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SHAPE OF THE CHROMATOGRAPHIC ELUTION CURVE DERIVED FROM THE NORMAL DISTRIBUTION OF SAMPLE IN THE COLUMN

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SUMMARY

The elution curve in linear chromatography has been derived from the assumption that the distribution of sample along the column is normal. From this normal distribution, the elution curve (the distribution of sample as a function of time) can easily be derived. This approach gives nearly the same result as other, mathematically more complex, derivations.

INTRODUCTION

Recently, the shape and the statistical moments of the elution curve have been thoroughly studied[1–3]. Even in the simplest case of a linear isotherm and complete equilibrium, the mathematical solutions to the problem of the elution curve are fairly complicated and lead to unsymmetrical peak shapes. However, the old and simple derivation of the molecular distribution along the column as a symmetrical normal curve of error is available[4,5]. It has been pointed out that this leads to an unsymmetrical elution curve (the curve which is, for example, drawn by a recorder)[6].

The purpose of the present work is to derive the equation of the elution curve, under the assumption that the distribution of molecules along the column is normal (Gaussian). It will be shown that this approach leads to a solution which is very similar to other solutions from mathematically more complex derivations.

THEORETICAL

Suppose that the distribution of molecules along the column (column profile) is given by:

$$P(x,t) = \frac{A}{\sqrt{2\pi b^2 t}} \cdot \exp\left[-\frac{(x - vt)^2}{2b^2 t}\right] \qquad (1)$$

Here $P(x,t)$ is the concentration on the column (number of molecules per unit length), A is the number of injected molecules, x is the room coordinate (distance from the column inlet) and t is the time. For a given t, eqn. 1 represent a normal distribution (where x is the independent variable, vt is the mean and b^2t is the variance) moving along the column with a linear speed v, and at the same time broadening proportional to $\sqrt{t}$.

If $x = a$, where a is the length of the column, the distribution of molecules at the outlet of the column as a function of time is given by:

$$P(a,t) = \frac{A}{\sqrt{2\pi b^2 t}} \cdot \exp\left[-\frac{(a - vt)^2}{2b^2 t}\right] \tag{2}$$

The retention time is the time for the maximum (and also for the centre of gravity and the median) of the Gaussian column profile to reach the end of the column[4]. Thus:

$$t_R = \frac{a}{v} \tag{3}$$

By definition, the number of theoretical plates, n, is given by:

$$n = \frac{a^2}{b^2 t_R} \tag{4}$$

Eqn. 2 can be transformed into:

$$P(a,t) = \frac{A}{a} \cdot \sqrt{\frac{n \cdot t_R}{2\pi t}} \cdot \exp\left[-\frac{n}{2t_R \cdot t} \cdot (t_R - t)^2\right] \tag{5}$$

The last part of the column (between $a - \mathrm{d}x$ and a) contains $P(a,t)\cdot \mathrm{d}x$ molecules. If these molecules are eluted during the time interval $\mathrm{d}t$, the function $Q(t) = P(a,t)\cdot \mathrm{d}x/\mathrm{d}t$ will describe the number of molecules per unit time which are eluted from the column. The quantity $\mathrm{d}x/\mathrm{d}t$ is the linear speed by which the sample molecules pass the last part of the column. This speed is greater for molecules which are eluted before the peak centre than for those which are eluted after the centre due to the peak broadening process.

Fig. 1 shows a room–time diagram intended to clarify this point. The line AB shows how the maximum of the Gaussian peak travels through the column: $x = vt$. A family of curves

$$x = vt + q \cdot b\sqrt{t} \tag{6}$$

can be constructed, describing how different parts of the peak travel through the column (AC, AD). Here q is the number of standard deviations ($b\sqrt{t}$) from the mean. For a given q, the area of the peak in the interval (vt, $vt + q \cdot b \cdot \sqrt{t}$) will be the same, independent of t. Thus for any q the area of the peak is divided into two parts, each having a constant area throughout the column. Differentiation of eqn. 6 yields $\mathrm{d}x/\mathrm{d}t = v + q\cdot b/2\sqrt{t}$. At the end of the column, $a = vt + q\cdot b\sqrt{t}$; $q = (a - vt)/b\sqrt{t}$. Thus, $\mathrm{d}x/\mathrm{d}t = v/2 + a/2t$ or, with eqn. 3, $\mathrm{d}x/\mathrm{d}t = a/2t_R + a/2t$. Multiplication of this expression for $\mathrm{d}x/\mathrm{d}t$ by eqn. 5 gives

$$Q(t) = \frac{A}{2} \cdot \left(\frac{1}{t_R} + \frac{1}{t}\right) \cdot \sqrt{\frac{n \cdot t_R}{2\pi t}} \cdot \exp\left[-\frac{n}{2 \cdot t \cdot t_R} \cdot (t_R - t)^2\right] \tag{7}$$

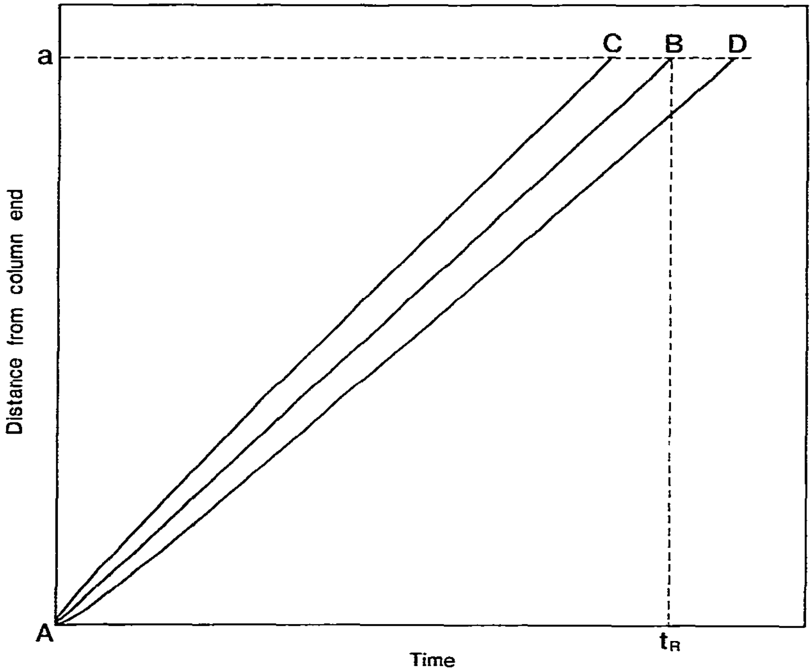

Fig. 1. Room–time diagram. For details see text.

which describes the distribution of the molecules as a function of time expressed as the number of molecules per unit time. This is the elution curve, which will be sensed by the detector and drawn on a recorder if the moving distribution along the column is normal (Gaussian).

Eqn. 7 can be written as

$$Q(t) = \frac{A}{2} \cdot \left(1 + \frac{t}{t_R}\right) \cdot \mathrm{f}(t) \tag{8}$$

where:

$$\mathrm{f}(t) = \frac{a}{\sqrt{2\pi b^2 t^3}} \cdot \exp\left[-\frac{(a - vt)^2}{2b^2 t}\right]$$

For the function f(t) above, Wasan[7] has given a formula for the calculation of the rth zero-point moment a_r:

$$a_r = \left(\frac{a}{v}\right)^r \cdot \sum_{s=0}^{r-1} \frac{(r-1+s)!}{s!(r-1-s)!} \cdot \left(2\frac{a \cdot v}{b^2}\right)^{-s} \tag{9}$$

Thus, zero-point moments and, by conventional procedures, central moments for the distribution in eqn. 7 can be calculated:

$$c_2 = t_R^2 \cdot \left(\frac{1}{n} + \frac{5}{4 \cdot n^2}\right)$$

$$c_3 = t_R^3 \cdot \left(\frac{3}{n^2} + \frac{11}{2 \cdot n^3}\right) \tag{10}$$

$$c_4 = t_R^4 \cdot \left(\frac{3}{n^2} + \frac{45}{2 \cdot n^3} + \frac{633}{16 \cdot n^4}\right)$$

Here c_r is the rth central moment. The mean, m (first zero-point moment), is calculated from:

$$m = t_R\left(1 + \frac{1}{2n}\right) \tag{11}$$

Parameters of interest are also the skew (g_1) and the excess (g_2). They are defined as:

$$g_1 = \frac{c_3}{c_2^{3/2}}; \qquad g_2 = \frac{c_4}{c_2^2} - 3 \tag{12}$$

It is interesting to study the moments and related quantities since the relations between them characterize the shape of the elution curve. Several workers[1,2] have found the following relations

$$g_1 = \frac{3}{\sqrt{n}} \text{ and } g_2 = \frac{15}{n} \tag{13}$$

which have been experimentally tested[2]. If eqns. 10 and 12 are combined, the following expressions result for the elution curve described in eqn. 7:

$$\begin{aligned} g_1 &= \frac{1}{\sqrt{n}} \cdot \frac{3 + \frac{11}{2n}}{\left(1 + \frac{5}{4n}\right)^{3/2}} \\ g_2 &= \frac{15}{n} + \frac{483 - \frac{375}{16n}}{n^2 + \frac{5}{2}n + \frac{25}{16}} \end{aligned} \tag{14}$$

Table I shows the effect of inserting a value of $n = 500$ into eqns. 13 and 14 to show the differences between these relations.

It is to be noted that, if the derivation of the elution curve is made under the assumption that $dx/dt = a/t$, a slightly different function than that in eqn. 7 results. This (questionable) function has exactly the same properties as given by eqn. 13. As can be seen from Table I, the differences between eqns. 13 and 14 are for most uses negligible. The second function can therefore be used and is given by:

$$R(t) = A\sqrt{\frac{n \cdot t_R}{2\pi t^3}} \cdot \exp\left[-\frac{n}{2t \cdot t_R} \cdot (t_R - t)^2\right] \tag{15}$$

TABLE I

COMPENSATION OF TWO DIFFERENT EXPRESSIONS FOR SKEW AND EXCESS ($n = 500$)

	From eqn. 13	*From eqn. 14*
g_1	0.13416	0.13415
g_2	0.030	0.0319

The parameters which are most usually calculated from an experimental chromatogram are the retention time, the number of theoretical plates, the skew and the excess. For this, the experimental parameters $\hat{m}$ and $\hat{c}_2$ — $\hat{c}_4$ are usually calculated according to the following formulae[8]

$$\hat{m} = \frac{\Sigma x_i y_i}{\Sigma y_i}; \qquad \hat{c}_r = \frac{\Sigma (x_i - m)^r \cdot y_i}{\Sigma y_i} \tag{16}$$

where x_i and y_i are the coordinates for points on the chromatogram and $r = 2, 3,$ or 4.

Determination of the retention time t_R

Usually, the retention time t_R is determined either from the mean $\hat{m}$ or the x coordinate of the maximum. From eqn. 11 it can be seen that:

$$t_R = \frac{m}{1 + \frac{1}{2n}} \tag{17}$$

The error, assuming $t_R = \hat{m}$, is thus 0.1% if $n = 500$. If the accuracy of the measurements justifies this, n can be calculated (see below) and inserted into eqn. 17 to obtain, a more accurate value of t_R.

In order to obtain the x coordinate of the maximum, eqn. 7 is differentiated to give:

$$\frac{dQ}{dt} = \frac{A}{2} \cdot \frac{n \cdot t_R}{2} \cdot \frac{1}{t} \cdot \exp\left[\frac{-n}{2t \cdot t_R} \cdot (t - t_R)^2\right] \cdot \frac{n}{t^3 \cdot 2t_R^2} \cdot$$
$$\cdot \left[t_R^3 + t \cdot t_R^2 \left(1 - \frac{3}{n}\right) - t^2 t_R \left(1 + \frac{1}{n}\right) - t^3 \right] \tag{18}$$

If this is equated with 0, a third-degree equation results. The non-imaginary solution is close to:

$$t_{\text{max.}} = t_R\left(1 - \frac{1}{n}\right) \tag{19}$$

The error, assuming $n = 500$, is 0.2%, if the maximum of the elution curve $t_{\text{max.}}$ is used as an estimation of t_R.

Determination of n

n is usually calculated from $\hat{m}^2/\hat{c}_2$. According to the above theory

$$\frac{m^2}{c_2} = \frac{t_R^2}{t_R^2} \cdot \frac{\left(1 + \frac{1}{2n}\right)^2}{\left(\frac{1}{n} + \frac{5}{4n^2}\right)} = n - \frac{1}{4} + \frac{9}{16\left(n + \frac{5}{4}\right)} \tag{20}$$

where the two last terms are negligible for realistic values of n, making the calculation of n from $\hat{m}^2/\hat{c}_2$ quite acceptable.

CONCLUSION

It has been shown that, if a normal (Gaussian) distribution of molecules along the column (column profile) is assumed, it is possible and relatively easy to deduce the equation for the elution curve (exactly in eqn. 7, approximately in eqn. 15). This elution curve has the following properties. Neither the maximum point nor the first moment corresponds exactly to the retention time (defined as the time when the maximum of the column profile appears at the outlet). The discrepancy is small (eqns. 17 and 19) and can be corrected for if the number of theoretical plates is known. The latter can be calculated from eqn. 20.

It should be noted that, for the case of a linear isotherm and complete equilibrium, the mathematical form for the column profile is much more simple than for the elution curve, and this behaviour is probably general. It is believed that, if it were possible to find, in the general case, a transformation from the elution curve to the column profile (to change the independent variable from time to distance from the column inlet) and *vice versa*, the equations for the chromatographic peaks could be more easily derived as column profiles and transformed into elution curves. Alternatively, the experimental elution data could be transformed and compared with theoretically derived column profiles.

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