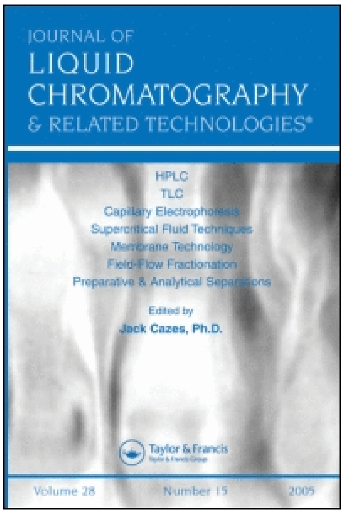

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Josef Janĉa[a]
[a] Institute of Analytical Chemistry Czechoslovak Academy of Sciences, Brno, Czechoslovakia

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RELATION BETWEEN THE ASYMMETRY OF THE ELUTION CURVE AND THE EFFICIENCY OF THE SEPARATION SYSTEM IN SIZE EXCLUSION CHROMATOGRAPHY

Josef Janča
Institute of Analytical Chemistry
Czechoslovak Academy of Sciences
611 42 Brno, Czechoslovakia

ABSTRACT

The shape of the elution curve provides information on different processes contributing to separation and dispersion. This information also includes the relation towards the concentration profile in the chromatographic column. If, e.g., the concentration profile is symmetrical, the resulting tailing of the elution curve is caused by the passage of the chromatographic profile through the column end with continuing zone broadening. The tailing will be the more marked the lower will be the efficiency of the chromatographic column. A quantitative description of the shape of the elution curve by means of the defined parameters of asymmetry and efficiency, based on statistical moments, made it possible to obtain very simple relationships between the efficiency of the chromatographic column and the asymmetry of the elution curve. In addition, the asymmetry of the elution curve was evaluated by slope analysis, which can be less complicated for practical application than the evaluation with the aid of statistical moments, and which also permits a graphical evaluation of an experimental chromatogram. It was found that an objective and regular relationship exists between the asymmetry parameters defined on the basis of statistical moments and those defined on the basis of the tangent slopes at the point of inflexion of the elution curve. The defined parameters are recommended for an objective evaluation of the shapes of elution curves instead of diverse, entirely empirical parameters, described in literature without physical basis. The results obtained are relevant particularly in those instances when the efficiency of the separation system for a given solute is relatively low, such as, e.g., in size exclusion chromatography of macromolecules.

INTRODUCTION

Various theoretical models describing chromatographic processes predict almost Gaussian elution curve in the limit of infinite elution time (1). A record of the response of a differential detector at the end of chromatographic columns (i.e., in a fixed length coordinate of the longitudinal axis of of chromatographic column or of their system) to an eluting solute as a function of the time (or of the volume of the mobile phase passed through) is called the elution curve. The limiting condition of the infinite elution time is a simplifying assumption which makes it possible to approximate the resulting elution curve by the Gaussian function. Taylor (2) derived mathematically and proved experimentally that if a soluble low-molecular-weight substance is introduced into a solvent flowing slowly in a tube of a small diameter (in a capillary), almost a Gaussian concentration profile originates in the limit of infinite time in the direction of the longitudinal axis. This is a consequence of combined influences of molecular diffusion and variation in the velocity across the capillary cross-section.

The concentration distribution along the longitudinal axis of the capillary or the chromatographic column at a given fixed time or towards a given fixed elution volume is called the concentration profile. If the concentration profile of a simple monodisperse solute is symmetrical, the resulting tailing of the elution curve originates due to the passage of this symmetrical concentration profile through the column end (detector) while zone broadening continues. Whereas a given point of the zone has already been detected, the part of the zone following this point in time development still moves in the column and is subjected to additional broadening. In other words, broadening of zone front is less than that of zone back, owing to a shorter time for which the front is present in the column. The greater will be the spreading of the concentration profile on its movement along the unit length of the column, the *more marked will be the tailing of the elution curve.* Hence it is obvious that the concentration profile and the elution curve are in mutual relationship (with respect to symmetry or asymmetry) associated with the efficiency.

The shape of the elution curve provides information on different processes contributing to separation and dispersion. In a number of instances, information of this kind is of high relevance. The information on molecular-weight distribution of the polymer sample under study is encoded in the shape of the elution curve in size-exclusion chromatography (SEC) of polymers. In the case of the flow in capillary systems (2), it is the information on the character of the concentration profile on the basis of which it is possible to estimate the shape of the velocity profile in the capillary or the value of the diffusion coefficient. A number of other possible informative data provided by the elution curve were assumed by Grushka and coworkers (3), e.g., the information on overlap and contamination of the elution curve, on conditions in the column etc. In principle it is possible to obtain some of the information mentioned above from the shape of the concentration profile, e.g., by column scanning in a given relevant instant. This way of obtaining information is, however, fairly complicated as far as the experimental equipment is concerned and cannot be realized under any arbitrary conditions.

The evaluation from the elution curves is easier. It is, however, necessary that internal relationship between the shape of the concentration profile and the shape of the elution curve should be known. The present work continues the previous study on the relationship between the efficiency of the chromatographic column system and the asymmetry of the resulting elution curves (4), generalizes the preceding conclusions and, besides the analysis by means of statistical moments, utilizes also the slope analysis, which simplifies the evaluation of experimental elution curves asymmetry by a graphical method.

Though the derived relationships are valid for elution chromatography in general, they are of practical importance only when mean or small number of theoretical plates is generated on the passage of a monodisperse solute through a separation system; in other words, when the efficiency characterized by the number of theoretical plates is relatively low for a given solute as in SEC of polymers.

THEORETICAL CONSIDERATIONS

Solution for a Gaussian Model of the Concentration Profile

In order to make mathematical operations easier, it is convenient to define the following dimensionless quantities

$$L = x/l, \quad T = t/t_R = V/V_R \qquad (1)$$

where x is a longitudinal coordinate in the direction of the chromatographic column, l is the total column length or the coordinate of the detector position, t and V are the time and the elution volume coordinates, t_R and V_R are the corresponding retention time and volume, resp. Assuming a Gaussian injection function for which it holds that $\xi_I \rightarrow 0$, the concentration profile or the elution curve can be described by the function (4)

$$F(L,T) = (1/\sqrt{\xi_T^2\ 2\pi T})\exp\left[-(L - T)^2/2T\xi_T^2\right] \qquad (2)$$

where ξ_T is the total standard deviation of the concentration profile provided that the maximum lies in the coordinate L = 1 (x = l). The concentration profile and the elution curve were described analogically in our earlier work (4). Function F(1,T) is the elution curve whereas function F(L,t) is the concentration profile at a given time, t = const.

Statistical moments (5) can conveniently be used to describe shapes of elution curves quantitatively. Let the n-th statistical moment with respect to zero μ'_n of the function F(1,T) in Eqn. 2, be defined by the following equation

$$\mu'_n = \int_0^\infty T^n \, F(1,T) \, dT \qquad (3)$$

then the n-th central moment, μ_n is defined by the relationship

$$\mu_n = \int_0^\infty (T - \mu'_1)^n \, F(1,T) \, dT \qquad (4)$$

The zeroth statistical moment with respect to zero is equal to one by definition

$$\mu'_0 = \int_0^\infty F(1,T) \, dT = 1 \qquad (5)$$

The first statistical moment with respect to zero μ'_1 of the function F(1,T), defined by Eqn. 2, has, as the only one, a direct and simple analytical solution (4)

$$\mu'_1 = 1 + \xi_T^2 \qquad (6)$$

Of interest are also the second and the third central moments, μ_2 and μ_3, respectively, which can be described by

$$\mu_2 = \int_0^\infty T^2 \, F(1,T) \, dT - (\mu'_1)^2 \qquad (7)$$

and

$$\mu_3 = \int_0^\infty T^3 \, F(1,T) \, dT - 3\mu'_1\mu_2 - (\mu'_1)^3 \qquad (8)$$

Physical meaning of the above moments is as follows: the first statistical moment with respect to zero, μ'_1, means the average elution time or volume, the second central moment, μ_2, is equal to the variation of the elution curve (i.e., to the second power of the standard deviation) and the third central moment, μ_3, is a measure of the elution curve asymmetry. For a symmetrical elution curve is $\mu_3 = 0$, for a tailed elution curve the value of μ_3 is positive and for an elution curve with a fronting it is negative.

In addition, normalized parameters were defined, making it possible to describe quantitatively the shapes of the elution curves and their mutual correlations even in such cases when they were obtained under different conditions. Parameter designated (4) asymmetry A is defined by the relationship

$$A = \mu_3/(\mu'_1)^3 \qquad (9)$$

Asymmetry A is in formal analogy with the reciprocal value of the theoretical plate number, N, which can be expressed in a similar manner

$$N = (\mu'_1)^2/\mu_2 \qquad (10)$$

Skew parameter, γ , can be defined by analogy (6)

$$\gamma = \mu_3/\mu_2^{1.5} \qquad (11)$$

A series of the values of asymmetry A of elution curves was calculated (4) numerically for given efficiencies of the chromatographic system, characterized by the number of theoretical plates, N. The resulting values of asymmetry A were correlated with the efficiencies, N, and the following relationship (4) was obtained by using the linear regression method.

$$A = c_1 N^{-c_2} \qquad (12)$$

The values obtained for constants c_1 and c_2 were 2.86816... and 1.99497..., respectively. The achieved regression coefficient, expressing the reliability of the calculated correlation, was high; $r = 0.999997$.

For practical applications the use of the approximative relationship will be feasible

$$A = 3\ N^{-2} \tag{13}$$

Correlation between the values of Υ and N led to the relationship (4)

$$\Upsilon = c_1\ N^{-c_3} \tag{14}$$

where $c_1 = 2.86816...$, $c_3 = 0.49497...$ with regression coefficient $r = 0.99994$. The quantitative description of the shapes of elution curves with the aid of the above parameters, based on the statistical moments, can be rather difficult in some cases of practical applications. In order to obtain a sufficient precision in the determination of the values of A, N or Υ , it is necessary that experimental chromatograms should be read fairly precisely particularly in both of their extreme parts. The use of elution curve in digital form, obtained with the aid of a converter of analogue output signal of the differential detector is advantageous. Sometimes the use of slope analysis may be more beneficial for both evaluation of the efficiency of the chromatographic system, characterized by parameter N, and the evaluation of the asymmetry of the elution curve obtained. Graphical evaluation of the chromatogram is also used for this purpose. In order to calculate the asymmetry, the slope of the tangent values at the points of inflexion of the frontal and back parts of the elution curve are utilized. This quantity will be designated as slope asymmetry SA

$$SA = \left(1 + \frac{S_B}{S_F}\right) = \left(1 - \frac{|S_B|}{S_F}\right) = \left(1 - \frac{a}{b}\right) \qquad (15)$$

where S_F is the slope of the tangent at the point of inflexion of the elution curve front and S_B is the slope of the tangent at the point of inflexion of the elution curve back. This evaluation may be carried out graphically in the manner demonstrated in Fig. 1.

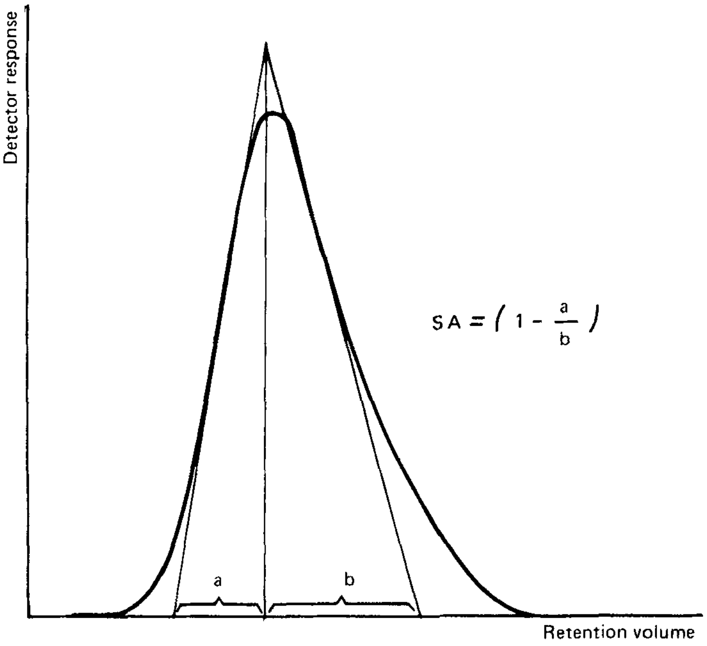

FIGURE 1.
Graphical evaluation of the slope asymmetry of the elution curve

For slope analysis one needs to know the slopes of tangents at the points of inflexion of the elution curve. First the positions of these points of inflexion in T coordinate must be found. This can be effectuated by making the second derivative of function F(1,T) equal to zero, hence

$$d^2 F(1,T)/(dT)^2 = 0 \tag{16}$$

By differentiating it is obtained

$$F(1,T)\left(\left(\frac{1}{2\xi_T^2 T^2} - \frac{1}{2T} - \frac{1}{2\xi_T^2}\right)^2 + \frac{1}{2T^2} - \frac{1}{\xi_T^2 T^3}\right) = 0 \tag{17}$$

It holds at the points of inflexion that F(1,T) $\neq 0$ and thus after the rearrangement it is obtained that

$$T^4 + 2\xi_T^2 T^3 + (3\xi_T^4 - 2)T^2 - 6\xi_T^2 T + 1 = 0 \tag{18}$$

For coordinates T of the points of inflexion, calculated by using Eqn. 18, the slopes of the elution curve are found from the first derivative of function F(1,T)

$$dF(1,T)/dT = F(1,T)(1/2\xi_T^2 T^2)(1-T^2- \xi_T^2 T) \tag{19}$$

By correlation analysis of the set of A and SA values, obtained by calculation for different values of the efficiencies of chromatographic systems it was found that

$$SA = c_4 A^{c_5} \tag{20}$$

Numerical values of constants are $c_4 = 1.140018$ and $c_5 = 0.234163...$, with the regression coefficient being $r = 0.9991$. Eqn. 20 can again be replaced for practical application with the approximative relationship

$$SA = 1.1 \sqrt[4]{A} \tag{21}$$

Although constants c_n are not integers and the procedure for their determination was based on numerical methods, the equations obtained have the meaning of a fundamental relationship between the efficiency of the chromatographic system and the contribution to the shape or asymmetry of the elution curve. The reliability of the constants obtained, expressed in terms of regression coefficient r, approaching unity, indicates justification of this statement.

The above calculations were based on the initial assumption that the injection function has a zero width and that the system of separation columns acts as a Gaussian operator. This approximation is adopted in the case of SEC when, e.g., correction of molecular-weight distribution for a longitudinal spreading is performed by solving a convolution integral the root of which is a Gaussian distribution function.

Solution for a General Dispersion Model of the Concentration Profile

Starting from a general mass balance of the infinitesimal segment of the separation column which can be considered homogeneous from the viewpoint of dispersion characteristics we can write

$$\frac{\delta C}{\delta T} = \frac{1}{Pe} \frac{\delta^2 C}{\delta L^2} - \frac{\delta C}{\delta L} \qquad (22)$$

where C is a dimensionless quantity, $C = c/c_o$, where c is the actual concentration, c_o is the concentration at the column inlet at the moment of injection and $Pe = ul/D_m$ is Peclet´s number, u is the linear velocity of the mobile phase and D_m is the diffusion coefficient of the solute in the mobile phase. Eqn. 22 can be solved with the boundary conditions

$$C(L,0) = 0 \qquad L \geq 0 \qquad (23)$$

and for the injection δ-function

$$C(T,0) = \delta(T) \qquad T \geq 0 \qquad (24)$$

For a bounded solution of function C(L,T) it must further hold

$$C(L,T) \varepsilon < 0, +\infty > \qquad L \geq 0 \qquad (25)$$

The known solution is obtained by employing the Laplace transformation

$$C(L,T) = \frac{L\sqrt{Pe}}{2T\sqrt{\pi T}} \exp\left(- \frac{Pe}{4T} (L - T)^2\right) \qquad (26)$$

The n-th derivative of the Laplace transform is equal to the n-th statistical moment of the function with the variables of the transformation approaching zero. This fact can formally be expressed by the relationship

$$\mu'_n = \lim_{p \to 0} \left((-1)^n \left[d^n \overline{C}(p)/dp^n \right] \overline{C}(p)^{-1} \right) \qquad (27)$$

where the Laplace transformation $\overline{C}(p)$ of the function C(1,T) is defined by the relationship

$$\overline{C}(p) = \int_0^\infty \exp(-pT)F(1,T)dT \qquad (28)$$

By solving Eqn. 27 for function C(1,T), the moments are obtained

$$\mu'_1 = \int_0^\infty C(1,T)T\,dT / \int_0^\infty C(1,T)dT = 1 \qquad (29)$$

where

$$\int_0^\infty C(1,T)dT = 1 \qquad (30)$$

Hence for the second and the third central moments it is obtained

$$\mu_2 = \int_0^\infty C(1,T)(T-1)^2 dT = \int_0^\infty C(1,T)T^2 dT - 1 = 2/Pe \qquad (31)$$

$$\mu_3 = \int_0^\infty C(1,T)(T-1)^3 dT = \int_0^\infty C(1,T)T^3 dT - 6/Pe - 1 = 16/Pe^2 \qquad (32)$$

By substituting from Eqns. 29-32 to definition relationships 9-11, the precise relations are obtained

$$A = 4N^{-2} \qquad (33)$$

and

$$\gamma = 4N^{-0.5} \qquad (34)$$

For slope analysis the second derivative of the function C(1,T)

$$\frac{d^2C(1,T)}{dT^2} = C(1,T)\left(\frac{Pe^2}{16T^4} - \frac{5Pe}{4T^3} + (\frac{15}{4} - \frac{Pe^2}{8})\frac{1}{T^2} + \frac{3Pe}{4T} + \frac{Pe^2}{16}\right) = 0$$

(35)

must again be known.

As at the points of inflexion it holds that $C(1,T) \neq 0$, after the rearrangement it is obtained that

$$T^4 + \frac{12T^3}{Pe} + (\frac{60}{Pe^2} - 2)T^2 - \frac{20T}{Pe} + 1 = 0 \qquad (36)$$

Coordinates of the points of inflexion are obtained by solving Eqn. 36. The slopes of tangents at these points of inflexion are obtained from the first derivative of the function C(1,T)

$$\frac{dC(1,T)}{dT} = C(1,T)\ (\frac{Pe}{4T^2} - \frac{3}{2T} - \frac{Pe}{4}) \qquad (37)$$

Hence for the relationship between SA and A it is finally valid

$$SA = 1 + \frac{2-3T_B\sqrt{A}-2T_B^2}{2-3T_F\sqrt{A}-2T_F^2}\sqrt{(\frac{T_F}{T_B})^7}\ \exp\left(\frac{-1}{\sqrt{A}}\ (\frac{1+T_B^2}{T_B} - \frac{1+T_F^2}{T_F})\right)$$

(38)

CONCLUSIONS

It follows from the above results that the relation between the shapes of the elution curve and of the concentration profile is given by a simple dependence of the contribution to the elution curve asymmetry on the efficiency of the chromatographic system. Additionally it can be stated that slope asymmetry parameter, SA, as defined, shows an obvious and precise functional dependence on the objective asymmetry parameter, A, defined on the basis of the statistical moments. This important finding permits a simple evaluation of an experimental chromatogram (also by graphical method) in an objective manner, i.e., by means of the parameter which has not (in contrast to different graphical methods used till now) only an empirical definition character. The use of SA for the evaluation of the elution curve asymmetry will be necessary particularly in those cases when the integration of the chromatogram in both marginal regions, required for the calculation of the statistical moments, would, with respect to a given chromatogram, bring about substantial inaccuracies into the calculation of the third central moment, and would thus make the accuracy of the resulting value of A doubtful.

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