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# THE CONCENTRATION PROFILE OF SUBSTANCES SEPARATED ON THIN-MEDIA CHROMATOGRAMS

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## SUMMARY

The present paper brings a theoretical study to determine the concentration profile in a zone of separated substance in thin-layer chromatography. If the solution is applied to the origin as a narrow band uniform over the entire width of the substrate, the theoretical concentration profile of the zone is a Gaussian curve. If the width of the applied band cannot be neglected, the zone profile becomes the integral of a Gaussian curve. In the case of the Gaussian profile the amount of solute retained in the zone is proportional to the peak concentration value. Several reasons why concentration profiles observed in practice may deviate from the Gaussian distribution are discussed.

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## INTRODUCTION

Computer processing is at present one of the most efficient ways of extracting information from experimental data. There is, however, a general rule of information theory which may be applied and that is, that results obtained by processing the primary information are the better, the more is known in advance about the general structure of the processed data. Applied to chromatography this means that any prediction which can be made and then utilized about the character and the distribution of the separated zones to be analyzed will improve both the sensitivity and accuracy of any efforts made to assess the chromatogram in a quantitative manner.

The concepts considered in this paper, though very general in nature, are intended to apply mainly to optical procedures of quantitative evaluation. The ultimate sensitivity of this method is limited by noise[1–3]. Though much can be done by appropriate design of the photometric equipment to reduce the noise content in the final output signal, there are, nevertheless, limits beyond which further improvement can be obtained only by expensive and inconvenient trade-offs in the design and operation of the equipment. And, of course, a certain amount of noise can never be avoided. This noise is determined by basic physical principles such as temperature, the atomic structure of matter, quantum effects, etc.; it is, however, far below the level likely to be achieved in any practical scanning device.

The difference between the amount of noise actually present in the final signal and the theoretical minimum is called 'excess noise'; subsequent processing in a suitable way makes it possible to remove part of this excess noise thus improving the fundamental parameters of the method (accuracy, sensitivity, threshold, resolving power, reproducibility). The most convenient processing procedure for measured data is with the aid of a digital computer[4,5]; in most cases this will prove to be more convenient and economic than the development of more sophisticated and complex primary measuring devices and procedures. The inherent flexibility of computer processing permits in addition to the removal of a large part of the excess noise also a variety of other operations, which would otherwise have to be performed manually probably with little enthusiasm.

## THEORETICAL PART

### *Curve fitting*

Efforts to reduce the noise content of the photometric scan of a chromatogram include two basic operations. One is the establishment of a flat and smooth baseline serving as a reference axis, from which all other photometric values are measured. All common computer methods of restoring the unperturbed baseline of a chromatogram recording are based upon the assumption that noise deviations to either side from the idealized baseline have equal probability of occurrence. At higher noise levels this assumption may not be totally justified and a small shift in the restored baseline may result. The baseline of the blank chromatogram is assumed to be a straight line usually at zero level.

A similar argument applies when a zone of separated substance is encountered. Here the noise amplitudes may be assumed to be evenly divided on both sides of the unperturbed photometric profile of the zone. In order to apply the highly efficient noise elimination techniques used for the smoothing of the baseline over the blank parts of the chromatogram the idealized photometric profile must be known.

The term "noise" can here be taken in a broader sense than usual. It can for example be considered to include the influence of zones other than the one under consideration. Knowledge of the idealized photometric profile is thus tantamount to an increase in the resolving power of the chromatogram.

### *Simplifying assumptions*

Information (*a priori*) about the photometric profile of a separated zone may be obtained both from empirical measurements and from theoretical predictions; empirical data must obviously be based upon a sufficiently large number of individual measurements in order to eliminate inevitable fluctuations. Although theoretical predictions do not suffer from the latter problem the very complex character of the physical processes involved in chromatographic separations makes a theoretical treatment feasible only if many simplifying assumptions are introduced. In individual cases these assumptions may be justified to a varying degree. Any results obtained, therefore, may show varying degrees of agreement with observed situations. The best practical approach therefore appears to be a combination of both approaches, that is an idealized curve as predicted by theory but with individual parameters corrected so as to yield the best agreement with observation. A considerable advantage of the analytic approach as compared with the purely empirical one is that analytically obtained zone profiles are usually determined by few parameters, for instance the Gaussian profile is fully determined by just two parameters, the peak value of concentration in the zone and the distance between *e.g.* the 40% amplitude points. Instead of the latter value the distance from the origin can sometimes be used with the same result as will be shown later (see eqn. 24); this distance is of course much easier to establish than the zone width.

The need for simplifying assumptions has already been argued and the most important ones so far as this paper is concerned are the following:

(1) It will be assumed that the chromatographic medium is macroscopically homogeneous and isotropic. End-effects (which have been discussed in a previous paper[6]) near the edges of the active part of the medium are neglected.

(2) The boundary surfaces between the mobile and the stationary phases shall in the average be parallel to the longitudinal axis (the $x$-axis) of the medium (Fig. 1).

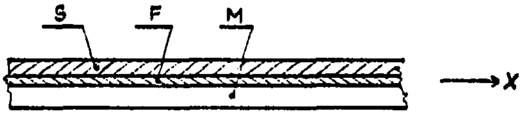


Fig. 1. Enlarged cross section of the medium (schematic). S = Solid phase; F = stationary liquid layer; M = mobile liquid.

The average direction of flow of the mobile phase shall also be parallel to the $x$-axis. The interchange of solute between stationary and mobile phases shall, however, in the average be perpendicular to $x$. Jointly these assumptions allow us to reduce the mathematical treatment to the one-dimensional case with the $x$-coordinate and time $t$ as the only independent variables. It is implied that the solute is applied as a uniform strip across the medium. It will also be assumed that the drift velocity $v$ of the mobile phase is sufficiently small so as to enable the distribution of solute between mobile phase and stationary phase to be everywhere near equilibrium. $v$ shall in the average be constant everywhere throughout the mobile phase.

*Mechanism of separation*

Two different mechanisms are in general responsible for the process of separation in thin-media chromatography; these are partition and adsorption. Nearly always both are involved though to varying degrees. Partition involves the liquid part of the stationary phase; adsorption occurs at the surface of the solid part of the stationary phase.

In the case of partition only the concentration of solute in the stationary liquid $c(\xi,t)_F$ is proportional to the concentration $c(x,t)_M$ in the adjacent elements of the moving phase. ($\xi$ refers to the longitudinal coördinate in the stationary phase, $x$ to that in the mobile phase). It will be assumed that both values are well below the limit of saturation. At constant temperature then the following simple relation holds[7]:

$$c(\xi,t)_F = \alpha \cdot c(x = \xi,t)_M \tag{1}$$

Let $\delta$ be the ratio between the volumes of the stationary liquid and of the moving solvent. The proportion of solute retained in the stationary liquid film is then $\alpha\cdot\delta$.

$$Q(\xi,t)_{\mathrm{F}} = \alpha\cdot\delta\cdot c(\xi,t)_{\mathrm{M}} \tag{2}$$

A similar relationship obtains for adsorption. The number of adsorbing sites available at the boundary surface between the liquid film and the solid substrate is finite. If the texture of the substrate is sufficiently homogeneous, the density of adsorbing sites can be conveniently referred to the unit of volume instead of to the unit of surface of the adsorbing medium.

From thermodynamic considerations it can be shown that the amount of solute adsorbed per unit of volume of the adsorbing medium is approximately proportional to the concentration in the adjoining elements of the liquid phase, provided that only a relatively small fraction of the available sites actually becomes occupied. This again means, of course, that the system is operating well below the saturation threshold.

$$c(\xi,t)_{\mathrm{a}} = \beta\cdot c(\xi,t)_{\mathrm{F}} \tag{3}$$

The coefficient $\beta$ in this expression depends upon the difference in the free energies of a particle of solute when dissolved in the solvent and when adsorbed and upon the total density of adsorbing sites in the solid phase.

Let $\varepsilon$ be the ratio between the volumes of the solid phase and the moving solvent. The proportion of solute retained through adsorption on the surface of the solid phase is then:

$$Q(\xi,t)_{\mathrm{a}} = \varepsilon\beta\cdot c(\xi,t)_{\mathrm{F}} = \varepsilon\alpha\beta\cdot c(\xi,t)_{\mathrm{M}} \tag{4}$$

Addition of expressions 2 and 4 yields the total proportion of solute retained in the stationary phase:

$$Q(\xi,t)_{\mathrm{S}} = c(\xi,t)_{\mathrm{M}}\cdot\alpha[\delta+\varepsilon\beta] \tag{5}$$

Consider now a volume element d$V$ in the shape of a parallelepiped with cross section unity and with length d$x$ (Fig. 2). The longitudinal axis of this parallelepiped is supposed to be parallel to the average direction $x$ of flow of the mobile phase.

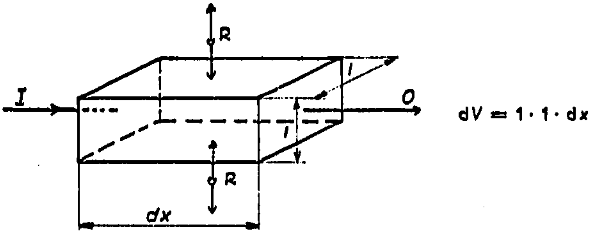


Fig. 2. A volume element d$V$ of the mobile phase. I = Inflow of solute (diffusion and/or convection); O = outflow of solute (diffusion and/or convection); R = inflow/outflow of solute (retention and release).

If the concentration of solute inside the element d$V$ is to change there has to be a net difference between inflow and outflow of solute. Three different processes are basically responsible for that: these are the flow of solvent in the mobile phase, diffusion both in the moving and in the stationary phases and finally interchange of solute between these two phases.

*Solvent flow.* The three processes mentioned above will now be treated separately with the changes due to solvent flow to be considered first. Implied is linearity of the system as a whole, so that the principle of superposition can be applied. Though certainly not rigidly valid, this assumption appears to be a good approximation to the real situation.

Let the longitudinal coordinate in the stationary part of the system be $\xi$, in the mobile part $x$. Obviously

$$x = \xi + v \cdot t \tag{7}$$

The total differential of $x$ is then:

$$\mathrm{d}x = \mathrm{d}\xi + v\mathrm{d}t \tag{7a}$$

The rate of change of concentration in the mobile phase due to the transport velocity $v$ alone is then:

$$v = \frac{\mathrm{d}x}{\mathrm{d}t}$$

$$-\frac{\mathrm{d}c(x)_{\mathrm{M}}}{\mathrm{d}t} = \frac{\partial c(x)_{\mathrm{M}}}{\partial x} \cdot \frac{\mathrm{d}x}{\mathrm{d}t} = \frac{\partial c(\xi,t)_{\mathrm{M}}}{\partial x} \cdot v \tag{8}$$

*Diffusion.* Diffusion is a process which quite generally tends to equalize all non-uniformities in the distribution of substance. It occurs here in the mobile phase as well as in the stationary phase. In the latter it operates both in the liquid and in the solid parts.

The rate of change of the concentration of solute due to thermal diffusion is determined by Fick's well known (second) law:

$$\frac{\partial c(x,t)}{\partial t} = D \cdot \frac{\partial^2 c(x,t)}{\partial^2 x} \tag{9}$$

Here $D$ is the coefficient of diffusion (the diffusivity) of the diffusing substance. It depends not only upon the diffusing substance itself but also upon the nature of the medium where diffusion takes place. The values of $D$ for a given solute in the solvent and on the surface of the solid phase will, therefore, in general be different.

Let the coefficient of diffusion in the liquid phase be $D_{\mathrm{L}}$ and the coefficient of surface diffusion at the boundary between solid and liquid phase be $D_{\mathrm{a}}$. Fick's law then yields the following relations:

$$\frac{\partial c(x,t)_{\mathrm{M}}}{\partial t} = D_{\mathrm{L}} \frac{\partial^2 c(x,t)_{\mathrm{M}}}{\partial x^2} \tag{10a}$$

In the liquid part of the stationary phase (see eqn. 3) the relation is:

$$\frac{\partial c(\xi,t)_{\mathrm{F}}}{\partial t} = D_{\mathrm{L}} \frac{\partial^2 c(\xi,t)_{\mathrm{F}}}{\partial \xi^2} \approx D_{\mathrm{L}} \frac{\alpha \partial^2 c(\xi,t)_{\mathrm{M}}}{\partial \xi^2} \tag{10b}$$

An analogous relation holds for the adsorbing surfaces of the solid phase:

$$\frac{\partial c(\xi,t)_{\mathrm{a}}}{\partial t} = D_{\mathrm{a}} \cdot \alpha \cdot \beta \cdot \frac{\partial^2 c(\xi,t)_{\mathrm{M}}}{\partial \xi^2} \tag{10c}$$

### *Retention and release of solute from the stationary phase*

When the concentration of solute in some part of the mobile phase changes due to diffusion or convection, the amount of solute retained in the adjoining elements of the stationary phase changes as well. The result is an exchange flow of solute between the two phases. The direction of this flow is perpendicular to the $x$-axis. Under quasi-equilibrium conditions the concentration gradient in the direction of the flow can be disregarded. With these assumptions the resulting change in the amount of stored solute can be calculated as follows. All values are again referred to the unit of volume of the mobile phase.

$$-[\Delta c(\xi,t)_{\mathrm{M}}]_{\mathrm{R}} = \Delta c(\xi,t)_{\mathrm{a}}\cdot\varepsilon + \Delta c(\xi,t)_{\mathrm{F}}\cdot\delta + \Delta c(\xi,t)_{\mathrm{M}}\cdot\alpha(\delta+\varepsilon\cdot\beta) \tag{11}$$

The index R of the left-hand term in the equation above refers to retention. The first two terms on the right-hand side of eqn. 11 are given in expressions 10b and 10c. The last term is determined by the sum of a diffusion and a convection term shown in expressions 10a and 8, respectively.

$$\Delta c(\xi,t)_{\mathrm{M}} = [\Delta c(\xi,t)_{\mathrm{M}}]_{\mathrm{D}} + [\Delta c(\xi,t)_{\mathrm{M}}]_{\mathrm{C}}$$

Substituting the partial results obtained in the equations quoted yields:

$$-\frac{\partial}{\partial t}[c(\xi,t)_{\mathrm{M}}]_{\mathrm{R}} = \frac{\partial^2 c(\xi,t)_{\mathrm{M}}}{\partial\xi^2}[D_{\mathrm{a}}\cdot\alpha\cdot\beta\cdot\varepsilon + D_{\mathrm{L}}\cdot\alpha\cdot\delta + D_{\mathrm{L}}\alpha(\delta+\xi\beta)] - \frac{\partial c(\xi,t)_{\mathrm{M}}}{\partial\xi}\cdot v\cdot\alpha(\delta+\varepsilon\beta)] \tag{12}$$

For simplicity the following abbreviations will be used:

$$D_{\mathrm{a}}\cdot\alpha\cdot\beta\cdot\varepsilon + 2D_{\mathrm{L}}\alpha\cdot\delta + D_{\mathrm{L}}\alpha\cdot\varepsilon\beta = K$$
$$\alpha(\delta+\varepsilon\beta) = L$$

Expression 13 is the final equation which determines the rate of change of solute content in the unit of volume (that is the rate of change of concentration) in the mobile phase due to the retaining action of the stationary phase.

$$-\frac{\partial}{\partial t}[c(\xi,t)_{\mathrm{M}}]_{\mathrm{R}} = \frac{\partial^2 c(\xi,t)_{\mathrm{M}}}{\partial\xi^2}\cdot K - \frac{\partial c(\xi,t)_{\mathrm{M}}}{\partial\xi}\cdot v\cdot L \tag{13}$$

To obtain the total rate of change of concentration there, the effects of pure convection and diffusion have to be taken into account. This results in the following equation:

$$\frac{\partial c(\xi,t)}{\partial t} = \frac{\partial^2 c(\xi,t)}{\partial\xi^2}[D_{\mathrm{L}} - K] - \frac{\partial c(\xi,t)}{\partial\xi}\cdot v\cdot(1-L) \tag{14}$$

The index M, indicating the mobile phase, is not any more required and has therefore, been discontinued.

The Laplace transform is commonly applied to the solution of equations of this type. Putting in eqn. 14

$$c(\xi,t) = A.e^{-u\xi}\cdot e^{pt} \tag{15}$$

yields

$$p = u^2(D_{\mathrm{L}} - K) - uv(1-L) \tag{16}$$

The substitutions $D_L - K = G$ and $1 - L = H$ serve to further shorten the notation; introducing them first into expression 16 and then into eqn. 15 yields:

$$u\xi + pt = u^2\cdot Gt - u\cdot(vHt - \xi) = Gt\left(u - \frac{vHt - \xi}{2Gt}\right)^2 - \frac{(vHt - \xi)^2}{4Gt} \qquad (17)$$

Eqn. 15 now becomes:

$$c(\xi,t,u) = A\cdot e^{-\frac{(vHt - \xi)^2}{4Gt}} \cdot e^{Gt\left(u - \frac{vHt - \xi}{2Gt}\right)^2} \qquad (15)$$

The desired system response can now be found by applying the inverse Laplace transform to eqn. 14a. It will be assumed that the mathematical prerequisites for this operation are met and that the operation is permissible. From the basic rules of the Laplace transform we then obtain:

$$c(\xi,t) = A\cdot e^{-\frac{(vHt - \xi)^2}{4Gt}} \int_{a-j\infty}^{a+j\infty} \exp\left[Gt\left(u - \frac{vHt-\xi}{2Gt}\right)^2\right]\mathrm{d}u \qquad (18)$$

Using the substitution:

$$u - \frac{vHt - \xi}{2Gt} = z$$

$$\mathrm{d}u = \mathrm{d}z$$

we can write the Laplace integral in eqn. 18 in the form:

$$\int_{a-j\infty}^{a+j\infty} e^{Gtz^2}\,\mathrm{d}z = \sqrt{\frac{1}{\pi Gt}} \qquad (19)$$

The solution of eqn. 18 is:

$$c(\xi,t) = A\cdot\frac{1}{\sqrt{\pi Gt}}\cdot e^{-\frac{(\xi - vHt)^2}{4Gt}} \qquad (20)$$

The coefficient $A$ can be determined from the boundary conditions of the system at $t = \xi = 0$. It turns out to be equal to the concentration of solute in the original strip applied to the origin.

$$A = c(0,0) \qquad (21)$$

## DISCUSSION

Examination of expression 20 shows that the density profile of the separated zone is represented by a Gaussian curve. This result is valid under the assumptions made, that is if the band of solvent applied to the medium is very (theoretically vanishingly) narrow. In practice this is frequently not the case. The density profile then becomes slightly wider and flattened; this will be shown at the end of this paragraph.

The axis of the zone moves along the medium with a speed $C$ equal to

$$C = vH \simeq v[1-\alpha(\delta+\varepsilon\beta)] \tag{22}$$

The right-hand part of the term in the brackets represents, however, the proportion of solute retained in the stationary phase. It is expressed as a fraction of the amount of solute contained in the unit of volume of the mobile phase, that is as a proportion of the concentration there. The more solute the stationary phase is able to accept, the more is the solute retarded relative to the solvent. A comparison with the common definition of the $R_F$ value shows that

$$R_F = \alpha(\delta+\varepsilon\beta) \tag{23}$$

The width $W$ of a separated zone measured at 40% amplitude is approximately equal to two times the square root of the denominator in the exponent of eqn. 20.

$$W = 4\sqrt{Gt}$$

The term $G$ depends partly upon the diffusivity of the solute in the various phases involved and upon the retention volume of the stationary phase. No simple interpretation is available. The term $\sqrt{t}$, on the other hand, indicates that a separated zone flattens out with increasing distance $d$ from the origin.

$$d = v \cdot t \tag{24}$$

The coefficient $\sqrt{\frac{1}{Gt}}$ in front of the Gaussian function in eqn. 20 shows that the peak value of concentration in a zone declines with its width. It is easy to show, however, that the total area of the zone remains unchanged. In consequence of this the amount of solute contained in a zone with ideally Gaussian concentration profiles is fully determined by the peak concentration in the zone. The width $W$, which is difficult to measure, is not needed to this purpose nor is integration required.

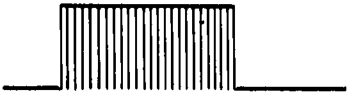
More often than not the applied band of solution at the origin is relatively wide; the approximation by a very narrow pulse (a Dirac function) used in the derivation of eqn. 20 thus requires some correction. For this purpose it will be assumed that the initial pulse is rectangular in shape with width $W_1$. A pulse of this shape can, however, be considered as the sum of closely adjoining elementary pulses of the Dirac type (Fig. 3). The concentration profile produced can then be found as the sum (integral) of similarly spaced responses.

$$c(\xi,t)_r = 0 = \int_{\theta=-W_1/2}^{\theta=+W_1/2} c(\xi+\theta,t)\,\mathrm{d}\theta \tag{25}$$

As long as $W_1$ is not too large the concentration profile retains the general character of the Gaussian distribution defined by eqn. 20 (Fig. 4).

### *Skew symmetry of the concentration profile*

In practice sometimes a skew symmetrical shape of the concentration profile is observed. The reason for this deviation from the theory leading to eqn. 20 is the limited validity of some of the assumptions made. The most important one in this



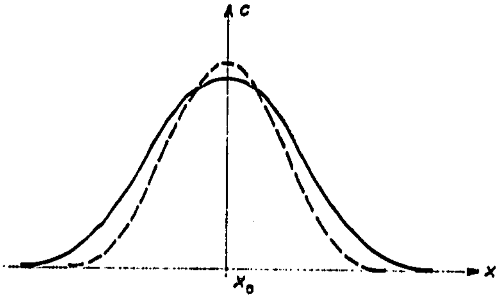


Fig. 3. Rectangular pulse of finite width as sum of narrow pulses (Dirac functions).

Fig. 4. Zone profile for a band of finite width applied at the origin. — — —, Gaussian response for infinitely narrow band.

regard seems to be the assumption that the concentrations in the mobile and in the stationary phases are always and everywhere in thermodynamic equilibrium. At higher solvent speeds deviations from equilibrium seem, however, to exist. These deviations will be the larger, the larger the relative velocity of the moving particles of solvent against the accepting volume elements of solvent (and partly the adsorbing surface elements in the solid phase). The reason is of course that less time is then available to reach a quasi-equilibrium state.

The relative velocity of particles diffusing in the mobile phase against the acceptor elements in the stationary phase is largest at the leading edge of a zone. Oppositely particles of solute diffusing in the stationary phase have the largest relative velocity against their presumptive acceptor elements in the mobile phase at the trailing side of a zone (Fig. 5). In general diffusion in the mobile zone prevails. The result of increased relative velocity at the leading side of a zone is a decrease of the coefficient $K$ (see eqn. 13) and consequently an increase of coefficient $G$. From eqn. 23 it can be seen that this causes the concentration profile of the leading part of the zone to become wider than that of the trailing side. The concentration profile of the zone tends, therefore, to become skew symmetrical.

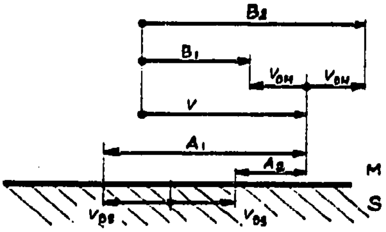


Fig. 5. The relative velocities of diffusing particles and the retaining elements. S = Stationary phase; M = mobile phase; $A_1$,$A_2$ = relative velocity of particles diffusing in the stationary phase against volume elements of solvent in the moving phase; $B_1$,$B_2$ = relative velocity of particles diffusing in the mobile phase against retaining volume elements in the stationary phase.

## ACKNOWLEDGEMENTS

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