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## Separation Science and Technology

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

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**To cite this Article** Kawasaki, Tsutomu(1982) 'A Theory of Stepwise Elution Chromatography with Small Sample Loads: Effect of the Top of the Column', Separation Science and Technology, 17: 5, 695 — 716
**To link to this Article: DOI:** 10.1080/01496398208068561
**URL:** http://dx.doi.org/10.1080/01496398208068561

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SEPARATION SCIENCE AND TECHNOLOGY, 17(5), pp. 695–716, 1982

# A Theory of Stepwise Elution Chromatography with Small Sample Loads: Effect of the Top of the Column*

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## Abstract

A theory of stepwise elution chromatography was developed on the basis of a continuity equation for a simplest case of linear chromatography with small sample loads. Longitudinal diffusions taken into consideration are limited to both thermal Brownian diffusion occurring in the mobile phase and diffusion caused by the flow heterogeneity. However, account is taken of the existence of the column top, where longitudinal Brownian diffusion can be assumed to occur associated with a diffusion that is provoked by a type of flow heterogeneity. This paper provides a fundamental theory that was necessary for the earlier development of the theory of gradient hydroxyapatite chromatography.

## INTRODUCTION

Theories of chromatography on the basis of a continuity equation were first developed over 40 years ago by Wilson (*1*), DeVault (*2*), and Weiss (*3*) for stepwise chromatography. In these theories, both assumptions of (a) instantaneous equilibrium of mobile and stationary phases and (b) no longitudinal diffusion in the column are involved; the two assumptions are intimately related (*1–3*). Theories in which account is taken of longitudinal molecular diffusion were developed later by a number of authors (*4–14*). However, longitudinal diffusion that was mainly investigated in these

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*The terminology "stepwise" can, of course, be applied to the special case where the development of the solute is carried out on the column by using the same solvent as that of the sample solution that was initially loaded.

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theories is a type of diffusion that would occur provided that the transit rate of a molecule is finite through an energetical barrier that presumably lies between the mobile and stationary phases in chromatography. As a typical example that would be explainable by this diffusion, Bak (*9*) refers to the asymmetrical shape of the chromatographic spot occurring in paper chromatography which, in general, is more extended in the migration direction.

It can be assumed, however, that this type of diffusion is negligible if the flow rate of chromatography is small in comparison with the transit rate of the molecule through the energetical barrier. This can, in fact, be deduced to be the case (at least) with hydroxyapatite (HA) adsorption chromatography, where no deformation of the chromatogram or the change in elution position is observed when the flow rate is changed (*15*).

In the present theory, longitudinal diffusions taken into account are limited to (a) thermal Brownian diffusion occurring in the mobile phase and (b) diffusion due to the flow heterogeneity occurring in each vertical section of the column. [Diffusion due to the flow heterogeneity is a concept that is intimately related to the concept of "eddy diffusion." Here, we avoid the use of this terminology, however (*15*).] Diffusion that might occur in the stationary phase in the case of absorption chromatography (see, for instance, ref. *10*) is not taken into consideration. We also limit ourselves within the case of "linear" chromatography with small sample loads when a narrow band of molecules is formed initially at the top of the column. The assumptions that are involved in the present theory are summarized in Theoretical Section A.

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The main purpose of the present work consists in proposing (under the assumptions in Theoretical Section A) an initial boundary condition to the fundamental continuity equation of chromatography which would fit the actual situation where a narrow molecular band is formed initially at the top of the column. Account is taken of the existence of a column top where longitudinal Brownian diffusion can be assumed to occur associated with a diffusion that is provoked by a type of flow heterogeneity (see Theoretical Section A). Intuitively, it might be assumable that the inital condition to the fundamental continuity equation can approximately be described (a) replacing the actual column by a hypothetical column with inifinite length that extends upward beyond the top of the column, and (b) representing the initial molecular band occurring at the column top in terms of a delta function that has a value only at a given longitudinal position on the infinite column (see Ref. *15*, Appendix III); this type of assumption, in fact, is involved explicitly or implicitly in the chromatographic theories (*4–14*). From this assumption, however, an unreasonable general conclusion is attained that a part of the molecular band should be leaking upward beyond the column top in early stages of the development process (Ref. *15*, Appendix III). Further, the relationship between the shape of the molecular band migrating on the hypothetical infinite column and the elution chromatogram obtained for the actual column with finite length (with the bottom as well as the top) is unclear except for the special case when the longitudinal molecular diffusion in the column is provoked essentially only by the flow heterogeneity (Ref. *15*, Appendix III; cf. Remark 1 in Theoretical Section A).

In earlier papers (*15–18*) a theory of gradient HA chromatography was developed. In this theory the assumption of the delta function occurring initially on the infinite column (see above) is also involved. In contrast with stepwise chromatography, with gradient chromatography it is impossible for a chromatographic process to be described on the basis of a continuity equation for the actual molecular flux occurring on the column itself; it is an abstract flux occurring on the gradient that is fundamental (*17*). As a result, the assumption of the delta function on the infinite column leads to a different unreasonable conclusion from that attained with stepwise chromatography (see above); a limit of the application of this assumption to gradient chromatography was discussed (*18*). In Refs. *19* and *20* it was shown that the initial boundary condition to the continuity equation for stepwise chromatography that is proposed in the present paper can be applied with modifications to the abstract continuity equation for gradient chromotography. A second purpose of the present work is to provide a fundamental theory that was necessary for further developing the earlier theory of gradient HA chromatography (*15–18*) to the new theory (*19, 20*). Therefore, terminologies that are adequate only to HA chromatography are often used. Nevertheless, the present theory is valid for wider chromatographies.

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## THEORETICAL

### A. First Principle and Fundamental Assumptions

Coexistences of mobile and stationary phases in the column are a necessary condition for chromatography. Under this condition, a first principle of chromatography is realized that, within a vertical section at any longitudinal position $L$ on the column, the ratio $R_F$ of the migration rate of the molecules (of the chromatographic component under consideration) to that of the solvent, *on average*, is equal to the partition $B$ of the molecules in the mobile phase. The average is taken for all molecules under consideration that pass the column section during the whole process of chromatography. This principle can also be stated in such a way that, provided the column is long enough for the total molecular band to exist on it at the same time, then at any instant $t$, the mean relative migration rate $\bar{R}_F$, of the band is equal to the partition $\bar{B}$ in solution concerning all molecules that constitute the total band. $R_F$ and $B$ are equal to $R_F$ and $B$ occurring at the mean part of the band, respectively; at this part, $R_F(=\bar{R}_F)$ is equal to $B(=\bar{B})$. (Cf. Ref. *10* and Remark 1 below.)

Let us introduce the following assumptions. (a) Molecular diffusion can occur only in the interstitial liquid in the column or the mobile phase. (b) The pore volume $\alpha$ per unit column length is macroscopically constant; as a result, at a given instant $t$, the mean flow rates of the solution are equal to one another among any different vertical column sections. (c) The mean flow rate is also constant with respect to time $t$. (d) Due to microscopical heterogeneity in interspaces among crystals packed in the column, however, flow heterogeneity, in general, does occur in the column. Therefore, the longitudinal diffusion in the column, in general, is the addition of two types of diffusion: thermodynamic diffusion and diffusion provoked by the flow heterogeneity. Thermodynamic diffusion is defined as any diffusion occurring, provided there is no flow heterogeneity. Under Assumption (g) below, however, thermodynamic diffusion is identical with the diffusion occurring caused by a thermal Brownian motion of molecules in solution. Now, if the column is divided into a number of parallel hypothetical columns with diameters of the order of magnitude of the interdistances among the crystals being packed, the flow heterogeneity would be classifiable into two types. Thus, when caused by the heterogeneity in interspaces among packed crystals, the flow rate would fluctuate at random not only among different longitudinal positions on the same microcolumn but also among parts of different microcolumns existing within the same vertical section of the total column; this brings about the first type of flow heterogeneity. Due to a viscous property of the solution, however, it might be possible that the flow rate in an interstice in the column depends upon the distance from the crystal surface. Therefore, even within a microcolumn, flow heterogeneity is realizable (the second type of flow heterogeneity). (e) The effect of the second type of flow heterogeneity, if it occurs, is so small that it does not virtually provoke heterogeneity in molecular density in the interstice between the crystals. Nevertheless, the movement of the molecules in an interstice in the column, in general, is disturbed by the second type of flow heterogeneity. Under this hypothesis, it is impossible to distinguish chromatographically the molecular diffusion occurring caused by the second type of flow heterogeneity from thermal Brownian diffusion; the addition of the two diffusions would behave as an element of the chromatographic mechanism (cf. Remarks 2 below). (f) The average time that is necessary for a molecule to traverse the interdistance $d$ between crystals in the column by thermal Brownian diffusion (to which the effect of the second type of flow heterogeneity is added) is much shorter than that necessary for it to move the same distance with a rate that is

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equal to the mean flow rate, $|\mathbf{v}_0|$, of the solvent (occurring in the longitudinal direction of the column). (g) The time averages that are necessary for a molecule to transit the energetical barriers from the mobile to the stationary phase and from the latter to the former (cf. Introduction Section) also are much shorter than the time necessary for it to move distance $d$ with the rate equal to $|\mathbf{v}_0|$. Under this assumption, thermodynamic diffusion is identical with thermal Brownian diffusion (cf. Ref *15*, Discussion Section). (h) Both energetical and geometrical interactions among sample molecules are negligible; chromatography is "linear," and the behavior of the molecular component under consideration is independent of the behaviors of the other components in the mixture. Under Assumptions (e)–(h), the partition $B$ of molecules in solution is constant for any vertical column section; $B$ is constant also with respect to time $t$, and it is equal to the mean partition $\bar{B}$. (i) A minute volume of the sample solution that is smaller than, or equal to, the critical volume $\Delta L'$ (see Section B) is initially loaded at the top of the column; at the beginning of the development process the sample also occupies a volume that is smaller than, or equal to, $\Delta L'$ at the top of the column. In other words, the total width of the molecular band occuring at the column top at the beginning of the development process is smaller than, or equal to, $\Delta L = \Delta L'/\alpha$.

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***Remark 1.*** The quasi-static chromatographic process treated in the earlier papers (*15–20*) is a special case of chromatography when a thermodynamic equilibrium is locally realized within any elementary volume $\delta V$ in the column and when the longitudinal molecular diffusion is carried out, caused essentially only by flow heterogeneity (see Ref. *15*, Introduction Section). In this instance the ratio $(R_F)_{\delta V}$ of the migration rate of the sample molecules to the migration rate of the solvent at any part of the molecular band is equal to the partition $B_{\delta V}$ of the molecules in solution occurring in the elementary volume $\delta V$ existing at that part (see Ref. *15*, Introduction Section). It should be noted, however, that the migration rate of the solvent in a $\delta V$, in general, is different from the mean rate occurring in the vertical column section in which the $\delta V$ is involved. As a result, even when $B_{\delta V}$ or $(R_F)_{\delta V}$ is constant for any $\delta V$, the ratio $(R_F')_{\delta V}$ of the migration rate of the sample molecules *in $\delta V$* to the mean migration rate of the solvent *in the column section in which $\delta V$ is involved* is not constant, whereas it can be assumed that the mean migration rate of the solvent in a column section is constant for any section of the total column. This means that the ratio $R_F$ of the mean migration rate of the sample molecules in a vertical column section to the mean migration rate of the solvent in the same column section (this latter being constant for any column section; see above), in general, is not equal to the mean value $B$ of $B_{\delta V}$ in the corresponding column section; these are equal to each other only in the column section in which the mean part of the molecular band exists. This is because the molecular densities in different $\delta V$'s within the same column section, in general, are different from one another; this is the reason why longitudinal diffusion occurs caused by flow heterogeneity.

***Remark 2.*** In the theory in Refs. *15–18*, the effect of the second type of flow heterogeneity *a priori* is neglected. However, this theory treats the special case of the quasi-static process when thermodynamic longitudinal diffusion (being identical with thermal Brownian diffusion; see Ref. *15*, Discussion Section) is negligible in comparision with the diffusion occurring due to the total flow heterogeneity (cf. Remark 1 above). If Assumption (e) is applied to the theory in Refs. *15–18*, a conclusion is attained that the second type of flow heterogeneity should also be negligible; this is because it is the addition of Brownian diffusion and diffusion due the second type of flow heterogeneity that behaves as an element of the chromatographic mechanism. This conclusion is consistent with the assumption that *a priori* is involved in Refs. *15–18* (see above; cf. the argument in Ref. *19*).

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## B. Chromatography in the Absence of the First Type of Flow Heterogeneity; Critical Width $\Delta L$ and Critical Volume $\Delta L'$ at the Column Top*

It would, in general, be possible to represent the thermal Brownian diffusion coefficient (to which the effect of the second type of flow heterogeneity is added; denoted by $\hat{D}$) as

$$\hat{D} = \overline{\Delta l^2}(\tau)/2\tau \qquad (1)$$

where $\tau$ is a time interval, and $\overline{\Delta l^2}(\tau)$ is the mean-square-displacement of a molecule occurring in $\tau$. Equation (1) is called as an Einstein equation in the case of thermal Brownian diffusion only. Due to Assumption (e) in Section A, however, Eq. (1) would be valid even taking into account the effect of the second type of flow heterogeneity. Equation (1) can be rewritten as

$$\sqrt{\overline{\Delta l^2}(\tau)}/\tau = 2\hat{D}/\sqrt{\overline{\Delta l^2}(\tau)} \qquad (1')$$

where the left-hand term would have a meaning of the mean migration rate of a molecule occurring in a distance $\sqrt{\overline{\Delta l^2}}$; this rate decreases with an increase in $\sqrt{\overline{\Delta l^2}}$ (see Eq. 1′). Assumption (f) is Section A states that the rate

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*Considerations made in both Sections B and C are partially originated in the considerations made in Ref. *21* and Appendix II in Ref. *22*.

$\sqrt{\overline{\Delta l^2}/\tau}$ is much higher than the mean flow rate $|\mathbf{v}_0|$ of the solvent if $\sqrt{\overline{\Delta l^2}}$ is of the order of magnitude of the distance $d$ between crystals packed in the column. Let us estimate the distance $\sqrt{\overline{\Delta l^2}}$ occurring when $\sqrt{\overline{\Delta l^2}/\tau}$ is equal to $|\mathbf{v}_0|$. This estimation is easy; we have for the distance $\Delta\tilde{L}$ that is twice as large as this distance:

$$\Delta\tilde{L} = 4\hat{\Theta}_0 \tag{2}$$

where

$$\hat{\Theta}_0 = \hat{D}/|\mathbf{v}_0| \tag{3}$$

has a dimension of length.

Our purpose here and in Section C is to represent an idealized chromatographic process occurring in the absence of the first type of flow heterogeneity in terms of the fundamental continuity equation for the molecular flux in the column. By solving this equation under a suitable initial condition, the distribution in the total molecular density $\Omega$ in the column (i.e., the density concerning both mobile and stationary phases) would be representable as a function of both time $t$ (or elution volume $V$) and the longitudinal distance $L$ from the column top. We mention below, however, that provided (a) that the molecular band with a width that is smaller than, or of the same order of magnitude as, $\Delta\tilde{L}$ is initially formed at the top of the column [It should be noted, however, that in the arguments below, the existence of the column top plays a role of a sufficient condition. The argument below is valid provided a narrow molecular band is initially formed at an intermediate position on the column with or without the top. However, this column should definitively be distinguished from the hypothetical infinite column without the top (see Section C). For the argument in the presence of the first type of flow heterogeneity, the existence of the column top is a necessary condition, since it is only near the top of the column that the effect of the first type of flow heterogeneity is negligible (see Sections D and E).] and (b) that the longitudinal distance on the column where the center of the molecular band migrates is still smaller than, or of the same order of magnitude as, $\bar{R}_F\Delta\tilde{L}/2$, then it, in principle, is impossible to represent the molecular distribution $\Omega$ on the basis of the continuity equation. Under this situation, the general initial boundary condition to the continuity equation is inconceivable. Thus, due to thermal Brownian motion of molecules (to which the effect of the second type of flow heterogeneity is added), the partition $B$ of the molecules in the mobile phase occurs, whereas it is $B$ that determines the mean migration velocity $\mathbf{v}_0\bar{R}_F$ of the molecular band. $\mathbf{v}_0\bar{R}_F$ is equal to $\mathbf{v}_0 B$ because $\bar{R}_F$ is equal to $\bar{B}$ (first principle of chromatography; Section A), and $\bar{B}$ is equal to $B$ [result

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arising from Assumptions (e)–(h) in Section A]. On the other hand, again due to the random Brownian motion of molecules (to which the effect of the second type of flow heterogeneity is added), the initial distribution of the molecules at the column top (occurring immediately after the sample load has been finished) would be random. It can further be assumed that, while the center of the molecular band migrates a distance that is smaller than, or of the same order as, $\bar{R}_F \Delta\tilde{L}/2$ or $B\Delta\tilde{L}/2$, the molecules (which formed initially the band with the width that was smaller than, or of the same order of magnitude as, $\Delta\tilde{L}$) move almost at random, *around* their initial positions, a mean distance that is larger than, or almost equal to, the migration distance of the center of the band. [In the presence of the column top, however, the probability of the migration of the molecules in the direction of the flow due to Brownian motion (to which the effect of the second type of flow heterogeneity is added) would be higher than the probability that they migrate in the opposite direction.] As a result, the molecular distribution $\Omega$ still is random. A random general initial condition to the continuity equation that finally produces a random general distribution $\Omega$ is inconceivable.

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We mention below, however, that if $B$ is small, an initial boundary condition to the continuity equation is conceivable. Thus, let us introduce a critical width

$$\Delta L = \gamma \Delta\tilde{L} = 4\gamma\hat{\Theta}_0 \tag{4}$$

at the top of the column where $\gamma$ is a positive constant that is greater than, or equal to, unity. The $\gamma$ value is of the order of magnitude of unity, however. Now, if the partition $B$ of molecules in the mobile phase is small, a large volume of the solvent in comparison with the total interstitial volumes

$$\Delta L' = \alpha \Delta L \tag{5}$$

within $\Delta L$ would pass $\Delta L$ while (almost) all molecules are eluted out of $\Delta L$. Under this situation, the amount of molecules that is eluted out of $\Delta L$ while a volume of the solvent that is equal to $\Delta L'$ passes would be virtually equal to the mean amount of molecules that stay in the mobile phase in $\Delta L$ during this time interval. This hypothesis, in fact, is representable in terms of an ordinary differential equation, which should be solvable under conservation conditions. The solution can be applied as a boundary condition to the fundamental continuity equation (see Section C). The problem now is how the parameter $\gamma$ (Eq. 4) can be evaluated. This also will be considered in Section C.

### C. Continuity Equation of Chromatography, Boundary Condition, and the Solution of the Equation

Modifying Eq. (1) in the earlier paper (*15*), the fundamental continuity equation in the absence of the first type of flow heterogeneity can be written as

$$\mathrm{div}_L\,(\mathbf{v}_0 C - \hat{D}\,\mathrm{grad}_L\,C) + \frac{\partial \Omega}{\partial t} = 0 \tag{6}$$

where

$$C = B\Omega \tag{7}$$

represents the molecular concentration in the interstitial liquid (or the mobile phase) in the column. Since

$$|\mathbf{v}_0| = \frac{1}{\alpha}\,\frac{dV}{dt} \tag{8}$$

where $V$ is the elution volume, and since $B$ is constant (Section A), Eq. (6) can be rewritten, by using Eq. (3), as

$$\hat{\Theta}\,\frac{\partial^2 \Omega}{\partial L'^2} = \frac{\partial \Omega}{\partial L'} + \frac{\partial \Omega}{\partial W} \tag{9}$$

where

$$L' = \alpha L \tag{10}$$

$$W = BV \tag{11}$$

and

$$\hat{\Theta} = \alpha \hat{\Theta}_0 \tag{12}$$

$L'$ (Eq. 10), with dimensions of volume, represents the sum of interstitial volumes that are involved between position $L$ and the column top; $W$ (Eq. 11) has a physical meaning of the sum of interstitial volumes involved between the column top and the position of the infinitesimal molecular band occurring, provided there is no longitudinal diffusion. $W$, therefore, gives the distance (expressed in units of volume) between the column top and the mean part of the actual molecular band migrating on the column (see Ref. *15*,

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Appendix III); $W$ increases linearly with an increase in $V$ (Eq. 11). $\Theta$ (Eq. 12), with dimensions of volume, is a positive constant under both Assumptions (b) and (c) in Section A.

Provided the initial distribution

$$\Omega(W=0, L') = \Omega_0(L') \tag{13}$$

is given on a hypothetical column with an infinite length that extends even to minus values of $L$ or $L'$, then Eq. (9) has a solution

$$\Omega(W, L') = \frac{1}{\sqrt{4\pi\hat{\Theta} W}} \int_{-\infty}^{\infty} \Omega_0(L'') e^{-(L'-W-L'')^2/4\hat{\Theta} W} dL'' \tag{14}$$

the derivation of which is shown for a more general case in the Appendix in Ref. *20*. Especially when

$$\Omega_0(L') = \delta(L') \tag{15}$$

where $\delta$ represents a delta function, Eq. (14) reduces to

$$\Omega_\delta(W, L') = \frac{1}{\sqrt{4\pi\hat{\Theta} W}} e^{-(L'-W)^2/4\hat{\Theta} W} \tag{16}$$

On the other hand, on the basis of the consideration made in the last paragraph in Section B, the boundary condition to Eq. (9) should be given as a solution of the following differential equation:

$$-\frac{d\Omega}{d(V/\Delta L')} = C \tag{17}$$

or

$$-\frac{d\Omega}{dW} = \frac{1}{\Delta L'}\Omega \tag{17'}$$

Due to linearities in both Eqs. (9) and (17'), it is possible to give $\Omega$ in Eq. (17') not only the *intensive* meaning of the molecular density at position $L = \Delta L$ on the column but also an *extensive* meaning of the amount of molecules existing within the critical width $\Delta L$ (cf. the argument in Ref. *22*, Theoretical Section D). Under a normalized conservation condition

$$\int_0^\infty C\, dV = \int_0^\infty \Omega\, dW = 1 \tag{18}$$

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Eq. (17′) has a solution

$$\Omega = \frac{1}{\Delta L'} e^{-W/\Delta L'} \tag{19}$$

which represents the boundary condition to Eq. (9) fulfilled at $L' = \Delta L'$ (see above). What we should do now is (a) estimate the value of $\Delta L'$, $\Delta L$, or $\gamma$ (Eq. 4), and (b) rewrite Eq. (19) into a form of Eq. (13); how the actual situation where the column has a top can be consistent with the hypothesis behind Eq. (13) that the column extends even to minus values of $L$ or $L'$ will also be considered.

The value of $\Delta L'$ can be estimated on the basis of a hypothesis that, when the center of the molecular band is far below the position $L = \Delta L$ on the actual column, or when $W \gg \Delta L'$, then the molecular density $\Omega$ observed at $L = \Delta L$ (although it should be very low) should coincide asymptotically with the hypothetical density at postion $L = 0$ on the infinite column occurring, provided the initial molecular distribution is given by the delta function (Eq. 15). Under this situation, the total molecular band should have a very large width; both the width $\Delta L$ and the initial width of the band within $\Delta L$ should be negligible. In other words, they should be virtually infinitesimal. This would mean that the right-hand side of

$$\frac{1}{\Delta L'} e^{-W/\Delta L'} \xrightarrow[W \to \infty]{} e^{-W/\Delta L'}$$

and the extreme right-hand side of

$$\lim_{L' \to 0} \Omega_\delta (W, L') = \frac{1}{\sqrt{4\pi\hat{\Theta} W}} e^{-W/4\hat{\Theta}} \xrightarrow[W \to \infty]{} e^{-W/4\hat{\Theta}}$$

coincide asymptotically. It follows from this that

$$\Delta L' = 4\hat{\Theta} \tag{20}$$

$$\Delta L = 4\hat{\Theta}_0 \tag{21}$$

and

$$\gamma = 1 \tag{22}$$

(see Eqs. 4, 5, and 12).

Equation (19) can now be rewritten as

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$$\left.\begin{aligned} &\Omega(W \geq 0, L' = 4\hat{\Theta}) = \frac{1}{4\hat{\Theta}}\, e^{-W/4\Theta} \\ \text{and} \\ &\Omega(W < 0, L' = 4\hat{\Theta}) = 0 \end{aligned}\right\} \tag{23}$$

where the second equality has been added only for convenience' sake. We consider below the rewriting of Eq. (23) into the form of Eq. (13). Thus, introducing a transformation

$$\omega = \Omega e^{[W-2(L'-4\hat{\Theta})]/4\hat{\Theta}} \tag{24}$$

Eq. (9) can be rewritten as

$$\frac{\partial \omega}{\partial W} = \hat{\Theta}\frac{\partial^2 \omega}{\partial L'^2} \tag{25}$$

Under any boundary conditions:

$$\omega(W \geq 0, L' = 4\Theta) = 1/4\Theta \tag{26}$$

and

$$\left.\begin{aligned} &\omega(W = 0, L' < 4\hat{\Theta}) = 1/2\hat{\Theta} \\ &\omega(W = 0, L' = 4\hat{\Theta}) = 1/4\hat{\Theta} \\ \text{and} \\ &\omega(W = 0, L' > 4\hat{\Theta}) = 0 \end{aligned}\right\} \tag{27}$$

Equation (25) has the same solution:

$$\omega(W \geq 0, L') = \frac{1}{4\hat{\Theta}} \operatorname{erfc}\left(\frac{L' - 4\hat{\Theta}}{\sqrt{4\hat{\Theta}W}}\right) \tag{28}$$

where

$$\operatorname{erfc}(u) = \frac{2}{\sqrt{\pi}}\int_u^\infty e^{-u'^2}\,du' \tag{29}$$

is called as a complimentary error function. On the other hand, by using Eq. (24), Eq. (26) can be rewritten as the first equality in Eq. (23), whereas Eq. (27) becomes

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$$\left.\begin{array}{l}\Omega(W=0,\,L'<4\hat{\Theta})=\Omega_0(L'<4\hat{\Theta})=\dfrac{1}{2\hat{\Theta}}\,e^{(L'-4\Theta)/2\Theta}\\[2ex] \Omega(W=0,\,L'=4\hat{\Theta})=\Omega_0(L'=4\hat{\Theta})=1/4\hat{\Theta}\\[1ex] \text{and}\\[1ex] \Omega(W=0,\,L'>4\hat{\Theta})=\Omega_0(L'>4\hat{\Theta})=0\end{array}\right\}\qquad(30)$$

This means that Eq. (30) (with the form of Eq. 13) is a boundary condition to Eq. (9), which is equivalent to the boundary condition given by Eq. (23) (but see below). By substituting Eq. (30) into Eq. (14), the solution of Eq. (9):

$$\Omega(W\geq 0,\,L')=\frac{1}{4\hat{\Theta}}\,e^{[2L'-4\hat{\Theta})-W]/4\hat{\Theta}}\,\mathrm{erfc}\left(\frac{L'-4\hat{\Theta}}{\sqrt{4\hat{\Theta}W}}\right)\qquad(31)$$

is obtained; Eq. (31) can also be obtained if Eq. (28) is substituted into Eq. (24).

It should be emphasized, however, that Eq. (31) was originally derived under the boundary condition given by Eq. (19) or the first equality in Eq. (23), and not Eq. (30). From the physical meaning of Eq. (19) (see the last paragraph in Section B), Eq. (31) has meaning only when $L'\geq 4\hat{\Theta}$ or $L\geq 4\hat{\Theta}_0$. Thus, writing

$$\left.\begin{array}{l}\Omega(W\geq 0,\,L'\geq 4\hat{\Theta})=\dfrac{1}{4\hat{\Theta}}\,e^{[2(L'\ 4\hat{\Theta})-W]/4\hat{\Theta}}\,\mathrm{erfc}\left(\dfrac{L'-4\hat{\Theta}}{\sqrt{4\hat{\Theta}W}}\right)\\[3ex] \Omega(W\geq 0,0<L'<4\hat{\Theta})=\dfrac{1}{L'}\,e^{-W/L'}\\[2ex] \quad\Omega(W\geq 0,\,L'\leq 0)=0\\[1ex] \text{and}\\[1ex] \qquad\Omega(W<0,\,L')=0\end{array}\right\}\qquad(32)$$

the first equality in Eq. (32) states that the part $L'\geq 4\hat{\Theta}$ of the distribution $\Omega$ on the actual column *with the top* is identical with the corresponding part of the distribution occurring on the hypothetical infinite column *without the top*. On the hypothetical column, the distribution $\Omega$ even extends to minus $L'$ values (see Eq. 31). Further, provided $B$ is small (the last paragraph in Section B), $\Omega$ in the first equality in Eq. (32) can also have the meaning of the relative concentration $C/B$ (see Eq. 7) of molecules in solution that have just been eluted out of the column with length $L$ [$= L'/\alpha$ (Eq. 10)]. $L$ is larger

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than, or equal to, $4\hat{\Theta}_0$. In fact, when $B$ is small, the volume of the solvent over which a chromatogram appears is much larger than $\Delta L' = 4\hat{\Theta}$ (the last paragraph in Section B). This means that the shape of the total chromatogram is hardly influenced by the flow of molecules that proceed backward on the column *caused by thermal Brownian diffusion* (*to which the effect of the second type of flow heterogeneity is added*).

The molecular distribution $\Omega$ occurring in the part $0 < L < 4\hat{\Theta}_0$ or $0 < L' < 4\hat{\Theta}$ of the actual column is indescribable due to the importance of random motions of molecules (Section B). However, if the meaning of the relative concentration $C/B$ of molecules in solution that have just eluted out of the column is given to $\Omega$, the second equality in Eq. (32) can be derived on the basis of the same consideration as in the derivation of Eq. (19). The last two equalities in Eq. (32) have been added only for convenience' sake.

Finally, in the extreme case when $\hat{\Theta}_0$ or $\hat{\Theta}$ tends to zero or when there is absolutely no longitudinal diffusion, Eqs. (23) and (30) reduce to

$$\lim_{\hat{\Theta}\to+0} \Omega(W, L' = 4\hat{\Theta}) = \lim_{\hat{\Theta}\to+0} \Omega(W, 0) = \delta(W) \tag{33}$$

and

$$\lim_{\hat{\Theta}\to+0} \Omega(W=0, L') = \lim_{\hat{\Theta}\to+0} \Omega_0(L') = \delta(L') \tag{34}$$

respectively; Eq. (32) reduces to

$$\lim_{\hat{\Theta}\to+0} \Omega(W, L') = \delta(L' - W) \tag{35}$$

### D. Chromatography in the Presence of the First Type of Flow Heterogeneity; Continuity Equation and Some Assumptions for Boundary Conditions

The fundamental continuity equation can be written, slightly modifying Eq. (1) in Ref. *15*, as

$$\mathrm{div}_L(\mathbf{v}_0 C - DB\,\mathrm{grad}_L\,\Omega - \hat{D}\,\mathrm{grad}_L\,C) + \frac{\partial \Omega}{\partial t} = 0 \tag{36}$$

where $D$ is the diffusion coefficient for diffusion due to the first type of flow heterogeneity, which is proportional to $|\mathbf{v}_0|$ (see Ref. *15*, Appendix III). Therefore, introducing a positive proportionality constant $\Theta_0$ with a dimension of length, $D$ can be represented as

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$$D = \Theta_0 |\mathrm{v}_0| \tag{37}$$

Corresponding to Eq. (9), Eq. (36) can be rewritten as

$$(\Theta + \hat{\Theta}) \frac{\partial^2 \Omega}{\partial L'^2} = \frac{\partial \Omega}{\partial L'} + \frac{\partial \Omega}{\partial W} \tag{38}$$

where

$$\Theta = \alpha \Theta_0 \tag{39}$$

is a positive constant with dimensions of volume (cf. Eq. 12).

Let us consider a boundary condition of Eq. (38). From its mechanism it is reasonable to assume that the first type of flow heterogeneity cannot occur near the top of the column (cf. Remark 1 below). This means that, near the column top, the value of the parameter $\Theta$ tends to zero, and that Eq. (38) reduces to Eq. (9); Eq. (23), which is the boundary condition of Eq. (9), should be fulfilled at position $L = 4\hat{\Theta}_0$ even in the presence of the first type of flow heterogeneity (cf. Remark 1 below). It would, therefore, be a good approximation to apply Eq. (23) directly to Eq. (38). In order to solve Eq. (38) under this boundary condition, it is necessary to rewrite Eq. (23) into a form of Eq. (13). Unfortunately, however, this generally is not easy. This is because, in order for the aspects in both parts $L \geq 0$ and $L < 0$ of the hypothetical infinite column to be describable in terms of a common continuity equation, the total diffusion coefficient occurring in the part $L < 0$ should be $D + \hat{D}$ rather than $\hat{D}$, so that Eq. (30) cannot be applied. We therefore treat only the special case where longitudinal Brownian diffusion (to which the effect of the second type of flow heterogeneity is added) is negligible in comparison with diffusion due to the first type of flow heterogeneity (Section E; cf. Remark 2 below).

***Remark 1.*** With thermal Brownian diffusion, the diffusion coefficient $D_T$ can be represented as

$$D_T = \frac{\overline{\Delta\lambda^2(\tau)}}{2\tau} = \frac{a^2}{2\tau^\circ} = \frac{a}{\tau^\circ}\,\frac{a}{2} \tag{a}$$

where $\overline{\Delta\lambda^2(\tau)}$ is the mean-square-displacement of a molecule occuring in a time interval $\tau$, and the first equality represents the Einstein equation (cf. Eq. 1). However, by using a mean-free-pass $a$ occurring in a mean-free-time $\tau^\circ$, $D_T$ can also be represented as the second or the last term in Eq. (a); it is assumed that the molecular collision occurs instantaneously. In the extreme right-hand side in Eq. (a), $a/\tau^\circ$ represents the mean migration rate of a molecule occurring in the free-pass. Equation (a) has a precise meaning only

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when $\tau \gg \tau^{\circ}$ or when $\sqrt{\overline{\Delta\lambda^2}} \gg a$. Equation (a) can be compared with the case of diffusion occurring due to the first type of flow heterogeneity. Thus, introducing positive constants

$$\bar{a} = 2\Theta_0 \tag{b}$$

and

$$\overline{\tau^{\circ}} = 2\Theta_0/|\mathbf{v}_0| \tag{c}$$

with a dimension of length and a dimension of time, respectively, Eq. (37) can be rewritten as

$$D = \frac{\bar{a}}{\overline{\tau^{\circ}}} \frac{\bar{a}}{2} \tag{d}$$

It now is possible to give $\bar{a}$ and $\overline{\tau^{\circ}}$ meanings of apparent free-pass and apparent free-time of a molecule concerning longitudinal diffusion in the column that is provoked by the first type of flow heterogeneity, respectively. In parallel with the case of Brownian diffusion, it is only when the square-root of the mean-square-displacement is larger than $\bar{a}$ that the parameter $D$ can have a precise meaning of the diffusion coefficient. Diffusion due to the first type of flow heterogeneity with diffusion coefficient $D$, therefore, is conceivable only at column positions where the distances from the column top are larger than $\bar{a}$ or $2\Theta_0$. It can be assumed that the first type of flow heterogeneity begins to decline when the distance from the column top decreases to attain the order of magnitude of $2\Theta_0$; at the column top, it vanishes completely. Diffusion due to the first type of flow heterogeneity, in general, is conceivable only when $\Theta_0 \gg \hat{\Theta}_0$ since, unless $\Theta_0 \gg \hat{\Theta}_0$, it cannot be distinguished from diffusion due to the second type of flow heterogeneity. This means that, at column position $L = 4\hat{\Theta}_0$, the effect of the first type of flow heterogeneity is negligible.

***Remark 2.*** Equation (23) can also be applied with modifications to the abstract continuity equation for gradient chromatography (see Introduction Section). In this instance, due to the form of the continuity equation, it is unnecessary to rewrite Eq. (23) (modified form) into an Eq. (13)-type equation; the former can be used as a direct boundary condition to the differential equation. As a result, a chromatogram can be calculated by taking into account all effects of the first and second types of flow heterogeneities, and thermal Brownian diffusion (*19, 20*).

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### E. The Case When Longitudinal Thermal Brownian Diffusion (to which the effect of the second type of flow heterogeneity is added) Is Negligible in Comparison with Diffusion Due to the First Type of Flow Heterogeneity

This is the case similar to that treated in earlier papers (*15–18*). Equation (38) reduces to

$$\Theta \frac{\partial^2 \Omega}{\partial L'^2} = \frac{\partial \Omega}{\partial L'} + \frac{\partial \Omega}{\partial W} \tag{40}$$

It can be assumed that, at the top of the column, the first type of flow heterogeneity is negligible, and that Eq. (23) is fulfilled (Section D). In the absence of longitudinal thermal Brownian diffusion (to which the effect of the second type of flow heterogeneity is added), however, Eq. (23) reduces to a delta function (Eq. 33).

Now, in order for the hypothesis above to be realized, it is sufficient that the relationships

$$\left.\begin{aligned} &\lim_{\substack{L' \to +0 \\ \Theta \to +0}} \Omega(W, L'; \Theta) = \delta(W) \\ \text{and} \\ &\int_0^\infty \lim_{L' \to +0} \Omega(W, L'; \Theta)\, dW = 1 \end{aligned}\right\} \tag{41}$$

be fulfilled, where the second equality represents a conservation condition (cf. Eq. 18). Further, in order for Eq. (41) to occur, it is sufficient that the relationships

$$\left.\begin{aligned} &\lim_{L' \to +0} \Omega(W \geq 0, L'; \Theta) = \frac{1}{\sqrt{4\pi\Theta W}} e^{-W/4\Theta} \\ \text{and} \\ &\lim_{L' \to +0} \Omega(W < 0, L'; \Theta) = 0 \end{aligned}\right\} \tag{42}$$

be fulfilled. Under the boundary condition given by Eq. (42), Eq. (40) has a solution

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$$\left.\begin{aligned}&\Omega(W \geq 0, L') = \frac{1}{\sqrt{4\pi\Theta W}}\, e^{-(L'-W)^2/4\Theta W}\\ \text{and}\\ &\Omega(W < 0, L') = 0\end{aligned}\right\} \tag{43}$$

where the second equality appears only formally.

The first equality in Eq. (43) shows that, at the beginning of chromatography when $W \rightarrow +0$, a band of molecules with an infinitesimal width is formed at the top ($L' = 0$) of the column. It also shows formally that, when $W > 0$, the band appears even in minus values of $L'$. It should be emphasized, however, that Eq. (43) has been derived for the column *with the top* occurring only when $L' \geq 0$, since Eq. (42) has been derived as a sufficient condition for the occurrence of Eq. (33) or (23) (see above), and Eq. (23) is concerned with the column with the top. An interpretation should therefore be introduced that the part of the distribution $\Omega$ that formally occurs in minus values of $L'$ in Eq. (43) actually appears at the top ($L' = 0$) of the column forming a band with an infinitesimal width. The intensity of this band decreases gradually with the development process. At early stages of the development process, the infinitesimal band remains at the column top as part of the total band with a finite width.

In spite of the hypothesis that, in the part $L < 0$ of the infinite column, the diffusion coefficient should be $D + \hat{D}$ (= $D$) rather than $\hat{D}$ (= 0) (see Section D), it is possible to solve Eq. (40) by using Eq. (34) (which has been derived from Eq. 30) as a boundary condition. Here, again, the first equality in Eq. (43) is obtained as a solution. This arises from another hypothesis that the flow heterogeneity should not occur in the neighborhood $[-0, +0]$ of the position $L = 0$ on the infinite column, whereas Eq. (34) (derived from Eq. 30) shows that the initial molecular distribution occurs only in an infinitesimal range $[-0, 0]$.

***Remark.*** Earlier (Ref. *15*, Appendix III), the first equality in Eq. (43) was derived by using Eq. (34) (identical with Eq. A40 in Ref. *15*) as a boundary condition (see Eq. A34 in Ref. *15*). In Fig. 8 in Ref. *16*, two theoretical chromatograms calculated on the basis of Eq. (A34) in Ref. *15* (i.e., the first equality in Eq. 43) are illustrated. In Ref. *15*, however, it was simply assumed that the length of the column is infinity, extending even in minus $L$ values, and that the theory is valid only when the part of the distribution $\Omega$ occurring in minus $L$ values is negligibly small, viz., when the position of the molecular band is far below the column top. A new interpretation for Eq. (A34) in Ref. *15* (i.e., the first equality in Eq. 43) has been proposed above.

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## NUMERICAL CALCULATIONS OF IDEALIZED CHROMATOGRAMS IN THE ABSENCE OF THE FIRST TYPE OF FLOW HETEROGENEITY AND DISCUSSION

Instead of considering probabilities $\Omega\, dW$ (Eq. 32) and $\Omega_\delta\, dW$ (Eq. 16) occurring between the value $W$ and $W + dW$ of the parameter $W$ (Eq. 11) when the total interstitial volumes $L'$ (Eq. 10) of the column is given, it is more convenient to consider probabilities $\Omega^\circ\, dW_0$ and $\Omega_\delta^\circ\, dW_0$ occurring between the value $W_0$ and $W_0 + dW_0$ of the parameter

$$W_0 = W/\alpha \tag{44}$$

when the length $L$ of the column is given. $W_0$ (Eq. 44) has a dimension of length. Thus, by modifying Eqs. (32) and (16), $\Omega^\circ$ and $\Omega_\delta^\circ$ can be written as

$$\left.\begin{aligned} \Omega^\circ(W_0 \geq 0, L \geq 4\hat{\Theta}_0) &= \frac{1}{4\hat{\Theta}_0}\, e^{[2(L-4\hat{\Theta}_0)-W_1]/4\hat{\Theta}_0} \\ &\qquad \times \operatorname{erfc}\left(\frac{L - 4\hat{\Theta}_0}{\sqrt{4\hat{\Theta}_0 W_0}}\right) \\ &\text{and} \\ \Omega^\circ(W_0 \geq 0, 0 < L < 4\hat{\Theta}_0) &= \frac{1}{L}\, e^{-W_0/L} \end{aligned}\right\} \tag{45}$$

and

$$\Omega_\delta^\circ(W_0 \geq 0, L) = \frac{1}{\sqrt{4\pi\hat{\Theta}_0 W_0}}\, e^{-(L-W_0)^2/4\hat{\Theta}_0 W_0} \tag{46}$$

respectively. Equation (45) represents $\Omega^\circ$ as a function of $W_0$ when the length $L$ of the column is given. $W_0$ is proportional to elution volume $V$ since $B$ is constant (Theoretical Section $A$; see Eqs. 44 and 11). Provided the partition $B$ of molecules in solution in the column is small, $\Omega^\circ$ can, in general, represent the normalized concentration (with respect to $W_0$) of molecules in solution that have just been eluted out of the column (cf. the explanation of Eq. 32 and below). Under this consideration, Eq. (45) can represent a chromatogram for a column of length $L$.

Equation (46) is an approximate expression of Eq. (45) obtained by assuming (a) that the length of the column is infinity extending even in minus $L$ values upward beyond the top, and (b) that the molecules are adsorbed initially at position $L = 0$ of the infinite column, forming a band with an infinitesimal width. The physical meaning of Eq. (46) (or Eq. 16) is different from the meaning of the first equality in Eq. (43) in spite of the same mathematical form (but see below).

Figure 1 illustrates theoretical chromatograms for several different column lengths calculated from both Eqs. (45) and (46). It is always assumed that $\hat{\Theta}_0 = 0.005$ cm, or that $\Delta L (= 4\hat{\Theta}_0) = 0.02$ cm (see Eq. 21; for this assumption, see Ref. *20*). Part (a) of Fig. 1, therefore, represents the case when $L = \Delta L$. It can be seen in Fig. 1 that the width in the chromatographic peak increases with an increase in length $L$ of the column due to molecular diffusion. (Note the scale differences in the abscissa for different parts of the figure.) It is only when $L$ is extremely small that the chromatogram calculated from Eq. (46) is considerably different from that calculated from Eq. (45). When $L$ exceeds the order of magnitude of 1 cm (the usual experimental condition), the coincidence of the two chromatograms is almost complete (Figs. 1c and d). The approximate equation, Eq. (46), is useful for practical purposes.

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The first equality in Eq. (43) has the same mathematical form as that of Eq. (46) (or Eq. 16). This means that, if the parameter $\Theta$ is replaced by $\Theta + \hat{\Theta}$, the first equality in Eq. (43) can represent an approximate chromatogram in which account is taken of all the effects of the first and second types of flow heterogeneities, and thermal Brownian diffusion. The interpretation given to the first equality in Eq. (43) in the absence of longitudinal Brownian diffusion (to which the effect of the second type of flow heterogeneity is added; Theoretical Section E) can be applied even in its presence, since the original mathematical form (Eq. 23)) of the boundary condition (Eq. 33) that has been used for the derivation of Eq. (43) is independent of the value of $\hat{\Theta}$. When $\hat{\Theta} \rightarrow +0$, Eq. (23) tends to Eq. (33).

Equation (45) or (32) has been derived for a small $B$ value (the last paragraph in Theoretical Section B). However, the width in the molecular band migrating on the column can be considered to be almost independent of the $B$ value. In fact, if $\Omega^\circ$ is considered as a function of longitudinal position $L$ on the column, $W_0$ represents the mean position of the band (cf. the explanation of Eq. 11). Equation (45) shows that the width in the band depends upon $W_0$, but that it is independent of $B$. With an increase in $B$, however, the migration rate of the band increases, and the width in the chromatogram (as a function of elution volume $V$) decreases. In contrast to Eq. (45), Eq. (46) is fulfilled independently of the $B$ value. It should be added that, if $B$ is large or close to unity, the adsorption of molecules on the crystal surfaces does not occur, so that "chromatography" does not occur.

***Remark.*** Equation (23) is also based on the assumption of a small $B$ value. In Remark 2 in Theoretical Section D it was mentioned that Eq. (23) can be applied with modifications to the abstract continuity equation for gradient chromatography. Gradient chromatography actually is used only for

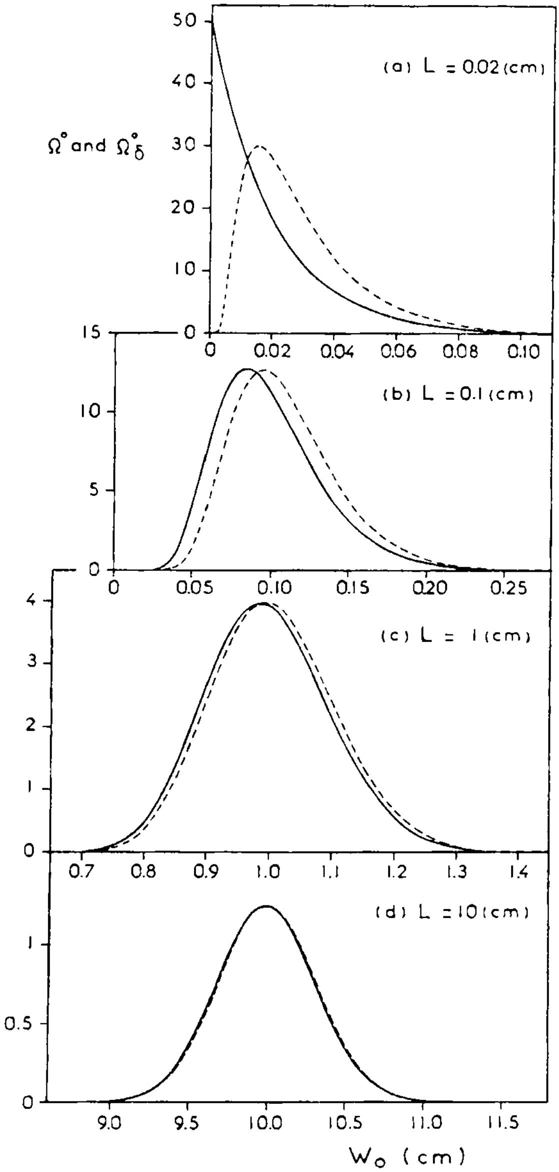

FIG. 1. Theoretical chromatograms in the absence of the first type of flow heterogeneity for several different lengths, $L$, of the column, calculated from both Eq. (45) ( ) and the approximate equation, Eq. (46) (- -). It is assumed that $\hat{\Theta}_0$=0.005 cm or that $\Delta L$ (= $4\hat{\Theta}_0$) = 0.02 cm. Therefore. Part (a) represents the case when $L = \Delta L$. (For details, see text.)

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molecules that are initially retained on the column since, unless this is the situation, it is unnecessary to apply the gradient. In order for the initial molecular retention to occur, it is necessary that the initial $B$ value at the top of the column be close to zero. This means that Eq. (23) (in modified form) is valid for any gradient chromatography.

## Acknowledgments

The author is grateful to Dr G. Bernardi for his interest in this work. The calculation for Fig. 1 was performed on the CDC 6600 computer of the Faculty of Sciences, University of Paris.

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*Received by editor January 29, 1981*

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