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HIGH PRECISION MEASUREMENT OF SPECIFIC RETENTION VOLUMES

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SUMMARY

A statistical analysis has been performed of the errors incident to the individual experimental parameters necessary for expressing the specific retention volume. A gas chromatograph, in which the working conditions can be controlled with a high accuracy, has been constructed. When using packed columns in this apparatus, the precision of a single measurement corresponds to a coefficient of variation of about 0.2 %; with capillary columns the coefficient amounted to about 0.3 %. The width of the confidence interval of the V_g obtained in two chromatographic runs was about $\pm$ 3 ml/g, which corresponds to a resolving power of about 45,000 theoretical plates at a given relative retention. The reliability attained renders the V_g values useful for calculating the excess thermodynamic properties.

INTRODUCTION

The physico-chemical as well as analytical significance of gas chromatographic retention data have been recognized since the very beginning of gas chromatography[1]. However, the problem of the reliability of measuring retention data has only lately come to the fore as a matter of interest. There are roughly three trends in this field, relating to the schools of KEULEMANS, CRUICKSHANK and GUIOCHON. The objectives of the above trends have been, in the main, the use of precise retention data for fine correlation[2–10] and structure elucidation[11–16], calculation of thermodynamic properties[17–23], and precise measurement of the detector response[24].

A common feature of all the aspects of the problem is the need for a specially designed gas chromatograph, as both the quality and the arrangement of the individual components in conventional analytical gas chromatographs are inconvenient for the above purposes.

The most important gas chromatographic retention characteristic is the specific retention volume, V_g. However, the necessity of specifying and measuring the great number of parameters needed to determine the V_g presents difficulties in the precise and, particularly, the accurate measurement of this retention quantity. From this viewpoint, the determination of the V_g is much more of a problem than the determination of the retention time or relative retention data.

The aim of the present paper is to carry out a detailed analysis of the errors associated with the experimental parameters necessary for calculating the specific retention volume, and to construct a gas chromatograph which will allow measurements with a reliability of at least one order higher than has been attained with commercially available instruments.

THEORETICAL

According to the respective definitions[25], the specific retention volume can be expressed through use of experimental parameters by

$$V_g = \frac{(l_R - l_0) v_f P_f 273 j}{r w P_2 T_f} \tag{1}$$

where l_R and l_0 are the distances from the start line of the substance in question and of a nonsorbed component, respectively, as measured in the chromatogram, r is the chart speed, v_f is the carrier gas volume flow rate as measured under a pressure P_f and temperature T_f in the flowmeter, P_2 is the column outlet pressure, w is the weight of the effective sorbent in the column, and j is the JAMES–MARTIN factor[26], given by $j = 3\,P_2(P_1^2 - P_2^2)/2(P_1^3 - P_2^3)$, where P_1 is the column inlet pressure.

As it is hardly possible to define a standard value of the specific retention volume, it is difficult to check the accuracy of V_g values measured directly. Therefore, attention has been given to the problem of eliminating all the possible systematic errors. The accuracy of the data obtained has been checked[27] by comparing the thermodynamic properties calculated from the V_g with those determined by the calorimetric method. The precision has been expressed by the standard deviation and demonstrated in terms of the resolving power of the apparatus; the data on precision express the repeatability of the measurements.

The estimation of the standard deviation (briefly standard deviation) of a V_g value, s_{Vg}, can be obtained from the standard deviations of the individual parameters. It follows from the rules of statistics[28] that

$$s_{Vg} = \left[\left(\frac{\partial V_g}{\partial l_R}\right)^2 s_{l_R}^2 + \left(\frac{\partial V_g}{\partial l_0}\right)^2 s_{l_0}^2 + \cdots + \left(\frac{\partial V_g}{\partial j}\right)^2 s_j^2\right]^{1/2} \tag{2}$$

where

$$s_j^2 = \left(\frac{\partial j}{\partial P_1}\right)^2 s_{P_1}^2 + \left(\frac{\partial j}{\partial P_2}\right)^2 s_{P_2}^2. \tag{3}$$

Relations (2) and (3) serve as guides in the analysis of errors, unless it is possible to exclude the latter by appropriate measures.

Analysis of the problem

The nature, as well as the magnitude, of the errors to be considered obviously depend on the design of the apparatus. In our arrangement, we have used a specially designed capillary flowmeter with an oblique manometric tube, placed ahead of the injection port. This type of flowmeter[29] yields continuous and pressure-independent information on the flow rate. The column inlet pressure was measured by a multiple U-tube mercury manometer[30,31] situated between the flowmeter and the

inlet port; the resultant mercury level difference, Δh_m, is given by $\Delta h_m = \sum_i \Delta h_{mi}$. The temperatures of the column, flowmeter, and other items were measured by mercury thermometers graduated by 0.1 °C and, in some cases, a Beckman thermometer was used.

The errors of the individual experimental parameters as well as the arrangements used for eliminating some of them are summarized below.

Potential sources of error	*Elimination or estimation of the errors*
Measurement of the carrier gas flow rate	
Changes of temperature	Thermostating of both the measuring capillary and manometric tube
Measuring the angle of positioning the manometric tube	Fixed positioning
Nonuniformity of the internal diameter of the manometric tube	Reading out from both arms of the tube
Readout of the level difference (Δh) in the manometric tube	$s_{\Delta h} = 0.35$ mm
Measurement of the column inlet pressure	
Changes of temperature	Expressing the mercury column length at 0° C
Parallax	Covering the rear parts of the manometric tubes with a mirror
Nonuniformity of the internal diameter of the manometric tubes	Reading out from all the arms
Deviation from the standard acceleration of gravity	Correction
Readout	$s_{\Delta h} = \sqrt{3 \Delta h_1^2} = 0.24$ mm
Measurement of the atmospheric pressure (The measurement[32] was carried out by means of a conventional mercury barometer)	
Changes of temperature	Recalculation of the density of Hg at 0° C
Deviation from the standard acceleration of gravity	Correction
Readout (using a vernier)	$s_h = 0.1$ mm $\equiv 1.31 \times 10^{-4}$ atm.
Measurement of temperature (ref. 32)	
Errors in the original calibration	Recalibration
Difference in the conditions in calibration and measurements	Correction
Zero point depression	Avoiding the measurement of lower temperatures immediately after having measured high temperatures
Readout	$s_{temp.} = 0.05$° C

Measurement of the retention parameters

The net retention time, $t_R - t_0$, was determined from the respective $l_R - l_0$ and r. The peak maximum was defined as a half distance between the peak shoulders just below the apex, and the point so obtained was transferred to the zero line of the chromatogram. The standard deviation of the difference $l_R - l_0$, $s_{\Delta l}$, determined by the above method was $s_{\Delta l} = \sqrt{s_{l_R}^2 + s_{l_0}^2} = 0.28$ mm. The standard deviation of the chart speed was determined experimentally and amounted to 2×10^{-4} mm/sec (*cf.* Table I).

Determination of the weight of the stationary phase in the column

The accuracy and precision of determining the weight of sorbent in the column

is of basic importance in measuring V_g. In our measurement, the total weight of the column packing was determined as the difference obtained by weighing an appropriate amount of the packing before and after filling the column. The standard deviation of a single weighing was 0.01 g, so that the standard deviation of the determination of the weight of the column packing, $s_{w(p)}$, is 0.014 g.

Although the solid support had been thoroughly dried before coating it with the stationary phase and the solvent used in the coating procedure carefully evaporated, it was not possible to determine the amount of stationary phase simply from the weight and supposed composition of the packing, owing to undefined changes in the support and stationary liquid proportions during the procedure. Therefore, the true amount of the stationary phase was determined by extracting the stationary phase from a sample of the packing, evaporating the solvent, and weighing the residue. Under the given conditions, the standard deviation when determining the weight of the liquid in the packing by the above procedure, s_{ex}, was 0.0038 g. If $w(p)^*$ and $w(ex)^*$ are the weights of the packing sample and of its respective extract, respectively, the standard deviation of the weight of the stationary liquid in the column, s_w is given by $s_w = [s^2_{ex} + (w(ex)^*/w(p)^*)^2 s^2_{w(p)}]^{1/2}$ and amounts to 0.0052 g.

The above analysis of the problem served as the basis for designing the final concept of the chromatograph. Using this arrangement and hexane chromatographed on squalane at 50° C, the repeatability of the specific retention volume and of measurements of individual parameters was determined. The respective data are summarized in Table I.

TABLE I

STANDARD DEVIATIONS AND PERCENTAGE COEFFICIENTS OF VARIATION ESTIMATED THEORETICALLY FOR RATED VALUES OF THE EXPERIMENTAL PARAMETERS CORRESPONDING TO THE CHROMATOGRAPHY OF HEXANE ON SQUALANE AT 50° C

Variables	*Dimensions*	*Rated value*	*s(theor)*	*V*
Δl	mm	437.9	0.28	0.06
r	mm/sec	0.3339	2×10^{-4}	0.06
v_f	ml/sec	0.3535	2×10^{-4}	0.06
T_f	°K	303.15	0.06	0.02
$P_f = P_1$	atm	1.8360	2.237×10^{-4}	0.01
P_2	atm	0.9917	1.316×10^{-4}	0.01
j		0.68119	1.4×10^{-4}	0.02
w	g	3.4817	0.0052	0.15
T	°K	323.16	0.001	0.001
V_g	ml/g	151.30	0.284	0.19

EXPERIMENTAL

Apparatus

A flow diagram of the apparatus is shown in Fig. 1. The carrier gas as well as the H_2 and air were taken from storage cylinders (the controlling and measuring devices for the air and H_2 paths have not been indicated in the diagram). The pressure of the carrier gas was reduced to about 4 atm by a coarse pressure controller (1) after which the carrier gas enters a flow controlling unit. This unit comprises two high pre-

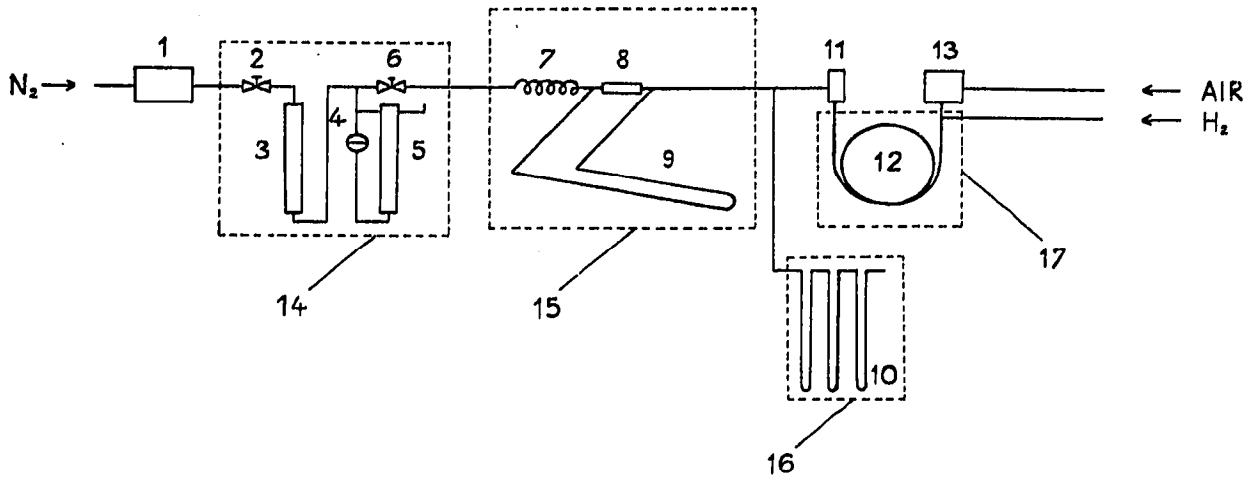

Fig. 1. Flow diagram of the gas chromatograph.

cision needle valves (2,6), a drying tube with molecular sieve 5A (3), and a Cartesian manostat (Hoke-Manostat Corp., Cresskill, N.J., U.S.A.) (4,5). The elements 2–6 were enclosed in a polystyrene-foam box (14) to protect the former against the effects of variations in the ambient temperature. It is evident from the diagram that the controls can function as a source of either constant pressure or flow.

The measuring part of the apparatus is represented by the capillary flowmeter (7,8,9) and multiple U-tube mercury manometer (10). Before entering the measuring capillary (8), carrier gas is tempered by passing it through a 2 m long copper capillary of 1 mm inner diameter (7). The pressure drop across the measuring capillary was measured by a 1 m long U-tube (9) filled with dinonylphthalate. The tube was situated in an oblique position, forming an angle of 15° with the horizontal. Both controlling and measuring capillaries as well as the manometric tube were kept at a constant temperature by a water ultrathermostat (15). The measuring capillaries are exchangeable in order to take measurements in various flow rate ranges, this permits for the measurement of various flow rates with an identical relative error. The capillaries were calibrated by means of a Mariotte flask.

The manometer (10) consisted of three 120 cm long U-tubes filled with mercury; the voids between the neighbouring Hg levels were filled with water. The individual tubes were interconnected by brass capillary joints attached to the glass by means of appropriate fittings with silicone rubber sealing rings. The whole manometer was bedded in a polystyrene-foam case (16).

The injection port (11) has been designed with regard to the need for minimizing the dead space while ensuring a sufficient flow-through cross section in order to prevent any appreciable pressure drop across the inlet port. This was achieved by using a special insert liner. The chromatographic columns (12) were stainless steel tubes, 120–190 cm in length and 4 mm I.D., coiled into helices of about 12 cm diameter. The columns were immersed in a glycerol thermostat (17) permitting a working temperature range of 30–150°C. Fine temperature control and vigorous stirring of the liquid ensured good temperature stability and homogeneity within the thermostat.

The set-up can also be used for work with capillary columns. For this purpose the lower part of the injection block can be provided with a fitting communicating

with a fine needle valve, thus forming an inlet splitter affording an adjustable split ratio. The column fitting on the detector side is the same as with packed columns, however, it is necessary that the capillary column outlet extends beyond the hydrogen intake.

A flame ionization detector (13) was used for detection, the signal being processed by means of a conventional amplifier and recorded by an EZ-3 recorder (Laboratory Equipment, n.e., Prague). The hydrogen and air flow rates were controlled, stabilized, and measured using fine needle valves and capillary flowmeters.

Procedures and materials

The performance of the apparatus was tested by chromatographing pentane, hexane, and benzene (B.D.H. Ltd., Great Britain) on a packed and a capillary column with squalane (Carlo Erba Co., Italy) as the stationary phase; and *m*- and *p*-xylene on a packed column with benzylbiphenyl (May & Becker Ltd., Great Britain) as the stationary phase. The purpose of the measurements was to carry out a comparison of the actual errors of V_g's obtained in replicate chromatographic runs with those predicted theoretically according to eqn. 2.

In the work with packed columns, about 25 wt. % of both squalane and benzylbiphenyl were used on Chromosorb W 60/80 mesh (Carlo Erba Co., Italy). Chromatography of the squalane on an SE-30 column showed that it contained 6.1 % of impurities mostly lower hydrocarbons.

After putting the instrument into operation and setting all the working conditions, the apparatus was left running for 3 h to ensure a steady state. The hydrocarbons were injected in the form of 0.1 % solutions in CS_2 with a 10 μl Hamilton syringe, each charge containing 1–2 μl of the solution and *ca.* 1 μl of CH_4 to obtain data on the dead volume.

In the work with the capillary column, the amount of stationary phase in the column was determined from the specific retention volume known from the measurements with packed columns, net retention volume, and the other experimental parameters (*cf.* eqn. 1). The carrier gas flow rate through the capillary column was measured with the help of a Mariotte flask.

RESULTS AND DISCUSSION

The specific retention volumes (arithmetic means of fifteen values) of pentane, hexane, and benzene on squalane, measured at different temperatures, along with the standard deviations of single measurements and the percentage coefficients of variation are summarized in Table II. The data at the bottom of the table refer to the measurements on the capillary column.

It is evident that the actual error in measuring the V_g agrees well with that estimated theoretically. Hence it follows that there were no essentially unpredicted sources of error in the measurements. A higher error ($V = 0.33$ %) can be noticed in the measurement with the capillary column; this is obviously due to a higher error in the measurement of the very low flow rates (10^{-3} ml/sec).

With the precision attained, the effects of impurities present in the stationary phase on the V_g measured became evident. It has been found that the amount of lower hydrocarbons in the squalane used as the stationary phase rose from the initial

TABLE II

V_g VALUES (ARITHMETIC MEANS OF 15–20 MEASUREMENTS) OF PENTANE, HEXANE, AND BENZENE ON SQUALANE AT VARIOUS TEMPERATURES, EXPERIMENTAL STANDARD DEVIATIONS OF SINGLE MEASUREMENTS OF V_g, AND THE CORRESPONDING PERCENTAGE COEFFICIENTS OF VARIATION

Packed column: column length and inner diameter 190 cm and 4 mm, respectively, weight of the column packing 13.5509 g (3.4817 g of squalane), carrier gas flow rate 0.33–0.41 ml/sec at a constant column inlet overpressure of 650 mm Hg; capillary column (last two lines): column length and inner diameter 60 m and 0.2 mm respectively, weight of the squalane 0.01157 g, carrier gas flow rate 3.8×10^{-3} ml/sec at an overpressure of 780 mm Hg, split ratio 1:1000.

Temperature (°K)	*Pentane*			*Hexane*			*Benzene*		
	V_g	sV_g	V	V_g	sV_g	V	V_g	sV_g	V
303.14	106.23	0.244	0.23	327.66	0.315	0.10	476.17	1.53	0.32
313.19	75.75	0.197	0.26	219.58	0.316	0.14	319.60	0.68	0.21
318.19	64.83	0.095	0.15	182.81	0.480	0.26	266.61	0.51	0.19
323.21	55.16	0.117	0.21	151.64	0.288	0.19	220.75	0.55	0.23
328.17	47.71	0.110	0.23	127.91	0.189	0.15	187.82	0.21	0.11
333.15	41.40	0.081	0.20	108.36	0.222	0.20	159.23	0.23	0.14
338.21	36.35	0.060	0.16	92.38	0.285	0.31	135.62	0.32	0.24
323.10	54.95	0.230	0.42				223.52	0.88	0.39
328.20				126.36	0.308	0.24	186.48	0.49	0.26

6.1 % to as much as 14.7 % upon heating the packing for some hours at 80°C, obviously to the detriment of the squalane content. This resulted in lowering the V_g values of pentane and hexane by 1.0 and 2.5 %, respectively, but the V_g of benzene increased by 1.5 %, in spite of carrying out the correction for loss of stationary phase owing to its volatilization. The above changes in retention are most likely due to changes in the activity coefficients, brought about by the changes in the composition of the stationary phase.

The V_g values of *m*- and *p*-xylene on benzylbiphenyl are in Table III. These data have been used to demonstrate the significance of the precision of V_g values with respect to using them for identification purposes. The separation efficiency of the column employed is apparently insufficient for the resolution of the above pair of substances when injected as a mixture. On the other hand, however, the difference in the V_g values obtained on chromatographing the individual components may or may not be decisive for distinguishing between the two substances. The reliability of identifying a substance from its specific retention volume is given by the reliability

TABLE III

V_g VALUES (MEAN VALUES OF n MEASUREMENTS) OF *m*- AND *p*-XYLENES ON BENZYLBIPHENYL AT 104.8 °C, STANDARD DEVIATIONS OF SINGLE MEASUREMENTS (sV_g) AND THE MEAN, VALUES, sV_g, AND THE RESPECTIVE WIDTHS OF THE CONFIDENCE INTERVAL (tsV_g)

Column length and inner diameter 190 cm and 4 mm, respectively, weight of the column packing 10.4864 g (2.6216 g of benzylbiphenyl), carrier gas flow rate 0.42 ml/sec at a column inlet overpressure of 440 mm Hg.

Component	V_g	sV_g	V	sV_g	tsV_g	n
p-Xylene	216.77	0.635	0.30	0.1456	0.3059	19
m-Xylene	223.99	0.684	0.30	0.1529	0.3200	20

of the V_g measurement and can be expressed statistically. Namely, two values of V_g may be considered as different only if the difference exceeds the respective width of the confidence interval, *i.e.*, if the expression below holds[33].

$$|\overline{V}_{g2} - \overline{V}_{g1}| > t_\alpha s \sqrt{(1/n_2) + (1/n_1)} \quad (4)$$

where

$$s = \{[1/(n_1 + n_2 - 2)]\,[(n_2 - 1)s_2^2 + (n_1 - 1)s_1^2]\}^{1/2} \quad (5)$$

1 and 2 denote two different substances, t_α is the critical Student coefficient for the confidence level α and the given number of degrees of freedom, and n stands for the number of measurements. The symbol $\overline{V}_g$ signifies the arithmetic mean.

To illustrate the case in chromatographic terms, let us express the number of theoretical plates that would be necessary for perceptibly resolving two substances with V_g values differing from each other just by the width of a confidence interval. Using KAISER's method[34] for determining the number of theoretical plates N necessary to attain a degree of separation, v, it is possible to write

$$N = 2\left(\frac{V_{g2} + V_{g1} + 2V_{g0}}{V_{g2} - V_{g1}}\right)^2 \ln \frac{2}{1 - v} \quad (6)$$

where V_{g0} is the column dead volume. Taking account of eqns. 4, 5 and 6 and supposing that $v = 0.1$ is sufficient for perceptible resolution, it can be concluded that identification may be carried out with V_g values differing from each other by about ± 3 ml/g with only two chromatographic runs for each substance; this precision corresponds to a resolving power of more than 40,000 theoretical plates at a given V_{g2}/V_{g1} ratio and negligible V_{g0}. The number of theoretical plates used in the above conception obviously rises on increasing the number of measurements.

CONCLUSIONS

Judicious design of the gas chromatograph, careful calibration of all the measuring units, and accurate control of the working conditions have permitted the minimization of systematic errors in measuring the specific retention volume and have made it possible to measure V_g values with a precision corresponding to a coefficient of variation of about 0.2 %.

The actual standard deviation of the V_g values measured on an apparatus of the above type agreed well with the standard deviation estimated theoretically by statistical analysis of the individual experimental parameters. The reliability attained in measuring the V_g makes it possible to calculate excess thermodynamic functions from the V_g values successfully.

In the case of only two chromatographic runs with the substance under analysis, the width of the confidence interval of the respective V_g's corresponds to a resolution power equivalent to about 45,000 theoretical plates at a given relative retention.

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