



ELSEVIER

Journal of Chromatography A, 760 (1997) 219–226

JOURNAL OF
CHROMATOGRAPHY A

Hold-up time in gas chromatography I. New approach to its estimation

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Received 23 July 1996; revised 16 September 1996; accepted 17 September 1996

Abstract

A new procedure to reach a good estimate of the hold-up time value (t_M) in gas chromatography is presented. The value of t_M obtained lies close to the experimental retention time of neon, the gas which has shown the shortest retention time in the columns studied. The new method, based on the retention of *n*-alkanes, is easily applied with any personal computer and may be used with detectors which do not respond to permanent gases. The procedure is reliable, produces very reproducible hold-up times and the value of t_M obtained may be safely used to calculate both chromatographic and thermodynamic parameters.

Keywords: Hold-up times; Retention times; Alkanes; Permanent gases

1. Introduction

The accurate and precise determination of the retention time of an unretained solute in a chromatographic column (hold-up time, t_M) is fundamental in gas chromatography (GC), since it is related to important chromatographic (k , I , V_g , t'_R) and thermodynamic (δ , $\chi_{1,2}$, ΔH_m , etc.) parameters. For some time after the presentation of GC by James and Martin [1], the retention time of the air peak of a chromatogram obtained with thermal conductivity detection (TCD) was taken as the hold-up time. However, the widespread use of flame ionization detection (FID) forced chromatographers to look for other ways of estimating the t_M value. Thus, methane was proposed [2] as a marker of t_M , but it has been proved that it is retained in GC columns even at high temperatures [3–6]. The use of the retention time of

neon as an indicator of t_M has been recommended [3,6–9], but this implies the use of mass spectrometry (MS) or another universal detection method, not always available. Different methods to calculate the hold-up time were developed, most of them based on a linear relationship between the logarithm of the adjusted retention time (t'_R) and the carbon number of a series of *n*-alkanes [5,10–15]. The value of t_M thus obtained is often referred to as mathematical dead time (t_{MM}). However, all these methods give a value of t_M without real meaning [3,16,17], not related to the retention of a true unretained substance, being useful only for obtaining retention indices, which may be used for interlaboratory comparisons.

Any method proposed to estimate the hold-up time in a chromatogram, should fulfil at least two conditions: it must provide, with good precision, a value of t_M that really represents the retention time of a real non-retained gas, and also it must be useful for

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chromatograms obtained with detection methods that do not give a signal with permanent gases, as is the case with FID. With those two conditions in mind, a new approach to the problem of estimating the t_M is proposed. The method is based on the assumption that a mathematical relationship exists between the experimental retention times (t_R) of the n -alkanes and their carbon number (n). Under this assumption, the new procedure assumes that the hold-up time is the retention time of a hypothetical n -alkane with zero carbon atoms (obtained by extrapolation in the mathematical model). The t_M value thus obtained must be equal to, or even smaller than, the retention time of the least retained gas in the chromatographic column, considering that it must be free of any retention mechanism. The procedure to select a mathematical expression to estimate the hold-up time in chromatograms implies three steps: (a) the least retained substance under any chromatographic condition must be selected as a reference; (b) a mathematical expression must be chosen so that it offers a good fit of the experimental retention times of the substances used as standards; (c) the value of t_M deduced from the chosen mathematical expression must be close to the experimental retention time of the selected reference gas, but only slightly shorter, considering that any reference real gas will have some, but not much, retention.

2. Experimental

2.1. Apparatus

Most experiments were carried out on a Fisons 8035 gas chromatograph coupled to a Fisons MD 800 quadrupole mass filter. The retention times used in this paper were obtained on a stainless-steel packed column [column 1, laboratory-made, 19.4% (w/w) PS-255 (Petrarch) on Chromosorb W AW DMCS, 4 m \times 2.2 mm] and on a fused-silica capillary column [column 2, CPSIL-5CB (Chrompack), 50 m \times 0.32 mm, film thickness 0.43 μ m]. Both stationary phases are polydimethylsiloxane, the former with 1–3% of vinyl groups. The gas leaving the chromatographic columns was fed to the mass spectrometer through a direct line of fused-silica tubing of 0.1 mm I.D., used as a sort of open split interface. In this

way, column outlet pressure was maintained at ambient pressure in all cases (i.e., normal chromatographic conditions). A few experiments were carried out in a Hewlett-Packard 5890A gas chromatograph.

2.2. Chromatograms

Experiments were carried out isothermally at 30, 60, 90, 120, 150 and 180°C. Hydrogen and helium were used in most cases as carrier gases at the inlet pressures given in Table 1. A few additional experiments with nitrogen and argon as carrier gases were performed in the HP chromatograph. Solutes injected include n -alkanes from 1 to 15 carbon atoms and several permanent gases: hydrogen, helium, neon, nitrogen, oxygen and argon.

Chromatograms recorded with the mass filter were obtained by the selected ion recording mode (SIR), except for experiments carried out at 30°C in the capillary column where the full scan mode was used. Table 1 also shows the m/z values monitored and the cycle times in both chromatographic columns.

2.3. Mathematical treatment

Data were fitted by a regression procedure that obtains least squares estimates of the parameters in a nonlinear regression model, by minimizing the residual sum of squares. The algorithm used was developed by Marquardt [18]. All calculations were carried out using the Statgraphics program (Statistical Graphics) in an Epson computer equipped with a 486-DX processor at 33 MHz.

3. Results and discussion

3.1. Retention of gases

In order to check which is the gas with the smallest retention, to be used as an indicator of t_M , different gases were injected in both columns. Mean retention times of five runs are shown in Table 2. It may be observed that the gas showing the smallest retention time under any of the experimental conditions tried is neon. Therefore, neon was used as a reference to check the validity of the method proposed here.

Table 1
Experimental conditions

Column	Carrier gas	Inlet pressure (p.s.i.)	Temperature (°C)	Scan mode	Cycle time (s)	Substance	Monitored m/z	
1 (packed)	He	17	30	SIR	0.2	H ₂ , Ne, N ₂ , O ₂ , Ar C _n H _{2n+2} ($n=1-4$)	2, 20, 28, 32, 40 15, 30, 43	
			60	SIR	0.2	Ne, N ₂ , O ₂ , Ar C _n H _{2n+2} ($n=1-x^a$)	20, 28, 32, 40 15, 30, 43	
			90					
			120					
			150					
	H ₂	8	30	SIR	0.2	He, Ne, N ₂ , O ₂ , Ar C _n H _{2n+2} ($n=1-4$)	4, 20, 28, 32, 40 15, 30, 43	
			120	SIR	0.2	He, Ne, N ₂ , O ₂ , Ar C _n H _{2n+2} ($n=1-12$)	4, 20, 28, 32, 40 15, 30, 43	
2 (capillary)	He	11	30	Full scan	0.04	H ₂ , Ne, N ₂ , O ₂ , Ar C _n H _{2n+2} ($n=1-4$)	1–59	
			60	SIR	0.04	Ne C _n H _{2n+2} ($n=1-x$)	20 15, 30, 43	
			90					
			120					
	H ₂	6	30		0.04	He, Ne	4, 20	
			120	SIR	0.04	He, Ne C _n H _{2n+2}	4, 20 15, 30, 43	

^a $x=7$ to 15 depending on column temperature.

3.2. Selection of the mathematical model

The dependence of the retention time of a n -alkane on its carbon number n , may be expressed as:

$$t_R(n) = f(A, B, C, \dots, n) \quad (1)$$

where A, B, C, \dots are adjustable parameters that depend on the experimental conditions (column type, stationary phase, flow-rate, temperature, etc.)

In order to describe the dependence of t_R on n , 25 different equations, using from two to five parameters, were selected. One chromatogram from the packed column, and one from the capillary column were arbitrarily chosen, and their retention data (see Table 3) were adjusted to the different expressions by the nonlinear regression procedure, to find out the A, B, C, \dots parameters and the corresponding goodness of fit. For each type of column (packed and capillary) the root mean square (RMS) values served to select eight expressions which produced reasonable results. The t_M value deduced from each

expression was then calculated and the corresponding Δt_M value was obtained by deriving the expression for t_M [19]. The eight chosen expressions and the corresponding results are gathered in Table 4. Note that Eq. (8) is the basis of most of the methods used so far to estimate adjusted retention times in chromatograms. The equation is normally used as $\ln(t_R - A) = B + Cn$, and the value of A is taken as the hold-up time estimation. The final selection was based on the following considerations: the mathematical model corresponding to some of the equations produces Δt_M values which are unacceptable (low precision in the value deduced for t_M). Using this criterion, three equations were rejected based on results on the packed column (Eqs. (5–7)), and two other were rejected according to results obtained on the capillary column (Eqs. (8,9)). The remaining equations (Eqs. (2–4)) were then compared by examining the difference $t_R(\text{Ne}) - t_M$, and Eq. (4) rejected, due to the result of the packed column. The plot of expression (Eq. (4)) shows an

Table 2

Retention times (s) of several gases under different experimental conditions (each figure is the average of 5 values)

Column	Carrier gas	Substance	Temperature (°C)					
			30	60	90	120	150	180
1 (packed)	He	Ne	129.4	136.8	145.2	152.7	161.3	170.5
		N ₂	130.7	137.9	146.3	153.9	162.5	172.2
		O ₂	132.0	139.5	147.8	155.5	164.4	173.8
		Ar	132.3	139.6	148.2	156.2	164.8	174.1
		H ₂	132.7					
		Methane	136.1	142.8	151.1	158.6	166.6	176.4
		Ethane	171.6	167.9	170.3	173.9	180.1	187.8
		Propane	258.0	217.3	202.4	196.1	196.4	200.5
		<i>n</i> -Butane	521.6	345.0	274.4	240.9	224.8	218.7
		H ₂	Ne	129.5			156.5	
	N ₂		130.4			157.9		
	He		131.2			159.8		
	O ₂		132.2			159.4		
	Ar		132.5			160.1		
	Methane		136.7			162.9		
	Ethane		171.5			178.1		
	Propane		258.2			201.2		
	<i>n</i> -Butane		521.1			246.6		
	2 (capillary)		He	Ne	181.88	194.83	210.94	224.15
		N ₂		181.94				
O ₂		182.04						
Ar		182.04						
H ₂		182.06						
Methane		182.30		195.25	211.38	224.58	236.03	
Ethane		184.81		197.00	212.69	225.60	236.86	
Propane		190.98	200.53	215.03	227.40			
<i>n</i> -Butane		209.64	209.71	220.32	230.27	239.62		
H ₂		Ne	143.99			182.64		
		He	144.10			182.88		

excessive curvature for values below $n=1$, giving a very reproducible value of t_M but clearly out of range.

Therefore, Eqs. (2,3) have been selected as the only two equations (from a total of 25) that properly describe the dependence of the retention times of the normal alkanes on their carbon number and at the same time allow a good estimate of the hold-up time in the chromatogram.

Table 5 shows the results of fitting Eqs. (2,3) to the data shown in Table 3. The value of t_M in the table is the value deduced from Eqs. (2,3) for $n=0$, that is:

$$t_M = A + \exp B \quad (10)$$

As mentioned earlier, the values of t_M deduced by any procedure should lie close to the experimental value of the least retained gas (neon, according to values shown in Tables 2 and 3). In order to check this point, chromatograms run at all temperatures were randomly taken for both columns, and the hold-up time deduced with either equation compared with the experimental retention time of neon in the same chromatogram. Results are presented in Table 6, showing that all deduced values are lower than (and very close to) the experimental retention time of

Table 3
Retention times subjected to a nonlinear regression procedure

Substance	Retention time (s)	
	Packed column 150°C	Capillary column 90°C
Ne	161.7	210.96
N ₂	162.8	
O ₂	164.8	
Ar	164.8	
Methane	166.9	211.38
Ethane	180.5	212.64
Propane	196.8	214.98
<i>n</i> -Butane	225.4	220.32
<i>n</i> -Pentane	277.3	230.94
<i>n</i> -Hexane	358.9	252.18
<i>n</i> -Heptane	493.7	294.72
<i>n</i> -Octane	714.1	379.32
<i>n</i> -Nonane	1076.6	547.38
<i>n</i> -Decane	1668.7	880.02
<i>n</i> -Undecane	2630.8	1536.24
<i>n</i> -Dodecane		2828.46

the gas. Differences are smaller for the capillary column, very likely due to the higher importance of the residual adsorption of neon in the case of the packed column. Values deduced from either equation are equivalent. In both instances differences decrease with increasing temperature, as expected. Therefore, we may deduce that the estimation of t_M using either Eqs. (2,3) for $n=0$ is a reasonable procedure of obtaining a reliable value of the hold-up time in chromatograms.

Reproducibility of results was checked by inject-

ing five successive samples at all temperatures. An example is shown in Table 7. Results show that the value of Δt_M remains similar for the different injections, and lies close to the experimental error (the cycle time of the mass spectrometer). On the other hand, the value of $t_R(\text{Ne}) - t_M$ is very small and roughly constant. The reliability of the procedure seems, thus, confirmed.

3.3. Generalization of the method to other GC detectors and conditions

The most popular detection method in GC (analytical and inverse) is FID. On the other hand, capillary columns or high temperatures in packed columns produce chromatograms with a poor or nonexistent separation between methane and *n*-alkanes of up to, say, four carbon atoms. Besides this, the injection of one gas (methane) at the same time as the liquid sample is simple and may be carried out by filling the syringe with the gas after the sample has been taken. The use of all other gaseous *n*-alkanes in the same injection might complicate the preparation of the mixture to be introduced in the syringe, although such mixtures are commercially available.

In order to test the validity of the method for cases where the light hydrocarbons are absent, 50 injections of samples including the light *n*-alkanes were carried out at different temperatures on both columns. Values of the hold-up time (t_M) were calculated in all chromatograms twice: considering

Table 4
Equations selected (out of 25) from results of a nonlinear regression procedure (Section 3.2)

Eq.	Mathematical expression		Packed column			Capillary column		
			RMS	Δt_M	$t_R(\text{Ne}) - t_M$	RMS	Δt_M	$t_R(\text{Ne}) - t_M$
2	$t_R = A + \exp(B + Cn + Dn^2)$	(2)	0.8343	1.430	0.393	0.1064	0.067	0.418
3	$t_R = A + \exp(B + Cn^D)$	(3)	0.8443	1.748	0.420	0.0688	0.051	0.313
4	$t_R = A + n^B \exp(C + Dn)$	(4)	0.8235	1.749	5.237	0.0588	0.046	0.510
5	$t_R = A + \exp(B + Cn + Dn^2 + En^3)$	(5)	0.8225	5.450	0.771	0.0689	0.098	0.315
6	$t_R = A + \exp(B + Cn + Dn^E)$	(6)	0.8440	10.42	0.374	0.1094	0.579	0.413
7	$t_R = A + \exp\left(B + Cn + \frac{D}{n+E}\right)$	(7)	0.8190	15.36	0.743	0.7542	1.861	0.980
8	$t_R = A + \exp(B + Cn)$	(8)	1.4520	1.041	2.540	0.8739	0.418	1.421
9	$t_R = A + Bn^{(C-Dn)}$	(9)	1.0068	1.185	-1.971	0.3026	0.210	-0.615

Table 5
Results of the fitting of Eqs. (2,3) to the data of Table 3

Column	Parameter ^a	Equation	
		2	3
1 (packed)	RMS	0.8443	0.8443
	r^2	0.999999	0.999999
	$A \pm \Delta A$	151.72 ± 1.08	153.08 ± 1.23
	$B \pm \Delta B$	2.264 ± 0.036	2.109 ± 0.063
	$C \pm \Delta C$	0.5191 ± 0.0071	0.590 ± 0.020
	$D \pm \Delta D$	-0.00131 ± 0.00037	0.09461 ± 0.0096
	$t_M \pm \Delta t_M$	161.3 ± 1.4	161.3 ± 1.7
2 (capillary)	RMS	0.1064	0.0688
	r^2	1	1
	$A \pm \Delta A$	209.925 ± 0.065	210.133 ± 0.047
	$B \pm \Delta B$	-0.4826 ± 0.0040	-0.6649 ± 0.0073
	$C \pm \Delta C$	0.71348 ± 0.00048	0.7964 ± 0.0021
	$D \pm \Delta D$	-0.0014510 ± 0.0000066	0.95451 ± 0.00068
	$t_M \pm \Delta t_M$	210.54 ± 0.07	210.65 ± 0.05

^a $RMS = \text{SQR}[\sum(t_{R \text{ exp}} - t_{R \text{ calc}})^2/n_i]$ where $t_{R \text{ exp}}$ is the experimental value, $t_{R \text{ calc}}$ is the calculated value by the nonlinear procedure and n_i is the total number of *n*-alkanes in the fitting; r^2 = square of the correlation coefficient; t_M and Δt_M in s.

all *n*-alkanes and ignoring the presence of the light hydrocarbons. A typical example corresponding to the capillary column is shown in Table 8. It may be observed that the absence of ethane, propane and *n*-butane does not alter the value of t_M substantially at any temperature, provided that methane is always included in the injection. The maximum difference between t_M values deduced from the same chromato-

graphic run, when the light hydrocarbons are, or are not included, represents a 0.03% of the value of t_M [leading to a change of about 0.2% in the value of the retention factor (*k*) for a chromatographic peak with *k* equal to 0.2]. Peaks with higher values of *k* would not be affected.

The number of *n*-alkanes necessary to obtain a reliable value for t_M might depend on the chromato-

Table 6
Hold-up times obtained by Eqs. (2,3) and retention time of neon

Column	Carrier gas	Temperature (°C)	t_M (s) ^a		
			Ne	Eq. 2	Eq. 3
1 (packed)	He	60	136.3	133.3	133.5
		90	145.1	142.9	142.9
		120	153.2	152.0	151.9
		150	161.7	161.3	161.3
		180	170.2	169.9	169.8
	H ₂	120	157.0	156.5	156.5
2 (capillary)	He	60	195.24	194.75	194.83
		90	210.96	210.54	210.65
		120	224.28	224.13	224.67
		150	235.92	235.76	235.86
		H ₂	120	183.12	182.88

^a Values shown correspond to one chromatogram at each temperature.

Table 7
Reliability of the method for column 2 at 90°C with He as carrier gas (retention times in s)

Run	$t_R(\text{Ne})$	Equation 2		Equation 3	
		$t_M \pm \Delta t_M$	$t_R(\text{Ne}) - t_M$	$t_M \pm \Delta t_M$	$t_R(\text{Ne}) - t_M$
1	211.02	210.71 ± 0.08	0.31	210.80 ± 0.06	0.22
2	210.90	210.62 ± 0.04	0.28	210.66 ± 0.06	0.24
3	210.96	210.54 ± 0.07	0.42	210.65 ± 0.05	0.31
4	211.26	210.92 ± 0.06	0.34	211.03 ± 0.05	0.23
5	210.54	210.14 ± 0.07	0.40	210.22 ± 0.06	0.32

Table 8
Effect of the elimination of ethane, propane and *n*-butane from the sample, on the value of t_M (capillary column)

Carrier gas	Temperature (°C)	Equation	$t_M \pm \Delta t_M$ (s)	
			$C_1 - C_{12}$	$\text{CH}_4 + C_5 - C_{12}$
He	60	2	194.75 ± 0.12	194.80 ± 0.13
		3	194.83 ± 0.10	194.90 ± 0.08
	90	2	210.54 ± 0.07	210.58 ± 0.12
		3	210.65 ± 0.05	210.69 ± 0.06
	120	2	224.13 ± 0.06	224.18 ± 0.09
		3	224.17 ± 0.08	224.25 ± 0.14
H_2	120	2	182.88 ± 0.06	182.96 ± 0.04
		3	182.88 ± 0.07	182.92 ± 0.06

graphic conditions. Methane should always be present, and the lightest liquid *n*-alkane included should show a well-resolved peak. The last *n*-alkane eluted should not present any peak distortion (overloading, skewing, etc.). Table 9 shows results that prove that as few as four *n*-alkanes, plus methane, are sufficient to obtain a good estimate of t_M . The maximum

Table 9
Effect of the number of *n*-alkanes on the t_M value (Column 2, 90°C)

Substance	$t_M \pm \Delta t_M$ (s)	
	Equation 2	Equation 3
$\text{CH}_4 + C_5 - C_{12}$	210.58 ± 0.12	210.69 ± 0.06
$\text{CH}_4 + C_6 - C_{12}$	210.59 ± 0.12	210.71 ± 0.07
$\text{CH}_4 + C_7 - C_{12}$	210.66 ± 0.13	210.73 ± 0.07
$\text{CH}_4 + C_8 - C_{12}$	210.70 ± 0.15	210.74 ± 0.09
$\text{CH}_4 + C_9 - C_{12}$	210.72 ± 0.14	210.75 ± 0.10
$\text{CH}_4 + C_5 - C_{12}$	210.58 ± 0.12	210.69 ± 0.06
$\text{CH}_4 + C_5 - C_{11}$	210.69 ± 0.06	210.84 ± 0.08
$\text{CH}_4 + C_5 - C_{10}$	210.72 ± 0.07	210.76 ± 0.05
$\text{CH}_4 + C_5 - C_9$	210.75 ± 0.07	210.75 ± 0.06
$\text{CH}_4 + C_5 - C_8$	210.78 ± 0.01	210.77 ± 0.03

deviation between values of t_M in the table is only a 0.09%, representing 0.5 to 0.6% error for values of k as low as 0.2, and smaller errors for higher values of the retention factor. It may be concluded that the number or the chain length of the *n*-alkanes injected is not critical, provided that methane and other four hydrocarbons are included and the other two conditions are fulfilled.

Chromatograms run on the same apparatus with the same column at the same temperature using hydrogen, helium, nitrogen and argon as carrier gases show values of the retention factors (as deduced according to the procedure here proposed) for the *n*-alkanes that are independent of the carrier gas.

4. Conclusions

For the first time, a procedure of estimating the hold-up time in a chromatogram is based on the assumption that a hypothetical *n*-alkane of zero carbon atoms is not retained by the stationary phase. The procedure here proposed may best be applied

using the two equations selected in this paper. In this way, the method proposed fulfils the two basic conditions that any mathematical method of estimating the hold-up time should have: the value of t_M obtained represents the retention of a gas which is not subject to any retention mechanism by the stationary phase of the column and may be used with detectors that do not produce a signal with permanent gases. The first of these conditions is not fulfilled by the well established methods available. The new procedure is reliable; the value deduced is very reproducible, and is very simple to use. Results show that the method may be applied with any normal carrier gas (hydrogen, helium or nitrogen), produces sound results at any temperature, and may be used both with packed and capillary columns.

Acknowledgments

This work has been carried out with the financial assistance of the Dirección General de Investigación Científica y Técnica of Spain (DGICYT), under Projects PB91-0077 and PB94-0218.

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