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MICROCOMPUTER SYSTEM FOR DATA ACQUISITION AND SOFTWARE HANDLING IN CHROMATOGRAPHY

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SUMMARY

A complete, powerful yet simple and inexpensive microcomputer system for chromatography is described. It consists of an electronic interface card and an exploiting software, both controlled by an independent microcomputer. The interface includes all necessary circuits for digitizing the chromatographic signal and for controlling the actuators and the sensors connected with the chromatograph. The software, clock-interrupted, allows simultaneous data acquisition, mathematical treatment, connected plotting, and printing, and drives injection valves and gradient elution pumps. This system is both suitable for high-speed capillary gas chromatography and for high-performance liquid chromatography.

INTRODUCTION

For the application of microcomputer systems to data acquisition, signal treatment, and process control in chromatography, commercially available equipment is generally expensive and not flexible enough for the needs of research laboratories. Moreover, this equipment does not fully use the capacities of the computer and its interface.

Among the inexpensive microcomputer systems designed for chromatography that have been described[1–3], none fulfills all the necessary requirements for high-performance liquid or gas chromatography such as real-time peak treatment, process control, digital accuracy, high-speed sampling and large-capacity storage.

The analog signal of a chromatographic detector is generally continuous and has a low high frequency content. It is distorted by periodic interferences, such as line noise or the pulsation of solvent pumps. The relative chromatographic peak amplitudes lie between 1 and 10^6, depending on the component concentrations during a single analysis. These characteristics of the chromatographic signal determine the specifications of the data acquisition system.

Digitization leads to a loss of information and corresponds to an additional noise with a standard deviation equal to the quantification. A 12-bit A/D converter

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is generally sufficient for a chromatographic signal to reduce the digitization noise; *i.e.*, the resolution is limited to 2.44 mV for the least significant bit on a 10-V input. The full measuring scale of the acquisition system must be adjusted to the range of the input signal with an amplifier. However, for greatest efficiency, a programmable-gain amplifier is essential in a chromatographic system for automatic correction of the gain amplification, as a function of the signal magnitude and of its derivative, at any moment during the data acquisition.

According to the Shannon theorem, the sampling frequency must be at least twice the maximum value of the frequency to be recorded[4,5]. This means that at least 5 points are required across the peak width. However, it is generally admitted that the number of data points taken per peak is 4–5 times this value to achieve a precision of 1% in the determination of the zeroth and first moments[5]. Therefore, in high-speed capillary chromatography, where the peak width is 500 msec, the maximum sampling period should be 20 ms, *i.e.* the basic sampling rate of the A/D converter clock should be set at a minimum value of 50 Hz in order to allow data acquisition for all kinds of chromatography, including high-speed chromatography. The possibility of a higher-frequency sampling rate allows us to perform numerical filtering to reduce noise. For that purpose, a 1000-Hz base frequency has been selected for the sampling converter clock.

In order to perform simultaneous data acquisition from several chromatographs or to achieve auxiliary measurements from independent sensors, it may be interesting to include an analog multiplexer in the data acquisition system. It is also useful to add an input/output digital port and a digital-to-analog converter. These components allow control of most of the actuators (relays, motors) and the reception of external signals. Thus, control may be achieved during data acquisition and adapted to the experiment according to the collected data.

The aim of this work was to build a simple system, combined with an independent microcomputer, to allow data acquisition, on-line control, and real-time treatment of the chromatographic signal. This system includes a single electronic interface card with software incorporating a main module, adapted for a microcomputer, written in BASIC and address subroutines written in Assembly language (Fig. 1). We thus developed a research tool for real-time applications, where the software is divided in two parts. The first one, in Assembly language, is only accessible to a software specialist. It performs the data acquisition and some of the high-priority controls. The second one, in BASIC, communicates with the first part to introduce the data parameters and perform low-priority controls and real-time treatment. The BASIC programming can easily be modified by a non-specialist.

EXPERIMENTAL

The components necessary for digitizing the analog chromatographic signal (multiplexer, amplifier, A/D converter, clock), the interface logic with the Apple IIe microcomputer (Apple, Cupertino, CA, U.S.A.), the control logic of external devices (numerical inputs/outputs) and two D/A 8-bit converters are contained on a single electronic interface card (Fig. 2). The circuit board is placed in one of the I/O slots of the microcomputer.

The electronic components are in accordance with the specifications for chromatographic experiments which have been described above.

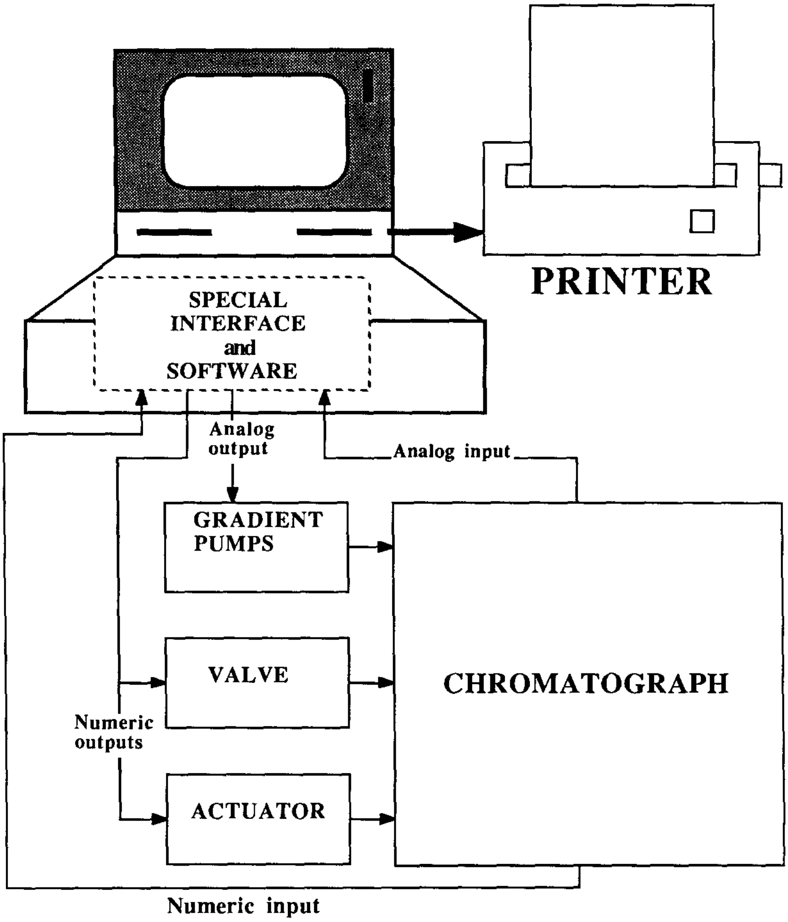

Fig. 1. General description of the system.

Data acquisition module

The data acquisition module consists of an analog multiplexer with 8 numerically selected, differential inputs AD 7507JD (Analog Devices, Norwood, MA, U.S.A.), an instrumentation amplifier AD 524AD (Analog Devices) which is equipped with a gain commutation logic (values: 1, 10, 10^2, 10^3) and an A/D converter AD 574AKD (Analog Devices) with 12-bit resolution and a conversion time of 25 μs. The input signal is 0–10 V in the unipolar mode and -5 V to $+5$ V in the bipolar mode. A manual switch allows selection between these input modes.

The total input dynamic range is therefore $4 \cdot 10^6$, with a minimum voltage resolution of 2.44 μV with a gain of 10^3 and 10-V maximum input with unity gain.

Control module

The control module contains the following components: (i) Two D/A 8-bit converters AD 558KN (Analog Devices), having a resolution which is sufficient for

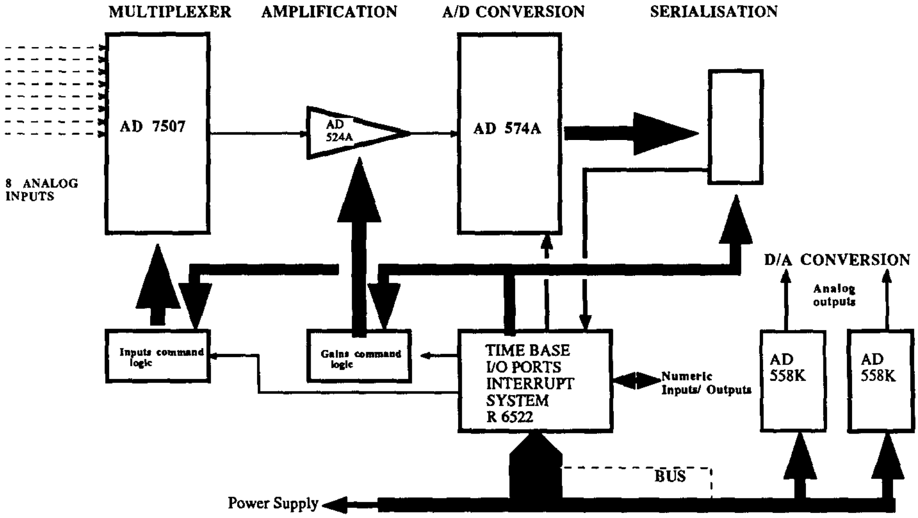

Fig. 2. Hardware description.

many purposes, such as control of solvent pumps in liquid chromatography; (ii) an interface circuit VIA Rockwell R6522 (Rockwell, Anaheim, CA, U.S.A.) possessing several programmable modules; (iii) two bidirectional 8-bit parallel ports, needed to control the ADC circuits and the actuator or sensor devices; (iv) two 16-bit timers, used in the oscillating, impulse, or counting mode. One of the timers is the time-base generator of the interface card; (v) a shift register for serial I/O, which is used in connection with the timer to generate the base time but may also be used for file transmissions between computers; (vi) an interrupt system with 4 external interrupt inputs, a status register for internal interrupts (timers) and external ones, and a mask register.

The control module is connected to the high-priority "non-masquable interrupt" (NMI) level of the microprocessor.

RESULTS

The working software of the interface consisted of two programs. The first, coded in Assembly language, runs the data acquisition according to the chosen criteria (acquisition windows, sampling etc.) and commands the solvent pumps for gradient elution by using the D/A converters.

The second program, written in BASIC, is interrupted at every clock pulse and the high-priority Assembly language program is executed. The BASIC program performs real-time data handling (peak detection; determining the retention time at the peak maximum and the peak area), controls the graphic display, and the printing of the chromatogram with the results. This real-time procedure allows the operator to judge the quality of his experiment and to make changes in BASIC, if necessary. Automatic control of fraction collectors, for instance, is made possible by the rapid detection of peaks. It is also possible to adjust calculations and peak treatments as needed, since programming is done in an easily accessible language.

Software modules (Fig. 3)

Entering the parameters. This program, written in BASIC, opens a dialogue with the operator for entering the experimental parameters: choice of the analog input; windows for collecting data from a defined moment; the sampling period and its evolution in time; the parameters connected with monitoring the elution gradient. All these parameters are coded, and stored in a memory area, which is shared by two modules: the data acquisition and control program and the data handling program.

Initialization and start timer. This subroutine, written in Assembly language, is called after the parameters have been entered. It initializes the interface card: I/O ports, selection of the analog input and of the gain, timer and interrupt system programming. It generates a digital signal (*e.g.* for actuating a valve) and waits for an automatically or manually produced pulse which determines the start of the timing. It starts the 1-ms time-base generator and returns to the main real-time data handling program (Module 4).

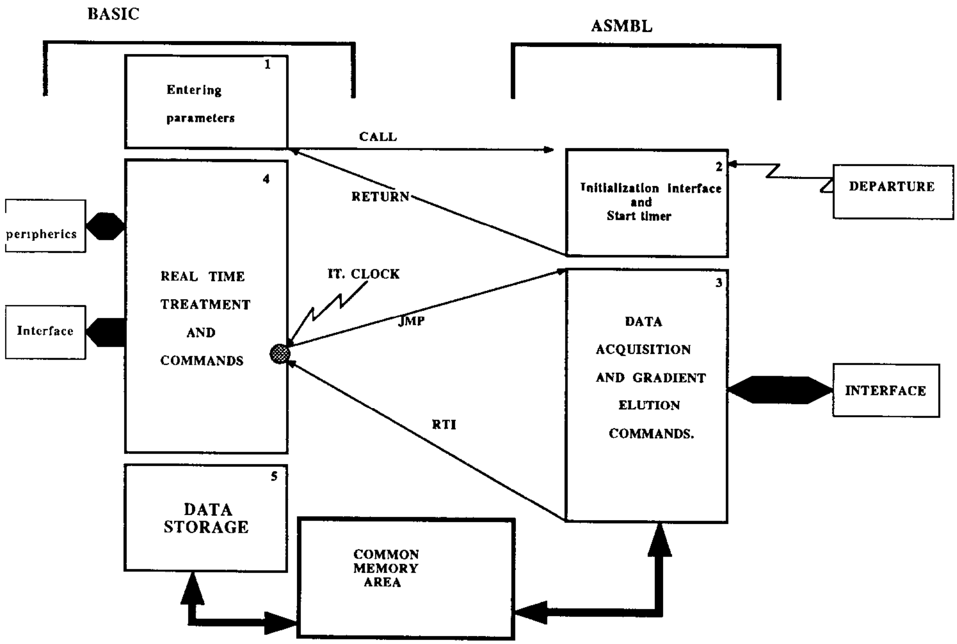

Fig. 3. Software description.

Data acquisition and gradient elution monitoring (Fig. 4). This module, written in Assembly language, is called and executed at every timer interrupt, *i.e.* every millisecond. The program measures the elapsed time by counting the number of clock pulses. By comparison with the input parameters, the program will decide whether the data are to be collected or rejected depending on whether they are inside or outside of the preestablished acquisition-time domain. The collected data are stored in two 8-bit memory words. An average of the data, calculated over a 20-ms period, allows an efficient reduction of the 50-Hz line-frequency noise. The control com-

Fig. 4. Data acquisition and gradient-elution command module (Assembly language).

mands are sent to the D/A converters in relation with input parameters and the elapsed time.

Real-time peak treatment algorithm (Fig. 5). This module, written in BASIC, reads the data stored in memory by the data acquisition program. It first determines the maximum of every elution peak, and then the peak start and end by calculating

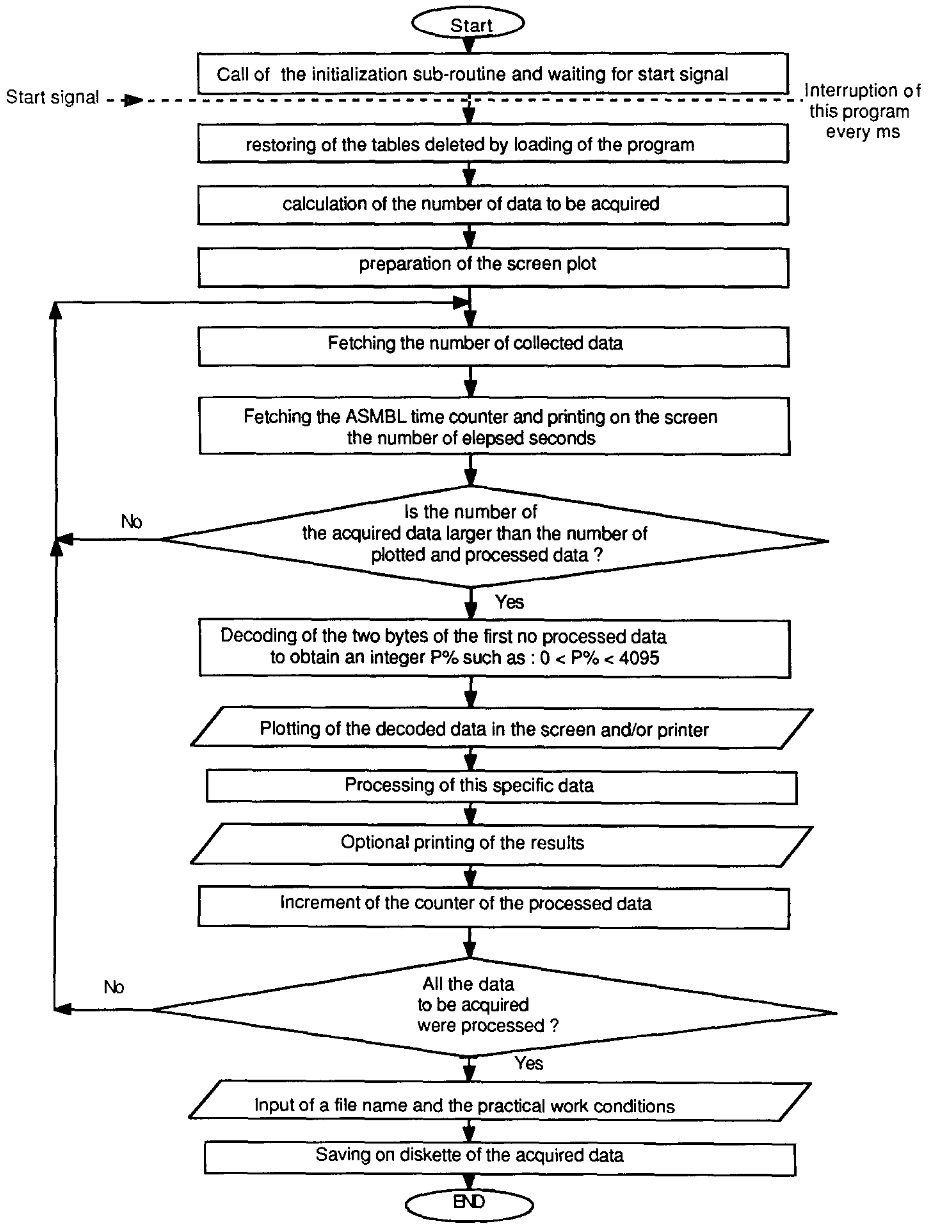

Fig. 5. Main program, including real-time peak treatment (written in BASIC).

the derivative of the signal and comparing it to a predetermined threshold for base-line noise. It calculates area, height, retention time at the peak maximum, standard deviation (or plate height) and takes into account the base-line drift.

The basic module for peak detection and determination of endpoints is shown in Fig. 6. The derivative is calculated from every two successive points and compared to a predetermined value for detection of the peak maximum. This test is repeated for confirmation on consecutive data points until 10% of the peak width is reached.

In the present program, we perform an integration from valley to valley. This has limitations, but chromatographers can program it with their own criteria, for example valley-to-base-line interpolation or curve-fitting methods to distinguish overlapping peaks.

Fig. 7 shows an example of the real-time peak treatment for a complex chromatogram, obtained with a capillary column. We first detect the peak maxima and

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1000  REM  I=DATA COUNTER
1010  REM  LI=EVENT COUNTER(MAX OR VALLEY)
1020  REM  B%(I)=SIGNAL DATA
1030  REM  N3=MAXIMUM NUMBER OF DATA
1040  REM  BR=NOISE LEVEL
1050  REM  BT=(NUMBER OF PEAK POINTS )/10
1060  REM  IM=DATA  NUMBER  OF PEAK  MAXIMUM
1070  REM  FI= DATA NUMBER OF ENDING PEAK  VALLEY
1080  REM  ID=DATA NUMBER OF BEGINNING OF PEAK  VALLEY
1500  REM  PEAK  MAXIMUM ROUTINE
1520 L1 = 0
1530  REM
1540  I = I + 1
1550  IF (I + 2)   N3 THEN 2100
1560 Y1 = B%(I + 2) - B%(I)
1570  IF Y1    - BR THEN 1530
1580 L1 = L1 + 1
1590  IF L1   BT THEN 1530
1600 IM = I - BT
1620 I = I + BT
1630  REM  ENDIG PEAK  VALLEY
1650 L1 = 0
1660 Y1 = B%(I + 2) -- B%(I)
1670 I = I + 1
1680  IF (I + 2)   N3 THEN 2200
1690  IF Y1 < 0 THEN 1660
1700 L1 = L1 + 1
1710  IF L1   BT THEN 1660
1720 FI = I - (I)
1750 I = IM - BT
1755  REM  BEGINNING PEAK  VALLEY
1760 L1 = 0
1770  IF I   10 THEN 1760
1772  GOTO 1777
1775  IF I   . THEN 1780
1777 ID = I + L1
1778  GOTO 1900
1780 Y1 = B%(I   2) - B%(I)
1790 I = I   1
1800  IF Y1   0 THEN 1775
1810 L1 = L1 + 1
1820  IF L1   BT THEN 1775
1830 ID = I + BT
2200  
```

Fig. 6. Listing of peak detection algorithm in the real-time peak treatment program.

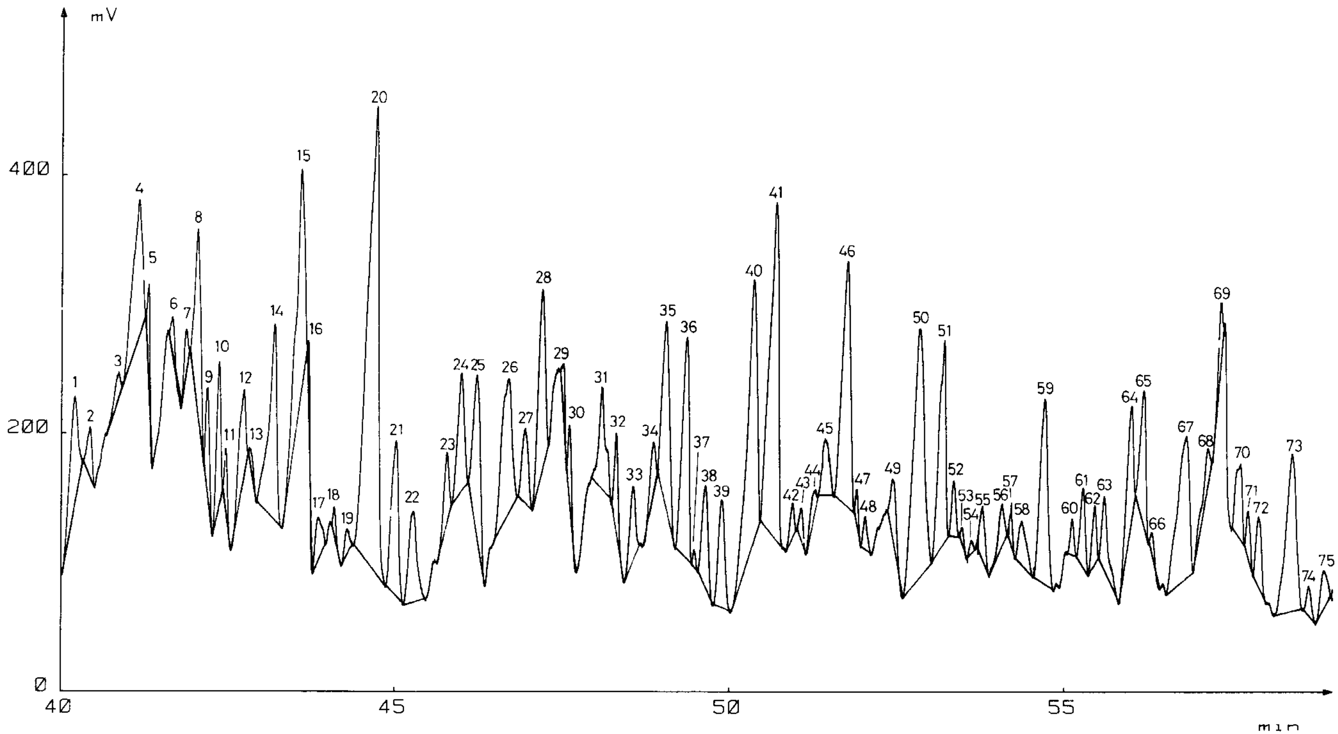

Fig. 7. Real-time peak detection. Portion of the gas chromatogram of the aromatic hydrocarbons extracted from a coking gas oil. GC conditions: glass capillary column, 50 m × 0.25 mm I.D., coated with OV-73, temperature-programmed from 50 to 280°C at 4°C/min, isothermal at 280°C.

then the valleys. It is obvious that for a good integration, we need to determine the base-line evolution. This can be done from an independent chromatogram.

Storage of the data. When the data acquisition and the real-time processing is over, this unit is able to transfer all data from the central memory to a magnetic diskette or to a hard disk so that they remain available for further treatment.

DISCUSSION

The advantages of the data acquisition system include several features. From a hardware point of view, a single card using only one slot of the computer achieves several functions generally executed by a set of several different interface cards such as the base time generator, the analog to digital converter and the digital I/O port. A single card, which combines the base time generator with the converters, allows several tasks to be performed simultaneously, for example data acquisition and the controlling external devices (pump control for gradient elution liquid chromatography; fraction collection in preparative chromatography; column switching). The programmable-gain amplifier with automatic gain selection allows a wide dynamic range which is useful for trace analysis.

The use of computer interrupts enables the simultaneous achievement of several functions to which priority levels are assigned. For example in gradient elution, the first priority is to collect the data, the second priority to command the pumps. Also, in between executing the interrupts the computer can perform some other treatments, such as screen visualization, ordinary data processing (*e.g.* detection of peak maxima and printing the results). Moreover, the use of the last generation of disk operating systems allows large capacities (up to 1 Mbyte) of storage on RAM cards under the interrupt program, *i.e.* a capacity of 10000 peaks with a definition of 50 data points per peak.

This microcomputer system is useful for the development of new chromatographic techniques, when fast data acquisition is required, while the association with a high-level computer language such as PASCAL should allow the building of a versatile family of programs for special applications. For example, in fast capillary chromatography[9,10] a 50-Hz sampling is sufficient for describing a peak with 20 data points, but it is necessary to use a 1000-Hz sampling for physico-chemical studies on peak shapes or for an appropriate noise treatment. Moreover, in fast capillary chromatography the loadability of 50-μm I.D. columns is low[10] and analyses are performed with sample amounts between 0.02 and 2 ng which corresponds to flame ionization detector currents below 10^{-11} A. This means that peaks are generally blurred in the background noise and that on-line noise treatment such as an average smoothing requires fast sampling rates such as those of the present system (1000 Hz).

Our system may also be useful in the development of zone electrophoresis in open tubular capillaries[11] where $5 \cdot 10^5$ plates are achieved with analysis times of about 10 min, and with peak standard deviations of about 1 s. Therefore, a minimum of 20 points per peak requires a sampling rate of 50 Hz. But elaborate peak treatment, in order to study theoretical models requires 1000 Hz sampling rate.

CONCLUSION

The system we have just described consists of a single autonomous printed circuit and a diskette, containing the software for chromatography. An inexpensive and readily available microcomputer controls this system and can be very quickly incorporated into conventional equipment by non-expert programmers in analytical chemistry laboratories. Its flexibility permits adjustment for various kinds of experiments.

The real-time treatment allows immediate evaluation of the quality of experiments and generally gives adequate results, but storage of the data file also permits further computing with more sophisticated software on another computer, if required. This may include integration with choice of bounds, filtering with the Savitsky-Golay method, the use of Fourier transforms, etc.

This system is not an integrator apparatus but may be used as one. It is mostly presented as a development tool for researchers in the field of special chromatographic techniques, such as high-speed chromatography, which requires a high data acquisition rate, gradient programming, various fraction collections in preparative chromatography, evaluation of noise levels in detections, and correction of non-linear responses.

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