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Journal of Chromatography A, 803 (1998) 197–202

JOURNAL OF
CHROMATOGRAPHY A

The hold-up time in gas chromatography¹

III. Linearity of the plot of $\ln t'_R$ of the n -alkanes vs. carbon number questioned

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Received 16 October 1997; received in revised form 12 December 1997; accepted 19 December 1997

Abstract

Three arguments are presented to show that the plot of the logarithms of the adjusted retention times of n -alkanes eluted under isothermal conditions versus their chain length is not a straight line, as generally accepted, even for n -alkanes of seventeen carbon atoms. Comparison of values of t'_R deduced by methods based on the linearity assumption ($\ln t'_R = mz + n$) and on a different approach [$t_R = A + \exp(B + Cz^D)$] shows that experimental retention times do not fit the first expression, independently of the value used for the hold-up time, even if the correlation coefficient of the fit is better than 0.99999. © 1998 Elsevier Science B.V.

Keywords: Hold-up times; Retention times; Alkanes

1. Introduction

The specific retention volume (V_g) of a solute is the fundamental retention parameter in gas chromatography (GC). This parameter depends only on temperature and the particular solute–stationary phase pair, being independent of the apparatus, type of column or other chromatographic conditions such as flow-rate, etc. In everyday practice, however, adjusted retention volumes (V'_R) or, in the case of chromatograms run under constant flow conditions, adjusted retention times (t'_R) are used. In order to

obtain the value of t'_R , the exact point of the chromatogram where an infinitesimally small amount of a nonretained gas would appear, must be known. This point would give notice of what is termed the 'gas hold-up time' of the chromatogram (t_M), the time used by the mobile phase to pass through the column. For some time, the injection of air or other permanent gases has been taken as a good way of finding this point of the chromatogram. The widespread use of flame ionization detection (FID), and the further evidence of the retention of methane under most chromatographic conditions [1,2], forced chromatographers to find indirect ways of deducing the hold-up time, mostly by mathematical procedures based on the idea of the linearity of the semilog plot of the specific retention volumes of the n -alkanes versus carbon number (hereafter the 'semilog plot'). The value thus produced has often been referred to

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¹ Paper presented at the 26th Meeting of the Group of Chromatography and Related Techniques of the Spanish Royal Society of Chemistry, Almería, October 1997.

as ‘mathematical dead time’ (t_{MM}). Bearing in mind the recommendations of IUPAC [3], the name should be changed to ‘mathematical hold-up time’.

It has long been accepted that the addition of a methylene group to the chain of a n -alkane produces an increment in its general properties that is roughly independent of the actual number of carbon atoms of the chain. The principle, often referred to as the additivity principle, is generally accepted for homologues of say, a minimum of seven or eight carbon atoms. This principle has been used in other branches of chemistry to predict values of properties of molecules when they are not available [4]. In GC, this increment is reflected in the observed linearity of the semilog plot of the n -alkanes mentioned earlier. This is also observed for any other chromatographic parameter related to V_g , such as adjusted or net retention volumes or times. This fact has led, among other things, to the establishment of the Kováts’ retention index system [5]. The minimum number of carbon atoms of the lightest n -alkane which may be included in the linearity plot seems to depend on the conditions of the chromatogram. Although five carbon atoms have been mentioned to be the accepted minimum, results of experiments have been published [6,7] in which a straight line may be drawn using retention data of n -alkanes from three carbon atoms (propane).

The need for a reliable method to deduce the point of the chromatogram where the ‘ideal’ nonretained gas would appear has become important since the publication of the report of the retention of ‘permanent’ gases such as nitrogen, air or neon [2]. Methods used to calculate the hold-up time have been reviewed [8,9]. Some of the methods use three homologues eluted in the same chromatogram. In order to reduce the uncertainty derived from the variations of the experimental retention times, a higher number of homologues has been recommended. The two methods based on this principle with a higher acceptance are perhaps those of Guardino et al. [10] and García-Domínguez et al. [11]. Both methods correct an initial hold-up time estimation until the adjusted retention times (or volumes) produce a sufficiently good straight line adjusted by a least squares method. The value of t_{MM} deduced by the two methods is practically the same [2].

All procedures proposed so far to deduce the value of the hold-up time assume a correct determination of the origin of the retention times of the chromatogram. However, what they really give is the point of the chromatogram where the nonretained gas should appear: the point of the chromatogram from which the adjusted retention times must be measured. In other words: the value is claimed to represent the hold-up time, but it does so, only if no extra-column volumes exist, or if an error has not been produced in the computing of the origin of retention times. In order to avoid confusion, the retention of an unretained substance, deduced by a mathematical procedure, will be referred to as $t_{MM(X)}$ in this paper, where X refers to the method used to deduce it (see below).

The idea of a straight line defined as mentioned above has recently been questioned [1,2], as it was found that the retention times of the n -alkanes in an isothermal chromatogram are better defined by Eq. (1) below, than by Eq. (2):

$$t_{R,z} = A + \exp(B + Cz^D) \quad (1)$$

$$t_{R,z} = A + \exp(B + Cz) \quad (2)$$

In these expressions, $t_{R,z}$ is the experimental retention time of the n -alkane of z carbon atoms, and A , B , C and D , the parameters corresponding to the best fit of the expression. Eq. (2) is the base for the accepted linearity of the semilog plot mentioned.

In this paper, we will show that the straight line of the semilog plot based on Eq. (2) is actually a curve. The subtle difference between the curve and the straight line has so far passed unnoticed because the small differences between theoretical and experimental retention indices of the n -alkanes have been attributed to normal experimental errors. Correlation coefficients of the order of 0.99999 and better are often found for the fit (a value of 0.999999993 for C_3 – C_7 n -alkanes has been reported [6]), and this has contributed to the general acceptance of the linearity of the plot. The use of capillary columns and modern ways of computing retention times, produce data that clearly show that the semilog plot is really a curve. Experimental results will also be presented to show that no value of t_{MM} selected in any way will give a straight line for the aforementioned semilog plot.

2. Experimental

2.1. Apparatus

Some of the experiments were carried out on a Fisons 8035 gas chromatograph coupled to a Fisons MD 800 quadrupole mass filter. Other experiments were carried out in a Hewlett-Packard HP 5890A Series II gas chromatograph, with nitrogen as the carrier gas. Retention times were computed with a precision of 0.01 min, or 0.1 s. Further details may be found in Parts I and II of this series [1,2].

2.2. Chromatograms

Isothermal chromatograms of *n*-alkane mixtures on packed and capillary columns with polar and

apolar stationary phases were obtained at various temperatures and head pressures. Once the conditions of the experiment were established (temperature, column head pressure, etc.), sufficient time was allowed to elapse, in order to reach equilibrium [12]. Although the facts described here are general, only three chromatograms have been used to produce the plots of the figures of this paper, using the same set of experimental retention times for different representations. The chromatograms selected correspond to a commercial Hewlett-Packard HP-5 capillary column of 60 m × 0.25 mm I.D. and a film thickness of 0.25 μm, because in them appear *n*-alkanes from 7 to 17 carbon atoms, while in other examples the range of chain lengths included in the same chromatogram is smaller. Data used have been taken from one single chromatogram in each one of the examples given. Therefore, they have the natural dispersion of any experimental measure. The values used are presented in Table 1.

Table 1
Experimental retention times (min) and parameters of the fitting of Eqs. (1) and (2) used in the elaboration of Figs. 1–5

Substance	150°C	160°C	200°C
Methane	14.58	14.97	15.92
<i>n</i> -Heptane	15.49	15.74	16.37
<i>n</i> -Octane	16.10	16.23	16.60
<i>n</i> -Nonane	17.10	17.02	16.94
<i>n</i> -Decane	18.73	18.28	17.43
<i>n</i> -Undecane	21.39	20.27	18.15
<i>n</i> -Dodecane	25.72	23.43	19.20
<i>n</i> -Tridecane	32.73	28.43	20.71
<i>n</i> -Tetradecane	44.12	36.32	22.91
<i>n</i> -Pentadecane	62.45	48.74	26.08
<i>n</i> -Hexadecane		68.20	30.65
<i>n</i> -Heptadecane		98.81	37.25

Parameters

Eq. (1)

<i>A</i>	14.548	14.930	15.880
<i>B</i>	−3.8360	−3.8224	−3.6596
<i>C</i>	0.6071	0.5876	0.4799
<i>D</i>	0.93832	0.93258	0.93162
RMS ^a	0.0080	0.0083	0.0025

Eq. (2)

<i>A</i>	14.512	14.886	15.855
<i>B</i>	−3.4315	−3.3750	−3.2777
<i>C</i>	0.4871	0.4596	0.3734
<i>r</i> ^a	0.999998	0.999995	0.999996

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

3. Results and discussion

In the discussion which follows, the idea of a straight line defined by the ‘semilog plot’ of the *n*-alkanes eluted in the same isothermal chromatogram will be examined, by calculating ‘adjusted’ from experimental retention times. Three methods will be used to calculate the point of the chromatogram where the ideal nonretained gas should appear:

3.1. Method A: the three homologues method

This method is based on Eq. (2), written in a different way:

$$\ln(t_{R,z} - A) = B + Cz \quad (3)$$

For three homologues of carbon numbers *z*, *z* + *n* and *z* + 2*n*, we may write [6,13]:

$$A = t_{R,z+n} - \frac{[t_{R,z+n} - t_{R,z}][t_{R,z+2n} - t_{R,z+n}]}{t_{R,z+2n} - 2t_{R,z+n} + t_{R,z}} \quad (4)$$

The value of *A* will be mentioned in this paper as $t_{MM(A)}$.

3.2. Method B: the linear iterative method

The method of García-Domínguez et al. [11] is being used here. It is also based on Eq. (2) but uses a larger number of *n*-alkanes. The mathematical hold-up time deduced by this method (parameter A of the equation) will be referred to as $t_{MM(B)}$.

3.3. Method C: the LQG method

This method [1,2] is based on Eq. (1), and does not presuppose the linearity of the semilog plot repeatedly mentioned. For the sake of clarity, the value of t_{MM} found by this method will be mentioned here as $t_{MM(C)}$.

3.4. First evidence of the nonlinearity of the semilog plot

Accepting the validity of Eq. (2), Method A will produce different values of $t_{MM(A)}$, depending on the actual group of *n*-alkanes selected, and their particular experimental retention time errors. Applying the method to all possible retention times of the same chromatogram (three at a time), all values of $t_{MM(A)}$ will fall round a mean value, that should be the 'correct' hold-up time according to the method. However, if the semilog plot describes a curve, then the $t_{MM(A)}$ values will change gradually as the chain length of the *n*-alkanes increases, allowing of course for the natural experimental variation of t_R , and keeping *n* constant in Eq. (4).

The same type of test may be used with Method B, employing a short number of *n*-alkanes for the fit (say four), but successively increasing by one the carbon number of the lightest hydrocarbon used.

Many chromatograms obtained along the years on packed and capillary columns in our laboratories show results similar to those described in Fig. 1 for three chromatograms from the same column, at three temperatures and the same carrier gas head pressure. For Method A, a value of $n=2$ (Eq. (4)) has been used. For each value of *z*, Methods B and C use the same set of four *n*-alkanes (*z* to *z*+3 carbon atoms), including methane in Method C. The horizontal dotted lines have been drawn to enhance differences.

The plots clearly show that the values from methods based on Eq. (2) change gradually with the

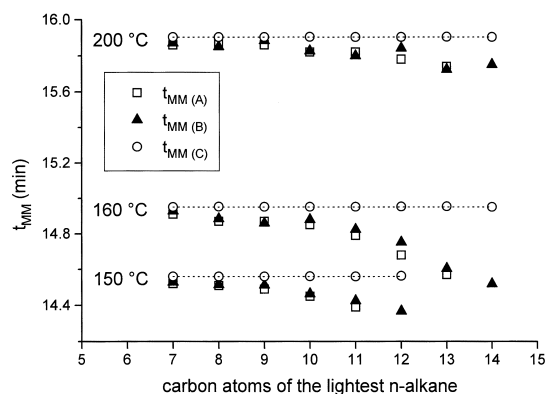


Fig. 1. Values of the retention time of an unretained substance, obtained by Method A (Eq. (2), $n=2$); Method B (*n*-alkanes *z* to *z*+3); and Method C (*n*-alkanes *z* to *z*+3, plus methane). The horizontal dotted lines have been drawn for comparison purposes. Column: HP-5, 60 m×0.25 mm I.D., 0.25 μm film thickness.

chain length of the *n*-alkanes used. As expected, larger retention time differences (lower temperatures) make the effect of the nonlinearity more evident. The temperatures chosen are sufficiently high to prevent overloading of the higher *n*-alkanes.

3.5. Second evidence of the nonlinearity of the semilog plot

Methods B and C, produce values of the parameters of the corresponding equations, and a value of $t_{MM(X)}$ ($X=B$ or C), so an adjusted retention time, $t'_{R,exp}$ may be deduced. By plotting the differences $\ln t'_{R,exp} - \ln t'_{R,model}$ versus carbon number, values should be randomly distributed along a horizontal straight line if the model describes the behaviour of the *n*-alkanes in the chromatogram, but will show a trend if it does not. Fig. 2 shows the results obtained on the HP-5 capillary column at 160°C, using the hold-up time values deduced by applying Method B to *n*-alkanes of 7 to 17 carbon atoms, and Method C to the same *n*-alkanes plus methane. The points corresponding to Method B clearly indicate that the model chosen is not correct. In the case of the LQG model (Method C), all points lie close to zero, with the exception of the first one. The reason for this is that Eq. (1) represents a curve which is almost parallel to the abscissas axis for *n*-alkanes eluting

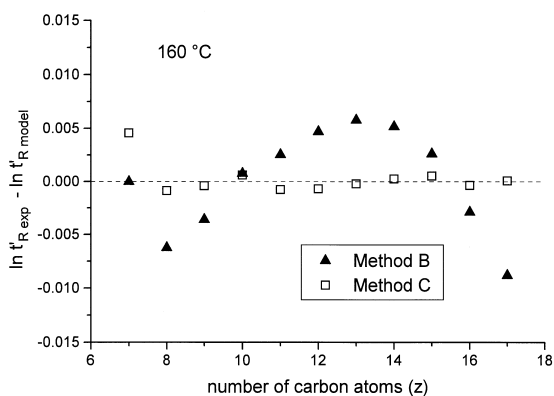


Fig. 2. Differences between the logarithms of adjusted retention times, $t'_{R,exp}$ and those deduced from the model, $t'_{R,model}$, according to Eqs. (1) and (2), for the same set of values (Table 1, 160°C).

close to methane, and consequently, small experimental variations are magnified in this region of the plot.

It might be argued that the chain length of n -heptane is too short to be included in the linearity zone of the semilog plot (Method B), causing the strange shape of the distribution of differences shown in the graph. Fig. 3 shows that this is not the case: the elimination of the first few n -alkanes does not improve the representation.

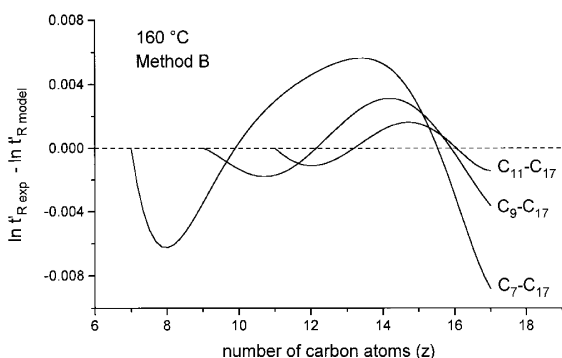


Fig. 3. Representation of the best fits of the differences between the logarithms of the adjusted retention times of n -alkanes, $t'_{R,exp}$, and those deduced from the model described by Eq. (2), $t'_{R,model}$. Each curve uses all n -alkanes between the two indicated in the figure, from the same chromatogram (Table 1, 160°C). The experimental points have been omitted for clarity.

3.6. Third evidence of the nonlinearity of the semilog plot

Applying Eq. (2) to n -alkanes of z and $z+1$ carbon atoms, we may deduce that the ratio of the adjusted retention times of any two consecutive n -alkanes, or the logarithm of that ratio, is independent of the actual chain length. From Eq. (2) we may write:

$$\ln \frac{t'_{R,z+1}}{t'_{R,z}} = C \quad (5)$$

Plotting the left hand side of Eq. (5) versus carbon number, a horizontal straight line with an intercept equal to the slope of the semilog plot (parameter C in Eq. (2)) should be obtained.

From Eq. (1), bearing in mind that the retention time of an unretained substance is given by the value of the expression for z equals zero, we may write:

$$t'_{R,z} = (\exp B)[(\exp Cz^D) - 1] \quad (6)$$

and

$$\ln \frac{t'_{R,z+1}}{t'_{R,z}} = \ln \frac{\{\exp[C(z+1)^D]\} - 1}{[\exp(Cz^D)] - 1} \quad (7)$$

where B , C and D are the parameters of Eq. (1). The value of the left-hand side of Eq. (7) will change with z .

An example of what is obtained, deducing adjusted retention times by methods B and C, is offered in Fig. 4. The two dotted lines are the representations according to the model. Once more, the plot confirms that the 'semilog plot' is not a straight line. Fig. 5 has been prepared to show that there is no value of the hold-up time that would make the representation look as predicted. The different lines have been drawn through experimental points (t'_R), obtained from data of the same chromatogram, by successive additions of arbitrary times to an initial mathematical hold-up time. For the sake of clarity, the experimental points have been eliminated from the graph.

4. Conclusions

It has been clearly shown that the plot of the logarithms of the adjusted retention times, or any

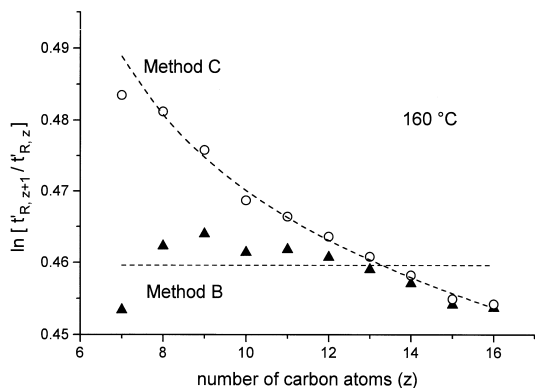


Fig. 4. Representation of the logarithms of the ratios of adjusted retention times of adjacent n -alkanes, obtained by Methods B and C. The two dotted lines represent the theoretical values obtained by the use of the parameters A , B , C and D (Table 1) deduced for the best fits of the experimental points to Eq. (1), Method C, and 2, Method B.

other related retention parameter, versus the carbon number of the n -alkanes is not a straight line, although for some applications it may be taken as such without fear. The examples shown here correspond to only a few chromatograms. However, the experience of years of work in our laboratories,

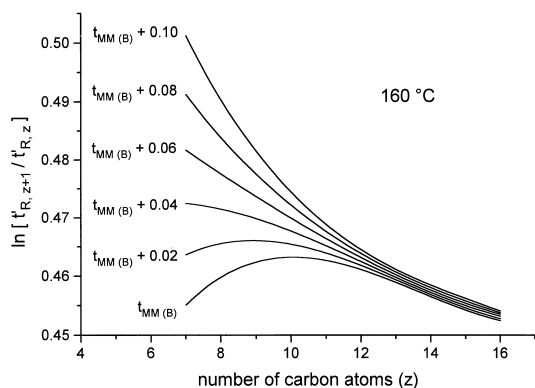


Fig. 5. Representation of the logarithms of the ratios of adjusted retention times of adjacent n -alkanes, obtained by adding different times (minutes) to the retention of an unretained substance (t_{MM}) obtained by Method B applied to n -alkanes from 7 to 17 carbon atoms. The experimental points have been omitted for clarity. The lowest curve corresponds to the points of Fig. 4, Method B.

including thousands of chromatograms in all kinds of packed and capillary columns, commercial and made in the laboratory, show that the facts presented in this paper have always been evident.

Acknowledgements

The work presented in this paper has been carried out under Project PB94-0218 of the Dirección General de Investigación Científica y Técnica (DGICYT) of Spain. The authors thank A.M. Tello, M.A. de Diago and A. Fernández-Torres for their help with some of the chromatograms and numerical calculations.

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