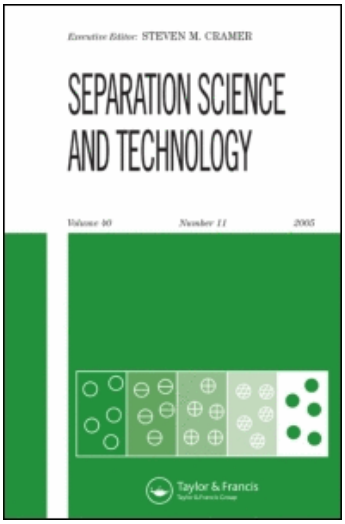

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A Specification of the General Theory of Quasi-Static Linear Gradient Chromatography: Relationship with the Overload Chromatography Theory in the Presence of Mutual Molecular Interactions

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Abstract

The earlier theory of quasi-static linear gradient chromatography with small sample loads is partially specified. On the basis of this study, the relationship between the theory of small load gradient chromatography and the approximate theory of overload gradient chromatography which was developed several years ago is also specified; this will promote a better understanding of the overload theory.

INTRODUCTION

Earlier (*1*, *2*), a general theory of quasi-static linear gradient chromatography was developed. Quasi-static chromatography as finally been defined in Ref. *2* represents chromatography in which (a) the transition rate of a molecule between the mobile and the stationary phase is so high that the phase transition effect hardly contributes to the longitudinal molecular diffusion in the column; (b) the longitudinal contribution of the Brownian diffusion occurring in the mobile phase is also negligible; and (c) no diffusion occurs in the stationary phase. (This definition of quasi-static chromatography is less severe than that proposed in Appendix I of Ref. *1*.) The theory developed in Refs. 1 and 2, however, is limited to the case where mutual interactions among sample molecules are negligible, or, from a practical point of view, the theory is only valid

for the case of small sample loads. The small load theory is outlined in the Appendix.

The first purpose of the present work is to analyze in detail the derivation process of Eq. (A19) from Eq. (A17) in the Appendix; this will promote a better understanding of the small load theory. In the earlier papers (*3–5*), the approximate theory of gradient chromatography in which account is taken of mutual molecular interactions occurring in the stationary phase has been developed for the purpose of elucidating the mechanism of hydroxyapatite chromatography; the interactions occurring in the mobile phase are negligible since the molecular concentration in solution is usually low.* This theory can be called overload gradient chromatography theory since, under the overload condition, the mutual molecular interactions occurring in the stationary phase are very important. The second purpose of the present work consists in specifying the relationship between the small load theory (Appendix) and the overload theory, and the specification is made in connection with the derivation analysis of Eq. (A19) from Eq. (A17) with small sample loads, which will promote a better understanding of the theory of overload gradient chromatography (*3–5*).

Physical meanings of any symbols involved in the equations are given in the Appendix.

THEORETICAL

(A) Physical Meaning of Eq. (A17) in the Appendix

It should be recalled (*1*) that Eq. (A8) in the Appendix represents the continuity equation for the molecular flux (of the chromatographic component under consideration) occurring in the column provided there are no longitudinal diffusions in the column except Brownian diffusion. If Fick's second law (Eq. A9 or A12) is substituted into Eq. (A8), then Eq. (A14) is derived. Equation (A14) states that, provided the longitudinal molecular diffusion in the interstitial liquid in the column occurs

*The approximate theory of gradient chromatography in which account is taken of mutual molecular interactions (*3–5*) has been developed in connection with the competition model for the adsorption and desorption phenomena occurring in the column (For the model, see the Introduction Section in Ref. *6*). The theory is valid even in the case when the competition model is not applicable. For instance, by modifying the expression in the intermediate or the right-hand side of Eq. (5) in Ref. *5*, the theory is applicable to any gradient chromatography.

independently of the chromatographic mechanism following Fick's second law (Eq. A9 or A12), then the chromatography is carried out independently of molecules existing in the interstitial liquid, following Eq. (A14) (cf. Ref. *6*, Appendix II). Equation (A17) is another expression of Eq. (A14) obtained by substituting both Eqs. (A15) and (A16) into Eq. (A14). In gradient chromatography, it is Eq. (A17), rather than Eq. (A14), that has a fundamental physical meaning since Eq. (A17) involves the minimal number, two, of variables (s and m) whereas Eq. (A14) involves three (L', V, and m).

Equation (A17) is self-consistent with the physical meaning, which is involved in itself, that the chromatography is carried out independently of molecules existing in the interstitial liquid. This is because both variables s and m are intensive quantities with a dimension of concentration (for the gradient element), respectively, and Eq. (A17) shows the change in molecular density, χ, in the stationary phase occurring with changes in concentrations (s and m) of the gradient element, independently of molecules existing in the interstitial liquid (for details, see Ref. *6*, Appendix II). Equation (A17) represents the idealized chromatographic process occurring in the absence of any longitudinal diffusion in the column since no diffusion occurs in the stationary phase (see Introduction Section).

Equation (A17) does not represent the conservation of the amount of molecules in a section of the column. In fact, the conservation occurring in the column section can be represented by using Eq. (A8), so that the conservation realized in the absence of any longitudinal diffusion in the column can be represented by using Eq. (A8) in which θ_{therm} is replaced with zero, or by using the equation

$$\mathrm{div}_{L'}\,[\overrightarrow{B(m)}\Omega] + \frac{\partial \Omega}{\partial V} = 0 \qquad (1)$$

By using both Eqs. (A13) and (A18), the term $\overrightarrow{B(m)}\Omega$ in Eq. (1) can be rewritten as $\overrightarrow{B(m)}/[1 - B(m)]\}\chi$. It can now ben seen that Eq. (1) is different from another expression, Eq. (A14), of Eq. (A17); this means that Eq. (A17) does not represent the conservation of the amount of molecules in the column section. (For further arguments on Eq. A17, see Ref. *6*, Appendix II, and Ref. *7*.)

(B) Analysis of the Derivation of Eq. (A19) from Eq. (A17)

Introducing a new parameter, C, defined as

$$C = \frac{B(m)}{1 - B(m)} \chi \tag{2}$$

Eq. (A17) can simply be rewritten as Eq. (A19), and, whereas Eq. (A17) has a general solution:

$$\chi = \Phi(\dot{r} - s) \tag{3}$$

Eq. (A19) has a general solution:

$$C = \frac{B(m)}{1 - B(m)} \Phi(\dot{r} - s) \tag{4}$$

where

$$\dot{r} = \int_{m_{in}}^{m} \frac{B(m)}{1 - B(m)} dm \tag{5}$$

and Φ is any function. On the other hand, Eq. (2) is equivalent to both Eqs. (A13) and (A18). This means that the parameter C defined by Eq. (2) has the same physical meaning as C apearing in Fick's second law (Eq. A9 or A12), representing the molecular density in the interstitial liquid in the column.

It can now be understood that the process of deriving Eq. (A19) from Eq. (A17) consists of two steps of (a) estimating, by using Eq. (2) (or both Eqs. A13 and A18), the molecular density C in the mobile phase that is in equilibrium with the molecular density χ in the stationary phase, and (b) substituting C for χ in Eq. (A17). It should be pointed out, however, that Procedure (a) is inconsistent with the physical meaning involved in Eq. (A17) that the chromatography should be carried out independently of molecules existing in the interstitial liquid in the column (see Section A). This is because Procedure (a) is based upon the hypothesis that the molecular density C in the mobile phase is in equilibrium with molecular density χ in the stationary phase even in the ideal case of no longitudinal diffusion in the column; this means that the chromatographic mechanism is dependent upon molecules existing in the interstitial liquid in the column even in this ideal case.

Let us examine, however, the case when a molecular band with an infinitesimal width is migrating on the column in the absence of longitudinal diffusion. In this instance, Eq. (3) can be represented by using a delta-function:

$$\chi = \delta(\dot{r} - s) \tag{6}$$

We consider the flow of molecules at a given position L' ($= s/g'$; see Eq. A16) on the column. Then, it will be observed that the value of the parameter $\dot{r}$ increases with a lapse of time because m increases with a lapse of time (see Eq. (5) in which B is a monotonical function of m increasing from ≈ 0 to 1 with an increase in m from m_{in} to ∞), and that all the molecules appear at a certain value of $\dot{r}$. The probability that the molecules appear between the value $\dot{r}$ and $\dot{r} + d\dot{r}$ of the parameter $\dot{r}$ can be given by $\chi d\dot{r}$ or $\delta(\dot{r} - s)d\dot{r}$ (to be precise,

$$\int_{\dot{r}}^{\dot{r}+d\dot{r}} \delta(\dot{r} - s)d\dot{r}$$

see Eq. 6). This means that the probability (written as Cdm) that the molecules appear between concentration m and $m + dm$ of the gradient element is equal to $\delta(\dot{r} - s) \cdot (d\dot{r}/dm) \cdot dm$, thus leading to

$$C = \delta(\dot{r} - s)\frac{d\dot{r}}{dm} = \chi\frac{d\dot{r}}{dm} \tag{7}$$

where C is normalized in such a way that

$$\int_{m_{in}}^{\infty} Cdm = 1 \tag{8}$$

It can simply be *interpreted* that the molecules appearing between the values m and $m + dm$ of the parameter m represent those existing in the interstitial liquid in the column since m represents the concentration (of the gradient element) occurring in the interstitial liquid. By differentiating Eq. (5) with respect to m,

$$\frac{d\dot{r}}{dm} = \frac{B(m)}{1 - B(m)} \tag{9}$$

is obtained, and, from both Eqs. (7) and (9), Eq. (2) can be derived. Hence, as far as the molecular band with an infinitesimal width is concerned, the parameter C endowed with a physical meaning of the probability density of the existence can be defined by using Eq. (2), and, on the basis of Eq. (2), Eq. (A19) can be derived from Eq. (A17). This derivation procedure is not inconsistent with the fundamental hypothesis that the chroma-

tography should be carried out independently of molecules existing in the interstitial liquid in the column.

In an earlier paper (*2*) it was mentioned that, in general, it is possible to divide the column into an infinite number of quasi-parallel microcolumns with an infinitesimal cross-sectional area in such a way that molecular bands with an infinitesimal width are distributed among the respective microcolumns. The phases of infinitesimal bands belonging in different microcolumns are always different from one another, at least by an infinitesimal magnitude, and a given infinitesimal band migrates within the same microcolumn throughout the whole process of chromatography [see Ref. *2*; the argument can be compared with that for the case when there are mutual molecular interactions (Discussion Section B)]. This means that the migration of the infinitesimal molecular band occurring within each microcolumn is carried out independently of molecules existing in the interstitial liquid in the microcolumn, following Eq. (A19). The derivation of Eq. (A21) from Eq. (A19) can be interpreted to represent the accumulation procedure of the microcolumns to make up a total column. In each microcolumn (or in Eq. A19), the parameter C is endowed with a physical meaning of the probability density of the existence (see above). In the total column (or in Eq. A21 or A24), however, C has a physical meaning of the molecular density in the interstitial liquid.

In connection with Eq. (A21), Eq. (A19) insists on its validity only for the case of the infinitesimal molecular band while, due to the physical meaning of Eq. (A17), Eq. (A19) can have a physical meaning only when the infinitesimal molecular band is concerned. As a result, a self-consistent theoretical system as represented in the scheme in the Appendix is completed, provided there are no mutual molecular interactions in the column.

DISCUSSION

(A) General Discussion

Equation (A17) represents a chromatographic process occurring in the stationary phase independently of molecules existing in the interstitial liquid in the column (Theoretical Section A). In accordance with the physical meaning of Eq. (A17), it can, in general, be stated that the molecular density, C, in the interstitial liquid occurring at a given position, L', on the column in the absence of longidutinal diffusion can

be calculated as a decrease in molecular density, χ, in the stationary phase (given by Eq. 3 as a solution of Eq. A17) occurring at the position L' with an increase in concentration, m, of the gradient element. Thus, taking into account the fact that the value of the parameter s is determined if the position L' is given (since the slope g' of the gradient is constant; see Eq. A16), C can be represented as

$$C = -\left(\frac{\partial \chi}{\partial m}\right)_s \tag{10}$$

where the constant s (with the same dimension as m; see Eq. A16) has been added in order for the dimension of C to be identical with that of χ; this is necessary for Eq. (10) to be compatible with Eq. (A13). In an earlier paper (*5*), it was shown that Eq. (7) can, in fact, be derived from Eq. (10) as an extreme case when the width of the molecular band migrating on the column tends to infinitesimal; in this extreme case the parameter C is endowed with the physical meaning of the probability density of the existence of the molecules in the interstitial liquid in the column. (Cf. Eq. 31 in Ref. *5* in which the parameter C/s is used, instead of C, to represent the molecular concentration in the interstitial liquid of the column; cf. also Eq. 7 in Ref. *5*.)

(B) Relationship with the Case When There Are Mutual Interactions among Sample Molecules

From their physical meanings, it can be understood that Eqs. (A1)–(A18) in the Appendix are even valid for the case when mutual interactions occur among sample molecules existing in the stationary phase; the interactions occurring in the interstitial liquid in the column are negligible since the molecular concentration in solution is usually low. In the presence of molecular interactions, parameter B is a function of the molecular densities, χ, for the respective components of the sample mixture. Therefore, Eqs. (A1), (A4), (A6), (A8), (A14), and (A17) are simultaneous equations for the respective components of the mixture.

In the absence of mutual molecular interactions, it is possible, in general, to divide the total column into an infinite number of microcolumns in such a way that a given infinitesimal molecular band migrates within the same microcolumn not only at a given instant but also throughout the whole process of chromatography (Theoretical Section B). In the presence of repulsive molecular interactions (usual

case; cf. Refs. *3–5*), however, the situation is different. Under this situation, although the total column can be divided into microcolumns in such a way that molecular bands with an infinitesimal width are distributed among the respective microcolumns at a given instant, the infinitesimal band occurring within each microcolumn at the given instant will have a finite width immediately after the migration of the band begins; this is due to interactions among molecules. (At any given instant, however, it is possible to redefine the microcolumns in order for molecular bands with an infinitesimal width to be redistributed among the respective microcolumns.)

In the presence of mutual molecular interactions, let us divide the total column into microcolumns in such a way that infinitesimal molecular bands are distributed among the respective microcolumns initially or at the beginning of the development process of chromatography. The chromatographic process occurring in each microcolumn can be described by Eq. (A17), which is simultaneous equations in this case; in Eq. (A17), B is a function of not only m but also of χ's for the respective components of the sample mixture. Equation (10) also holds when χ represents one of the solutions of the simultaneous equations, Eq. (A17), and C represents the molecular density in the interstitial liquid in the microcolumn for the molecular component under consideration. In earlier papers (*3*, *5*), the molecular density C (or the theoretical chromatogram) occurring in a microcolumn was calculated on the basis of Eq. (10) for a mixture of molecules with the same dimensions and the same shape, taking into account repulsive molecular interactions (see Eqs. 43 and 44 in Ref. *5* in which the parameter C/s is used, instead of C, to represent the molecular concentration in the interstitial liquid in the column; cf. also Eq. 7 in Ref. *5*). Further, it was shown (*5*) that the equations (Eqs. 43 and 44 in Ref. *5*) occurring in the presence of molecular interactions converge into Eq. (31) in Ref. *5*, i.e., Eq. (7) at the limit of no molecular interactions.

In the presence of mutual molecular interactions, it is mathematically difficult to derive from Eq. (A17) a simple equation like Eq. (A19) occurring in the absence of molecular interactions. In an earlier paper (*4*), a method of the approximate calculation of chromatograms on the basis of Eq. (A17) was developed in which account was taken of both mutual molecular interactions and longitudinal diffusion occurring in the column.

APPENDIX

An outline of the earlier theory of quasi-static linear gradient chromatography with small sample loads (*1*, *2*) is given in Scheme 1, where:

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 gradient exists.

V = elution volume.

W = distance between the column inlet and a mean position of the band of the sample molecules (of the chromatographic component under consideration) migrating on the column; this is expressed as the sum of interstitial volumes involved between the column inlet and the longitudinal mean position of the band under consideration.

m = mean concentration of the gradient element in the mobile phase within any vertical section of the column. In some instances, m also represents the mean concentration 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 of the gradient element in the mobile phase at the inlet of the column.

m_{in} = initial concentration of the gradient element in the mobile phase at the beginning of the concentration gradient.

g' = positive constant (with a dimension of concentration/volume) representing the slope of the concentration gradient in the column. This is expressed as the increase in the concentration 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 representing "time" in the second point of view on gradient chromatography; in the first point of view, s represents the product of g' and L'.

$$\operatorname{div}_{L'}\left[\overbrace{B(L',m)\underset{\sim}{v}_0\Omega}^{\underset{\sim}{j}} - D'B(L',m)\operatorname{grad}_{L'}\Omega\right] + \frac{\partial\Omega}{\partial t} = 0 \qquad (A1)$$

$$\theta = D'/(dV/dt) \qquad (A2)$$

$$|\underset{\sim}{v}_0| = dV/dt \qquad (A3)$$

$$\operatorname{div}_{L'}\left[\overbrace{B(L',m)\Omega}^{\underset{\sim}{j}} - \theta B(L',m)\operatorname{grad}_{L'}\Omega\right] + \frac{\partial\Omega}{\partial V} = 0 \qquad (A4)$$

(fundamental differential equation for isocratic chromatography when m = constant)

$$\theta \gg \theta_{therm} \qquad (A5)$$

$$\operatorname{div}_{L'}\left[\overrightarrow{B(L',m)}\Omega - \theta_{therm}\operatorname{grad}_{L'}C - \theta B(L',m)\operatorname{grad}_{L'}\Omega\right] + \frac{\partial\Omega}{\partial V} = 0 \qquad (A6)$$

$$\theta \ll \theta_{therm} \qquad (A7)$$

$$\operatorname{div}_{L'}\left[\overrightarrow{B(L',m)}\Omega - \theta_{therm}\operatorname{grad}_{L'}C\right] + \frac{\partial\Omega}{\partial V} = 0 \qquad (A8)$$

$$D_{therm}\Delta_{L'}C = \partial C/\partial t \quad \text{(Fick's second law)} \qquad (A9)$$

$$D'_{therm}\Delta_{L'}C = \partial C/\partial t \qquad (A10)$$

$$\theta_{therm} = D'_{therm}/(dV/dt) \qquad (A11)$$

$$\theta_{therm}\Delta_{L'}C = \partial C/\partial V \qquad (A12)$$

$$\Omega = C + \chi \qquad (A13)$$

$$\operatorname{div}_{L'}\left[\frac{\overrightarrow{B(m)}}{1-B(m)}\chi\right] + \frac{\partial\chi}{\partial V} = 0 \qquad (A14)$$

$$W = BV \qquad (A25)$$

$$\operatorname{div}_{L'}(\vec{\Omega} - \theta\operatorname{grad}_{L'}\Omega) + \frac{\partial\Omega}{\partial W} = 0 \qquad (A26)$$

(universal differential equation for any chromatography)

$$L' = r/g' \qquad (A27)$$

$$W = s/g' \qquad (A28)$$

$$\begin{cases} V = L_0' - L' = (m - m_{in})/g' & \text{(A15)} \\ s = m_0 - m = g'L' & \text{(A16)} \end{cases}$$

$$\Uparrow \quad \mathrm{div}_s\left[\frac{\overrightarrow{B(m)}}{1-B(m)}\chi\right] + \frac{\partial \chi}{\partial m} = 0 \qquad \text{(A17)}$$

(intermediate abstract flow)

First point of view

$$\begin{cases} C = B\Omega \\ \text{eq.(A13)} \end{cases} \qquad \text{(A18)}$$

Second point of view

$$\Downarrow \quad \mathrm{div}_m\left[\frac{\overrightarrow{1-B(m)}}{B(m)}C\right] + \frac{\partial C}{\partial s} = 0 \qquad \text{(A19)}$$

$$\theta = 0 \qquad \text{(A20)}$$

$$\mathrm{div}_m\left[\frac{\overrightarrow{1-B(s,m)}}{B(s,m)}C - \frac{g'\theta}{B(s,m)}\mathrm{grad}_m\frac{C}{B(s,m)}\right] + \frac{\partial C}{\partial s} = 0 \qquad \text{(A21)}$$

(fundamental differential equation for gradient chromatography)

$$\begin{cases} g'\theta = D^*/(ds/dt) & \text{(A22)} \\ |\underset{\sim}{v}_0^*| = ds/dt & \text{(A23)} \end{cases}$$

$$\mathrm{div}_m\left[\frac{1-B(t,m)}{B(t,m)}\underset{\sim}{v}_0^* C - \frac{D^*}{B(t,m)}\mathrm{grad}_m\frac{C}{B(t,m)}\right] + \frac{\partial C}{\partial t} = 0 \qquad \text{(A24)}$$

$$\mathrm{div}_r(\vec{\Omega} - g'\theta\,\mathrm{grad}_r\Omega) + \frac{\partial \Omega}{\partial s} = 0 \qquad \text{(A29)}$$

$$\left(\frac{\partial r}{\partial s}\right)_m = \left(\frac{\partial r}{\partial m}\right)_s = B(s,m) \qquad \text{(A30)}$$

$$B(s,m) = \widetilde{B}(r) \qquad \text{(A31)}$$

$$dr/\widetilde{B}(r) = ds + dm \qquad \text{(A32)}$$

r = parameter with a dimension of concentration. r/g' (with dimensions of volume) represents the longitudinal position on the column expressed as the sum of the interstitial volumes involved between the column inlet and the longitudinal position under consideration.

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

C = mean density of sample molecules (of the chromatographic component under consideration) in mobile phase in a vertical column section. C is related to Ω by Eq. (A18).

χ = mean density of sample molecules (of the chromatographic component under consideration) in stationary phase in a vertical column section. χ is related to both Ω and C by Eq. (A13).

$B(s,m)$, $\tilde{B}(r)$, and 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.

$\mathbf{v}_0'$ = migration velocity (represented in units of volume/time) of the concentration gradient in the L' direction on the (L',m) plane. (See Fig. 1 in Ref. *1*, Appendix II.)

$\mathbf{v}_0^*$ = migration velocity (represented in unit of concentration/time) of the "gradient" in the m direction on the (L',m) plane; "gradient" is defined as an assembly of longitudinal positions on the column which migrates on the concentration gradient. (See Fig. 1 in Ref. *1*, Appendix II.)

D' = diffusion coefficient for the longitudinal diffusion in the column that is directly provoked by the 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_{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 as $(L'/L)^2 \cdot D_{therm}$.

D^* = parameter (with dimensions of concentration^2/time) defined by Eq. (A22).

θ = parameter (with dimensions of volume) defined by Eq. (A2).

θ_{therm} = parameter (with dimensions of volume) defined by Eq. (A11).

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