PEAK CAPACITY CALCULATION IN CAPILLARY GAS LIQUID CHROMATOGRAPHY

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A method is suggested for the determination of the peak capacity in isothermal and temperature-programmed high resolution gas chromatography (HRGC). The calculation is based on the integral by Grushka and Giddings, requiring that the dependence of the peak width on the retention time be known. Regression analysis of experimental data gave evidence that in isothermal and linear temperature-programmed HRGC where the temperature rate is the single variable parameter, the dependence of the peak width on retention time can be approximated by a linear equation. Substitution of this linear dependence in the integrand gives an integral which can be solved analytically. For temperature-programmed HRGC with two variable parameters, viz. the time of initial isothermal period and the temperature rate, the above dependence can be fitted with a cubic equation. The resulting integral is more complex and has to be solved numerically. The peak capacities calculated by the procedure suggested and by the use of the separation number (*TZ*) are in a good agreement.

Separation of complex mixtures by gas chromatography requires the use of capillary columns possessing a sufficiently high separation power. Although several criteria characterizing the separation power of capillary columns exist for isothermal conditions[1], criteria applicable to procedures where the column temperature is varied are lacking[2]. Among criteria that can be applied to isothermal as well as non-isothermal conditions is the peak capacity n_c, which gives the number of peaks separable at a required resolution between two chosen retention times[3]. Although conventionally used to determine the number of peaks separated between the gas hold-up time t_M and a given retention time t_R, the peak capacity can also provide the number of peaks separable between two compounds with retention times t_{R1} and t_{R2}. Based on the approach by Giddings[3] and Grushka[2], the n_c value can be calculated by using the integral

$$n_c = 1 + \int_{t_M}^{t_R} \frac{dt}{4\sigma} \quad , \qquad (1)$$

where σ is the standard deviation for a chromatographic peak.

Within this concept, all adjacent peaks, *i* and *j*, are resolved in a chromatogram with a resolution factor of $R_{j,i} = 1.0$ because the adjacent peak maxima differ then by 4σ (assuming that $\sigma_i = \sigma_j$ and the peaks possess Gaussian profiles). Clearly, the half peak widths $w_{0.5}$ or the peak base widths *Y* can be measured more easily than the σ values. Statistical treatment shows that for a Gaussian peak, $w_{0.5} = 2.355\sigma$ and $Y = 4\sigma$. The peak width obtained from the integrator is usually calculated as $w = A/h$ where *A* is the peak area and *h* is the peak height. For a Gaussian profile, $w = \sigma\sqrt{2\pi} = 2.507\sigma$. Since such peak widths are obtained easily, the following formula can be applied to calculate the peak capacity:

$$n_c = 1 + \int_{t_M}^{t_R} \frac{dt}{1.596w} \quad . \tag{2}$$

Peak capacity calculations in isothermal or temperature-programmed high-resolution gas chromatography (HRGC) often include simplifying assumptions. Since such assumptions may be different for the two chromatographic modes, they will be discussed separately now.

Isothermal Conditions

In isothermal HRGC it is frequently assumed that the column possesses a given number of theoretical plates, *N*, which is identical for all solutes[2,3]. The peak capacity then can be calculated as

$$n_c = 1 + \int_{t_M}^{t_R} \frac{\sqrt{N}}{4t}\,dt = 1 + \frac{\sqrt{N}}{4} \ln \frac{t_R}{t_M} \quad . \tag{3}$$

We have demonstrated[1,4] that the number of peaks resolved between two adjacent n-alkanes (with *z* and *z* + 1 carbon atoms, respectively) at $R = 1.0$ can be calculated based on the separation number (*TZ* – Trennzahl) concept[5,6] by means of the formula

$$n_c - 1 = 1.177\,(TZ + 1) \quad , \tag{4}$$

For this, *TZ* is calculated from Kaiser's equation[5] as

$$TZ = \frac{t_{R,z+1} - t_{R,z}}{w_{0.5,z} + w_{0.5,z+1}} - 1 \quad . \tag{5}$$

To evaluate the separation power of the column, Kaiser proposed[6] calculating the number of peaks resolved between the hold-up time and the tenfold longer retention time ($t_R = 10t_M$). Usually, however, the Giddings' concept[2,3] is applied, allowing the number of peak resolved at $R_{j,i} = 1.0$ between any two retention times to be calculated.

It is well known that neither the number of theoretical plates nor the *TZ* value is constant in isothermal capillary GC, and both of them depend on the retention factor k_i of the compounds involved[1]. If the dependence of the number of theoretical plates on the retention factor is considered, the peak capacity can be calculated numerically by modifying Eq. (*1*) (ref.[4]).

Non-Isothermal Conditions

In non-isothermal conditions, the standard deviation is assumed to be constant for all peaks in the chromatogram or in a segment of the chromatogram[7]. This assumption is supposed to hold true in linear temperature-programmed GC (ref.[8]) and in linear gradient elution LC (refs[7,9]). Within this concept, the peak capacity can be calculated as

$$n_c = 1 + \frac{t_R - t_M}{1.596w} \quad . \tag{6}$$

In fact, the assumption of identical peak widths is generally considered to be only valid over limited retention time ranges in linear temperature-programmed gas chromatography[7,8,10,11] as well as in reverse-phase liquid chromatography with suitable linear programming of the mobile phase composition[7,9]. In view of this, the total number of components in sample is best determined by dividing the whole chromatogram into smaller segments within which the peak widths can be assumed to be constant[7].

For the peak capacity calculation from the *TZ* values in HRGC, Eq. (*4*) is used as in isothermal conditions.

However, even in the simplest version of temperature-programmed HRGC (a single temperature gradient), the *TZ* values fail to be constant; instead, they depend on the number of carbon atoms in the n-alkane employed for the *TZ* calculation[1].

This paper describes a procedure for estimating the peak capacity in non-isothermal HRGC.

THEORETICAL

As follows from the text above, the peak capacity can easily be calculated for practical purposes by means of Eq. (*2*), which can be applied to isothermal as well as non-isothermal conditions provided that the dependence of the peak width *w* on the retention time t_R is known.

In isothermal gas chromatography, this dependence can be found by using an equation for the calculation of the number of theoretical plates. Combination of such an equation with Eq. (*2*) gives an integral which can be solved analytically provided that N is independent of the retention time (Eq. (*3*)) or this dependence is not very complex[1], or numerically if the dependence is complex[1].

Frequently, regression analysis of experimental data suggests that the dependence of w on t_R in isothermal gas chromatography is basically linear[6],

$$w = A + B\,t_R \qquad (7)$$

(A and B are constants).

Combination of Eqs (*2*) and (*7*) gives the following expression:

$$n_c = 1 + \frac{1}{1.596}\int_{t_M}^{t_R} \frac{1}{A + B\,t}\,\mathrm{d}t \ . \qquad (8)$$

Coefficients A and B can be found by regression analysis of experimental data for homologous series (usually n-alkanes) so as to fit Eq. (*7*).

Integration of Eq. (*8*) leads to the formula

$$n_c = 1 + \frac{1}{1.596B}\ln\frac{A + B\,t_R}{A + B\,t_M} \ , \qquad (9)$$

which is identical with Eq. (*3*) if $A = 0$.

In temperature-programmed gas chromatography the dependence of the peak width on the retention time is more or less complex, in relation to the number of parameters varied during the run. If this dependence is inserted in Eq. (*2*), an integral is obtained, which can be solved either analytically or numerically.

EXPERIMENTAL

Capillary Columns

Column A: a glass capillary column 96 m long, 0.3 mm i.d., coated with OV-01 polydimethylsiloxane stationary phase 0.2 μm thick. Column B: a fused silica capillary column 100 m long, 0.25 mm i.d., coated with polydimethylsiloxane stationary phase 0.5 μm thick.

Apparatus

A Fractovap 4180 gas chromatograph equipped with an FID and a Grob type cold on-column injection port (Carlo Erba, Milan, Italy). Carrier gas: hydrogen, inlet pressure 200 kPa for column A and 250 kPa for column B. A C-R3A integrator (Shimadzu, Kyoto, Japan).

Samples

A model mixture of 32 hydrocarbons dissolved in chloroform (1 : 10) and a model mixture of $C_6 - C_{12}$ alkanes dissolved in pentane (1 : 10).

RESULTS AND DISCUSSION

The peak width dependence on the retention time has to be known for the calculation of the peak capacity in temperature-programmed HRGC based on Eq. (*2*). This depends on the number of variable parameters: the simplest relationship will apparently emerge for linear temperature-programmed HRGC where the temperature rate is the only variable.

Linear Temperature-Programmed HRGC

Isothermal HRGC can be regarded as trivial linear temperature-programmed HRGC with a zero temperature rate. If the temperature is increased at a chosen rate along the entire chromatogram, the dependence of the peak width on retention time can be expected to be similar to that in isothermal HRGC.

The dependence of the recorded peak width w on the retention time t_R for well-resolved components of the model hydrocarbon mixture on column A in temperature-programmed HRGC over the 50 – 150 °C region at a rate of 0.5 °C/min is shown in Fig. 1.

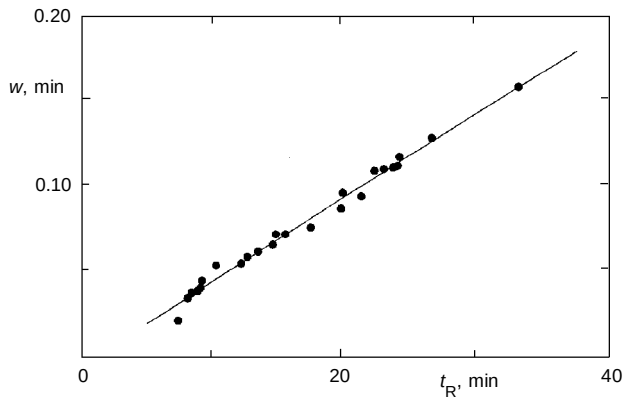

FIG. 1
Dependence of peak width on retention time for a hydrocarbon model sample. Column A operated with linear temperature programmed HRGC from 50 °C with 0.5 °C/min gradient

The dependence is linear (Eq. (*7*)), so that the peak capacity can be calculated by means of Eq. (*8*). Slope *B* depends on the temperature rate (Fig. 1) and is highest in isothermal conditions; in temperature-programmed HRGC at a sufficiently high temperature, *B* is constant (Fig. 2).

Temperature-Programmed HRGC with the Initial Temperature Time Interval and Temperature Rate as Parameters

For the separation of the $C_6 - C_{12}$ alkane mixture on column B, 9 chromatograms were obtained by programming the temperature from 40 to 240 °C, varying the initial time interval t_0 from 5 to 19 min with a 7 min step and the temperature rate r from 0.5 to 3.5 °C/min with a 1.5 °C/min step, and following the 3-level experimental design given in Table I. The t_R and w values were derived from the chromatograms and the w vs t_R dependence was plotted (Fig. 3). Regression analysis revealed that this plot can be fitted by a cubic equation:

$$w = A_0 + A_1 t_R + A_2 t_R^2 + A_3 t_R^3 \ , \qquad (10)$$

where w and t_R are time quantities (seconds) and A's are constants. Since this equation emerged from multiple regression analysis based on the F- and t-tests, no physical meaning can be attached to the constants. Their values also depend on some experimental parameters which usually fail to be standardized precisely enough (stationary phase film thickness, carrier gas pressure drop along the column, carrier gas outlet pressure).

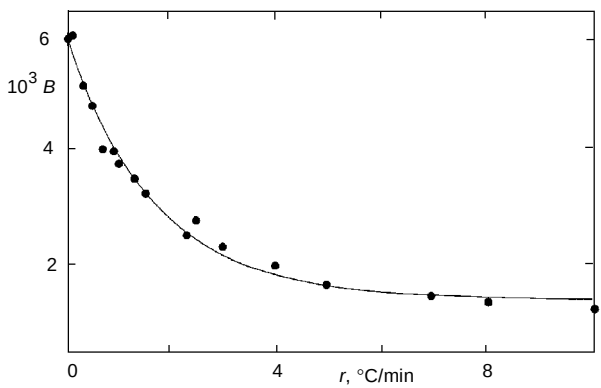

FIG. 2
Dependence of slope on the temperature rate for a hydrocarbon model sample. Column A operated with linear temperature programmed HRGC starting from 50 °C

For this reason, the values are not reported in this paper. Nevertheless, Fig. 3 demonstrates that the calculated line fits the experimental data acceptably.

Combination of the modified Eq. (*2*) and Eq. (*10*) gives the following expression:

$$n_c = 1 + \frac{1}{1.596} \int_{t_{R,1}}^{t_{R,2}} \frac{dt}{A_0 + A_1 t + A_2 t^2 + A_3 t^3} \quad . \tag{11}$$

TABLE I
Initial temperature time intervals and temperature rates for different experiments by three level experimental design

Experiment No.	t_0, min	r, °C/min
1	5	0.5
2	12	0.5
3	19	0.5
4	5	2.0
5	12	2.0
6	19	2.0
7	5	3.5
8	12	3.5
9	19	3.5

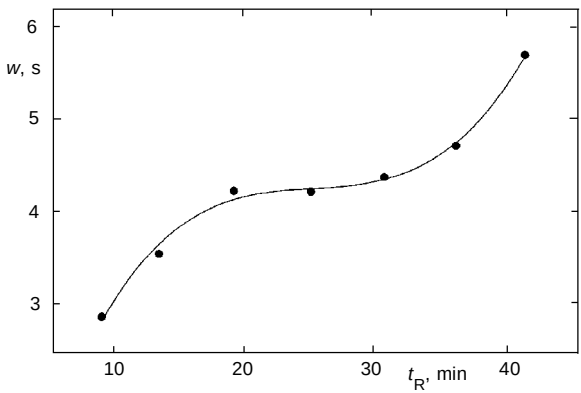

FIG. 3
Dependence of peak width on retention time for n-alkane C_6 – C_{12} sample separated on column B. Programmed temperature from 40 to 240 °C according to the experiment 8 in Table I (t_0 = 12 min, r = 3.5 °C/min)

Since mathematical integration would be rather difficult, we resorted to numerical integration by the Gauss' iterative method. Table II shows a comparison of the n_c values between hexane ($t_{R,1}$) and dodecane ($t_{R,2}$) for the nine experimental chromatograms,

TABLE II
Peak capacities calculated between hexane and dodecane at different working conditions from Eqs (*11*) and (*12*)

Experiment No.	n_c	$n_c(TZ)$	Δn_c
1	601.5	603.3	−1.8
2	596.4	595.6	0.8
3	610.0	607.2	2.8
4	532.5	533.1	−0.6
5	548.9	548.8	0.1
6	562.7	562.8	−0.1
7	464.2	465.6	−1.4
8	486.9	481.5	5.4
9	465.8	475.7	−9.9

TABLE III
Values of *TZ* determined between consecutive $C_6 - C_{12}$ n-alkanes using Eq. (*5*)

Experiment No.	Peak pairs					
	C_6–C_7	C_7–C_8	C_8–C_9	C_9–C_{10}	C_{10}–C_{11}	C_{11}–C_{12}
1	74.2	91.7	90.7	87.3	83.3	77.2
2	75.4	86.6	87.5	86.0	83.2	79.2
3	73.8	91.3	91.4	88.1	84.6	78.5
4	70.7	82.3	83.5	75.5	68.9	64.1
5	76.7	88.4	83.8	75.7	70.0	63.6
6	75.1	95.5	87.4	79.1	69.1	63.9
7	70.0	74.1	70.9	66.5	58.8	48.4
8	77.5	82.6	76.7	66.9	66.9	40.5
9	79.9	95.6	79.3	60.5	46.2	34.7

calculated by using Eq. (*11*) and by using the separation numbers *TZ* employing modified Eq. (*4*),

$$n_c(TZ) = 1 + 1.177 \sum_{j=7}^{12} (TZ_j + 1) \quad , \qquad (12)$$

where TZ_7 is the *TZ* value between n-hexane and n-heptane, TZ_8 between n-heptane and n-octane, etc. Table II demonstrates that the peak capacity depends strongly on the temperature-programmed run parameters and that a relatively good agreement is obtained between the peak capacities calculated from Eq. (*11*) and Eq. (*12*) ($\Delta n_c < 10$). Comparison of Table II with respect to Table I leads to the conclusion that the corresponding peak capacities between hexane and dodecane decrease with increasing temperature rate. The *TZ* values determined based on Eq. (*5*) for all adjacent n-alkane peak pairs are given in Table III. The *TZ* values from this table and the peak capacities from Eq. (*4*) exhibit a dependence on the number of carbon atoms in the alkane chain. The *TZ* values are found highest for experiments with low temperature rates; this is consistent with the published finding that the number of peaks separable between two selected n-alkanes decreases with temperature[1].

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