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Note

Direct measurement of gas chromatographic retention volume

III. Software

JAN ÅKE JÖNSSON

Department of Analytical Chemistry, Chemical Center, University of Lund, POB 704, S-220 07 Lund (Sweden)

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The construction of a precision gas chromatograph has been described in several articles in this journal¹⁻⁴. This paper presents a brief description of the computer-program system used to handle all the measurements, calculations and supervising needed to operate the equipment*.

In order to achieve direct measurement of retention volume, the computer reads sensors for several parameters (gas flow-rate, pressure, temperature) at short intervals. In every instance, the corrected gas volume that has passed through the column is calculated; thus a volume-scale is created instead of the conventional time-scale. During chromatographic runs, the computer also senses the detector signal and correlates it with the volume-scale; from these data, the retention volume and other peak parameters are calculated.

Provision is also made for creating and executing BASIC-language programs at a separate terminal during the period of chromatographic measurements.

COMPUTER SYSTEM

The computer (ALPHA LSI-2; Computer Automation, Irvine, Calif., U.S.A.) has a 32K memory of 16-bit words. It is connected, via standard interfaces, to a cathode-ray-tube terminal (CRT) (model 1520, Data Media Corp.), a matrix line-printer (model 6330, Data Recording Instruments), a teletypewriter (model 390, Data Dynamics), a paper-tape reader (model 601, Addmaster Corp.) and a double floppy-disc unit (model FD511, Pertec). The gas chromatograph and an *xy*-recorder are connected via a 16-bit input/output interface and "home-built" electronics². The system is depicted in Fig. 1.

OPERATIVE SYSTEM

For this work, the real-time FORTRAN system developed by Computer Automation is used. The central part of this system is the real-time executive (RTX)⁵,

* A detailed description is available from the author.

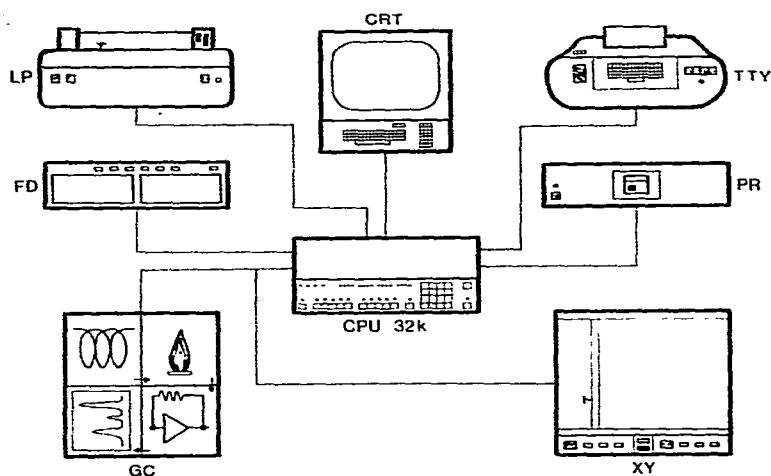


Fig. 1. Computer system. CPU = Central processor unit; CRT = cathode-ray-tube terminal; LP = line-printer; TTY = teletypewriter; PR = paper-tape reader; FD = floppy-disc drives; XY = xy-recorder; GC = gas chromatograph.

by means of which programs created with the FORTRAN-IV compiler or the MACRO assembler can be run in real-time in a time-sharing fashion. The RTX permits several programs, called tasks, to be run concurrently. Each task has its own priority, which defines the relative importance of the different programs. Thus, a task with a low priority will wait while a task with higher priority is executed; also a task with high priority can interrupt the execution of other tasks. The RTX contains a sub-system (IOX) that handles all input/output transfer to and from the system in a flexible way. By means of the RTX, complicated real-time program systems can be run in a moderately sized computer.

GENERAL SOFTWARE ORGANIZATION

GCMEAS

The measurement of flow as described in ref. 1 is conducted by a task called GCMEAS. It runs (with high priority) every 100 msec, is started by a clock pulse and is always operated when gas flows through the column. This program reads the sensors (for temperature, flow and pressure) and calculates a value for the volume of gas (corrected to column temperature and pressure¹) that has passed through the column during the 100-msec interval. These incremental flow values are summed, and the total amount of gas that has passed through the column is calculated.

PEAKM

At a slightly lower priority, a task called PEAKM is started by every pulse during a chromatographic run. This program senses the detector signal and is responsible for the detection of peaks during SCAN-runs and for the collection of data points during PEAK-runs (described below). It also controls the attenuation of the detector amplifier.

PEAKEND

When the end of a peak is reached, the PEAKM program initiates the action of the task PEAKEND (with an even lower priority). PEAKEND makes all the calculations to be performed on the data collected by PEAKM during the elution of the peak. In contrast to the other programs mentioned, PEAKEND is long, usually taking about 20 sec to execute; it is therefore interrupted many times by the short high-priority programs GCMEAS and PEAKM.

COMMAND

The task COMMAND has still lower priority. It handles the communication with the terminals, mainly the CRT and the line-printer. COMMAND is also the interpreter for the "GASIC" language, which is described below. As the response to certain GASIC commands, parameters are changed, new runs are initiated, result listings are generated on the line-printer, and the whole structure of higher-priority programs is administered.

BASIC

The standard BASIC-2 interpreter (slightly modified) runs as a separate task; BASIC uses the teletypewriter terminal with low priority. It contains several machine-language sub-routines (*xy*-recorder driver, floppy-disc driver, etc.). This arrangement makes it possible for the computer to be used for general calculations during the often week-long experiments conducted with the gas chromatograph.

METHODS OF CALCULATION

SCAN runs

For evaluation of the chromatographic peaks, the corresponding data for detector signal and gas volume must be collected in a number of points during a part of the chromatogram which contains one peak and some baseline on either side; such a part of the chromatogram is called a peak cell. Thus, before any chromatographic data can be calculated, the peak cell must be defined for every peak. The bounds of this cell are normally determined in a special run called a "SCAN" run. In this, a peak is considered to elute when the detector signal is greater than a certain level above the baseline. Thus, approximate bounds are found (points P_1 in Fig. 2a) for every peak. These bounds are used to calculate suitable bounds for the peak cell (points P_2 in Fig. 2a).

It is assumed that the peaks on the chromatogram are well separated, so that a portion of baseline exists between each peak. This program system is developed for research on chromatographic processes and not for analysis of complex mixtures; thus, samples can be so chosen that this assumption is valid.

After the SCAN run has been completed, the peak-cell boundaries, the cycle time (the time between the individual measurements in a peak cell) and the amplifier attenuation for each peak are collected in the so-called control table. The cycle time is determined so that the number of measurements in every peak cell is as great as possible without exceeding the available buffer space in memory (400 data points). The attenuation is defined for every peak so that the peak will have optimal height.

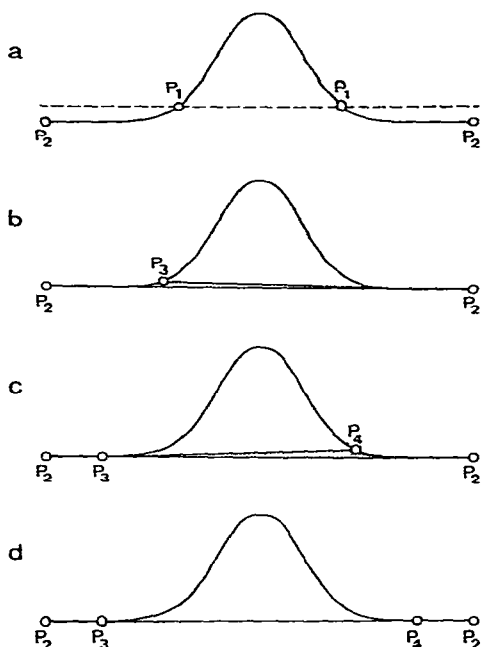


Fig. 2. Definition of points used for evaluating a gas chromatographic peak (see text).

When a SCAN run is made and a good control table has been generated, any number of measurement runs (PEAK runs) can be made.

PEAK runs

During this type of run, the program PEAKM collects data points in a buffer according to the directives given in the control table. At the end of each peak cell, the program PEAKEND is started to make all calculations on the stored data points. Two buffers are provided, so that one peak can be collected at the same time as calculations are made on another.

The operation of PEAKEND starts with a digital smoothing (11 point quadratic smooth)⁶ of the collected detector readings. After this, two points that separate the peak from the baseline must be found. To accomplish this, a preliminary baseline is drawn through the first and the last points of the peak cell (P_2 in Fig. 2b), and the area above this line is computed by addition of detector values; this is called the initial area. Next, the first end-point of the preliminary baseline is moved towards the middle of the peak, and a new area is calculated. This procedure is repeated until the difference between the new area and the initial area exceeds a specified value; the (exaggerated) area difference is shaded in Fig. 2b. When this condition is met, the point P_3 is moved backwards one step (exaggerated in Fig. 2c; normally, the number of steps is about 100). The process is repeated, letting the point P_4 move from the end of the peak towards the middle in the same way. The end-points of the preliminary baseline (P_3 and P_4 in Fig. 2d), which have now moved to the base of the peak, are considered as the boundaries of the peak; the final baseline is calculated by linear regression from those points in the peak cell outside these boundary points.

When the baseline has been defined, the zero-th and the first zero-point moments, together with the second, third and fourth central moments, are calculated⁷. From these moments, the retention volume to the centre of gravity of the peak, the area, the skew, and the number of theoretical plates are calculated as described by Grushka *et al.*⁷.

The retention volume to the maximum of the peak is calculated as the volume when the first derivative⁶ of the peak is zero. The retention volume to the median of the peak is found by adding the detector values until the sum is greater than half the area of the peak. The position of the median is then found by linear interpolation.

PRESENTATION AND STORAGE OF RESULTS

Every chromatographic run results in a table, which is written out on the line-printer (Fig. 3). It contains all the information calculated by PEAKEND, as well as information about column temperature, the total amount of gas that has passed through the column, and the date on which the run was made. This table can be stored on a floppy disc for further processing using BASIC.

```
PEAK REPORT - RUN NO 333
76 09 10 19 41 13

PEAK      1          2          3
NAME      METHANE   PENTHANE  ETMETKET
CODE      0 00       1 00       4 00
VR (GC)   11 43       29 73      181 84
VR (MAX)  11 19       29 39      140.08
VR (MED)  11 24       29 53      169 86
AREA      5 36        8 73       22.81
SKEW      1 61        -.29       2 17
N         128 00     357 00     124 00

TFLOW 13 45   TEMP 333 13
SECTOR 18 ON DISC NO 8
```

Fig. 3. Table containing results from a chromatographic run.

GASIC

To facilitate the operation of this system, a simple interactive "language" called GASIC was constructed; it consists of about 50 code-words. Most of these commands can be executed immediately, *i.e.*, when the command is given through the console terminal, the appropriate action is started. It is also possible to store programs consisting of several GASIC words in the memory for later execution, thereby making unattended operation possible. An example of a GASIC program is given in Table I; this program produces five pages, each containing results from two runs. Every run is made with a different injection time, *i.e.*, the amount of sample injected is gradually increased. Every result is stored on a floppy disc. Before this program is run, the control table and certain other parameters must be defined; this is done by using other GASIC commands in an immediate-execution mode.

TABLE I
TYPICAL PROGRAM IN GASIC

<i>GASIC commands</i>	<i>Comment</i>
INIT	Initialise gas chromatograph
NPAG	New page on line-printer
REPT	Repeat
5	5 times
PARP	Parameter print
1025	Code for printing heading
REPT	Repeat
2	2 times
PEAK	Run chromatogram
PARP	Parameter print
128	Code for printing peak results
CHIN	Increase injection time
STOR	Store on floppy disc
NEXT	End of inner loop
NPAG	New page
NEXT	End of outer loop
END	Terminate

CONCLUDING REMARKS

This program system was developed for a research program involving studies of retention mechanisms, and it is therefore not immediately suited to other applications. It is characterized by facilities for running the same sample repetitively (with changes in concentration if desired); dependence on completely separated peaks; high-precision volume measurement and an integrated BASIC interpreter. With this highly automated system, it is possible, with a minimum of routine work, to collect large numbers of accurate chromatographic results and to make all necessary calculations and plottings on the collected data; this facilitates studies of retention mechanisms and related topics and renders them more reliable.

ACKNOWLEDGEMENT

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