THE THEORY OF GAS ELUTION CHROMATOGRAPHY WITH LARGE SAMPLES AND CALCULATION OF THE NECESSARY SENSITIVITY OF THE DETECTION SYSTEM*

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Within the scope of elution chromatography the usual requirements concerning the sensitivity of the detector are determined not only by the initial concentration, C_0, of the substance to be determined in the mixture analysed and the accuracy of determination required (permissible relative error σ) but also by the dilution of the injected sample in the chromatographic column, as a result of which the concentration, C, in the chromatographic peak is usually significantly less than the initial concentration in the sample. An increase in the amount of sample injected (q), although resulting in a corresponding increase in C is, however, limited by the decrease in the resolution power of the chromatographic unit.

It is generally thought necessary[1] that the limiting permissible sample q_m should be so small that the width of a chromatographic peak, τ, would be determined only by processes of dilution of the sample in the column and independent of sample size, where τ is the duration of a chromatographic peak, measured between the moments of appearance on the elution curve of the points, corresponding to half the value of the maximum signal, expressed in seconds. However, as will be shown later, in the great majority of cases such a requirement is excessive and results in an unjustified decrease of the actual sensitivity of the method.

In practice, it is a sufficiently good resolution of the peak of a component analysed from the neighbouring peaks on the chromatogram that is of interest, rather than the duration of the peak, τ, as such. As it is known[2], the separation is characterized by the resolution factor:

$$K_1 = \frac{\Delta t}{\tau_1 + \tau_2} \qquad (1)$$

depending on the difference Δt of the retention times of neighbouring components.

The required accuracy of chromatographic analysis determines the necessary resolution. To evaluate it in a quantitative manner, the relationship should be established between the resolution factor, K_1, and the error due to the overlapping of the peak of the component measured and the signal from the neighbouring in-

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completely resolved component. Such a calculation for the case of equal peak heights of the components to be separated on the elution curve has been made by one of the authors and N. M. TURKEL'TAUB[3]. In the general case the purity of a fraction, *i.e.* the proportion of the main component of the fraction, may be expressed in terms of two dimensionless parameters, one of which is the K_1 factor, and the other, the ratio of maximum heights of the peaks analysed, W.

The calculation, performed on the assumption that the shape of either of the two neighbouring peaks on the chromatogram may be described by a Gaussian curve equation with one value of the decrement index, enable us to relate the relative error, σ, introduced by an unresolved component, with the resolution factor, K_1, and the parameter W. In the case of analysis according to peak heights:

$$\sigma_h = We^{-16\,K_1^2} \tag{2}$$

When the analysis is made on the basis of peaks areas:

$$\sigma_s = \frac{W}{2}\left[1 - \phi\left(2\,K_1 + \frac{\ln W}{8\,K_1}\right)\right] \tag{3}$$

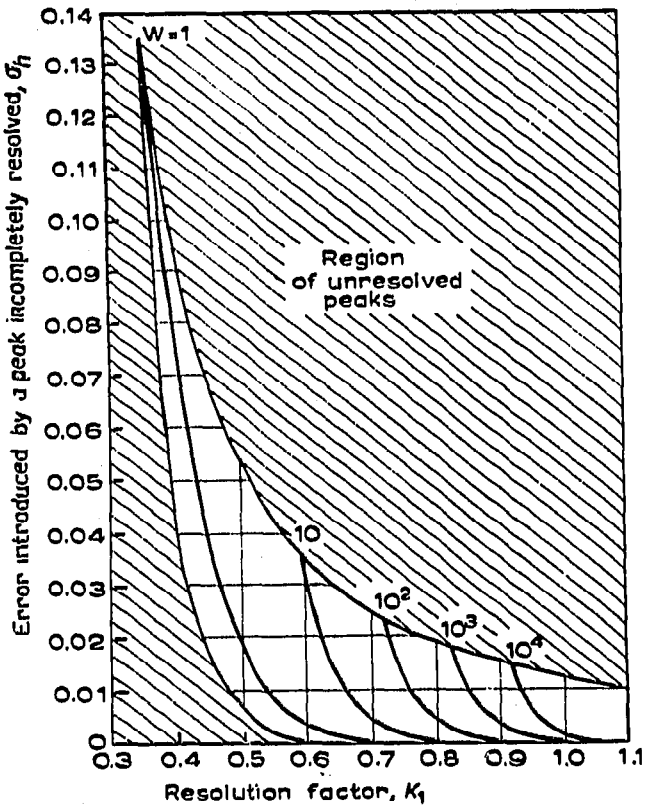
where:

$$\phi(x) = \frac{2}{\sqrt{\pi}}\int_0^x e^{-x^2}\,dx \text{ (the probability integral)}$$

These results, represented graphically in Figs. 1 and 2, make it possible to assess the necessary values of the resolution factor, K_1, from the precision of analysis required. The limiting values of K_1 and W, confining the region of unresolved peaks, have been found by determining the extremum of the function describing the result of superposition of two Gaussian curves, and by determining the regions where three extrema of this function are fused in one. An example of such a calculation may be found in the paper by GHENKIN[4].

The relation (3) permits the length of column necessary to secure the required purity of fraction to be calculated, depending on the relative content, W, of the interfering component. In Fig. 3 the results of such a computation are presented, showing precisely the dependence previously reported by one of the authors[5].

Let us now consider the dependence of the resolution factor K_1 and concentration C_m at peak maximum on sample size, q. If the initial peak width is comparable to the amount of diffusional dilution in the column, the shape of the peak cannot be described by the Gaussian curve equation. In this case, however, the elution curve may be considered as a result of superposition of a series of Gaussian curves, each of which corresponds to the elementary volume of sample, shifted in time with respect to each other. Therefore the shape of the peak may be found by multiplying the Gaussian curve by a function describing the distribution in time of concentration of the sample introduced and by integrating the product as a function of sampling time. Calculations of this type for different procedures of sample introduction are given in the literature[6]. To introduce the sample as a rectangular impulse with a concentration C_0 and duration B (the "plug" method), the relation describing the



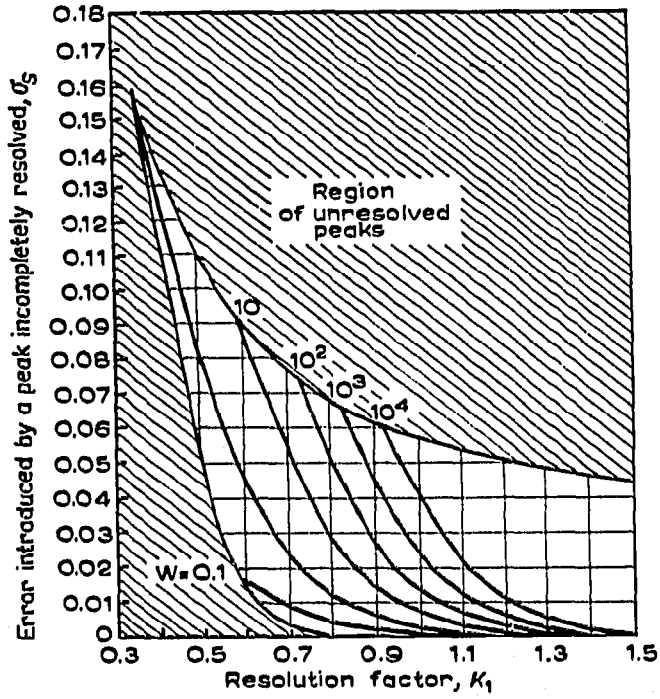


Fig. 1. Dependence of relative error (σ_h), introduced by the unresolved component, on the resolution factor (K_1), in quantitative analysis using peak heights. W = ratio of peak height of the interfering component, to that of the component to be determined.

Fig. 2. Dependence of relative error (σ_s), introduced by the unresolved component, on the resolution factor, (K_1), in quantitative analysis using peak areas. W = ratio of peak height of the interfering component, to that of the component to be determined.

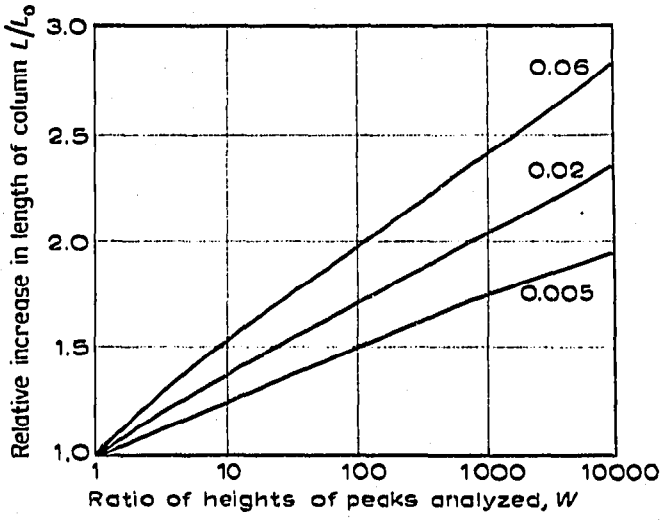


Fig. 3. Dependence of the relative increase in length of column (L/L_0) necessary for obtaining the required purity of fraction on the W parameter. Figures beside the curves indicate the selected value of the error σ_s due to incomplete resolution. L_0 = length of column securing the required purity of fraction (relative error σ_s) with W = 1.

shape of the peak as it emerges from the column may be represented as follows:

$$C(t) = \frac{C_0}{2}\left\{\phi\left[\sqrt{\frac{L\alpha}{4D}}\left(1 - \frac{t}{t_0} + \frac{B}{t_0}\right)\right] - \phi\left[\frac{L\alpha}{4D}\left(1 - \frac{t}{t_0}\right)\right]\right\} \quad (4)$$

where:

t_0 = retention time for an infinitely small sample size;
L = length of column;
α = linear velocity of carrier gas;
D = effective diffusion coefficient;
ϕ = probability integral.

The peak maximum emerges from the column at time $t_m = t_0 + B/2$. This is easily shown by differentiation of (4). Substitution in (4) gives the maximum concentration:

$$C_m = C_0\,\phi\left(\frac{B}{2\,t_0}\sqrt{\frac{L\alpha}{4D}}\right) \quad (5)$$

which is equivalent to:

$$C_0\,\phi\left(\frac{B}{4\,\Gamma}\sqrt{\frac{\alpha^3}{DL}}\right)$$

Considering that the duration of the peak, τ_0, observed at an infinitesimal sample size, q, is determined by the relation (3):

$$\tau_0 = 4\,\Gamma\sqrt{\frac{DL}{\alpha^3}}\ln 2 = 3.330\,\Gamma\sqrt{\frac{DL}{\alpha^3}} \quad (6)$$

where Γ is the Henry coefficient. Hence the expression (5) may be rewritten in the following form:

$$C_m = C_0\,\phi\left(0.8326\,\frac{B}{\tau_0}\right) \quad (7)$$

The peak width, τ, may be determined satisfying the equality $C(t) = 0.5\,C_m$. Using (4), (5) and (6) let us transform it to the following form:

$$\phi\left(Z + 1.6652\,\frac{B}{\tau_0}\right) - \phi\,(Z) = \phi\left(0.8326\,\frac{B}{\tau_0}\right) \quad (8)$$

where:

$$Z = \sqrt{\frac{L\alpha}{4D}}\left(1 - \frac{t}{t_0}\right) \quad (9)$$

Equation (8) has two roots, Z_1 and Z_2, to which correspond the moments, t_1 and t_2, of recording the points of half concentration. Obviously:

$$\tau = t_2 - t_1 \quad (10)$$

Since the probability integral $\phi(x)$ is an odd function, the roots Z_1 and Z_2 of eqn. (8) are related by the following relationship:

$$Z_2 = -Z_1 - 1.6652 \frac{B}{\tau_0} \tag{11}$$

Using the equalities (9), (10), and (11), the following expression may be obtained to determine the peak width, τ:

$$\frac{\tau}{\tau_0} = 1.201\ Z_1 + \frac{B}{\tau_0} \tag{12}$$

where Z_1 is the positive root of transcendent eqn. (8), which may be found by the common method for approximate solution.

From the known values of τ/τ_0 it is easy to calculate the relative impairment of resolution, K_1/K_0, due to the increase in sample size, by using the expression (1):

$$\frac{K_1}{K_0} = \frac{\tau_0}{\tau} \tag{13}$$

where K_0 is the maximum value of resolution factor for the particular conditions of the chromatographic run, corresponding to an infinitesimal sample.

The results of calculating C_m/C_0 (from (4)), τ/τ_0 (by an approximate solution (8)) and substitution of the value found (Z_1 into (12)), and K_1/K_0 (by using the relation-

TABLE I

DEPENDENCE OF THE RELATIVE CONCENTRATION (C_m/C_0) AT THE PEAK MAXIMUM, RELATIVE PEAK WIDTH (τ/τ_0) AND RELATIVE IMPAIRMENT OF RESOLUTION (K_1/K_0) ON THE RELATIVE SIZE OF THE SAMPLE INTRODUCED (B/τ_0)

B/τ_0	C_m/C_0	τ/τ_0	K_1/K_0
0.0	0.0000	1.0000	1.0000
0.1	0.0937	1.0042	0.9958
0.2	0.1862	1.0092	0.9909
0.3	0.2761	1.0209	0.9793
0.4	0.3624	1.0374	0.9639
0.5	0.4440	1.0590	0.9443
0.6	0.5201	1.0859	0.9209
0.7	0.5902	1.1179	0.8946
0.8	0.6538	1.1560	0.8651
0.9	0.7107	1.1997	0.8334
1.0	0.7602	1.2502	0.7999
1.2	0.8423	1.3662	0.7320
1.4	0.9007	1.5052	0.6644
1.6	0.9404	1.6633	0.6012
1.8	0.9660	1.8362	0.5446
2.0	0.9815	2.0196	0.4951
2.2	0.9904	2.2102	0.4525
2.4	0.9952	2.4050	0.4159

ship (13)) with different values of the parameter, B/τ_0, characterizing the size of the injected sample, q, are given in Table I and graphically represented in Fig. 4.

$$q = vC_0B \tag{14}$$

when v is the volume flow rate of carrier gas.

Obviously, an increase in sample size injected is an efficient method for increasing the sensitivity of chromatographic analysis, since with the consequent increase of concentration at the peak maximum there occurs only a relatively small increase in peak width and thus a relatively small impairment of the resolving power. Thus, at $B/\tau_0 = 0.4$ the concentration at peak maximum is about 36 % of the concentration in the sample, while the resolution factor decreases only to 96.4 % of the value of K_0 corresponding to an infinitesimally small sample. For the sake of comparison it may be noted that KEULEMANS[1] in his monograph regards $B/\tau_0 = 0.02$

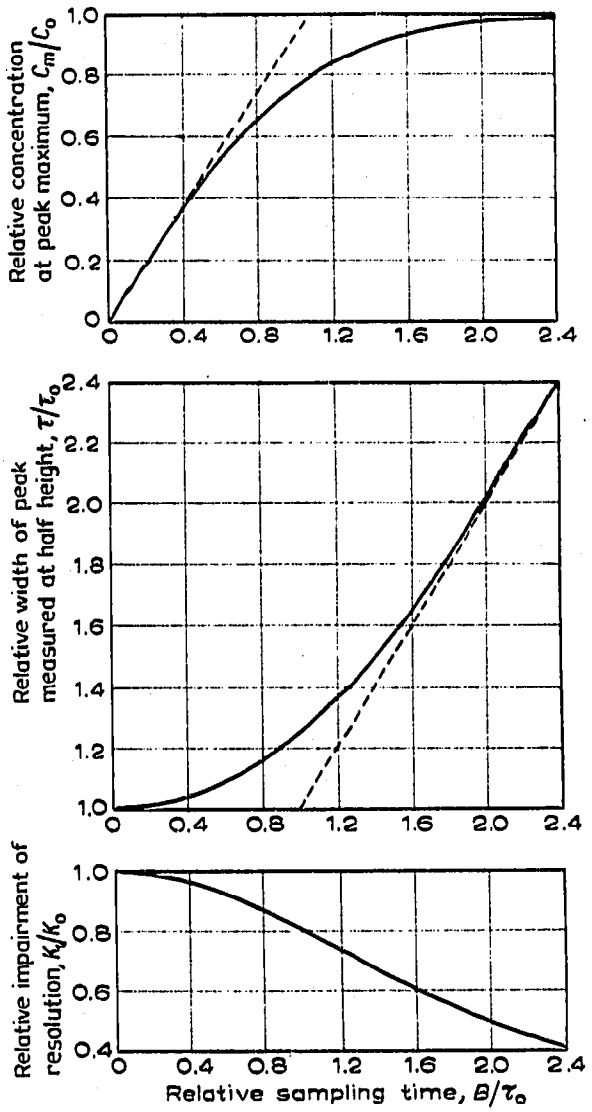


Fig. 4. Dependence of relative concentration (C_m/C_0) at peak maximum, relative peak width (τ/τ_0) and relative impairment of resolution (K_1/K_0), on the relative size (B/τ_0) of the sample introduced.

as the limiting permissible value for the sample injected, *i.e.* a value nearly 20 times less.

For an experimental verification of the relationships obtained, the height h and duration τ of chromatographic peaks were measured for each volume V of the liquid sample introduced into the sampling device. A study was made of the resolution mixture containing approximately equal amounts of benzene, octane, and nonane ($\sim$ 5% each), dissolved in diethyl ether. The analysis was made on a column 200 cm long, 5 mm in diameter, packed with Celite 545, 80–100 mesh, with 30% of silicone oil DC 550 at a temperature of 130°. Hydrogen was used as carrier gas; its rate was maintained at 170 ml/min. The liquid sample was injected into the vaporizer by means of a syringe with a micrometric movement of the piston.

A comparison of the experimental with the calculated values (presented in Table I) was made by plotting the two relations on a double logarithmic scale and superimposing the calculated curve on the experimental one until the best coincidence

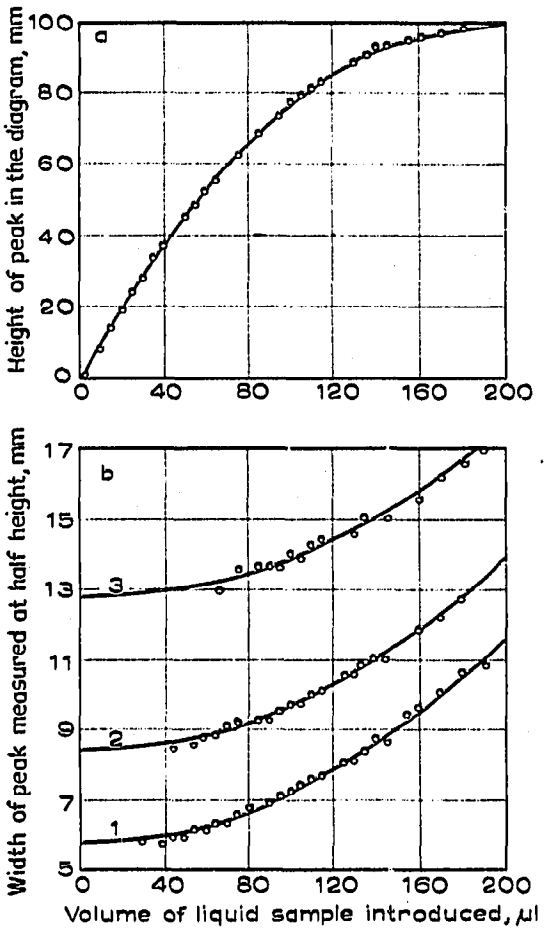


Fig. 5. Dependence of the height (h) and width (τ) of chromatographic peaks on the volume (V) of the liquid sample introduced. Solid lines show calculated curves plotted for the following values of the parameters: 1 = benzene, V_0 = 100.2 µl, h_0 = 102.3 mm, τ_0 = 5.74 mm; 2 = octane, V_0 = 125.9 µl, h_0 = 105.2 mm, τ_0 = 8.36 mm; 3 = nonane, V_0 = 162.2 µl, h_0 = 115.3 mm, τ_0 = 12.7 mm. The points show experimentally measured values. Column: 200 × 0.5 cm; inert support: Celite 545; grain size: 80–100 mesh; stationary phase: silicone DC 550, 30% of the support by weight; t = 130°; carrier gas: hydrogen; hydrogen flow rate: 170 ml/min. Thermal conductivity detection. Because of an overlapping of curves in Fig. 5(a), only the results for benzene are shown.

is reached, while the co-ordinate axes of the two curves are kept parallel; the position of the origin of co-ordinates of the calculated curve determined the values of the parameters h_0 and V_0, and τ_0 and V_0, respectively, for the dependence of the height h or duration τ of peaks on the sample volume V.

From the values of parameters thus found, calculated relationships were plotted, which were subsequently compared to the values experimentally measured.

The results of comparison are shown in Fig. 5, where the solid lines indicate the calculated values, and the points have been obtained by direct measurements. It is evident that the agreement of theoretical and experimental values is quite satisfactory. Therefore the use of the results of the theory described above for an assessment of the necessary values of sensitivity of detecting devices is fully justified.

To calculate the threshold sensitivity of a detecting system required for a particular chromatographic run the following quantities must be known:

(1) Concentration C_0 of the component to be determined in the system analysed.

(2) Limiting permissible relative error σ under the particular conditions of the analysis.

(3) The limiting value of the resolution factor K_0 obtained with a vanishingly small sample size. (The value of K_0 is determined by the physico-chemical characteristics of the components to be separated and by the choice of conditions for making the chromatographic run.)

(4) Relative concentration W of the neighbouring component on the elution curve.

The calculation is made as follows. From the given values of σ and W the value of the resolution factor, K_1, necessary for securing the required accuracy is determined from the curves presented in Figs. 1 or 2 (depending on the selected method of analysis from peak heights or areas). From the known value of the K_1/K_0 ratio, by using the curves in Fig. 4, the value of the maximum admissible sample and the corresponding value of C_m/C_0 are determined. Finally, the threshold sensitivity C_{min} is determined from the condition:

$$C_{min} = \sigma C_m \tag{15}$$

Some typical examples are discussed below.

EXAMPLES OF APPLICATIONS OF THEORY

(a) *Analysis of comparatively large concentrations on ordinary packed columns*

Concentration of the component to be determined, 10 %; with $M = 100$ g/mol this corresponds to $C_0 \approx 0.4$ mg/ml; permissible error $\sigma = 0.01$; $K_0 \approx 1$; $W \approx 10$; the calculation is made from peak areas.

From Fig. 2 it is shown that to secure the accuracy required a value of 0.95 is needed for the resolution factor, K_1, *i.e.*, the permissible impairment of resolution because of an increase in sample size, K_1/K_0, is 0.95. From the plot in Fig. 4 we determine that $B/\tau_0 = 0.46$ and $C_m = 0.41\ C_0$. Then from the equality (15) the necessary value of threshold sensitivity is calculated:

$$C_{min} = 0.01 \times 0.41 \times 0.4 \frac{mg}{ml} \approx 1.6 \times 10^{-3} \frac{mg}{ml}$$

The value obtained is suitable for thermal conductivity detectors, which are actually used for such analyses.

(*b*) *Analysis of impurities on ordinary packed columns*

Concentration of the component to be determined, 10^{-3} %; *i.e.* $C_0 \approx 4 \times 10^{-5}$ mg/ml; $\sigma = 0.05$; $K_0 \approx 1$; $W \approx 10^4$.

In this particular case from the plot in Fig. 2, we find $K_1 = 0.97$ and then from Fig. 4 we determine: $B/\tau_0 = 0.36$; $C_m = 0.33\ C_0$.

The threshold sensitivity required:

$$C_{min} = 0.05 \times 0.33 \times 4 \times 10^{-5}\ \text{mg/ml} \approx 7 \times 10^{-7}\ \text{mg/ml}$$

It is evident that for this analysis the use of high sensitivity ionization detector is required.

(*c*) *Relatively precise quantitative analysis of components difficult to separate on capillary columns*

Concentration of the component to be determined, 1 %; *i.e.*:

$$C_0 \approx 4 \times 10^{-2}\ \text{mg/ml};\ \sigma = 0.02;\ K_0 = 0.74;\ S = 10^2$$

Due to a poor resolution, it is advisable to treat the result using peak heights. From the plot in Fig. 1 it is seen that the required value of K_1 is near to K_0, and it would be difficult to determine it with sufficient precision. Therefore we calculated K_1 by using formula (2) and find $K_1 = 0.737$. Hence, $K_1/K_0 = 0.997$. From Table I we determine by interpolation:

$$\frac{B}{\tau_0} = 0.07 \text{ and } C_m = 0.06\ C_0$$

Consequently:

$$C_{min} = 0.02 \times 0.06 \times 4 \times 10^{-2}\ \text{mg/ml} \approx 5 \times 10^{-5}\ \text{mg/ml}$$

Considering that in capillary chromatography ionization microdetectors of the flow type are used, and the rate of carrier gas flow is near 1 ml/min, let us determine the required value of the flow type detector sensitivity:

$$j_{min} = 5 \times 10^{-5}\ \text{mg/ml} \times 1.7 \times 10^{-2}\ \text{ml/sec} \approx 8 \times 10^{-7}\ \text{mg/sec}$$

CONCLUSIONS

The proposed methods of calculation permit the assessment of the required values of the resolution factor and of the necessary length of column from the known conditions and also the calculation of the limiting sample size and of the necessary sensitivity of the detecting system.

The results obtained show that in practice it is advisable to work with samples of a substantially larger size than is usually recommended in the literature on chromatography.

SUMMARY

Formulae relating the error caused by incomplete resolution of peaks on the elution curve to the resolution factor and relative proportions of the components have been derived.

The dependence of peak height, peak width, and resolution factor on the size of the sample introduced has been theoretically established and experimentally confirmed. It is shown that increasing the sample size is an effective method for increasing the sensitivity of chromatographic analysis; the conditions for selecting the optimum sample size are formulated.

A method for an approximate calculation of the threshold sensitivity of a detector from the conditions of a given analytical problem is suggested and illustrated by typical examples.

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