GAS–LIQUID CHROMATOGRAPHY IN QUALITATIVE ANALYSIS

PART IV. A SIMPLE METHOD OF CALCULATING R_{x9} VALUES AT ELEVATED COLUMN TEMPERATURES

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INTRODUCTION

The R_{x9} method[1] of presenting relative retention ratios requires a knowledge of the retention of the n-alkane used as standard relative to that of n-nonane. At moderate column temperatures this can be obtained graphically from the plot of the logarithm of retention against carbon number (the "log plot") for the calibration series of n-alkanes[2]. At high column temperatures a graphical technique is impracticable and as "least squares" procedures are too time-consuming for routine application a simple method of calculating R_{N9} values (*i.e.* the retention of n-alkanes relative to n-nonane) from the slope of the "log plot" has been evolved with virtually no loss of precision.

PROCEDURE AND RESULTS

The method for obtaining R_{x9} values requires the following steps:
(1) Calculation of the column dynamic dead volume.
(2) Evaluation of a precise value of b (the slope of the "log plot") which is required to calculate R_{N9} values of the n-alkane standards from $\log R_{N9} = b(N - 9)$.
(3) Use of this data to calculate R_{x9} from $R_{x9} = R_{N9} \times R_{xN}$.

Calculation of column dead volume
The column dead volume must be calculated since it has been shown that even with detectors which do give an air peak, the volume to the air peak is not coincident with the dynamic column dead volume[3]. After recording the chromatogram of a suitable calibration series of n-alkanes[2] the column dead volume can be calculated using either of three methods.
(1) The static column interstitial volume can be calculated using the expression:

$$d_0 = \frac{2.54}{60} \frac{SV}{F} \left\{ 1 - \frac{B_p}{D_p} - \frac{PB_p}{D_L (100 - P)} \right\}$$

where
d_0 = distance from injection point to dead volume point on the recorder chart in cm,
S = the recorder chart speed in in./h,
V = volume of the column packed,

F = carrier gas flow rate in cm³/min at the average column pressure,
B_p = bulk density of the support,
D_p = true density of the support,
P = percentage (w/w) of stationary phase,
D_L = density of stationary phase.

This is subtracted from the observed retention distances of the n-alkanes measured between the point of injection and the peak maximum on the recorder chart. The adjusted retention distances are then plotted against carbon number using 2 or 3 cycle log-linear graph paper. Owing to the fact that the static and dynamic column dead volumes may not be identical (due to stream-lining of the gas flow for instance) a further correction is applied computed from the expression:

$$\delta a = \frac{2.303 \; \sum \delta \log R}{\sum 1/R_{\text{ext.}}}$$

where

$\delta \log R$ = $\log R - \log R_{\text{ext.}}$,
R = retention distance from the assumed dead volume,
$R_{\text{ext.}}$ = value obtained from the extrapolation of the linear part of the graph,
δa = the further correction to the retention distance to be subtracted or added to the first set of adjusted distances depending upon the sign of the deviation from the linear "log plot"[4].

The above expression is more convenient than that previously reported[2] and is more precise in the presence of random scatter. When the further adjusted retention distances are replotted they are generally found to give a straight line, however occasionally a second correction may be necessary to linearise the "log plot" (for example in the unlikely event of the calculated static dead volume being seriously in error).

(2) Alternatively the "log plot" may be linearised by graphical trial and error.

(3) Finally, the method reported by GOLD[5], which uses the retentions of three compounds of the same homologous series, which according to the author need not necessarily be n-alkanes, may be used. Care must obviously be exercised when using this method since in the presence of random deviations serious errors will result. Whereas GOLD states that his method can be used with any 3 members of a homologous series we have found that it is necessary to take the retentions of compounds near the beginning, middle and end of the calibration series in order to get consistently reliable results, by comparison with the other linearisation methods. For instance, with the calibration series[2] of n-alkanes C_{14} to C_{26} we would recommend the use of C_{16}, C_{20} and C_{24}. Since the lower polar homologues deviate systematically from linearity[6] dead volumes calculated by GOLD's method using non-hydrocarbon homologous series will be far less reliable.

Though the three methods yield comparable results the first is to be preferred on grounds of higher precision.

Calculation of R_{N9} values

The retentions of n-alkanes are given by the expression:

$$\log R = a + bN \tag{1}$$

where R is the retention measured in any convenient units, N the carbon number, and a and b are constants. When the standard is n-nonane, as with the R_{x9} method, $\log R$ for n-nonane is zero by definition, so that:

$$\log R_{N9} = b(N - 9) \tag{2}$$

Thus knowledge of the slope of the "log plot" (the b factor) enables one to calculate all R_{N9} values by means of the simple equation (2) so obviating the necessity of using very large graph paper at high temperatures.

The procedure for calculating b, which is illustrated by determinations on neopentyl glycol succinate polyester stationary phase at $160°$ and $183°$, is as follows:

(1) The retentions of an even number of consecutive n-alkanes are listed in order of increasing retention; if the calibration series involved an odd number of n-alkanes the first member is neglected as the errors in measuring retention are generally greater with the shorter retention components.

(2) The list is divided into two and the n-alkanes designated $Rm_1, Rm_2 \ldots Rm_n$, $Rn_1, Rn_2 \ldots Rn_n$ in order of increasing retention.

(3) The logarithms of the adjusted or corrected retentions are then recorded (using 5 figure tables) and the slope b calculated from:

$$\frac{\log Rn_1 - \log Rm_1}{\dfrac{\mathscr{N}}{2}}, \quad \frac{\log Rn_2 - \log Rm_2}{\dfrac{\mathscr{N}}{2}}, \quad \ldots \text{etc.,}$$

where $\mathscr{N}$ is the even number of n-alkanes in the calibration series. The range of n-alkanes needed for precise results is given in Part II of this series[2].

A consideration of the propagation of random errors reveals that the ratio of the probable error in b using the "simple mean" (as derived above) and the least squares method is defined by:

$$\frac{Pb^{\wedge}}{Pb^{\square}} = \frac{2}{\sqrt{3}} \frac{\sqrt{(n - m)(n - m + 2)}}{(n - m + 1)}$$

where
$Pb^{\wedge}$ = the probable error in b by the simple mean method,
$Pb^{\square}$ = the probable error in b by the least squares method,
n = the carbon number of the last member of the calibration series,
m = the carbon number of the first member used in the calculation of b.

This ratio lies between 1.0 when $n - m$ is small and 1.15 when $n - m$ is large. Thus, the insignificant improvement in the accuracy of b by use of the least squares method instead of the "simple mean" does not justify the use of this tedious technique.

The "weighting" of observations is frequently somewhat arbitrary, thus in order to calculate the best value for b we propose to weight directly according to retention. The weighting for the shorter retentions should more rigorously be replaced by retention squared whilst the higher retentions should have unit weighting as experience has shown that the reproducibility of highly retained materials comes down to a fairly constant level. Similarly, in correcting for column dead volume the expression:

$$\delta a = \frac{2.303 \sum \delta \log R}{\sum 1/R_{\text{ext.}}}$$

TABLE I

R_{N_9} VALUES FOR n-ALKANES ON NEOPENTYL GLYCOL SUCCINATE POLYESTER AT 160°

Carbon number	Observed retention (cm)	Corrected retention (cm)	Logarithm of corrected retention	b	Rm	b × Rm	R_{N_9} b = 0.1704 ± 0.0001*	R_{N_9}** b = 0.1702 ± 0.0001*
12	1.92	1.60	0.20412				3.25	3.24
13	2.67	2.35	0.37107				4.80	4.79
14	3.82	3.50	0.54407				7.11	7.10
15	5.48	5.16	0.71265	0.16968	1.60	0.27150	10.53	10.51
16	7.93	7.61	0.88138	0.17042	2.35	0.40049	15.59	15.53
17	11.62	11.30	1.05308	0.17036	3.50	0.59626	23.08	22.99
18	17.00	16.68	1.22220	0.17016	5.16	0.87802	34.17	34.02
19	25.07	24.75	1.39358	0.17069	7.61	1.29895	50.58	50.35
20	37.15	36.83	1.56620	0.17053	11.30	1.92699	74.9	74.5
21	54.47	54.15	1.73360				110.9	110.3
22	80.77	80.45	1.90553				164.2	163.1
23	119.50	119.18	2.07622				243.0	241.4
					Σ = 31.52	Σ = 5.37221		

$$\bar{b} = \frac{5.3722}{31.52} = 0.17044 \pm 0.00009*$$

* ± refers to the probable standard deviation of $\bar{b}$ (i.e. P_b) calculated from the expression $P_b^2 = \dfrac{\sum W^2 \, \delta b^2}{[\sum W]^2}$ where W = weighting factor for each observation and δb = deviation of each observation from the weighted mean.

** Calibration carried out on the same column 4 h later. The difference between the two values of b thus determined is consistent with the calculated probable standard deviation of $\bar{b}$ in each case thus implying a satisfactory constancy of experimental conditions.

TABLE II

R_{N9} VALUES FOR n-ALKANES ON NEOPENTYL GLYCOL SUCCINATE POLYESTER AT 183°

Carbon number	Observed retention (cm)	Corrected retention (cm)	Logarithm of corrected retention	b	Rm	b × Rm	R_{N9} b = 0.1498 ± 0.0001	R_{N9}* b = 0.1495 ± 0.0001
14	2.15	1.85	—				5.61	5.59
15	2.90	2.60	0.41497				7.92	7.89
16	4.00	3.70	0.56820				11.19	11.13
17	5.50	5.20	0.71600				15.79	15.70
18	7.65	7.35	0.86629	0.14999	2.60	0.38998	22.29	22.16
19	10.62	10.32	1.01368	0.14946	3.70	0.55300	31.48	31.26
20	14.95	14.65	1.16584	0.14969	5.20	0.77840	44.44	44.11
21	20.95	20.65	1.31492	0.14977	7.35	1.10082	62.75	62.23
22	29.47	29.17	1.46494	0.15011	10.32	1.54914	88.6	87.8
23	41.43	41.13	1.61416	0.14977	14.65	2.19413	125.1	123.9
24	58.50	58.20	1.76492				176.6	174.8
25	82.40	82.10	1.91434				249.4	246.6
26	116.30	116.00	2.06446				352.1	347.9
					$\Sigma = 43.82$	$\Sigma = 6.56547$		

$$\bar{b} = \frac{6.56547}{43.82} = 0.14983 \pm 0.00008$$

* Calibration carried out 4 h later; again the difference between the two values of b is consistent with the calculated probable standard deviation of $\bar{b}$ in each case.

which implicitly weights as the reciprocal of retention has been chosen rather than the more rigorous:

$$\delta a = \frac{2.303 \sum \dfrac{\delta \log R}{R_{\text{ext.}}}}{\sum 1/R^2_{\text{ext.}}}$$

on the grounds of computational convenience.

The "weighted mean" of b is given by the expression:

$$\bar{b} = \frac{\sum b \dfrac{Rm \cdot Rn}{Rm + Rn}}{\sum \dfrac{Rm \cdot Rn}{Rm + Rn}} \tag{3}$$

where Rm and Rn are the retentions of the two n-alkanes used in each case. This simplifies to:

$$\bar{b} = \frac{\sum b \cdot Rm}{\sum Rm} \tag{4}$$

since the ratio of Rm/Rn is effectively constant.

(4) The "weighted" mean value of b obtained from eqn. (4) is then used to calculate R_{N9} values using eqn. (2). The results are shown in Tables I and II.

(5) R_{x9} values for any unknown can be calculated using the n-alkanes as standards from:

$$R_{x9} = R_{xN} \times R_{N9} = R_{xN} \times 10^{b(N-9)}$$

where

R_{x9} = retention of the material being measured relative to n-nonane,

R_{xN} = retention of material being measured relative to the internal standard,

R_{N9} = retention of the standard relative to n-nonane.

The accuracy of retention values obtained using this technique are clearly dependent upon the precision with which b can be measured. The data in Tables I and II indicate that when suitable calibration series of n-alkanes are used with sensibly constant operating conditions R_{x9} values can be obtained with a reproducibility of within 1 % even at these elevated temperatures.

The factors which influence the long term reproducibility of b are to be investigated shortly.

<div align="center">EXPERIMENTAL</div>

Chromatograms were obtained using an apparatus consisting of glass columns (5 ft. long and 4 mm i.d.) with a modified flame ionisation detector[7]. The carrier gas was a 3:1 (by volume) mixture of hydrogen and nitrogen. The column was packed with a 20% w/w mixture of neopentyl glycol succinate polyester (Applied Science Laboratories) and acid-washed (1 % phosphoric acid) 60–72 mesh celite. The column was heated by means of a vapour jacket; cyclohexanol (160°) and 2-ethylhexanol (183°). In order to minimise the acid catalysed dehydration of the alcohol used in the vapour jacket ~ 2 % of triethanolamine was added along with ~ 0.01 % of topanol O.C as antioxidant. Samples (~ 10 γ) were added as ~ 5 % w/v solutions in n-heptane by

means of stainless steel capillary pipettes. The n-alkanes were obtained commercially except for certain members between C_{20} and C_{26} which were kindly supplied by Mr. C. G. SCOTT of Lobitos Oilfields Ltd.

SUMMARY

A method of calculating R_{N9} values for the higher n-alkanes using the slope of the "log plot" for the n-alkanes is described.

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