Journal of Chromatographic Science, Vol. 23, March, 1985

Technical Note

Modification of a Microprocessor-Controlled HPLC System (Perkin-Elmer)*

P. Kocna
Laboratory of Gastroenterology, Faculty of Medicine, Charles University, Karlovo náměstí 32, CS-121-11 Praha-2, Czechoslovakia
B.J. Mittermüller
Perkin-Elmer Service Department, Vienna, Austria

Abstract

Three modifications of a microprocessor-controlled high performance liquid chromatography system are described: initialization of peripheral devices by the injection valve enabling more precise estimation of retention constants, automatic return of the automatic switching valve to the RUN position after stop-flow spectroscopy, and solvent-saving by program cycling during overnight column stabilization.

Introduction

In the past few years, microprocessor- and microcomputer-control and data handling of the high performance liquid chromatographic (HPLC) separation have become prominent. To accomplish this, perfect synchronization of the whole system is necessary (1), especially in the use of high speed liquid chromatography (2). The modular system of HPLC instrumentation has the advantage of varying the interconnections of individual devices. Special modifications are required for individual combinations or modules (3).

The modifications described in this paper were aimed at automatic return of the automatic switching valve to the RUN position after stop-flow spectroscopy, actuation of peripheral devices by the injection valve, and solvent-saving by program cycling during column stabilization.

Experimental

Apparatus

The HPLC system (Perkin-Elmer) modified in this study consisted of the microprocessor-controlled pump module Series 3B, an LC-85 variable wavelength UV detector with Autocontrol accessory, an ASV-1 automatic switching valve, and a SuperRac 2211 fraction collector (LKB). The whole system was controlled by a Model 3600 data station with BASIC-programming of the pump module and a CIT-2 Interface for data handling.

Actuation of peripheral devices by injection valve

The actuation of individual devices is possible in modular systems either by keyboards or by using external commands by means of the system interconnections. An external signal, RUN/MARK (4), on the Series 3B module may be used for this purpose. The Model 7161 valve position sensing switch (Rheodyne) was used as an accessory for more precise synchronization of injection with the Series 3B program start. This magnetic switch was connected to the EXT/RUN terminals and the pump module is initialized in the same way as the RUN-key. However, this system of external actuation is available only during the program mode of the pumps. During independent use of the pumps, these external lines are not active.

The run-output signal may be obtained independently on the Series 3B mode if the Rheodyne switch is connected according to Figure 1. The relay K1 circuit is actuated by the Rheodyne switch through a capacitor 100 nF with a parallel resistor 10 kΩ. In this modification, the run-output signal was produced by any change of the valve position. This, however, may produce an artificial pulse during sample loop washing or it may actuate the peripheral devices even when the pump module is not equilibrated. To avoid this, it was necessary to modify the Rheodyne 7161 connection with an additional circuit, shown in Figure 2. The logical circuit SN 7402 (5) permits off-lining of the Rheodyne signal only if the Series 3B module is READY after equilibration or if it works in an independent mode. For this purpose, the check-signals from indicators READY and INDEPENDENT were used, the latter signal being inverted. One of the free NOT-gates in circuit M11 on the keyboard can be used for this function. The output pin 3 on M4 was connected to pin 11 on M11, and the inverted output from pin 10 on M11 was connected to the free pin 4 on J5.

The circuit described in Figure 2 was built onto an additional small PCB and mounted onto the microprocessor board. It was connected by means of six lines to the pins of J4, J5 and IC26.

Automation of stop-flow spectroscopy

During separation, momentary stop-flow spectroscopy is advantageous for the identification of the resolved peaks. The flow

*The complete interconnection diagram as well as a BASIC program for the Series 3B pump module is available from the authors on request.

Reproduction (photocopying) of editorial content of this journal is prohibited without publisher's permission.

through the column and the detector cuvette may be stopped either by the PAUSE command on the Series 3B pump module or diverted by electrically switching valve ASV-1 to waste. In either case it is possible to get an output signal for external actuation of scanning on the Autocontrol unit (6). The output of the ASV-1 valve was realized through two relays (7) whose contacts are closed according to the diagram in Figure 3. The triggering pulse for EXT/SCAN can be obtained by a combination of both relays but, when the valve motor reverses to the start, the pulse appears again and the scan is actuated for the second time.

A proposed change would allow the actuation of relay K1 input by using the signal for driving the valve motor instead of the photointerrupter. This change was carried out by connecting pin 1 on IC11 to the pin TP6.

The second modification of the ASV-1 valve concerns its automatic return to the run position after the spectral measurements have been completed. In the original device, the valve is returned only by manual switching if in the manual mode. In order to manually start the valve in the scan position, followed by automatic return to the run position by the output signal from the UV detector, it would be necessary to switch the valve to the on-line mode or modify the electric circuitry. This improvement was performed by connecting pin 2 on IC4 (i.e., EXT/RUN) to pin 6, relay K2. In this version, the flow of the mobile phase through the column and LC cuvette was stopped manually by valve switching to the scan position. The output pulse of the modified relay K1 (Figure 3) actuates EXT/SCAN on the LC-85 through terminals 3 and 5. The recorder is controlled by terminals 4 and 5 on TB 2. After the scan is completed, the EXT/RUN on valve ASV-1 is initialized through the relay output on TB 2 (terminals 2 and 3) in combination with terminals 2 and 6 on ASV-1, as an input for the modified relay K2, and the separation continues.

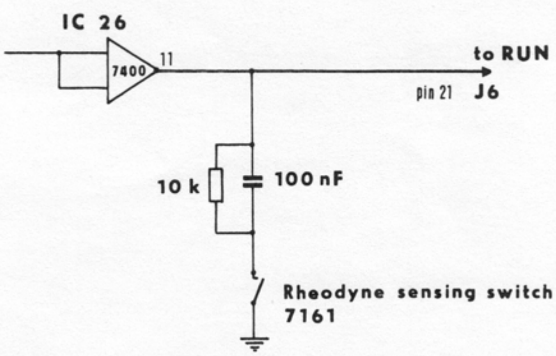

Figure 1. Circuit diagram for interconnection of the Rheodyne 7161 valve position sensing switch.

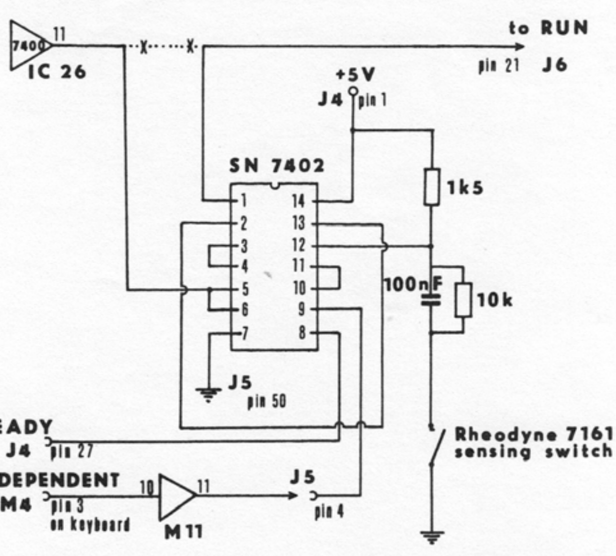

Figure 2. Circuit diagram for modification with additional SN 7402 logic circuit.

Solvent-saving modification for column stabilization

The overnight or extended equilibration and stabilization of a column are advantageous and/or necessary for many types of separation. A maximum of 7 × 99 mins may be set through 7 segments on the Series 3B pump module (i.e., less than 12 hrs) or the column can be equilibrated using the independent mode of both pumps with a minimal flow of 0.2 ml per min.

The modification recommended is very simple and was realized by a changeover switch connected to the relay-output READY, which is normally used for peripheral devices such as RETURN on a fraction collector or for EXT A/Z on an LC detector. In this second position the READY output was connected to terminals 10 and 11 for EXT/RUN. The output, actuated automatically, was run on the Series 3B module at each READY moment. In this way, the system was cycled.

Equilibration using the program mode of the Series 3B is not only solvent-saving but time-saving as well, because the initial conditions for separation may be set a few hours before work begins.

The second possibility is to use a Rheodyne 7161 sensing switch for this purpose, connected through relay output READY on EXT/RUN. This combination also automates the actuation of peripheral devices, and if the valve is constantly left in the INJECT position, the Series 3B pump is cycled. This modification is very simple, inexpensive, and does not require changing the original circuitry. It is, however, only active in the program mode and does not work in independent mode.

Conclusion

The modified automated system for high performance liquid chromatography contains a BASIC program for the Series 3B

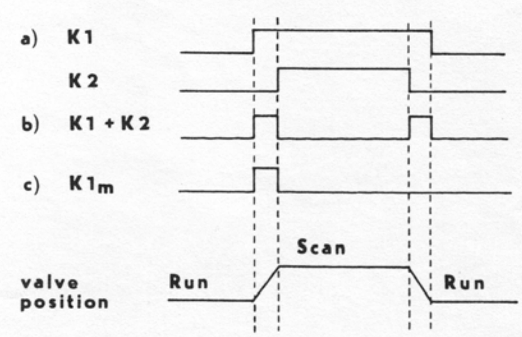

Figure 3. Timing sequence diagram for ASV-1 valve output: a) unmodified output of relays K1 and K2; b) pulse signal of both relays K1+K2 combined; and c) pulse signal of modified relay K1.

pump module and CIT-2 software for data processing. All parameters for separation and data handling are stored on floppy discs. The Series 3B module is actuated by sample injection. The signal RUN/MARK initializes peripheral devices, the data interface, and the fraction collector, whose event marker marks the fraction positions simultaneously with UV recording. At the end of the separation, the recorder is stopped by relay CIT-2 and through READY signals of the Series 3B EXT A/Z, the LC-85 detector is set. The fraction collector is also returned to its initial position.

Acknowledgment

We thank Dr. P. Frič for reviewing the manuscript and providing many helpful suggestions.

References

1. H. Engelhardt. *High Performance Liquid Chromatography.* Springer Verlag, 1979.
2. J.L. DiCesare, M.W. Dong, and L.S. Ettre. *Introduction to High Speed Liquid Chromatography.* Perkin-Elmer Corporation, 1981.
3. F.J. van Lenten and J.A. Pepitone. Modification of a microprocessor-controlled autosampler for LC to accept other injection valves. *J. Chromatogr. Sci.* **21:** 370-71 (1983).
4. *Instruction Manual for Series 3B Microcomputer Controlled Pump Module.* Perkin-Elmer Corporation, Norwalk, CT, 1981.
5. *National Semiconductor Logic Databook.* National Semiconductor Corporation, Santa Clara, CA, 1981.
6. *Instruction Manual for LC Autocontrol Accessory.* Perkin-Elmer Corporation, Norwalk, CT, 1981.
7. *Service Data Bulletin for Automatic Switching Valve ASV-1,* Perkin-Elmer Corporation, Norwalk, CT, 1981.

Manuscript received May 1, 1984.