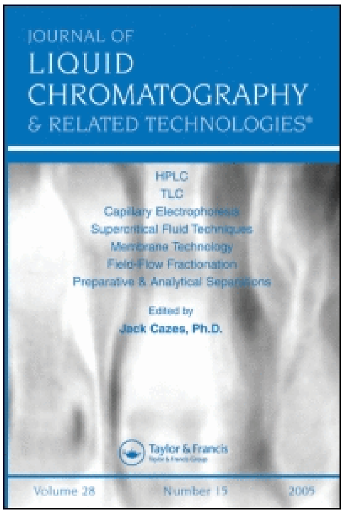

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The Shape of the Elution Curve in Gel Permeation Chromatography

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THE SHAPE OF THE ELUTION CURVE IN GEL PERMEATION CHROMATOGRAPHY*

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ABSTRACT

Asymmetry, or tailing, of the elution curve in elution chromatography, and particularly in gel permeation chromatography, is caused, among other things, by the passage of a symmetrical chromatographic zone through the end of the column system in proceeding zone spreading. Defined parameters of the asymmetry of elution curves, based on the theory of statistical moments, make possible a quantitative description of tailing and correlation of elution curves obtained under various experimental conditions. Simple relationship has been calculated between the efficiency of chromatographic columns and the asymmetry of elution curves for the case of the Gaussian concentration profile on the column. This may be applied to examine the symmetry of the concentration profile from experimentally determined elution curve.

INTRODUCTION

Gel permeation chromatography (GPC) has become one of the methods most in use in the determination of the molecular weight distribution of polymers. Molecules which are separated in solution according to their size leave the separation system in an order from the largest to the smallest ones, and their relative

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participation may be evaluated from a record of the concentration detector. A chromatogram in the coordinates elution volume or elution time vs. response of the detector (i.e. the elution curve) can then be recalculated to the distribution curve of molecular weights, using preceding calibration or a monitor which directly indicates molecular weight or a quantity proportional to it. However spreading must be taken into account, because owing to it, even a sample monodisperse with respect to the molecular weight distribution is eluted in a zone of finite width. Distribution curves of molecular weights closer to correct distribution curves are calculated using various mathematical methods (1) which serve to correct the effect of spreading. A certain shape of the elution curve of an ideal monodisperse sample is always assumed in such case. From the practical standpoint, such way of interpretation of GPC data seems satisfactory. On the other hand, however, for some fundamental studies, the width and shape of the resulting elution curve of an ideal monodisperse sample may be used to draw conclusions about various processes which contribute to both separation and dispersion in the chromatographic process. In such cases it is necessary to analyze with great care all factors which may affect the shape of the final elution curve.

Some factors which ensue from the passage of the chromatographic zone of given width through the end of the column system (concentration detector) under real chromatographic conditions and which are operative to a various extent in each kind of elution chromatography are dealt with in this paper.

THEORY

Let us assume a chromatographic column divided into unit volume elements in the direction of the longitudinal axis, as shown in Fig. 1.

If the concentration profile, i.e. the distribution of concentration of a monodisperse sample on the column in the direction

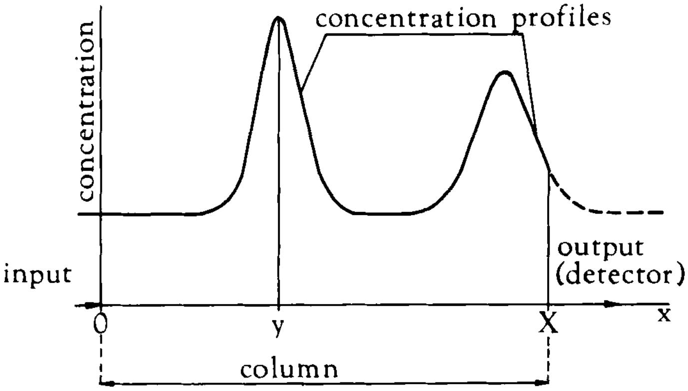

FIGURE 1. Spreading of chromatographic zone along the column

of the longitudinal axis, is symmetrical (related to the volume elements), the resulting tailing of the elution curve i.e. the detector response to concentration in different elution time or volume arises as a consequence of the passage of this symmetrical concentration profile through the end of the column in proceeding zone spreading. While a certain point of the zone is already detected, one part of the zone which follows in the time development after this point still moves in the column and undergoes further spreading. In other words, the front of the zone is less spread than the back due to a shorter time spent in the column. Using continuity equations, Halvorson and Ackers (2) derived a function describing the concentration profile or the elution curve, stated that if the concentration profile is Gaussian the elution curve would be a non-Gaussian, and called this physical finding an "end effect".

Let the concentration profile of a monodisperse compound on the column be expressed through the Gaussian function F(x), if the maximum is at the point y

$$F(x) = \frac{1}{\sqrt{2\pi\sigma_y^2}} \cdot e^{-\frac{1}{2}\left[\frac{(x-y)^2}{\sigma_y^2}\right]} \qquad (1)$$

Here, σ_y is the standard deviation of the Gaussian function valid for the position of the maximum in the coordinate y. The total variance of the concentration profile may be expressed as a function of the position of the maximum

$$\sigma_y^2 = \sigma_I^2 + y \cdot \sigma_u^2 \tag{2}$$

where the variances of the Gaussian functions σ_y^2 or σ_I^2 and $\sigma_u^2 = \frac{\sigma_D^2}{X}$ correspond to the total dispersion at the point y, or to the Gaussian contributions to dispersion due to injection and to the unit element of the column (σ_D^2 being a contribution to the variance caused by spreading on the column of length X). The resulting elution curve is then described by the function G(y)

$$G(y) = \frac{1}{\sqrt{2\pi(\sigma_I^2 + y\sigma_u^2)}} \cdot e^{-\frac{1}{2}\left[\frac{(X-y)^2}{\sigma_I^2 + y \cdot \sigma_u^2}\right]} \tag{3}$$

where the constant X is the coordinate of the position of the end of the column system (detector), the variable y is the coordinate of the position of the maximum of the concentration profile, which may in the real case assume values $y \leq X$, but hypothetically with respect to the solution of Eq.(3) may also become $y > X$. All quantities in Eq.(3) may be expressed in elution volume or time units.

For elution curves calculated from the given values σ_I, σ_u and X and from Eq.(3) we find the following quantities: the first statistical moment with respect to zero (3) μ_1' or the average elution volume (time) when expressing the variables in volume or time units.

$$\mu_1' = \int_0^{+\infty} y \, G(y) \, dy, \quad [y > 0] \tag{4}$$

where $\int_0^{+\infty} G(y)dy = 1$ by definition; the variance σ^2, equal to the second central moment μ_2

$$\mu_2 = \int_0^{+\infty} y^2\, G(y)\, dy - (\mu_1')^2 \tag{5}$$

and finally the third central moment μ_3, which is a measure of asymmetry of the elution curve (for the symmetrical function $\mu_3 = 0$)

$$\mu_3 = \int_0^{+\infty} y^3\, G(y)\, dy - 3\mu_1'\, \mu_2 - (\mu_1')^3 \tag{6}$$

It is also important to know the elution volume of the maximum of the elution curve V_{max}, which can be calculated if the first derivation in Eq.(3) is supposed to be zero:

$$\frac{d\, G(y)}{d\, y} = 0 \tag{7}$$

If, for the sake of simplicity, the contribution to total dispersion due to injection, $\sigma_I \approx 0$ is neglected, the solution of Eq. (7) has the form

$$\frac{1}{\sqrt{2\pi\sigma_u^2 \cdot y}} \cdot e^{-\frac{1}{2}\left[\frac{(X-y)^2}{y\,\cdot\,\sigma_u^2}\right]} \cdot \left(\frac{X^2 - y^2 - \sigma_u^2\, y}{2\,\sigma_u^2\, y}\right) = 0 \tag{8}$$

If Eq. (8) has to have a physical meaning, the condition $y > 0$ must be fulfilled. V_{max} may then be calculated from

$$V_{max} = \frac{\sqrt{(\sigma_u^4 + 4X^2)} - \sigma_u^2}{2} \tag{9}$$

Let statistical moments with respect to zero in Eqs (4,5,6) be denoted with

$$\mu'_n = \int_0^{+\infty} y^n \, G(y) \, dy \tag{10}$$

By substituting for G(y) from Eq.(3), into Eq.(10), for $n = 0, 1$ when $\sigma_I \approx 0$ and by substitution of the variables $y = z^2$, $dy = 2 z \, dz$ we obtain

$$\mu'_0 = \frac{2 \cdot e^{\frac{X}{\sigma_u^2}}}{\sigma_u \sqrt{2\pi}} \int_0^{+\infty} e^{\left[-\frac{z^2}{2\sigma_u^2} - \frac{X^2}{2\sigma_u^2 z^2}\right]} dz \tag{11}$$

$$\mu'_1 = \frac{2 \cdot e^{\frac{X}{\sigma_u^2}}}{\sigma_u \sqrt{2\pi}} \cdot \int_0^{+\infty} z^2 \cdot e^{\left[-\frac{z^2}{2\sigma_u^2} - \frac{X^2}{2\sigma_u^2 \cdot z^2}\right]} dz \tag{12}$$

$$\left[\frac{X^2}{2\sigma_u^2} > 0, \ \frac{1}{2\sigma_u^2} > 0\right]$$

The solution of μ'_0 and μ'_1 then is (4)

$$\mu'_0 = 1 \text{ (see also previous definition)} \tag{13}$$

and

$$\mu'_1 = X + \sigma_u^2 \tag{14}$$

The solution for μ'_2 and μ'_3 is obtained by calculating a general relationship (4)

$$\mu'_{n-1} = \int_{0}^{+\infty} y^{n-1} e^{\left[-\frac{\beta}{y} - \gamma y\right]} dy = 2\left(\frac{\beta}{\gamma}\right)^{\frac{n}{2}} K_n(2\sqrt{\beta\gamma}) \qquad (15)$$

$$\left[\beta > 0, \quad \gamma > 0\right]$$

where the function $K_n(q)$ may be expressed by the series (4)

$$K_n(q) = (-1)^{n+1} I_n(q) \ln\left(\frac{q}{2} + C\right) +$$

$$+ \frac{1}{2}(-1)^n \sum_{m=0}^{+\infty} \frac{\left(\frac{q}{2}\right)^{n+2m}}{m!\,(n+m)!} \left(\sum_{k=1}^{m} \frac{1}{k} + \sum_{k=1}^{n+m} \frac{1}{k}\right)$$

$$+ \frac{1}{2} \sum_{m=0}^{n-1} \frac{(-1)^m (n-m-1)!}{m!} \left(\frac{q}{2}\right)^{2m-n} \qquad (16)$$

in which C is the Euler constant. The function $I_n(q)$ may be expressed by the series of the Γ function (4)

$$I_n(q) = \sum_{k=0}^{+\infty} \frac{1}{k!\,\Gamma(n+k+1)} \left(\frac{q}{2}\right)^{n+2k} \qquad (17)$$

In practice it is more common, and with computer also more effective, to use an approximate numerical calculation of definite integrals in all cases where the search for primitive or tabulated functions or integration with power series is more difficult. Numerical integration is particularly advantageous for calculation of the μ'_n values in all cases where $G(y)$ is a more complicated distribution function, or where σ_y must be expressed by a polynomial.

Finally, let us define the parameter called asymmetry A by

$$A = \frac{\mu_3}{(\mu'_1)^3} \qquad (18)$$

The parameter A is a formal analogy of the reciprocal value of the number of theoretical plates N, which may be expressed in a similar way

$$N = \frac{(\mu_1')^2}{\mu_2} \tag{19}$$

The skew parameter γ may also be defined (5) by equation

$$\gamma = \frac{\mu_3}{\mu_2^{1.5}} \tag{19a}$$

Introduction of these parameters permits to compare the shapes of chromatograms obtained under different experimental conditions.

EXPERIMENTAL

The dispersion of a soluble low-molecular weight compound (toluene) in a solvent (tetrahydrofuran) flowing slowly through a capillary was measured using a very simple equipment. Constant flow of the solvent (0.038 ml/min) was provided by means of a home-made syringe type pump. Pure toluene (0.5 - 1 s) was injected into the stream of solvent through a six port valve (Waters Assoc., Milford, Mass., USA). Stainless-steel capillaries of various length 1 mm inner diameter, were used in the measurement. The eluate was detected with a Knauer differential refractometer, model 2025/50 (Knauer, Oberursel/Taunus, FRG), connected with a Linear model 355 recorder (Linear Instruments Corp., Irvine, Ca., USA). The measurement of the elution volume was derived from the movement of mechanical parts of the pump and was therefore very precise. The elution volume was recorded in intervals 1.25×10^{-2} ml each.

RESULTS

Eq. (5) was used in calculating the A values for various ratios $\frac{X}{\sigma_T}$ or N. Numerical integration of relationships (5) and (6) by means of a computer was carried out using Simpson's rule. Variable integration limits (upper and lower) were chosen in order to meet the condition of the smallest calculated (in the case of the computer, the smallest possible) value $G(y) = 1 \times 10^{-99}$. It was found, at the same time, that for the majority of calculated values it held that already at the minimum value $G(y) = 1 \times 10^{-20}$ and lower the result of the calculation was practically unaffected. The number of integration steps n, was chosen so that the eventual relative error was negligible. In the majority of cases it held that $10^3 < n < 10^5$. The result of the calculation namely, correlation of the N values with the asymmetry of the chromatogram A for different σ_I values, is shown in Fig. 2.

The relationship between the asymmetry A of the chromatogram and column efficiency expressed through N is linear for $\sigma_I = 0$ in the coordinates log(1/A) vs. log N, as follows from Fig. 2. The relationship may be formulated by the equation

$$A = c_1 \left(\frac{1}{N}\right)^{c_2} \qquad (20)$$

Using the method of linear regression of the values log(1/A) and log N, the constants $c_1 = 2.86816...$ and $c_2 = 1.99497...$ were calculated. The regression coefficient r = 0.999997 expresses the reliability of these constants.

For practical purposes it will be suitable to use an approximative relationship

$$A = 3\left(\frac{1}{N}\right)^2 \qquad (21)$$

If N and A were calculated using the value V_{max} from Eq. (9) instead of μ_1', the correlation of N with A gave a somewhat altered constants c_1 and c_2, and the regression coefficient of this correlation was r = 0.99993. The correlation between γ and N values obtained by the method of linear regression is formulated by

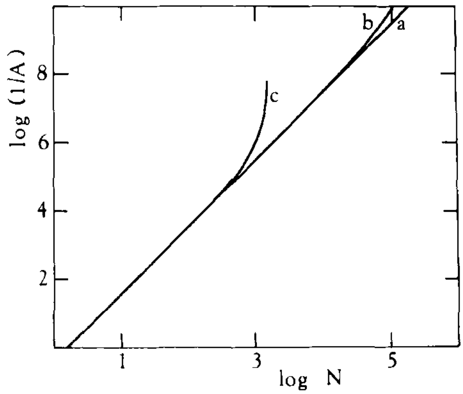

FIGURE 2. Relationship between asymmetry A of the elution curve and column efficiency characterized by the number of theoretical plates N for various injection widths σ_I.
a) $\sigma_I = 0$, b) $4\,\sigma_I = 0.01$ X, c) $4\,\sigma_I = 0.1$ X.

$$Y = c_1 \left(\frac{1}{N}\right)^{c_3} \tag{22}$$

where $c_1 = 2.86816...$ and $c_3 = 0.49497...$, but the regression coefficient is only $r = 0.99994$. The use of μ_1' values seems therefore more justified (5) although in the graphic evaluation of N the values of V_{max} are used as a rule, and the relationship (20) seems to be more correct then (22). The relationship between the logarith of the reciprocal asymmetry value and the logarithm of efficiency N under conditions where $\sigma_I > 0$ is not linear, as documented by Fig. 2. If we denote the values of A and of efficiency N in the cases of nonlinear dependence, i.e. at $\sigma_I > 0$, as A^* and N^*, then these values A^*, N^* may be converted into A, N, which correspond to the above linear dependence, using simple relationships

$$A = A^* \cdot \frac{\mu_2 - \sigma_I^2}{\mu_2} \tag{23}$$

$$N = N^* \cdot \frac{\mu_2 - \sigma_I^2}{\mu_2} \qquad (24)$$

Eqs (23) and (24) also express the fact that the injection width σ_I has no influence on the asymmetry of the elution curve A.

The procedure just described may be used in an opposite sense, i.e. to examine whether the experimentally determined elution curve has a corresponding Gaussian concentration profile on the column or not. The knowledge of the shape of the concentration profile may prove useful in e.g. the characterization of flow characteristics of the column.

An experimental demonstration of the utilizability of the above conception under real chromatographic conditions is very difficult. One of the possibilities consists in the determination of the A parameter of the elution curves during the dispersion of a soluble low-molecular weight compound introduced into a solvent which slowly flows in a tube of small diameter (in capillary). As has been shown by Taylor (6), after some time a Gaussian concentration profile is formed in such experimental system in the direction of the longitudinal axis under appropriate conditions, owing to the combined influence of molecular diffusion and variation of velocity across the cross-section of the capillary.

Experimental results obtained under conditions described in the Experimental part are summarized in Table 1 and Fig. 3. The parameters A in Table 1 were calculated using σ_I and σ_u obtained by the method of linear regression of experimental data in Fig. 3. Both Fig. 3 and Table 1 show that the validity of Eq.(2) has been sufficiently verified under the given conditions. The parameters A, both calculated and measured directly, agree fairly well, and the agreement is better with extending length of the capillary. It is quite likely that in the case of flow in a shorter capillary the concentration profile formed is not symmetrical (6); only after rather a long time the Gaussian profile is formed owing to dispersion. The cause of poorer

TABLE 1

Measured and calculated asymmetry parameters of elution curves in measurements of the dispersion of low-molecular weight soluble compound flowing in a capillary

Length of capillary (cm)	Experimental values $A \times 10^6$	Calculated values $A \times 10^6$
124	68.6	16.4
248	9.79	4.29
494	2.07	1.34

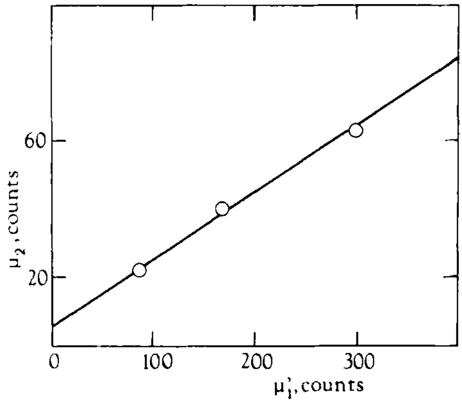

FIGURE 3. Dependence of the second central moment μ_2 on the first statistical moment μ_1' for the dispersion of soluble material in a solvent slowly flowing in the capillary (experimental conditions cf. text).

agreement between the calculated and experimental A values for a shorter capillary may also consist in additional extra column tailing. Obviously, this factor becomes the more operative the shorter the capillary is.

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