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General Theory of Quasi-Static Linear Gradient Chromatography

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Abstract

The earlier theory of hydroxyapatite chromatography is extended to a general theory of quasi-static linear gradient chromatography. The present theory is partially valid for the nonquasi-static case.

INTRODUCTION

In earlier papers (*1-11*) a theory of linear gradient hydroxyapatite (HA) chromatography was developed. The major part of the theory is valid for any quasi-static chromatographies (see Appendix I); some parts are even valid for the nonquasi-static case.

The purpose of the present paper is to rearrange the earlier theory (*1-11*), specifying the parts that are common to any chromatography, thereby establishing a general theory of linear gradient chromatography. In order to simplify the argumen, however, we here limit ourselves to the case where mutual molecular interactions are negligible.

Brief explanations of any symbols involved in the equations are given at the end of this paper.

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THEORETICAL

(A) Fundamental Continuity Equation for Gradient Chromatography

The purpose of this section is to confirm that Eq. (21) in Scheme 1 (pp. 123–125) (first derived as Eq. 17 in Ref. *3* for HA chromatography) is quite general, and that it represents the fundamental continuity equation for any quasi-static chromatography with linear gradient elution.

Scheme 1 briefly summarizes the derivation of Eq. (21). Thus, Eq. (21) can be derived from Eq. (1), a continuity equation for any quasi-static chromatography; Eq. (1) is based upon a spontaneous image understandable from the first point of view on gradient chromatography (Appendix II). However, although an isocratic chromatographic process can be *causally* described by Eq. (1), it is impossible in principle for a gradient chromatographic process to be described by Eq. (1); it is Eq. (21) that *causally* describes the gradient chromatography (see Ref. *3*). Equation (21) is based upon an abstract image understandable from the second point of view (Appendix II).

Let us follow the derivation of Eq. (21) (or Eq. 24) from Eq. (1) in Scheme 1. Thus, Eq. (4) is first derived from Eq. (1) by using elution volume V instead of time t; the transformation from t to V is possible since, in the first point of view, V increases with t (Appendix II). In other words, V is a chromatographic expression of time.

Equation (6) is a more precise expression of Eq. (4) in which account is taken of the thermal Brownian longitudinal diffusion in the interstitial liquid in the column that is negligible in the quasi-static case (Appendix I).

The derivation of Eq. (8) from Eq. (6) is carried out following a hypothesis according to which any type of flow heterogeneity (Appendix I) declines and becomes negligible in the column. As a result, with Eq. (8), no longitudinal diffusions survive in the column except Brownian diffusion.

Equation (14) represents the ideal case when even the Brownian longitudinal diffusion has declined completely and become null. The derivation of Eq. (14) from Eq. (8) is a key point in understanding the theory of gradient chromograpfy; the ideal equation with no longitudinal diffusion in the column (Eq. 14) can be obtained by substituting Fick's second law (Eq. 9 or 12) into Eq. (8). (The situation can be compared with the case of isocratic chromatography when the ideal equation with no longitudinal diffusion can be obtained if D_{therm} or θ_{therm} tends to 0. For details, see Ref. *1*, Appendix II.)

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$$\mathbf{J}$$

$$\|$$

$$\overbrace{\mathrm{div}_{L'}[B(L',m)\mathbf{v}'_0\Omega - D'B(L',m)\mathrm{grad}_{L'}\Omega]} + \frac{\partial\Omega}{\partial t} = 0 \qquad (1)$$

$$\Big\updownarrow \leftarrow \begin{cases} \theta = D'/(dV/dt) & (2) \\ |\mathbf{v}'_0| = dV/dt & (3) \end{cases}$$

$$\mathbf{j}$$

$$\|$$

$$\overbrace{\mathrm{div}_{L'}[\overrightarrow{B(L',m)}\Omega - \theta B(L',m)\mathrm{grad}_{L'}\Omega]} + \frac{\partial\Omega}{\partial V} = 0 \qquad (4)$$

$$\Big\updownarrow \leftrightarrow \theta \gg \theta_{\mathrm{therm}} \qquad (5)$$

$$\mathrm{div}_{L'}[\overrightarrow{B(L',m)}\Omega - \theta_{\mathrm{therm}}\mathrm{grad}_{L'}C - \theta B(L',m)\mathrm{grad}_{L'}\Omega] + \frac{\partial\Omega}{\partial V} = 0 \qquad (6)$$

$$\Big\updownarrow \leftrightarrow \theta \ll \theta_{\mathrm{therm}} \qquad (7)$$

$$\mathrm{div}_{L'}[\overrightarrow{B(L',m)}\Omega - \theta_{\mathrm{therm}}\mathrm{grad}_{L'}C] + \frac{\partial\Omega}{\partial V} = 0 \qquad (8)$$

$$\Big\uparrow \quad D_{\mathrm{therm}}\Delta_L C = \partial C/\partial t \quad \text{(Fick's second law)} \qquad (9)$$

$$\Big\updownarrow$$

$$D'_{\mathrm{therm}}\Delta_{L'}C = \partial C/\partial t \qquad (10)$$

(continued)

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$$\leftarrow \theta_{\text{therm}} = D'_{\text{therm}}/(dV/dt) \tag{11}$$

$$\leftrightarrow \theta_{\text{therm}}\Delta_{L'}C = \partial C/\partial V \tag{12}$$

$$\Omega = C + \chi \tag{13}$$

$$\operatorname{div}_{L'}\left[\frac{\overrightarrow{B(m)}}{1 - B(m)}\chi\right] + \frac{\partial \chi}{\partial V} = 0 \tag{14}$$

$$\leftarrow \begin{cases} V = L'_0 - L' = (m - m_{\text{in}})/g' & (15) \\ s = m_0 - m = g'L' & (16) \end{cases}$$

$$\Uparrow \quad \operatorname{div}_{s}\left[\frac{\overrightarrow{B(m)}}{1 - B(m)}\chi\right] + \frac{\partial \chi}{\partial m} = 0 \text{ (intermediate abstract flow)} \tag{17}$$

First point of view

Second point of view

$$\leftarrow \begin{cases} C = B\Omega & (18) \\ \text{Eq. (13)} \end{cases}$$

$$\Downarrow \quad \operatorname{div}_{m}\left[\frac{\overrightarrow{1 - B(m)}}{B(m)}C\right] + \frac{\partial C}{\partial s} = 0 \tag{19}$$

$$\leftrightarrow \theta = 0 \tag{20}$$

(continued)

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$$\mathbf{j}^* \equiv \overrightarrow{\frac{1 - B(s,m)}{B(s,m)} C - \frac{g'\theta}{B(s,m)} \operatorname{grad}_m \frac{C}{B(s,m)}} \quad \text{(21)}$$

(fundamental differential equation for gradient chromatography)

$$\leftarrow \begin{cases} g'\theta = D^*/(ds/dt) & \text{(22)} \\ |\mathbf{v}_0^*| = ds/dt & \text{(23)} \end{cases}$$

$$\mathbf{J}^* \equiv \overrightarrow{\frac{1 - B(t,m)}{B(t,m)} \mathbf{v}_0^* C - \frac{D^*}{B(t,m)} \operatorname{grad}_m \frac{C}{B(t,m)}} + \frac{\partial C}{\partial t} = 0 \quad \text{(24)}$$

SCHEME 1. Derivation of the fundamental differential equation for gradient chromtography.

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Equation (17) is another expression of Eq. (14) represented in terms of variables s and m instead of L' and V, respectively. Since g' is constant, s and m increase with increases in L' and V (i.e., t), respectively (see Eqs. 15 and 16). In other words, s and m still conserve the physical meanings of spatial coordinate and time, respectively. (The flow represented by Eq. 17 is called intermediate abstract flow; Eq. 17 is used for the approximate theory of gradient chromatography in which account is taken of mutual molecular interactions; see Refs. *8* and *11*.)

The derivation of Eq. (19) from Eq. (17) is another key point in understanding the theory of gradient chromatography. Thus, the transformation from Eq. (17) to Eq. (19) corresponds to the transfer from the first to the second point of view on gradient chromatography (Appendix II); spatial coordinate L' or s in the first point of view is reinterpreted as time in the second point of view, and time V or m is reinterpreted as spatial coordinate. Equation (19) (as well as Eq. 17, which is equivalent to

Eq. 19 except for the point of view) represents the fundamental continuity equation with linear gradient chromatography for the ideal case when there is no longitudinal diffusion in the column. Equation (19) (or Eq. 17) also represents the behavior of the mean part, with an infinitesimal width, of the actual molecular band migrating on the column; in this part of the band the effect of diffusion is always canceled out, and the relationship $R_F = B$ is fulfilled (see Appendix III).

The fundamental differential equation, Eq. (21), can be derived from Eq. (19) by adding a diffusive term to Eq. (19). For the determination of the form of the diffusive term, see below.

Equation (24) is another expression of Eq. (21) obtained by using time t instead of "time" s. The form of the diffusive term in Eq. (24) is determined by comparing Eq. (24) with Eq. (1); it is determined in order for the abstract flux **J*** (in Eq. 24) to coincide, at any instant t, with the actual flux **J** (in Eq. 1) at the outlet of the column (see Ref. *3*). The form of the diffusive term in Eq. (21) can be derived from the diffusive term in Eq. (24).

The process of deriving Eq. (24) from Eq. (1) is reversible, i.e., Eq. (1) can be derived from Eq. (24). This means that Eq. (24) or (21) is valid for any quasi-static chromatography with linear gradient elution since Eq. (1) represents a continuity equation for any quasi-static chromatography.

Scheme 1 itself constitutes a proof that the gradient chromatographic process can be *causally* described by Eq. (21) instead of Eq. (1) (see Ref. *1*, Appendix II, and Ref. *3*). Another proof is given in the section entitled "General Method of Finding the Flux in Any Type of Chromatography" in Ref. *3*

On the basis of the first principle of chromatography (Appendix III), it can be understood that Eqs. (14), (17), and (19) are even valid for the nonquasi-static case. In fact, even in the nonquasi-static case, the relationship $R_F = B$ is fulfilled in the mean part of the molecular band migrating on the column, and Eqs. (14), (17), and (19) represent the behavior of this part of the band.

The theoretical chromatogram can be obtained 1) if Eq. (21) is solved under a suitable initial condition, and 2) if a backtransfer is made from the second to the first point of view on gradient chromatography, giving s the physical meaning of the product of the slope g' of the concentration (or activity) gradient and the total length L' of the column (see Eq. 16). [Hereafter we simply use "concentration" instead of "concentration (or activity)".]

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(B) Form of the Function $B(s,m)$

The purpose of this section is to determine a possible form of the function $B(s,m)$ appearing in Eq. (21).

With quasi-static chromatography it is reasonable to assume (a) that the longitudinal diffusion of the sample molecules occurs in parallel with that of the gradient element in the column (since both diffusions are *directly* provoked by the first type of flow heterogeneity in the carrier liquid, see Appendix I; for gradient element, see Appendix II), and (b) that the linear concentration gradient is virtually undisturbed by 1) the (longitudinal) diffusion of the gradient element in the column (the diffusion effect is canceled out among different column sections), 2) the presence of the sample molecules, and 3) the fixation (if it occurs) of the gradient element on the packed particles in the column.

Now, let us suppose the case when a band of the sample molecules with an infinitesimal width is formed initially at the inlet of the column. When the migration of the molecular band begins, the width of the band becomes finite due to diffusion. Under this situation, the function $B(s,m)$ has the form

$$B(s,m) = B'[m_\lambda(s,m)] \tag{25}$$

where the function $m_\lambda(s,m)$ is represented implicitly as

$$m = m_\lambda + r(m_\lambda) - s \tag{26}$$

with

$$r(m_\lambda) = \int_{m_{in}}^{m_\lambda} \frac{B'(m_\lambda)}{1 - B'(m_\lambda)} dm_\lambda \tag{27}$$

From its physical meaning (see the explanation of the symbols at the end of the paper) it can, in general, be assumed that B' is a monotonical function of m_λ, increasing from ≈ 0 to 1 with an increase in m_λ from m_{in} to ∞.

Proof. (Step 1) Let us divide the column into a number of hypothetical microcolumns with diameters of the order of magnitude of the interdistances among packed particles in the column (cf. Appendix I). We

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characterize the microcolumns in such a way (a) that the volume of the liquid that flows into (and out of) any microcolumn is the same within any unit time interval, and (b) no longitudinal diffusion occurs within a microcolumn. [Actually, Hypothesis b is unrealizable. With quasi-static chromatography, however, the longitudinal diffusion within the microcolumns is negligible in comparison with the diffusion that is *directly* provoked by the first type of flow heterogeneity in the carrier liquid (Appendix I). This means that Hypothesis b, although it is untrue, is a reasonable assumption for the final result of the calculation.]

Let us consider the state within any cross section of the total column existing between positions L' and $L' + dL'$ at time t. When the mean concentration of the gradient element in this column section is between m and $m + dm$, then the concentrations m_λ of the gradient element within the cross sections of the microcolumns (belonging in this total column section) are distributed around the mean value m (as a result of longitudinal diffusion). Therefore, in some microcolumns the concentration of the gradient element is distributed within a certain infinitesimal range between m_λ and $m_\lambda + dm_\lambda$. On the other hand, since (a) a band of the sample molecules with an infinitesimal width is formed initially at the inlet of the column, (b) the longitudinal diffusion of the sample molecules occurs in parallel with that of the gradient element in the column (provoked by the first type of flow heterogeneity; Fundamental Assumption a), and (c) no longitudinal diffusion occurs within a microcolumn (Hypothesis b), then it is only in some microcolumns in which the concentration of the gradient element resides within a certain infinitesimal range (between m_λ and $m_\lambda + dm_\lambda$) that sample molecules appear at time t. This means that the function $B(s,m)$ has a form represented by Eq. (25); it can be considered that m_λ is a function of both m and s because m_λ depends upon both the mean concentration m and the distribution in the concentration. This latter depends upon "time" s.

(Step 2) Let us consider the state within a microcolumn in which a molecular band with an infinitesimal width is migrating. The migration of the band can be described (see Ref. *3*, Appendix) by

$$\frac{ds_\lambda}{dm_\lambda} = \frac{B'(m_\lambda)}{1 - B'(m_\lambda)} \tag{28}$$

where

$$s_\lambda = g' L'_\lambda \tag{29}$$

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and L'_λ represents the longitudinal position of the infinitesimal molecular band in the microcolumn; in Eq. (28), m_λ represents the concentration of the gradient element at position L'_λ. Since, at the inlet of the column, both relationships

$$L'_\lambda \text{ or } s_\lambda = 0 \tag{30}$$

and

$$m_\lambda = m_{in} \tag{31}$$

are initially fulfilled, Eq. (28) can be integrated to give

$$s_\lambda = r(m_\lambda) \tag{32}$$

where $r(m_\lambda)$ is defined by Eq. (27). On the other hand, s_λ can be written as

$$s_\lambda = m_0 - m_\lambda \tag{33}$$

(see Ref. *3,* Appendix), and, by substituting Eq. (32) into Eq. (33),

$$r(m_\lambda) = m_0 - m_\lambda \tag{34}$$

is obtained. Now, by eliminating m_0 between Eq. (34) and the left-hand side equation in Eq. (16), Eq. (26) can be obtained.

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(C) Solution of Eq. (21)

We here consider solving Eq. (21) when the function $B(s,m)$ has a form represented by both Eqs. (25) and (26). It should be recalled, however, that a physical meaning has been given to Eq. (25) only when the initial molecular band at the inlet of the column has an infinitesimal width (see Section B). We therefore solve Eq. (21) under the compatible initial condition:

$$C(s = +0,m) = \delta(m - m_{in}) \tag{35}$$

(for a more precise expression of Eq. 35, see Ref. *5*). It should be emphasized that, although Eq. (21) might have solutions under other

initial conditions [provided the function $B(s,m)$ is represented by both Eqs. 25 and 26], the physical meanings of these solutions are unclear.

Under the condition of Eq. (35), Eq. (21) has a solution

$$C = \frac{1}{\sqrt{4\pi g'\theta s}} \cdot e^{-\frac{(m_\lambda - m)^2}{4g'\theta s}} \cdot B'(m_\lambda) \tag{36}$$

(This can be confirmed if Eq. 36 is substituted into Eq. 21, although the calculation is somewhat laborious.) If the backtransfer is made from the second to the first point of view (giving s the physical meaning of the product of g' and L'), then Eq. (36) represents, with Eq. (26), a theoretical chromatogram as a function of m for a column of length L' and a slope g' of the concentration gradient.

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(D) Approximate Expression of Eq. (21) and the Theoretical Chromatogram Obtained under Any Initial Condition

The purpose of this section is to derive a simpler expression of Eq. (21); this can be solved under any initial condition, and the theoretical chromatogram under any initial chromatographic condition can be obtained.

Thus, in many instances, the range of the concentration gradient over which a chromatographic peak appears is small around the concentration μ at which the mean part of the peak exists. Under the approximation that the concentration over which a chromatographic peak appears is constant, being equal to μ, Eq. (21) reduces to a simpler equation:

$$g'\theta \cdot \frac{d}{ds}\left\{\frac{s}{[\tilde{B}(s)]^2}\right\} \cdot \frac{\partial^2 C}{\partial m^2} = \frac{1 - \tilde{B}(s)}{\tilde{B}(s)} \cdot \frac{\partial C}{\partial m} + \frac{\partial C}{\partial s} \tag{37}$$

where

$$\tilde{B}(s) = B'[\mu(s)] \tag{38}$$

and $\mu(s)$ can be implicitly represented by

$$s\,(= r(\mu)) = \int_{m_{in}}^{\mu} \frac{B'(\mu)}{1 - B'(\mu)}\, d\mu \tag{39}$$

(for the derivation of Eq. 37, see below and Ref. *6*). Under a general initial condition:

$$C(s = +0,m) = C_0(m) \tag{40}$$

where $C_0(m)$ is any function. Equation (37) has a solution:

$$C = \frac{1}{\sqrt{2\pi}\sigma(s)} \cdot \int_{-\infty}^{\infty} C_0(m') \cdot e^{-\frac{[m-m'+m_{in}-\mu(s)]^2}{2[\sigma(s)]^2}} \cdot dm' \tag{41}$$

where

$$\sigma(s) = \frac{\sqrt{2g'\theta s}}{\tilde{B}(s)} \tag{42}$$

[The solution (Eq. 41) can be obtained by using the Fourier transformation method; see Ref. *6*.] If the backtransfer is made from the second to the first point of view, Eq. (41) represents a theoretical chromatogram obtained under any initial chromatographic condition. In the special case when the initial condition, Eq. (40), has the form of Eq. (35), Eq. (41) reduces to a Gaussian form:

$$C = \frac{1}{\sqrt{2\pi}\sigma(s)} \cdot e^{-\frac{[m-\mu(s)]^2}{2[\sigma(s)]^2}} \tag{43}$$

(see Ref. *6*).

The approximate differential equation, Eq. (37), can be derived in the following way. First, Eq. (43) is derived from the pair of Eqs. (26) and (36) by carrying out a Taylor expansion of $r(m_\lambda)$ around μ, and neglecting high order terms (*4, 6*). Second, the form of the differential equation, Eq. (37), is determined in order for it to generate Eq. (43) as a solution under the initial condition given by Eq. (35) (*6*). What is important is that Eq. (37) can generate a solution (Eq. 41) under the general initial condition given by Eq. (40).

(E) Competition Model

If the fixation (adsorption) and dissociation (desorption) mechanism of the sample molecules in the column is specified, the concrete form of

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the function $B'(m')$ (where m' may be m_λ or μ; cf. Eqs. 26, 27, 36, 38, 39, 41–43) can be determined, and the concrete theoretical chromatogram can be obtained.

In earlier papers (*1–11*) a competition model was introduced for the adsorption and desorption mechanism in the HA column. The model states that adsorbing sites are arranged in some manner on the surfaces of the packed particles (HA crystals) in the column; the sample molecules (with adsorption groups) and the gradient element compete for adsorption onto the sites of the packed particles. A gradient element covers a single site when it is adsorbed, whereas a sample molecule, in general, covers plural sites. On the basis of the competition model, the function $B'(m')$ can be represented (see Ref. *1*, Appendix I) as

$$B'(m') = \frac{1}{1 + q(\varphi' m' + 1)^{-x'}} \tag{44}$$

where q, φ', and x' are positive constants. x' represents the number of adsorbing sites of the packed particle covered by an adsorbed sample molecule.

By using Eq. (44), both Eqs. (39) and (42) can be rewritten as

$$\mu(s) = \frac{1}{\varphi'}\{[(x' + 1)\varphi' q s + (\varphi' m_{\text{in}} + 1)^{x'+1}]^{1/(x'+1)} - 1\} \tag{45}$$

and

$$\sigma(s) = \sqrt{2g'\theta s}\{1 + q[\varphi'\mu(s) + 1]^{-x'}\} \tag{46}$$

respectively. If the sample molecule has large molecular dimensions ($x' \gg 1$), $\mu(s)$ (Eq. 45) tends to a constant value m^0 independent of s; $\sigma(s)$ (Eq. 46) tends to $\sqrt{2g'\theta s}$ at the same time (see Refs. *1* and *3*).

DISCUSSION

The present theory of gradient chromatography consists of (a) proposing an abstract continuity equation on the basis of the second point of view (Appendix II), (b) solving the equation under a suitable initial condition, and (c) carrying out a backtransfer from the second to the actual first point of view (Appendix II), giving the solution the physical meaning of the chromatogram. It is important to point out that, whereas the spatial coordinate of the actual flux in the spontaneous first

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point of view has an extensive dimension, the spatial coordinate of the abstract flux in the second point of view has an intensive dimension; this intensive coordinate represents a "force" that accelerates the actual flux occurring in the extensive spatial coordinate. It can be considered that this mode of the theory is quite general; the present theory would therefore be applicable to more general analytical methods in which a flux of the sample molecules participates and where a certain gradient of the "force" accelerates the flux.

Some experimental verifications of the theory are given in Ref. *2* for the case of HA chromatography. These verifications can be considered to be of general value since the basis of the theory is quite general (see above). Of course, the theory should still be verified by other experiments.

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APPENDIX I: LONGITUDINAL DIFFUSIONS IN THE COLUMN AND QUASI-STATIC CHROMATOGRAPHY

The longitudinal molecular diffusion occurring in the column can, in general, be classified into three types that are associated with one another: (a) thermal Brownian diffusion, (b) diffusion due to the flow heterogeneity in the carrier liquid,* and (c) diffusion due to a finite transition rate of a molecule between the mobile and the stationary phase in the column (for c, see Ref. *12*).

Due to the additive property of flux, the flow heterogeneity in the carrier liquid in the column (cf. b) is classifiable into two types. Divide the column into a number of hypothetical microcolumns with diameters of the order of magnitude of the interdistances among packed particles in the column. Because of heterogeneity in interspaces among packed particles, the flow rate (in the longitudinal direction of the column) 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. The flow heterogeneity (occurring in the longitudinal direction of the column) that is brought about by this mechanism is called the first type of flow heterogeneity. Due to a viscous property of the liquid, however, it might be possible that the flow rate in an interstice in the column depends upon the distance from the surface of the packed particle. Therefore, even within a microcolumn a flow heterogeneity is realizable (second type of flow heterogeneity).

*Diffusion due to flow heterogeneity is a concept that is intimately related to the concept of eddy diffusion. Here we avoid the use of this terminology (cf. Ref. *1*).

The molecular diffusion (b) can be *directly* provoked by both the first and the second type of flow heterogeneity in the carrier liquid. It can also be provoked in association with a reciprocal motion of molecules between neighboring microcolumns, and with a vertical motion of molecules within a microcolumn. The mechanism of diffusion (b) that is not directly provoked by the flow heterogeneity is similar to that of diffusion (c).

Quasi-static chromatography is here defined as chromatography in which the longitudinal molecular diffusion (b) that is *directly* provoked by the first type of flow heterogeneity in the carrier liquid is of overwhelming importance. Diffusion (b) which is *directly* provoked by the second type of flow heterogeneity, diffusion (b) which is provoked by the reciprocal motion of molecules between neighboring microcolumns, diffusion (b) which is provoked by the vertical motion of the molecules within a microcolumn, diffusion (a), and diffusion (c) all contribute only negligibly to the total longitudinal molecular diffusion in the column.

Define an elementary volume δV in the column, the cubic root of which is of the order of magnitude of the interdistances among packed particles in the column (which is of the same order of magnitude as the diameter of a microcolumn). δV has large enough dimensions for it to be a thermodynamic object. In the case of quasi-static chromatography it was previously assumed by its definition (see above) that a thermodynamic equilibrium is locally attained within any δV (in which the effect of the first type of flow heterogeneity is negligible) in any small time interval of the chromatographic process; the time interval is much smaller than the time that is necessary for the total molecular band to pass the longitudinal column position where δV exists, but it is large enough for equilibrium to be virtually attained within δV.

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APPENDIX II: TWO POINTS OF VIEW ON GRADIENT CHROMATOGRAPHY

We here specify the two points of view on gradient chromatography, the first on which Eq. (1) or (4) is based, and the second on which Eq. (24) or (21) is based. Figure 1 summarizes the two points of view, where the abscissa L' and the ordinate m represent the general longitudinal column position (with dimensions of volume; see the explanation of the symbols at the end of the paper) and the concentration (or activity) of a

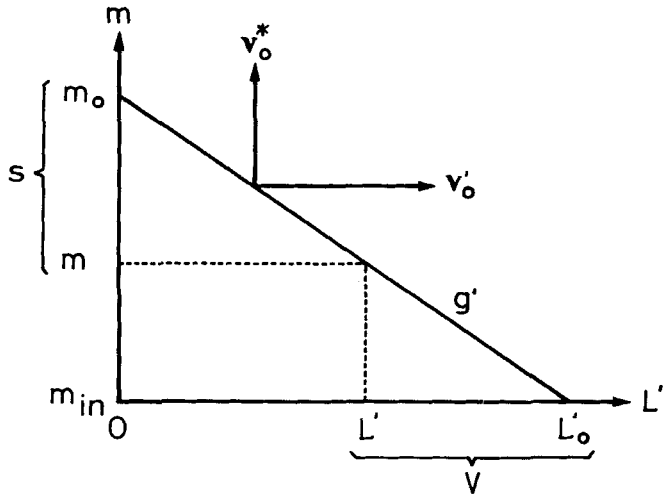

FIG. 1. Schematic representation of two points of view on gradient chromatography.

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component of the carrier liquid that constitutes a linear gradient in the column, respectively. The component of the carrier liquid (called the gradient element) usually represents inorganic ions in the cases of both ion exchange and HA chromatographies. In the case of reversed phase chromatography, it may be hydrophobic molecules (e.g., acetonitrile). The oblique line in Fig. 1 represents the linear concentration (or activity) gradient with slope g' occurring at time t. The slope g' is defined as positive in order for it to have a dimension of concentration/volume (or activity/volume) representing the increase in m from the outlet to the inlet of the column; g' is independent of time t. L_0' and m_0 show the column position at which the beginning of the gradient exists and the concentration (or activity) of the gradient element at the inlet of the column, respectively. m_{in} is the initial concentration (or activity) of the gradient element introduced at the inlet ($L' = 0$) of the column at time 0. This can be considered to be virtually equal to the concentration (or activity) at the beginning ($L' = L_0'$) of the gradient at time t.

In the first point of view, it is the column itself that is fixed. Both the concentration (or activity) gradient and the band of the sample molecules migrate in the L' direction on the (L',m) plane; we call v_0' the migration velocity (in units of volume/time) of the gradient observed at a given

longitudinal position L' on the column. Elution volume V occurring at position L' can be defined by Eq. (15) (see Fig. 1), where both L' and m_{in} are constant, and both L_0' and m increase with time t, m being defined as the concentration (or activity) of the gradient element at the given column position L'. Therefore, V increases with time t.

In the second point of view, it is the concentration (or activity) gradient that is fixed. Both the column and the band of the sample molecules migrate in the m direction of the (L',m) plane. It can be considered that the oblique line in Fig. 1 represents the linear "gradient" of column position L' (with slope $1/g'$) rather than the linear concentration (or activity) gradient (with slope g'). We call v_0^* the migration velocity [in units of concentration/time (or activity/time)] of the "gradient" along the concentration (or activity) gradient. Here, it is possible to find a parameter s [with a dimension of concentration (or activity)] that corresponds to V in the first point of view, occurring at a given position m on the concentration (or activity) gradient. This can be defined by Eq. (16) (see Fig. 1), where m is constant, and both m_0 and L' increase with time t, L' now being defined as the longitudinal position on the column at which the concentration (or activity) of the gradient element is always equal to m. Therefore, s increases with time t.

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APPENDIX III: FIRST PRINCIPLE OF CHROMATOGRAPHY IN GENERAL

In any chromatography a first principle is that, within a vertical section at any longitudinal position on the column, the ratio R_F of the migration rate of the sample molecules (of the chromatographic component under consideration) to that of the carrier liquid, *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 instant t, the mean relative migration rate $\bar{R}_F$ of the band is equal to the partition $\bar{B}$ in the mobile phase concerning all molecules that constitute the total band. $\bar{R}_F$ and $\bar{B}$ 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 ($= \bar{B}$). The mean part of the band is the part (with a virtually infinitesimal width) in which the effect of longitudinal molecular diffusion is always cnaceled out and where thermodynamic equilibrium is apparently realized between the mobile and the stationary phase.

SYMBOLS

t time

L any longitudinal position on the column, i.e., the distance from the column inlet

L' any longitudinal position on the column represented as the sum of interstitial volumes involved between the column inlet and the column position, L, under consideration. In some instances, L' represents the total column "length," i.e., the total interstitial volumes involved in the column

L'_0 longitudinal column position (represented in units of volume) at which the beginning of the concentration (or activity) gradient exists

L'_λ longitudinal position (represented in units of volume; for details, see Ref. *3*) of a microcolumn at which the infinitesimal band of sample molecules (of the chromatographic component under consideration) exists

V elution volume

m mean concentration (or activity) of the gradient element in the mobile phase within any vertical section of the column. In some instances, m also represents the mean concentration (or activity) in the mobile phase within the last infinitesimal vertical section at the outlet of the column, or the solution that has just been eluted out of the column

m_0 concentration (or activity) of the gradient element in the mobile phase at the inlet of the column

m_{in} initial concentration (or activity) of the gradient element in the mobile phase at the beginning of the concentration (or activity) gradient

m_λ concentration (or activity) of the gradient element in the mobile phase within the vertical section of the microcolumn at position L'_λ where the infinitesimal band of sample molecules (of the chromatographic component under consideration) exists. In some instances, m_λ also represents the concentration (or activity) of the gradient element in the mobile phase within any vertical section of the microcolumn

μ mean concentration (or activity) of the gradient element in the mobile phase within the vertical section of the column at which the mean part of the band of sample molecules (of the chromatographic component under consideration) exists

m' symbol representing m, m_λ, or μ, depending upon the case

g' positive constant [with a dimension of concentration/volume

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(or activity/volume)] representing the slope of the concentration (or activity) gradient in the column. This is expressed as the increase in the concentration (or activity) per unit "length" of the column, measured from the outlet to the inlet; the column "length" is represented in units of volume

s parameter [with a dimension of concentration (or activity)] defined by Eq. (16)

s_λ parameter [with a dimension of concentration (or activity)] defined by Eq. (29) or Eq. (33)

Ω mean total density of sample molecules (of the chromatographic component under consideration) in both the mobile and stationary phases in a vertical column section

C mean density of sample molecules (of the chromatographic component under consideration) in the mobile phase in a vertical column section. C is related to Ω by Eq. (18)

χ mean density of sample molecules (of the chromatographic component under consideration) in the stationary phase in a vertical column section. χ is related to both Ω and C by Eq. (13)

B partition of sample molecules (of the chromatographic component under consideration) in the mobile phase in a vertical column section, or the ratio of the amount of molecules in the mobile phase to the total amount in that column section. Therefore, B varies between 0 and 1

B' partition of sample molecules (of the chromatographic component under consideration) in the mobile phase in a vertical section of a microcolumn. Therefore, B' varies between 0 and 1

$\mathbf{v}_0'$ migration velocity (represented in units of volume/time) of the concentration (or activity) gradient in the L' direction on the (L',m) plane

$\mathbf{v}_0^*$ migration velocity [represented in units of concentration/time (or activity/time)] of the "gradient" (cf. Appendix II) in the m direction on the (L',m) plane

D' diffusion coefficient for the longitudinal diffusion in the column that is *directly* provoked by the first type of flow heterogeneity in the carrier liquid. D' is represented in units of volume^2/time (instead of length^2/time) since longitudinal column position L' is represented in units of volume. D' can be considered to be proportional to $|\mathbf{v}_0'|$, at least in the case of laminar flow

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D_{therm} thermal Brownian diffusion constant of sample molecules (of the chromatographic component under consideration) in the interstitial liquid in the column, represented in units of length^2/time

D'_{therm} positive constant (with dimensions of volume^2/time) defined by $(L'/L)^2 \cdot D_{therm}$

D^* parameter [with dimensions of concentration^2/time (or activity^2/time)] defined by Eq. (22)

θ parameter (with dimensions of volume) defined by Eq. (2). θ can be considered to be independent of the flow rate of chromatography, at least in the case of laminar flow.

θ_{therm} parameter (with dimensions of volume) defined by Eq. (11).

q positive constant

φ' positive constant

x' positive constant. For the physical meaning of x', see text

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REFERENCES

1. T. Kawasaki, *Sep. Sci. Technol., 16,* 325 (1981).
2. T. Kawasaki, *Ibid., 16,* 439 (1981).
3. T. Kawasaki, *Ibid., 16,* 817 (1981).
4. T. Kawasaki, *Ibid., 16,* 885 (1981).
5. T. Kawasaki, *Ibid., 17,* 319 (1982).
6. T. Kawasaki, *Ibid., 17,* 337 (1982).
7. T. Kawasaki, *Ibid., 17,* 407 (1982).
8. T. Kawasaki, *Ibid., 17,* 575 (1982).
9. T. Kawasaki, *Ibid., 17,* 695 (1982).
10. T. Kawasaki, *Ibid., 17,* 785 (1982).
11. T. Kawasaki, *Ibid., 17,* 1397 (1982).
12. J. C. Giddings, *Dynamics of Chromatography: Part I, Principles and Theory,* Dekker, New York, 1965.

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