USE OF VARIOUS HOMOLOGOUS SERIES IN DEAD-TIME CALCULATIONS

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(Received June 7th, 1978)

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SUMMARY

Dead-times have been calculated by an iterative procedure using data for four homologous series of different functional classes each determined on four stationary phases of varying polarity. No statistical difference was found between the mean dead-times calculated from the four homologous series on each stationary phase. The series included the *n*-alkanes which are the only compounds that have been previously considered for this determination.

The dead-time calculated from *n*-fatty acid methyl esters was computed and its usefulness in the computer processing of data obtained from an automated gas chromatograph described.

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INTRODUCTION

The introduction of automated gas chromatography (GC) equipment and the use of computer systems has simplified the processing and identification of GC data. Difficulties in admitting and measuring air and other gases into these and other systems have necessitated the development of alternate procedures capable of estimating the dead-time of a column in order to present the data accurately.

Simple procedures for estimating dead-time have been developed[1–4], however when computers are used, statistical procedures are easily calculated. Several procedures have been described using regression analysis. Grobler and Balizs[5] estimated dead-time, the slope and intercept of the regression line of the adjusted retention data of adjacent *n*-alkanes by the use of two linear regression calculations. Two iterative procedures have also been used by Guardino *et al.*[6] and Haken *et al.*[7] to minimise the sums of squares of the difference between the calculated and found Kováts index values of *n*-alkanes. In practice Haken *et al.*[7] and Smith *et al.*[8] have found very little difference between the accuracy of these procedures in calculating the mathematical dead-time.

The three statistical procedures reported[5-7] all attempt to straighten the retention data of a homologous series of n-alkanes. This is convenient if the retention data is to be reported as Kováts indices or relative to a particular n-alkane[9]. However, if the data is to be reported in terms of other schemes such as equivalent chain length (ECL)[10] in the case of fatty acids, or alcohols indices for alcohols[11], it is essential to run additional standards.

In this work we have determined the mathematical dead-time using several classes of homologous series on a variety of liquid phases of differing polarity.

MATERIALS AND METHODS

Gas chromatography

Data were obtained from a Hewlett-Packard 5700A gas chromatograph, fitted with dual flame-ionisation detectors. Nitrogen (30 ml/min) was used as the carrier gas, while hydrogen (30 ml/min) and air (200 ml/min) were supplied to the detector. All data were determined isothermally at 190°. Four stationary phases were used viz. in increasing order of polarity, 10% $C_{87}H_{176}$ (ref. 12), 10% SE-30, 3% OV-17 and 17% DEGS all coated on Chromosorb W AW DMCS (80–100 mesh) and packed into 2 m × 3 mm O.D. stainless-steel columns.

The gas chromatograph was connected to a Hewlett-Packard 3370B integrator with facilities for paper tape output on a teletype (STC, ASR-33). The chromatograph was also fitted with a Hewlett-Packard 7671A auto-sampler.

Four equimolar saturated homologous series of straight chain compounds were used to estimate dead-times: (a) methyl n-ketones (C11–C15); (b) n-fatty acid methyl esters (C10–20); (c) n-alcohols (C10–C20) and (d) n-alkanes (C12–C24), where carbon numbers, in brackets, do not include the methyl carbon adjacent to the functional group.

The ketone series were successive homologues, the other series were increased by increments of two carbon atoms i.e. $n + 2$. Series (b) to (d) were obtained from Applied Science Labs. (State College, Pa., U.S.A.) while series (a) was obtained from C.S.I.R.O. Division of Food Research (North Ryde, Australia).

Preparation of fatty acid methyl esters from wool wax

Wool from Merino sheep was extracted with petroleum spirit (b.p. 40–60°) using a Soxhlet apparatus. Small portions of wax were saponified with alcoholic NaOH. Unsaponifiable components were removed with petroleum spirit, the solution was then acidified and fatty acids together with hydroxy fatty acids were extracted with petroleum spirit. Fatty acids were separated from hydroxy fatty acids by thin-layer chromatography on silica gel G using the solvent system, hexane–ether–acetic acid (70:30:1). The bands were visualised under UV light after spraying with 0.1% ethanolic 2,7-dichlorofluorescein and identified using standards (Applied Science Labs.) run on the same plate. Fatty acid bands were scraped from the plate and extracted with ether. Methyl esters were prepared by adding diazomethane solution.

Calculations

Data were processed by means of a PDP11/15 computer fitted with a high-speed paper reader and disc pack (RK05).

Dead-times were calculated by the procedure of Guardino et al.[6], although as there were only minor differences between the three statistical procedures, the Simplex[7] or the Grobler et al.[5] procedures with modifications could have been se-

lected. While the iterative procedures require longer computing times[7] they did not require successive homologues as did the Grobler procedure, a disadvantage in the case of fatty acid analysis. ECL values were computed from the modified regression equation first obtained by the Guardino procedure from a homologues series of *n*-fatty acid methyl esters. In order to allow for the normal variations that occur with nett retention times with time (as up to 36 samples could be automatically processed) all experimental data were converted to retentions relative to methyl palmitate (C16). The nett retention time of methyl palmitate was constantly upgraded in each sample as it was either naturally occurring, or if not, added as an internal standard.

RESULTS AND DISCUSSION

Table I shows the mathematical dead-times, the means and standard errors calculated for four classes of homologous series on four stationary phases of varying polarity. From this table it is apparent that there are minor variations in the replicate analysis, but overall the differences in mathematical dead-time do not vary significantly from that calculated by the usual *n*-alkane system. The variations between replicate analysis can most probably be explained by the sampling technique applied. Haken *et al.*[7] has shown that small perturbances in retention times can cause large fluctuations in dead-times. This has been confirmed in this study where a 1/100-min difference in the retention time of only the C13 ketone on SE-30 (replicates 3 and 4) resulted in a 1.14-sec difference between the dead-time calculations. Consequently as our equipment was limited to the measurement of retention times to the nearest 1/100 min this could account for the variations observed, especially the higher standard errors obtained with the more rapidly eluted ketone series.

The values in Table I were obtained from either 4 or 5 components whose gross retention times were under 10 min. This was an attempt to improve the accuracy of the estimation as the error in measuring larger retentions would be expected to increase due to normal fluctuations in operating conditions. The use of 6 or more homologues did not improve the accuracy of estimation as measured by the sums of squares of the difference between the calculated and found retention time.

In Table II a summary of the results is shown obtained after the dead-time data was analysed by the technique of analysis of variance. From the variance ratios shown in this table it was apparent that there was no significant difference at the 1 % level between the dead-times calculated from data of the four different classes of homologous series for each stationary phase as determined by an F test. Naturally enough there were differences between dead-times calculated on different stationary phases.

The nature of the homologous series and stationary phases varied considerably from non-polar alkanes to more polar aliphatic alcohols and non polar $C_{37}H_{176}$ (ref. 12) to highly polar DEGS. It would appear that most homologous series of compounds could be used to calculate dead-times by this or similar procedures. However, care would have to be exercised in the case of lower homologues where the polar interactions that occur between the first few members of the homologuos

TABLE I

DEAD-TIMES (SEC) CALCULATED FOR FOUR HOMOLOGOUS SERIES ON FOUR STATIONARY PHASES

Stationary phase	Replication	Homologous series			
		Methyl ketone	Methyl ester	Alcohol	Hydrocarbon
$C_{87}H_{176}$	1	24.60	23.82	23.40	23.16
	2	23.52	23.34	22.86	23.16
	3	23.64	23.46	22.68	23.34
	4	21.60	22.50	23.22	22.80
	5	23.28	23.04	23.22	23.04
	6	24.24	22.26	23.64	22.44
	mean ± S.E.	23.48 ± 0.43	23.07 ± 0.24	23.17 ± 0.14	22.99 ± 0.13
SE-30	1	21.78	22.44	22.44	22.44
	2	21.48	22.56	23.52	21.72
	3	21.48	22.50	23.10	22.86
	4	22.62	22.80	22.68	22.86
	5	22.62	22.50	22.68	21.78
	6	22.86	22.50	22.68	22.86
	mean ± S.E.	22.14 ± 0.26	22.55 ± 0.05	22.85 ± 0.16	22.42 ± 0.22
OV-17	1	19.44	18.30	18.30	17.52
	2	15.96	18.90	18.30	18.06
	3	19.44	18.30	18.96	18.00
	4	15.96	18.90	18.96	18.48
	5	19.44	18.12	19.02	18.42
	6	15.96	17.88	19.38	17.22
	mean ± S.E.	17.70 ± 0.78	18.50 ± 0.19	18.82 ± 0.18	17.95 ± 0.20
DEGS	1	19.20	21.60	22.08	22.20
	2	24.18	21.18	22.80	22.20
	3	21.48	22.98	22.02	22.80
	4	24.36	23.04	22.02	22.44
	5	24.36	22.44	21.78	23.04
	6	22.08	22.32	21.84	22.86
	mean ± S.E.	22.61 ± 0.85	22.26 ± 0.30	22.08 ± 0.15	22.59 ± 0.15

TABLE II

ANALYSIS OF VARIANCE OF DEAD-TIMES

N.S. = Not significant.

Source of variation	Degrees of freedom	Sums of squares	Mean squares	Variance ratio	Significance level
Stationary phases	3	364.0051	121.3350	162.065	0.01
Homologous × series	3	0.9751	0.3250	0.434	N.S.
Phases × series	9	7.3039	0.8115	1.084	N.S.
Residual	80	59.8944	0.7487		
Total	95	432.1786	4.5492		
Grand total	95	432.1786			
Grand mean		21.574			
Total number of observations		96			

series and the more polar electron acceptor type stationary phases can cause significant deviation from linearity[13]. This deviation would result in a false estimate of dead-time; deviations were not apparent with the length of homologous series selected from this work.

The use of computers to identify accurately and calculate the composition of large numbers of complex mixtures, greatly simplifies the procedure. If the retentions are reported in terms of a related retention index system, an indication of the structure of the components is possible. The accuracy with which ECL values could be computed from experimental data obtained from wool wax fatty acid methyl esters on a DEGS column are shown in Table III. Here the dead-time was first estimated from a *n*-saturated fatty acid methyl ester series (C12–C22) as previously described. ECL values were then computed for 10 samples of wool wax methyl esters, the complexity of the chromatogram of which is shown in Fig. 1. The components were identified by comparison with the ECL of branched chain standard mixtures obtained from Applied Science Labs. In Table III very little deviation in the ECL values as shown by the magnitude of the standard errors was found using this procedure, as the mathematical dead-time was accurately known and the computer constantly updated the retention of the reference compound, in this case methyl palmitate. There were of course some variations in the proportion of some individual components as the 10 wool samples were obtained from 10 different sheep.

TABLE III
COMPOSITION OF FATTY ACIDS IN WOOL WAX
Data are means of separate wool wax samples from 10 sheep. a = Anteiso, i = iso, n = normal structures.

Fatty acid	ECL		Weight (%) *	
	Mean	S.E.	Mean	S.E.
a 11:0	10.671	0.008	0.29	0.09
i 12:0	11.438	0.006	0.47	0.15
a 13:0	12.688	0.003	1.92	0.58
i 14:0	13.463	0.002	3.18	0.68
n 14:0	13.973	0.003	2.03	0.72
a 15:0	14.699	0.002	9.42	0.77
i 16:0	15.472	0.002	8.48	0.76
n 16:0	16.000	—	1.86	0.48
a 17:0	16.713	0.002	9.56	0.64
i 18:0	17.481	0.001	9.18	1.63
n 18:0	18.011	0.001	1.03	0.27
a 19:0	18.713	0.002	10.52	0.78
i 20:0	19.482	0.001	6.36	0.87
n 20:0	20.011	0.002	2.74	1.76
a 21:0	20.713	0.002	8.42	1.02
i 22:0	21.467	0.003	3.16	0.29
n 22:0	21.993	0.002	0.38	0.13
a 23.0	22.693	0.002	3.12	0.41
i 24:0	23.439	0.002	2.66	0.48
n 24:0	23.961	0.001	1.66	0.43
a 25:0	24.652	0.002	4.81	0.84
i 26:0	25.390	0.003	3.04	0.79
n 26:0	25.911	0.002	0.93	0.30
a 27:0	26.593	0.002	3.60	0.83
i 28:0	27.326	0.002	0.76	0.31
n 28:0	27.849	0.006	0.91	0.07
a 29:0	28.525	0.003	0.54	0.19

* Assumes unity response by flame-ionization detection.
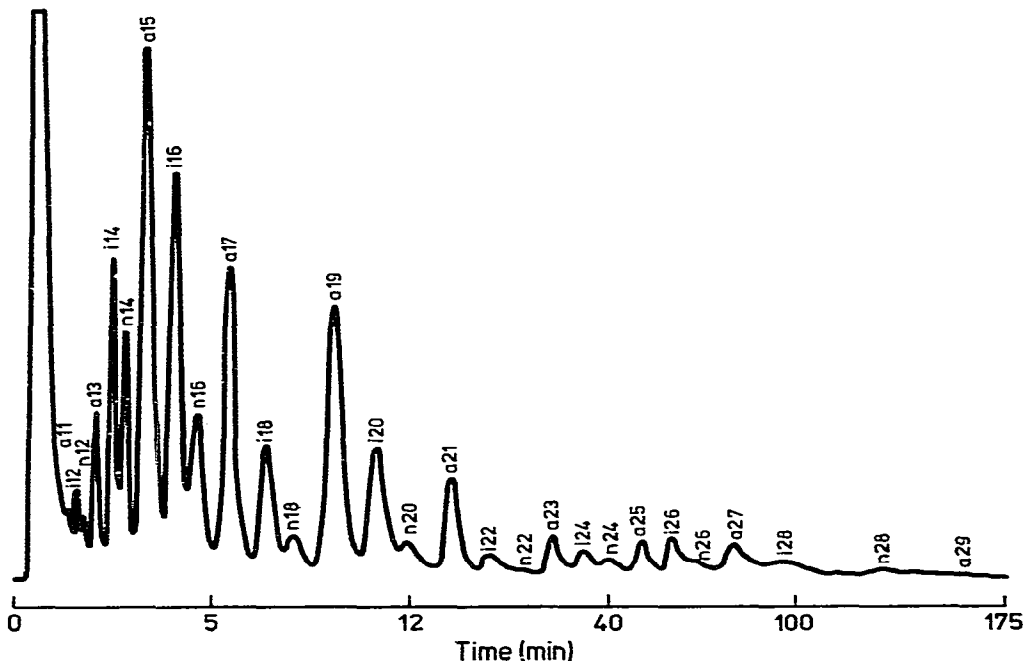
Fig. 1. Separation of wool wax fatty acid methyl esters achieved on a 2-m 17% DEGS column obtained isothermally at 190°.

CONCLUSIONS

Homologous series of compounds other than *n*-alkanes can be used to estimate the dead-volume of a GC column by an iterative procedure, provided the early homologues are of sufficient length to be free of polar interactions that occur with the stationary phase.

The value of dead-time in this manner is most useful in computer analysis of retention data obtained from automated instruments, as only a single standard needs to be run in order to identify and report the results in terms of an index system such as ECL.

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