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MICROPROCESSOR-CONTROLLED PEAK RETENTION TIME ANALYSER FOR THE DETERMINATION OF RETENTION INDICES

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SUMMARY

An inexpensive apparatus is described for the measurement of retention indices using an AIM-65 microcomputer. The dead volume is determined by the introduction of air together with the sample and the *n*-alkanes. The circuitry utilised pre-amplification of the air response to allow suitable measurement.

Use of the apparatus is demonstrated with both polar and non-polar stationary phases and the effect of sample size on retention is discussed.

INTRODUCTION

From the introduction of the retention index system by Kováts¹ 25 years ago, this concept has been developed to become the major gas chromatographic (GC) identification method for unknown compounds and is the basis for the classification schemes of stationary phase polarity.

Determination of the retention index (*I*) of a sample has involved the manual determination of the retention times for air, the sample and two *n*-alkanes under the chosen GC conditions and, at one time, it was believed that obtaining *I* values for an unknown on two columns of different polarities would permit positive identification by looking up the values in standard tables, the process being, in a way, analogous to the technique of two-dimensional paper chromatography. In fact, the variations in *I* values obtained for a particular substance, obtained by different workers and/or using different instruments, and particularly using stationary phases obtained from different suppliers, are so large that the production of standard tables has never been realistic. On inspection of published work, variations of 10 units or more for a substance are commonplace. Variations in excess of 50 units have been reported where workers have paid insufficient attention to the ageing processes and consequent chang-

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** Copies of the listings of the programs used in this work may be obtained by contacting Dr. F. Vernon.

es in polarity of the stationary phase. Major sources of error must be attributable to the operator in (a) accurately timing the introduction of the sample and (b) accurately timing the emergence of the peak maximum. Further large errors may be introduced by such practices as determining air, sample and *n*-alkane retention times separately and assuming that the instrumental conditions remain constants over this series of operations.

By the use of the computerized instrument described here, these errors may be minimized. Sample injection and peak emergence times are determined automatically using an internal clock with a resolution of 10 msec. Air is introduced together with the sample and *n*-alkanes and a secondary program requires only that the *n*-alkanes be identified to the computer to permit computation of retention index of the sample. The apparatus described here, which is similar to a microcomputer-based GC integrator reported recently², is relatively inexpensive, and the accuracy and reproducibility of the retention indices are at a level not previously achieved with such inexpensive equipment.

EXPERIMENTAL

The peak retention time analyser is required to automatically acquire data from the gas chromatograph to, process it and, from the results, determine a value for the Kováts retention index for an unknown compound. The output from the gas chromatograph is a d.c. voltage signal that is changing in amplitude with time. A wide variation in its amplitude occurs because of the diversity of the samples. For example, the response for, say, *n*-octane is a factor of at least 10 greater than that for air for equal volumes. It is therefore necessary to incorporate a "signal normalizing" arrangement in the analyses to compensate for the range of peaks encountered. In addition, the effects of noise, baseline drift, etc., must be overcome for adequate performance to be ensured.

An AIM-65 microcomputer (Rockwell, Anaheim, CA, U.S.A.) is used to control the analysis process, with operation as follows. The sample solution containing two "known" *n*-alkanes, an "unknown" and air is injected into the gas chromatograph using a syringe. This syringe has been modified with the inclusion of a normally open electrical switch, which is used to indicate to the AIM-65 microcomputer that the sample has been injected. An interval timer is started within the AIM-65 microcomputer when this occurs. An output from the gas chromatograph is applied to the input of the programmable gain amplifier used for signal-normalizing purposes. The amplified signal is applied to the threshold detector, which compares its amplitude with a manually pre-set voltage level that is set above the level of any "unwanted" peaks or baseline drift levels. The output from the threshold detector changes state whenever the pre-set level is exceeded by the analogue signal. This is identified by the AIM-65 microcomputer, which then commences to digitize the analogue signal. A Sample and Hold circuit is used to sample the analogue signal amplitude and "hold" it constant while it is digitized by the analogue to digital converter. The sampling rate is adjusted automatically to allow for increases in the peak half-width. A digital to analogue converter of 8-bit resolution (0.4%) in conjunction with a voltage comparator is used to perform the analogue to digital conversion. If two's complement binary code is used to represent both positive and negative chromatographic signals,

the accuracy is halved. The successive approximation algorithm is implemented by the AIM-65 microcomputer using an assembly language sub-routine. Sequential data values are smoothed by numerical methods before determining the first time-differential, which in turn is used to indicate the position of the peak maximum.

An algorithm³ using 17 data points, obtained at equal time intervals, is used. It is assumed that close to the peak maximum the peak's profile follows a quadratic curve. The process consists of multiplying the 17 points of data by a fixed set of numbers, summing the result and dividing it by a normalizing factor. The result is a new ordinate that is at the mid-point of the area of the curve sampled. New data points are added to the algorithm as the data are acquired and outdated data points are also discarded, to be replaced as the analysis continues. If the result of the calculation is a positive value, the signal is increasing towards the crest of the peak. If the result is negative, then the signal is decreasing away from the crest. A maximum is thus detected when the sign changes from positive to negative. The microcomputer prints out the peak retention times as each peak is located, and indicates the relative peak amplitude. The schematic circuit diagram of the peak retention time analyser is shown in Figs. 1 and 2. This inexpensive (approximately £35) addition to the GC-

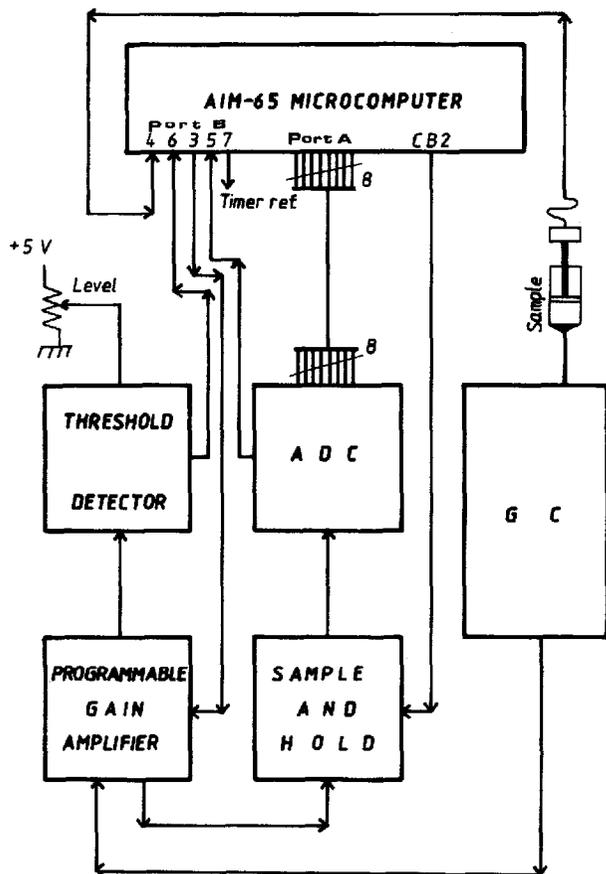


Fig. 1. Schematic diagram of the peak retention time analyser.

AIM-65 system was mounted within the casing of the AIM-65 microcomputer. The controlling programs for the peak retention time analyser are loaded into the microcomputer via its cassette recorder interface from audio tape.

Before commencement of the control procedures, the time interval for the variable time delay, and the initial sampling rate, are entered into the computer using a simple Basic program. This program sets the relevant parameters of the main assembly language program, which then performs the necessary sampling and data processing operations.

The sampling rate is set by a fixed time delay (FTD) unit in series with a variable time delay (VTD) within the microcomputer. The maximum rate, as given by the FTD, is 30 samples/sec. Using the VTD any initial rate in the range 13–30 samples/sec may be selected. The FTD is determined by the data processing procedures within the microprocessor. The VTD may be doubled at pre-selected time intervals (*e.g.*, every 100 sec). The sampling rate is then determined from FTD and VTD parameters and, below *ca.* 10 samples/sec, it will halve after the pre-selected time interval up to a maximum of eight times.

A flow diagram of the procedures is illustrated in Fig. 3. The microcomputer displays the message "Inject the Sample" and continuously monitors its PB4 input to determine when the sample is injected into the gas chromatograph. A modified syringe, incorporating a switch contact, is used to indicate this event to the microcomputer. Subsequent to the injection of the sample, a timer is started within the AIM-65 microcomputer. The timer 1 facility of the versatile SY 6522 interface adapter is utilized for this purpose. This adapter operates timer 1 in a "free running" mode, decrementing the counter at a rate determined by the 0 system clock pulse. The logical state of the PB7 output line is inverted whenever the timer counts down to zero, thus giving an indication of the update rate of the timer, which may be used for calibration purposes. The unit was updated 100 times per second, but this may be altered in the software by adjusting the "count", which is pre-loaded into the timer 1 counter. The elapsed time is then displayed by the AIM-65 microcomputer. The data sampling rate is determined by the time taken for the microcomputer to implement the procedures used to analyse the peak profile. With time, the sampling rate is decreased to compensate for the increase in peak half-width. This is achieved by incorporating a variable delay generated in the microcomputer software.

The analogue signal from the gas chromatograph is applied to a voltage follower (LM310) incorporating an active low-pass filter circuit to reduce signal noise. The output from this circuit is applied to the programmable-gain operational amplifier (LM741) where the initial peak (air) is amplified. The air is amplified by a gain factor that can be manually adjusted up to a value 13 times that of subsequent peaks. Automatic gain adjustments are accomplished by means of a CMOS analogue switch (40016B), which is connected across the feedback loop of the operational amplifier. Port PB3 line of the AIM-65 is designated as an output to energize or de-energize the analogue switch via suitable diode-transistor logic circuitry. One output from the programmable-gain amplifier is applied to a voltage comparator (LM311), which compares the GC signal amplitude with a pre-set threshold level. The threshold level is manually set to a value that is above the level of baseline drift, etc. When a peak exceeds the threshold level, the output of the comparator changes state, energizing an LED indicator and providing a visual indication of the evolution of a peak. The

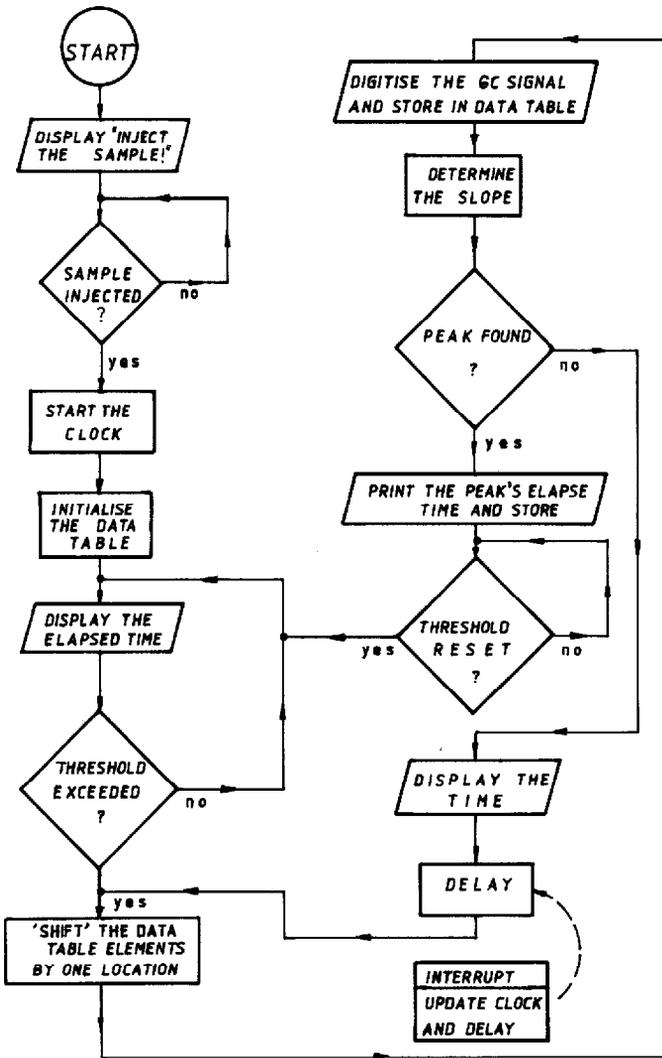


Fig. 3. Flow chart of control program.

AIM-65 recognizes this event via its PB6 input line and proceeds to sample the signal amplitude, utilizing the Sample and Hold circuit (LF398H). The Sample and Hold circuit receives an input from the second output of the programmable-gain amplifier. A third output from the programmable amplifier is applied to the pen recorder. The CB2 peripheral control line of the versatile interface adapted is used to allow the sampling of the Sample and Hold circuit. The Sample and Hold circuit provides a means of "freezing" the GC signal amplitude whilst the GC signal is digitized and stored in the microcomputer memory. Analogue to digital conversion is achieved by means of a digital to analogue converter, DAC (2N425E), and a voltage comparator (LM311) using a software-controlled successive approximation technique. This arrangement, although much slower than a commercial ADC integrated circuit, pro-

vides for greater flexibility by allowing analogue voltages to be generated by the microcomputer.

Port A of the AIM-65 is used to apply the digital codes to the DAC to produce a proportional voltage level at its output. This voltage is compared with the voltage from the Sample and Hold circuit. The output state of the voltage comparator is dependent on whether the digitally generated voltage exceeds the GC signal level. PB5 of the microcomputer is used to determine the output state of the voltage comparator.

Manipulation of the acquired data is undertaken with the AIM-65 microcomputer to determine the retention times of the GC peaks. An additional program permits the calculation of the retention index of the sample. This value is also printed by the AIM-65 microcomputer.

The computer was interfaced to a Carle gas chromatograph, a dual-column instrument with katharometer (filament-type) detectors.

The columns were 2 m \times 1/8 in. I.D. stainless steel, packed with 10% squalane or Carbowax 20 M on 100–120-mesh Chromosorb W AW DMCS. Initially the injected mixtures consisted of 1 μ l of 1:1:1 by volume of sample and two *n*-alkanes together with 3–4 μ l of air.

Audiodata recorder

The circuit shown in Fig. 4 was constructed as an inexpensive means of recording chromatograms on audio tape using a cassette tape recorder. Although the reproducible accuracy of the system is low (owing to variations in motor speed, etc.) it was found very useful in the development of the automatic retention time analyser. The record system incorporates a voltage to frequency converter integrated circuit and the GC signal is applied to its input. The output is applied to the microphone input of the cassette recorder, thus storing the chromatogram as a frequency-modulated signal on the audio tape. The chromatogram may be replayed at various speeds, allowing time-base compression to be achieved. In our case the output (taken from the extension speaker socket of the tape recorder) was applied to the low-pass filter input of the circuit shown in Fig. 2.

RESULTS AND DISCUSSION

Table I gives the values for 12 determinations of the retention index of toluene on a 10% squalane column at 100°C using *n*-hexane and *n*-octane. The average value is 760.49 with a standard deviation of 0.14, and all ten results lie in the range 760.30–760.68. The precision is very good but these data reveal nothing about the accuracy of the determination.

In order to study accuracy, in addition to precision, a sample of *n*-heptane was taken as the test substance (*I* by definition being 700) and injected with *n*-hexane and *n*-octane under the conditions used for toluene. As shown in Table II, the average of ten results was 701.33 with a standard deviation of 0.14, the result therefore being 1.33 units too high. Although a very small effect, it is known that increasing the amount of sample causes peak "fronting", which effectively delays the emergence of the peak maximum, thereby giving rise to increased *I* values. This effect was examined. As all samples prior to this had consisted of 1:1:1 mixtures by volume of the two *n*-alkanes and the test substance, this ratio was varied. As expected, the higher the

TABLE I
RETENTION INDICES OF TOLUENE ON A 10% SQUALANE COLUMN AT 100°C

<i>Determination No.</i>	<i>I</i>
1	760.30
2	760.40
3	760.47
4	760.40
5	760.37
6	760.40
7	760.62
8	760.68
9	760.57
10	760.58
11	760.41
12	760.55
Average	760.49
Standard deviation	0.14

ratio of test substance to *n*-alkanes, the higher was the *I* value obtained. As can be seen from Table II, using a 2:2:2 volume ratio of *n*-C₆:*n*-C₇:*n*-C₈ gave an average *I* value of 700.35 for *n*-heptane with standard deviation of 0.07. A 3:1:3 ratio gave *I* = 699.97, so it is important that high *n*-alkanes to test substance ratios are used in order to obtain the greatest accuracy.

TABLE II
EFFECT OF VARYING THE SAMPLE TO *n*-ALKANE RATIO ON THE RETENTION INDEX OF *n*-HEPTANE

Average values of 10 results and standard deviation, obtained on squalane at 100°C

<i>Ratio n-C₆:n-C₇:n-C₈</i>	<i>Mean retention index (n = 10)</i>	<i>Standard deviation</i>
1:2:1	701.94	0.09
1:3:1	701.33	0.14
2:2:2	700.35	0.07

Another effect examined was that of the sample size injected. Using 1:1:1 by volume mixtures of benzene with *n*-hexane and *n*-octane and injecting 1 μ l of sample and air on to the squalane column at 100°C, the retention index of benzene was 694.8. Increasing the sample size to 2 μ l gave *I* = 694.5, and from this it was considered that variations in sample size are not important. Determinations of *I* for benzene using *n*-octane and *n*-undecane were then carried out on a Carbowax 20 M column at 100°C. A 1- μ l sample gave *I* = 973.3 whereas a 2- μ l sample gave *I* = 944.8. It is known that retention indices are subject to higher errors on polar than on non-polar phases. The large variation seen would invalidate any attempt at correlation and the use of vary-

ing sample sizes accounts for the presence of conflicting data in the literature. This effect was studied further using 1:1:1 *n*-nonane-toluene-*n*-dodecane as the sample. The difficulty of accurately measuring the sample size in the vicinity of 1 μl was overcome by measuring the toluene peak height on the chromatograms. For small variations in sample size, the peak height will be proportional to the sample volume injected. Sample sizes could be determined by measuring peak heights relative to the toluene peak for a nominal 1- μl sample. Table III shows how the retention index of toluene apparently changes from 1053.7 for a 0.88- μl sample to 1046.3 for a 1.12- μl sample. An effective variation of 3 units per 0.1- μl change in sample size means that for highly accurate determinations on polar phases, a method of sample size control superior to that currently in use is required.

TABLE III

EFFECT OF SAMPLE SIZE INJECTED ON RETENTION INDEX OF TOLUENE ON CARBOWAX 20 M AT 100°C

Sample size (μl)	<i>I</i>	Sample size (μl)	<i>I</i>
0.88	1053.7	1.02	1048.5
0.91	1051.4	1.04	1049.9
0.91	1051.3	1.09	1047.2
0.96	1049.2	1.10	1046.4
0.97	1049.6	1.10	1046.5
1.00	1048.6	1.12	1046.3

In conclusion, as microprocessor technology has now developed to the point where computer-controlled GC is a relatively inexpensive technique, it has become possible to determine retention indices with high accuracy and a precision of less than 1 index unit. However, to obtain maximum utilization of the instrument it is necessary to have rigid control of sample parameters such as *n*-alkane to test substance ratios and the actual size of sample injected. Of equal importance is the careful characterization of the columns used in terms of their loading⁴ and the polarity of the stationary phase. Ageing of columns should be monitored on a regular basis and the packings discarded when changes in polarity become evident.

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