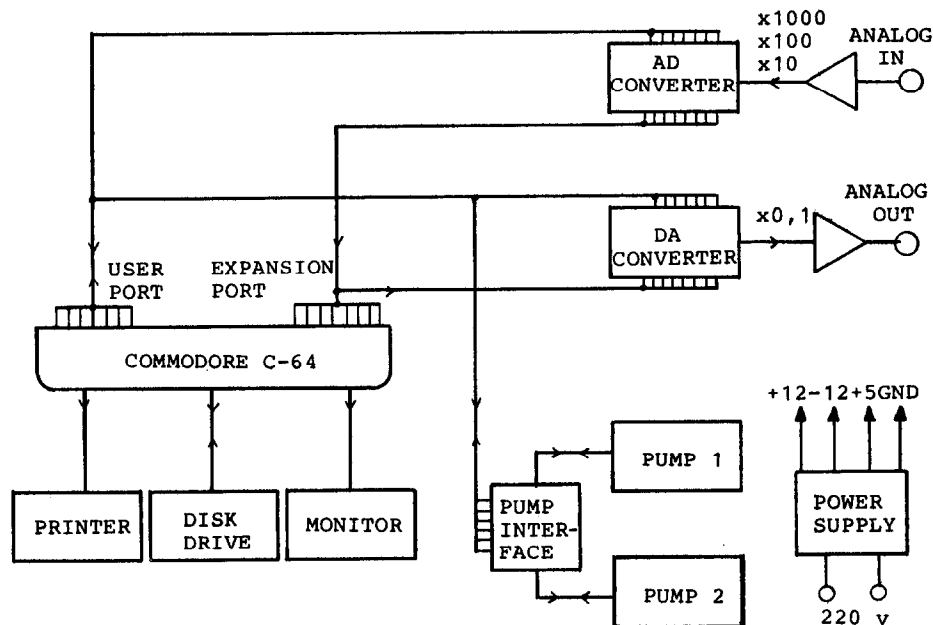

Fig. 1. Block diagram of HPLC pump, UV detector and Commodore 64 computer system. For explanation see text.

terface Adapters (CIAs) for communication with peripheral devices, including interfaces of the type discussed here. Our system includes only one disk drive and a monochrome monitor, but the Commodore 64 can support two or more disk drives, as well as composite colour monitors.

With respect to the HPLC interface, there are three tasks requiring hardware interfacing to the computer: (1) generation of signals to run the two solvent pumps in either isocratic or gradient mode; (2) analog-to-digital conversion of voltages from the UV detector during the chromatographic run; and (3) either output of digital data to a dot-matrix printer or the screen, or digital-to-analog conversion of stored chromatographic data for output to a chart recorder. These three tasks are handled in our system by an interface circuit contained on a single printed-circuit board, which makes connections to both the 24-pin User Port and the 44-pin Expansion Port on the back of the Commodore 64. The solvent pumps of our system, a Waters Model 45 and a Model 6000 A, are driven by 5-V square waves in the frequency range 0–2132 Hz. The required square waves are generated by software and buffered by a ULN2003 driver chip in the interface before being delivered to the pumps. The pump settings are updated once per second under program control. To protect the pumps from damage due to excessively high pressures in the solvent lines, it is also necessary to provide a means of shutting down the pumps if a pre-set pressure limit is exceeded. We found it more appropriate to take the pressure-limit signal from the pump circuit boards rather than from the interface cables on the pumps as supplied by Waters. As shown in Fig. 2, the pressure-limit signal for the Model 6000A solvent pump can be obtained from the junction of the collector of transitor T11 and resistor R20 in the Waters schematic. In the Waters design of the Model 6000A, the output of operational amplifier IC8 drives transistor T11. When the solvent pressure is less than the pre-set limit, the output of IC8 is negative and T11 is turned off. If the pressure exceeds the pre-set limit, the output of IC8 is latched positive, T11 turns on and signals the computer to shut down the pumps. The situation is similar for the Model 45 pump, but the installation of the circuit in Fig. 2 was required to derive a signal compatible with our interface.

The acquisition of data in our system is achieved using an Analog Devices Model AD570 analog-to-digital converter (ADC) as illustrated in Fig. 3. This unit is an 8-bit successive approximation ADC capable of an 8-bit conversion in 25 μsec. While an 11- or 12-bit ADC could be used in this system, the 8-bit resolution obtained here is more than adequate in view of the relatively large noise present in HPLC detector systems. No obvious differences can be discerned visually in our system between the original chromatogram and the digitized and plotted version of the same data.

The AD570 unit can be operated either in unipolar (0 to 10 V) or bipolar (-5 to $+5$ V) modes. Nearly all UV detectors operate as unipolar devices, as does the detector in use in our system (an LKB Model 2138 Uvicord S UV monitor). To make maximum use of the full range of the ADC, the detector output must be matched to the input range of the ADC. In our case, this required the insertion of a simple amplifier circuit between the UV detector (which has a full scale output of 100 mV) and the input of the AD570. The ADC is triggered by the Commodore 64's hardware interval timer (called a "jiffy clock") to collect and digitize a signal once per second during a normal chromatographic run. While this is frequent enough for
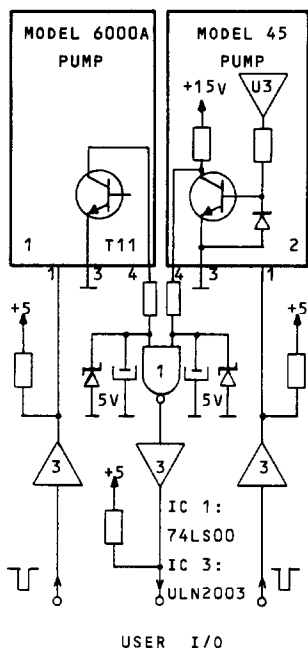
Fig. 2. Diagram of modification of Waters Model 6000 A and Model 45 pumps to transfer the pressure limit signal to the interface.

monitoring all but the sharpest of peaks, the data collection rate can of course be increased by software. Under these conditions, a 30-min chromatographic run generates 1800 data points. At the end of the chromatographic run, the data are stored on a floppy disk along with pertinent file parameters (see below), in a file established by the user at the start of the run.

Once stored in this manner, the data can be recalled for observation, and can be output to either a chart recorder or to a dot-matrix printer. Output to the chart recorder requires a digital-to-analog conversion, which is performed in our system by an Analog Devices AD 558 digital-to-analog converter (DAC). This unit accomplishes conversion of data on the 8-bit data bus of the Commodore 64 to an analog voltage in the range 0–10 V with a 1-μsec settling time. The rate of data output can be set by software to match the desired chart speed of the chart recorder. The dot-matrix printer in our system is a Commodore MPS801 printer, which connects directly to the Commodore 64 serial bus. Other suitable dot-matrix printers are available, but these will require the use of an interface on the Commodore 64 serial port. Our system offers full plotting versatility. Plot time, vertical scale and horizontal scale for any plot are under program control. One can choose to plot the full chromatogram or a selected portion of the run. We find that such horizontal expansion is helpful in sorting out closely spaced peaks in our chromatograms.

Software

The software for design and control of chromatographic runs has been written so as to provide the user with maximum flexibility in the selection of the chromato-
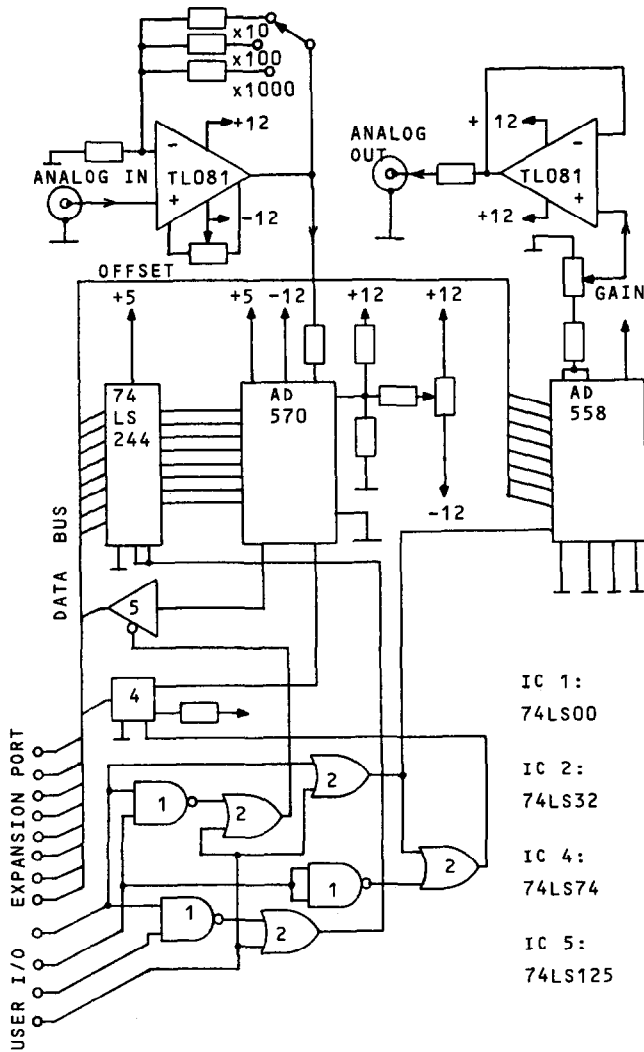
Fig. 3. Diagram of HPLC interface, showing connections of the AD 570 analog-to-digital and the AD 558 digital-to-analog converters. For explanation see text.

graphic protocols, while being simple enough for inexperienced operators to use with ease. At the beginning of the program HPLC-X, the user can choose either a gradient run or an isocratic run with data collection. If the gradient option is selected, the user is asked if he/she would like an isocratic run before or after the gradient run or both and is asked to select the times for the pre-run, gradient run and post-run periods. As an option for repetitive cycling of columns or for use with automatic sample-changing devices, the user may select to repeat the entire run as often as desired. The user is also asked to choose the desired gradient profile. The gradient profile subroutine selects profiles according to the formula $y = x^n$, where x is the time variable, normalized to the total time of the gradient, and y is the fraction of

the total solvent flow due to solvent 2 (solvent 1 is defined as the solvent composition at the start of the gradient, and solvent 2 is the desired solvent composition at the end of the gradient). The value of n is set by program control to any of the values 0.25, 0.33, 0.5, 0.67, 1.00, 1.50, 2.00, 3.00 or 4.00, corresponding to the user's selection of concave, linear or convex gradient profiles 1 through 9, respectively.

Having made these selections, the user is presented with a menu of additional parameters which will be stored on disk with the data file, including user name, date, flow-rate, length of the gradient run (if pertinent), composition of solvents 1 and 2, the gradient profile and the sample composition and volume. The user may either enter the parameters manually or call from disk (and then modify) the parameters from any previous run (selected by file name) or the most recently used set of parameters (stored on disk under the file name LAST FILE). Upon completion of the run, the data as well as these selected parameters are stored on disk. Transfer of a large number of data points is very slow in BASIC language, so we use 6510 machine code subroutes to load and save data files.

During isocratic runs, the program monitors the jiffy clock, triggering analog-to-digital conversions once per second and storing the obtained values in memory. During gradient operation, in addition to triggering analog-to-digital conversions, the program also updates the pump settings once per second, according to the previously described gradient equation. In both cases, the monitor displays messages to indicate whether the system is executing the isocratic pre-run, the gradient run or the post-run, the total length of the run, the updated time from the clock and the
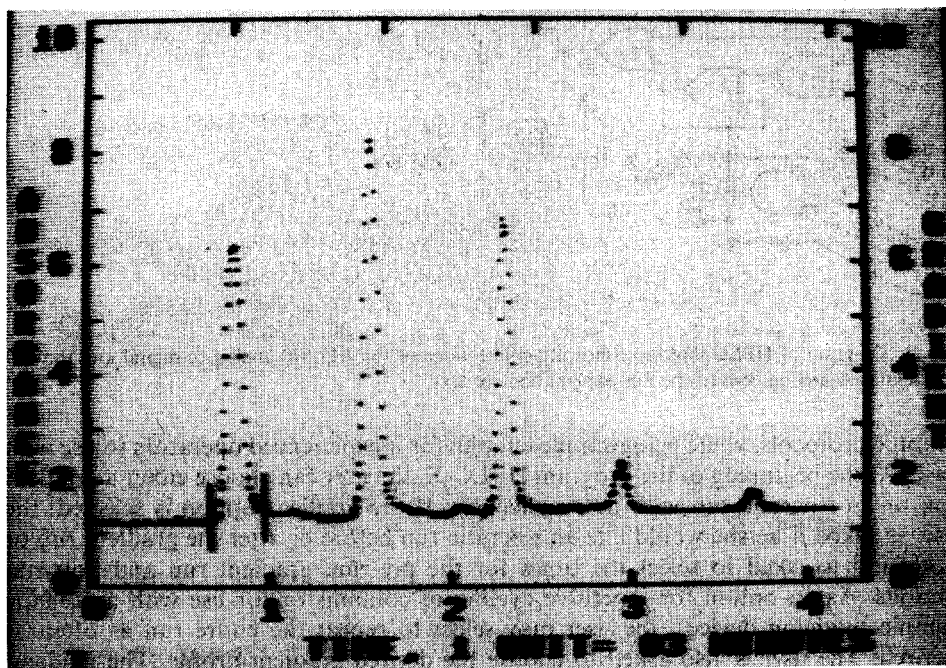

Fig. 4. Use of interactive cursors for area calculation of a peak in a horizontally expanded chromatogram of aromatics in methanol. Separation using a linear water–methanol gradient on a Nucleosil 5 C_{18} column.

number of the cycle being executed. At the end of the run, the data is stored on disk, and the user is asked if another run is desired. If so, the program allows for modification of the parameters and asks for a new file name before beginning a new run.

It is important to consider the accuracy of this or any method of solvent gradient control. Our software, which delivers square waves from the Commodore 64 to the two solvent pumps, permits 1-Hz resolution over the frequency range 0–880 Hz. This corresponds to a resolution of 0.0047 ml/min over a range of flow-rates from 0 to 4.1 ml/min. Above this point, the resolution declines gradually until at a flow-rate of 10 ml/min the resolution is 0.033 ml/min, or approximately one part in 300, more than adequate for most applications.

For display and analysis of chromatographic data on the monitor screen, we have used an enhanced BASIC wedge[2] which makes the design and plotting of graphics screens and maintenance of multiple screens particularly straightforward. Our screen display software permits us to plot an entire chromatogram or a part chosen by the user. When the plot is displayed, the user can call in two interactive cursor bars, and using the Commodore 64 function keys, can move the cursors right or left, and either spread or converge the two cursors to bracket a peak or a set of peaks for replotting or for area calculations as illustrated in Fig. 4. In this way, the user can select an area for expanded plotting or select peaks for tabulation on the printer of elution times and integrated intensities. Use of the interactive cursors in this way puts the user in full control of difficult area determinations, eliminating as far as possible many of the common problems associated with baseline drift and unsymmetrical or overlapping peaks.

ACKNOWLEDGEMENTS

REFERENCES

1 L. G. M. T. Tuinstra, W. A. Traag, H. J. Keukens and R. J. van Mazijk, *J. Chromatogr.*, 279 (1983) 533–542.
2 M. C. Kees, *Compute!*, 5 (1983) 198–214.