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

Further Investigations on the General Theory of Quasi-Static Linear Gradient Chromatography

Tsutomu Kawasaki[a]
[a] CHROMATOGRAPHIC RESEARCH LABORATORY, KOKEN CO. LTD., TOKYO, JAPAN

To cite this Article Kawasaki, Tsutomu(1988) 'Further Investigations on the General Theory of Quasi-Static Linear Gradient Chromatography', Separation Science and Technology, 23: 4, 451 — 471
To link to this Article: DOI: 10.1080/01496398808060716
URL: http://dx.doi.org/10.1080/01496398808060716

SEPARATION SCIENCE AND TECHNOLOGY, 23(4 & 5), pp. 451-471, 1988

Further Investigations on the General Theory of Quasi-Static Linear Gradient Chromatography

TSUTOMU KAWASAKI

CHROMATOGRAPHIC RESEARCH LABORATORY
KOKEN CO. LTD.
3-5-18 SHIMO-OCHIAI, SHINJUKU-KU, TOKYO 161, JAPAN

Abstract

The earlier theory of quasi-static linear gradient chromatography is still generalized. The present theory is valid whatever may be the initial location of the sample molecules on the column, and the condition imposed upon the quasi-static chromatography is less severe in this theory than earlier. The earlier theory represents a special case of the present theory. The universal continuity equation for any quasi-static chromatography is derived, from which the continuity equation that *causally* describes the chromatographic process can be derived for both gradient and isocratic (or stepwise) chromatographies.

INTRODUCTION

Earlier (*1*) a general theory of quasi-static linear gradient chromatography was developed. However, the theory was only valid for the case when a molecular band with an infinitesimal width is formed initially at the inlet of the column (*1*). In the present work, the fundamental continuity equation of gradient chromatography that appeared in Ref. *1* is studied in detail. It is shown that the continuity equation can be solved under a general initial condition. The present theory is valid whatever may be the initial location of the sample molecules on the column, and the condition imposed upon the quasi-static chromatography is less severe in this theory than earlier. The earlier theory (*1*) represents a special case of the present theory.

The universal continuity equation for any quasi-static chromatography

is derived, which only represents the conservation of the amount of solute molecules in a vertical column section. From the universal equation, the continuity equation that *causally* describes the chromatographic process can be derived for both gradient and isocratic (or stepwise) chromatographies.

Physical meanings of any symbols involved in the equations are given at the end of this paper.

THEORETICAL

(A) General Consideration on the Fundamental Continuity Equation of Gradient Chromatography

The fundamental continuity equation of gradient chromatography (Eq. 21 in Ref. *1*) can be written as

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

A proof is given below that a function $r(s, m)$ exists which fulfills the relationship

$$\left[\frac{\partial r}{\partial s}\right]_m = \left[\frac{\partial r}{\partial m}\right]_s = B(s, m) \qquad (2)$$

and, in Section D, it will be shown that B can, in general, be represented as a function of r as

$$B(s, m) = \tilde{B}(r) \qquad (3)$$

Proof of Eq. (2). In general, it is possible to find a function $r(s, m)$ that fulfills only the right-hand side equality in Eq. (2). Similarly, it is possible to find another function $r(s, m)$ that fulfills only the equality between the extreme left-hand side term and the extreme right-hand side term in Eq. (2). It is therefore sufficient to show that the left-hand side equality in Eq. (2) is generally fulfilled. Figure 1 illustrates another expression of Fig. 1 in Ref. *1*, Appendix II. Thus, in Fig. 1, the abscissa L' in Fig. 1 of Ref. *1* is transformed to s, which is defined as $s = g'L'$ on the basis of the first point of view on gradient chromatography (see Ref. *1*, Appendix II). It can be

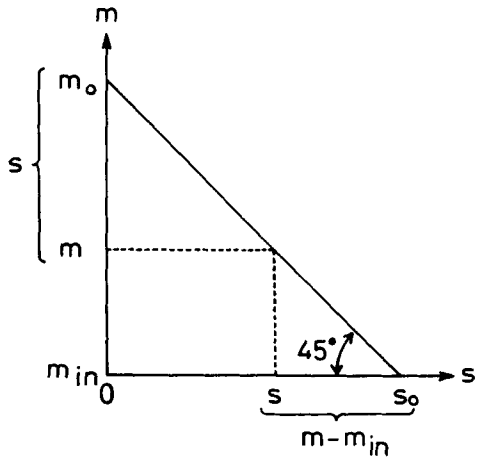

FIG. 1. Schematic representation of the principle of gradient chromatography. s in the abscissa s represents the relative longitudinal column position, $g'L'$ (first point of view) whereas s in the ordinate m represents "time" (second point of view). Cf. Fig. 1 in Ref. *1*, Appendix II.

seen that Fig. 1 is symmetric between the s and the m axis. This means that the expression $(\partial r/\partial m)_s$ (which is based on the first point of view) is equal to the other expression $(\partial r/\partial s)_m$ (which is based on the second point of view on gradient chromatography).

From both Eqs. (2) and (3)

$$dr/\tilde{B}(r) = ds + dm \tag{4}$$

is derived. Assuming that, when both $s = 0$ and $m = m_{in}$, then $r = 0$, Eq. (4) can be integrated to give

$$\int_0^r \frac{dr}{\tilde{B}(r)} = s + m - m_{in} \tag{5}$$

Introducing the parameters

$$\Omega = C/B(s, m) \tag{6}$$

and

$$F = \frac{1 - B(s, m)}{B(s, m)} C - \frac{g'\Theta}{B(s, m)} \cdot \left[\frac{\partial \Omega}{\partial m}\right]_s \tag{7}$$

Eq. (1) can be rewritten as

$$\left[\frac{\partial F}{\partial m}\right]_s + \left[\frac{\partial C}{\partial s}\right]_m = 0 \tag{1'}$$

in which, if C, Ω, and F are denoted by a common symbol f, f is considered to be a function of s and m ($f = f(s, m)$). Due to Eq. (5), however, it is possible to consider that f is a function of s and r ($f = f[s, r(s, m)]$) from which it follows that

$$\left[\frac{\partial f}{\partial s}\right]_m = \left[\frac{\partial f}{\partial s}\right]_r + \left[\frac{\partial f}{\partial r}\right]_s \left[\frac{\partial r}{\partial s}\right]_m$$

$$= \left[\frac{\partial f}{\partial s}\right]_r + \left[\frac{\partial f}{\partial r}\right]_s \tilde{B}(r) \tag{8}$$

and that

$$\left[\frac{\partial f}{\partial m}\right]_s = \left[\frac{\partial f}{\partial r}\right]_s \left[\frac{\partial r}{\partial m}\right]_s = \left[\frac{\partial f}{\partial r}\right]_s \tilde{B}(r) \tag{9}$$

where both Eqs. (2) and (3) have been used.

By replacing f with C, Ω, and F, and applying both Eqs. (8) and (9) to Eq. (1'),

$$g'\Theta \frac{\partial^2 \Omega}{\partial r^2} = \frac{\partial \Omega}{\partial r} + \frac{\partial \Omega}{\partial s} \tag{10}$$

or

$$\mathrm{div}_r(\Omega - g'\Theta \, \mathrm{grad}_r \, \Omega) + \frac{\partial \Omega}{\partial s} = 0 \tag{10'}$$

can be derived. Under a general initial condition:

$$\Omega(s \to 0, r) = \Omega_0(r) \tag{11}$$

where $\Omega_0(r)$ is any function, Eq. (10) or (10') has a solution:

$$\Omega(s, r) = \frac{1}{\sqrt{4\pi g'\Theta s}} \int_{-\infty}^{\infty} \Omega_0(r') e^{-\frac{(r-s-r')^2}{4g'\Theta s}} dr' \tag{12}$$

Especially when

$$\Omega_0(r) = \delta(r) \tag{13}$$

Eq. (12) reduces to

$$\Omega(s, r) = \frac{1}{\sqrt{4\pi g'\Theta s}} e^{-\frac{(r-s)^2}{4g'\Theta s}} \tag{14}$$

By using Eqs. (2), (3), and (6), Eq. (11) can be rewritten as

$$C(s \to 0, m) = C_0(m) \equiv \Omega_0(r) \cdot [\tilde{B}(r)]_{s\to 0} = \Omega_0(r) \cdot \left[\frac{dr}{dm}\right]_{s\to 0} \tag{15}$$

and Eq. (13) can be rewritten as

$$C_0(m) = \delta(m - m_{\text{in}}) \tag{16}$$

since when $s = r = 0$, then $m = m_{\text{in}}$ (Eq. 5). By applying both Eqs. (3) and (6) to Eqs. (12) and (14),

$$C(s, m) = \frac{\tilde{B}(r)}{\sqrt{4\pi g'\Theta s}} \int_{-\infty}^{\infty} \Omega_0(r') e^{-\frac{(r-s-r')^2}{4g'\Theta s}} dr' \tag{17}$$

and

$$C(s, m) = \frac{\tilde{B}(r)}{\sqrt{4\pi g'\Theta s}} e^{-\frac{(r-s)^2}{4g'\Theta s}} \tag{18}$$

can be derived. Equation (17) is a solution to Eq. (1) obtained under the initial condition given by Eq. (15) or (11); Eq. (18) is a solution obtained when the function $C_0(m)$ or $\Omega_0(r)$ has a special form represented by Eq. (16) or (13). (It will be understood later that the initial condition represented in the form of Eq. 15 is not convenient for practical purposes. The initial condition given in the form of Eq. 11 or that given in the form of Eq. 22 is adequate. See Section C and Discussion Section B.) With Eq.

(5), both Eqs. (17) and (18) represent C as a function of both s and m by using r as an intermediate parameter.

(B) Universal Continuity Equation for Any Quasi-Static Chromatography

Introducing transformations:

$$L' = r/g' \tag{19}$$

and

$$W = s/g' \tag{20}$$

Eq. (10) or (10′) can be rewritten as

$$\Theta \frac{\partial^2 \Omega}{\partial L'^2} = \frac{\partial \Omega}{\partial L'} + \frac{\partial \Omega}{\partial W} \tag{21}$$

or

$$\mathrm{div}_{L'}(\vec{\Omega} - \Theta \, \mathrm{grad}_{L'} \, \Omega) + \frac{\partial \Omega}{\partial W} = 0 \tag{21'}$$

Equations (11)–(14) become

$$\Omega(W \to 0, L') = \Omega_0(L') \tag{22}$$

$$\Omega(W, L') = \frac{1}{\sqrt{4\pi\Theta W}} \int_{-\infty}^{\infty} \Omega_0(L'') e^{-\frac{(L'-W-L'')^2}{4\Theta W}} dL'' \tag{23}$$

$$\Omega_0(L') = \delta(L') \tag{24}$$

and

$$\Omega(W, L') = \frac{1}{\sqrt{4\pi\Theta W}} e^{-\frac{(L'-W)^2}{4\Theta W}} \tag{25}$$

respectively.

It can be considered that, in Eqs. (19)–(25), L' represents the general longitudinal position on the column expressed as the sum of interstitial volumes involved between the column inlet and the column position under consideration.

W has a physical meaning of the longitudinal column position of the molecular band with an infinitesimal width occurring, provided that an infinitesimal molecular band was initially introduced at the inlet of the column and that no longitudinal diffusion occurs on the column ($\Theta = 0$). In other words, W represents the distance (expressed in units of volume) between the column inlet and the position of the infinitesimal molecular band. In the actual case when the initial molecular band has a finite width and when molecular diffusion occurs on the column, the position W would always be involved within the range over which the band is extending. This means that W, in general, represents the distance between the column inlet and a mean position of the molecular band migrating on the column. W therefore increases monotonically with a lapse of time.

It can be considered that Eq. (21) or (21′) represents a universal continuity equation for any quasi-static chromatography including both gradient and isocratic (or stepwise) chromatographies. It should be pointed out, however, that, although W increases with time (see above), how W increases with time is not at all described by Eq. (21) or (21′). This means that, although the conservation of the amount of solute molecules in a vertical column section can be represented by Eq. (21) or (21′), it is impossible in principle for any chromatographic process to be *causally* described by Eq. (21) or (21′). (For further arguments, see Section F and Discussion Section A.)

(C) Interpretation of the Parameter r and the Physical Meaning of the Initial Condition, Eq. (11)

It can be interpreted that the parameter r that has finally been defined by Eq. (5) represents the relative longitudinal position on the column since r is proportional to L' (Eq. (19). This means that Eq. (11) (which is equivalent with Eq. 22) represents the initial location of the sample molecules on the column; in this concept, the idea of the existences of the mobile and the staionary phase is not involved, and the total molecular density Ω is considered instead of the density C occurring in the mobile phase. It is $\Omega_0(L')$ or $\Omega_0(r)$, rather than $C_0(m)$, that can experimentally be realized. Therefore, on treating the fundamental continuity equation, Eq.

(1), it is convenient to use the initial condition given in the form of Eq. (11) rather than that given in the form of Eq. (15). (For further arguments, see Discussion Section B.)

(D) Consideration on Eq. (3)

We show below that Eq. (3) is valid with any gradient chromatography. Thus, the parameter r represents the longitudinal position on the column (Section C), and it can be considered that the property of a longitudinal column position is characterized by the mean concentration, m, of the gradient element (see Ref. 1) existing within the vertical section at that position. This means that r is a function of m. The property of the position r also changes with time or "time" s (second point of view). In fact, if the position r at which m is constant is pursued with a lapse of time or "time" s (the position r migrates on the column with a velocity equal to the migration velocity of the gradient itself), then it will be observed that the variance in concentration of the gradient element around the mean value m (within the vertical column section at the position r) increases because of the longitudinal diffusion occurring in the column; this will, gradually, change the property of the position r. This means that r also depends upon s, thus leading to $r = r(s, m)$ or Eq. (3).

It should be recalled (*1*) that the concentration gradient is defined as the gradient obtained by connecting m values occurring within respective column sections, which is linear with linear gradient chromatography. The linear concentration gradient is virtually undisturbed by the longitudinal diffusion of the gradient element in the column since the diffusion effect is canceled out among different column sections (*1*).

(E) Relationship with the Earlier Theory (*1*)

Earlier (*1*) it was shown that (a) if the longitudinal diffusion of the sample molecules occurs in parallel with that of the gradient element in the column, *directly* provoked by the first type of flow heterogeneity in the carrier liquid (Ref. *1*, Appendix I), and (b) if a band of the sample molecules with an infinitesimal width is formed initially at the inlet of the column,* then the function $B(s, m)$ is characterized (see Eqs. 25–27 in Ref. *1*) by

*For the other assumptions, see the earlier paper (1).

$$B(s, m) = B'[m_\lambda(s, m)] \tag{26}$$

$$m = m_\lambda + \dot{r}(m_\lambda) - s \tag{27}$$

and

$$\dot{r}(m_\lambda) = \int_{m_{in}}^{m_\lambda} \frac{B'(m_\lambda)}{1 - B'(m_\lambda)} dm_\lambda \tag{28}$$

Under the initial condition given by both Eqs. (15) and (16) (i.e., that given by both Eqs. 11 and 13), Eq. (1) has a solution:

$$C(s, m) = \frac{B'(m_\lambda)}{\sqrt{4\pi g' \Theta s}} e^{-\frac{(m-m_\lambda)^2}{4g'\Theta s}} \tag{29}$$

(see Eq. 36 in Ref. *1*). With both Eqs. (27) and (28), Eq. (29) represents C as a function of both s and m by using m_λ as an intermediate parameter (see Ref. *1*).

The purpose of this section is to show 1) that Eq. (26) is generally valid even in the absence of both Assumptions (a) and (b), and that Eq. (26) is equivalent with Eq. (3); 2) the function $B(s, m)$ can be characterized by Eqs. (26)–(28) only if Assumption (a) is fulfilled, i.e., independently of the initial chromatographic condition; and 3) the argument in this section is involved in the argument made in Section A. This means that Eq. (29) represents a special case of Eq. (18), and that, under the general initial condition given by Eq. (15) (i.e., that given by Eq. 11), Eq. (29) can be extended to have a general expression:

$$C(s, m) = \frac{B'(m_\lambda)}{\sqrt{4\pi g' \Theta s}} \int_{-\infty}^{\infty} \Omega_0(r') e^{-\frac{(m-m_\lambda-r')^2}{4g'\Theta s}} dr' \tag{30}$$

(cf. Eq. 17).

Proof that Eq. (26) is generally valid in the absence of both Assumption (a) and (b), and that Eq. (26) is equivalent with Eq. (3). Let us recall the proof of Eq. (26) (i.e., Eq. 25 in Ref. *1*) given in the paragraph "Proof. (Step 1)" in Theoretical Section B of Ref. *1*. The key point of this proof can be stated in the following way. Thus, a molecular band with an infinitesimal width migrates in each microcolumn (for microcolumn, see Ref. *1*), and the longitudinal positions of infinitesimal bands belonging in different microcolumns are always different from one another on the total column.

As a result, the partition, B, in solution of sample molecules occurring within a vertical section (with an infinitesimal thickness) of the total column represents the partition, B', occurring in the vertical section of one of the microcolumns; the vertical section of the microcolumn under consideration is part of the vertical section of the total column. In the vertical section of the microcolumn the concentration of the gradient element is m_λ while the mean concentration of the gradient element in the vertical section of the total column is m. Equation (26) is fulfilled because the relationship between m_λ and m depends upon "time" s.

In Ref. *1* it was stated, however, that the situation where an infinitesimal molecular band is present in each microcolumn and where the phases of the bands belonging in different microcolumns are different from one another (except just at the beginning of chromatography) is realizable only if the width of the initial molecular band introduced at the inlet of the column is infinitesimal (Assumption b). This is because, due to its physical meaning, the cross-sectional area of a microcolumn should be finite with a diameter of the order of magnitude of the interdistances among packed particles in the column (see Ref. *1*).

Provided the cross-sectional area of any microcolumn is infinitesimal, however, it would, in general, be possible to fill the interior space of the total column with microcolumns in such a way that, at any instant, each microcolumn captures only solute molecules (with infinitesimal dimensions) existing within an infinitesimal space in the total column. The other part of the microcolumn under consideration runs in the interior of the total column while avoiding the spaces filled with the other solute particles. This means that, independently of the shape of the molecular band migrating in the total column (therefore, independently of the initial location of the molecules on the column), the total column can be divided into an infinite number of microcolumns in such a way that the molecular bands with an infinitesimal width are distributed among the respective microcolumns and that the phases of infinitesimal bands belonging in different microcolumns are always different from one another, at least, by an infinitesimal magnitude. It would, in general, be possible to hypothesize that longitudinal positions of any solute particles (with infinitesimal dimensions) on the total column are different from one another, at least, by an infinitesimal magnitude. [This hypothesis is related to the theorem of the continuity of the real numbers or Dedekind's theorem. The hypothesis that a microcolumn can capture only solute molecules existing within an infinitesimal space in the total column and that the other part of the microcolumn can run in the interior of the total column while avoiding the spaces filled with the other solute particles (see above) is also related to Dedekind's theorem.]

The concept of the microcolumn with an infinitesimal cross section is quite general. [This can be applied more widely than the concept of the microcolumn with a finite cross-sectional area of the order of magnitude of the interdistances among packed particles in the column (see above).] This means that Eq. (26) is fulfilled quite generally, and that Eq. (26) is equivalent with Eq. (3), thus leading to

$$B'(m_\lambda) = \tilde{B}(r) \tag{31}$$

Proof that the function B(s, m) can be characterized by Eqs. (26)–(28) only if Assumption (a) is fulfilled independently of the initial chromatographic condition. Due to Assumption (a), the migration of a molecular band with an infinitesimal width occurring in a microcolumn can be described (see Appendix) by

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

where $\dot{r}\,(m_\lambda)$ is here defined as

$$\dot{r}\,(m_\lambda) = \int \frac{B'(m_\lambda)}{1 - B'(m_\lambda)}\, dm_\lambda \tag{28'}$$

and, on the basis of the first point of view on gradient chromatography, s_λ can be represented as

$$s_\lambda = \frac{g'}{\delta\lambda} \cdot L'_\lambda \delta\lambda = g' L'_\lambda \tag{33}$$

In Eqs. (32), (28'), and (33), $L'_\lambda\delta\lambda$ or $(s_\lambda/g') \cdot \delta\lambda$ has a physical meaning of the position of the infinitesimal molecular band on the microcolumn, and m_λ represents the concentration of the gradient element at the position where the infinitesimal band exists. $[\dot{r}\,(m_\lambda)/g'] \cdot \delta\lambda$ represents the position of the band as a function of m_λ (Eq. 32).

Since s_λ and s can be written (*1*) as

$$s_\lambda = m_0 - m_\lambda \tag{34}$$

and

$$s = m_0 - m \tag{35}$$

respectively, then, by substituting Eq. (32) into Eq. (34), and eliminating

m_0 between Eqs. (34) and (35), Eq. (27) can be derived. (For Eq. 35, cf. Fig. 1.)

The integration constant in Eq. (28′) can be determined in order for Eq. (28′) to be fulfilled under a condition where a molecular band with an infinitesimal width is formed at the inlet of the total column initially or at 'time" $s = 0$ (second point of view). Thus, both Eqs. (34) and (35) show that the concentration, m_0, of the gradient element in the first infinitesimal vertical section at the inlet of the total column is always homogeneous (cf. Fig. 1). Therefore, writing m_{in} for the m_0 value occurring at "time" $s = 0$, it can be stated that, when $s = 0$, then an infinitesimal molecular band is formed at the inlets, $(\dot{r}/g') \cdot \delta\lambda = 0$, of the respective microcolumns where $m_\lambda = m = m_{in}$. This means that, when $m_\lambda = m_{in}$, then $\dot{r} = 0$, thus leading to Eq. (28).

Hence, both Eqs. (27) and (28) have been derived by using only Assumption (a); this means that the function $B(s, m)$ can be characterized by Eqs. (26)–(28) only if Assumption (a) is fulfilled.

Proof that the argument in this Section is involved in the argument made in Section A. By using Eq. (31) it is easy to derive, from Eqs. (26)–(28), Eqs. (2)–(5), and the relationship,

$$\dot{r}\,[m_\lambda(s, m)] = r(s, m) \tag{36}$$

Further, by applying Eqs. (27), (31), and (36) to Eqs. (17) and (18), Eqs. (30) and (29) can be derived, respectively.

The argument in this section is involved in the argument made in Section A since an additional assumption (Assumption a) is present only in the argument in this section.

(F) Relationship with Isocratic (or Stepwise) Chromatography

With isocratic (or stepwise) chromatography, introducing a transformation:

$$W = BV \tag{37}$$

where B is constant, the universal continuity equation, Eq. (21) or (21′), can be rewritten as

$$\Theta B \frac{\partial^2 \Omega}{\partial L'^2} = B \frac{\partial \Omega}{\partial L'} + \frac{\partial \Omega}{\partial V} \tag{38}$$

or

$$\mathrm{div}_{L'}(\vec{B}\Omega - \Theta B \,\mathrm{grad}_{L'}\,\Omega) + \frac{\partial \Omega}{\partial V} = 0 \tag{38'}$$

(Equation 38′ represents the same equation as Eq. 4 in Ref. *1*.) Under a general initial condition:

$$\Omega(V \to 0, L') = \Omega_0(L') \tag{39}$$

Eq. (38) or (38′) has a solution:

$$\Omega(V, L') = \frac{1}{\sqrt{4\pi\Theta BV}} \int_{-\infty}^{\infty} \Omega_0(L'') e^{-\frac{(L'-BV-L'')^2}{4\Theta BV}} dL'' \tag{40}$$

Especially when

$$\Omega_0(L') = \delta(L') \tag{41}$$

Eq. (40) reduces to

$$\Omega(V, L') = \frac{1}{\sqrt{4\pi\Theta BV}} e^{-\frac{(L'-BV)^2}{4\Theta BV}} \tag{42}$$

Equations (38), (38′), (39)–(42) correspond to Eqs. (10), (10′), (11)–(14) for gradient chromatography, respectively.

Further, introducing the parameter

$$C = \Omega \cdot B \tag{43}$$

Eq. (38) or (38′) can be rewritten as

$$\Theta B \frac{\partial^2 C}{\partial L'^2} = B \frac{\partial C}{\partial L'} + \frac{\partial C}{\partial V} \tag{44}$$

or

$$\mathrm{div}_{L'}(\vec{B}C - \Theta B \,\mathrm{grad}_{L'}\, C) + \frac{\partial C}{\partial V} = 0 \tag{44'}$$

Equations (39)–(42) become

$$C(V\to 0, L') = C_0(L') \equiv \Omega_0(L') \cdot B \tag{45}$$

$$C(V, L') = \frac{1}{\sqrt{4\pi\Theta BV}} \int_{-\infty}^{\infty} C_0(L'') e^{-\frac{(L'-BV-L'')^2}{4\Theta BV}} dL''$$

$$= \sqrt{\frac{B}{4\pi\Theta V}} \int_{-\infty}^{\infty} \Omega_0(L'') e^{-\frac{(L'-BV-L'')^2}{4\Theta BV}} dL'' \tag{46}$$

$$C_0(L') = \delta(L') \cdot B \tag{47}$$

and

$$C(V, L') = \sqrt{\frac{B}{4\pi\Theta V}} e^{-\frac{(L'-BV)^2}{4\Theta BV}} \tag{48}$$

respectively. [Equations 46 and 48 can also be obtained by replacing Ω in Eqs. 40 and 42 by C/B (see Eq. 43), respectively.] Equations (44′), (45)–(48) correspond to Eqs. (1), (15)–(18) for gradient chromatography, respectively.

In contrast to the fact that the abstract flow in gradient chromatography (*1*) can be adequately represented by Eq. (1) rather than Eq. (10′), the actual flow in isocratic (or stepwise) chromatography (*1*) can be adequately represented by Eq. (38′) rather than Eq. (44′).

DISCUSSION

(A) Universal Continuity Equation, Eq. (21) or (21′)

The universal continuity equation, Eq. (21) or (21′), is valid for any quasi-static chromatography. However, the equation only represents the conservation of the amount of solute molecules in a vertical section of the column. Equation (21) or (21′) can, in general, be transformed to an equation that *causally* describes the chromatographic process. Thus, with gradient chromatography, the transformation is carried out by introducing both Eqs. (19) and (20), and, with isocratic (or stepwise) chromatography, by introducing Eq. (37).

(B) Initial Chromatographic Condition, Eq. (11)

The initial chromatographic condition, Eq. (11) (which is equivalent with Eq. 22 representing the initial location of the sample molecules on the column), is valid only when the concentration gradient is previously formed over longitudinal column positions where the molecules are initially located. In most actual cases, however, the sample molecules dissolved in a solvent (in which the initial concentration, m_{in}, of the gradient element is also dissolved) is loaded on the column, and the molecules flow into the column inlet; this is followed by a rinsing process carried out by using the same solvent as that of the sample solution. During this process, virtually all the molecules under consideration stay in the stationary phase in the vicinity of the column inlet, usually forming a uniform band; this is because, under the experimental condition as such, the molecules have very low B values. The concentration gradient now flows into the column, and the gradient begins to pass through the position of the molecular band. In this instant, the B values are usually still very low. As a result, the formation of the concentration gradient, in fact, occurs over the longitudinal column positions where the sample molecules are located conserving the initial state. This means that Eq. 11 is even valid in the actual case. (It should be noted, however, that, in the present theory, account is not taken of mutual molecular interactions that can be expected to take place within the initial molecular band. For the mutual molecular interactions, cf. Ref. *2*.)

It should be emphasized that another expression, Eq. 15, of the initial chromatographic condition does not generally represent the density of the sample molecules in the mobile phase existing just over the longitudinal column positions where the molecular band is present. In fact, Eq. (15) represents the molecular distribution occurring on the m axis of Fig. 1 whereas Eq. (11) represents that occurring on the s axis (or the L' axis of Fig. 1 in Ref. *1*, Appendix II). The intuitive understanding of the physical meaning of Eq. (15) is difficult except when the function $C_0(m)$ can be represented by using a delta function (Eq. 16).

(C) Relationship with the Experiment

In an earlier paper (*3*), the earlier theory (*1*) was experimentally confirmed for hydroxyapatite (HA) chromatography with good fits between the theory and the experiment. As far as dependences of the width (standard deviation, σ) of the chromatographic peak upon both L' and g' are concerned, however, the theory did not completely explain the

experiment: whereas the theory (*1*) predicts that the dependences be quantitatively explained by using a constant Θ value, the experiment showed that Θ increases slightly with a decrease in g' (see Fig. 3b in Ref. *3*, where the parameter Θ_0 which is proportional to Θ is considered instead of Θ). Further, the theory predicts that, with small sample loads, the shape of a chromatographic peak be almost Gaussian. However, the experimental chromatogram of a single component is often slightly asymmetrical with a slower decrease in height on the right-hand side of the pattern than on the other side (for instances, see Fig. 4 in Ref. *4* and Fig. 2 in Ref. *5*). (To be precise, the theoretical chromatogram is very slightly asymmetrical with a slower decrease in height on the left-hand side of the pattern than on the other side. The asymmetry is negligible from a practical point of view, however; for instance, see Fig. 1 in Ref. *6*. For the mathematical procedure for the Gaussian approximation of the theoretical chromatogram, see Refs. *1*, *6*, and *7*.)

It can be suggested that the slight difference between the theoretical prediction (*1*) and the experimental results (*3–5*) arises at least partially from the fact that Assumption (a) in the Theoretical Section E involved in the earlier theory (*1*) is not exact enough. As far as HA chromatography of macromolecules is concerned (as is the case with Refs. *3–5*), it can be suggested that, in contrast to Assumption (a), the longitudinal diffusion of the gradient element (competing ions in the experiments in Refs. *3–5*) in the column occurs more slowly than the sample molecules; the longitudinal diffusion effect provoked by the first type of flow heterogeneity (see Ref. *1*, Appendix I) is less important with competing ions than with sample molecules. Competing ions with a small particle size would have a large thermal Brownian diffusion constant, thus repeating frequent reciprocal motions between neighboring microcolumns (with a finite cross-sectional area of the order of magnitude of the interdistances among packed particles in the total column). As a result, the longitudinal diffusion effect occurring *directly* caused by the first type of flow heterogeneity would decrease (cf. Ref. *1*, Appendix I). With macromolecules with a large particle size and a small thermal Brownian diffusion constant, the longitudinal diffusion effect would not decrease so much due to reciprocal motions between neighboring microcolumns since the motions would be repeated less frequently. In general, the longitudinal diffusion effect *directly* provoked by thermal Brownian diffusion occurring in the mobile phase can be assumed to be negligible.

In Ref. *1*, Appendix I, the quasi-static chromatography was defined as chromatography in which the longitudinal diffusion that is *directly* provoked by the first type of flow heterogeneity in the carrier liquid is of overwhelming importance. As a result, the longitudinal diffusion of the

sample molecules occurs in parallel with that of the gradient element in the column both *directly* provoked by the first type of flow heterogeneity in the carrier liquid (Assumption a in Theoretical Section E). In the present work the definition of quasi-static chromatography is extended to the case when the reciprocal Brownian motion occurring between neighboring microcolumns contributes effectively to the longitudinal diffusion in the column; the contribution is negative, and it occurs differently between the gradient element and the sample molecules.*

(D) Looking Back upon the Earlier Developments of the Theory of Gradient Chromatography

The first theoretical investigation on gradient chromatography goes back to 17 years ago, when it was carried out for the purpose of elucidating the mechanism of HA gradient chromatography (*8*, *9*). In this investigation a series of a finite number of vertical sections with a finite thickness was used as a model representing a chromatographic column, and the behavior of sample molecules on the column was studied only numerically with the aid of a competition model occurring between the gradient element and the sample molecules for adsorption onto the HA crystals packed in the column (*8*, *9*).

Over 13 years ago, a combined form of both Eqs. (28) and (32) was first derived on the basis of a primitive consideration on the chromatographic mechanism (Eq. 12 in Ref. *10* or Eq. 1 in Ref. *11*).

A fundamental study of gradient chromatography that directly leads to the present work began 6 years ago, again in relationship with HA chromatography (*12*). It should be recalled that, in Ref. *12*, the same theoretical expression of a chromatogram as that derived in this paper (Eqs. 5 and 18, or Eqs. 27–29) was derived without using the fundamental continuity equation, Eq. (1), at all (see Eqs. 34 and 36 in Ref. *12*).

*In Ref. *1*, Appendix I, we mentioned the longitudinal diffusion that is provoked in association with the reciprocal motion of molecules occurring between neighboring microcolumns, and that is provoked by a vertical motion of molecules within a microcolumn. As far as the former diffusion is concerned, the phenomenon is apparent, occurring at the expense of the diffusion *directly* provoked by the first type of flow heterogeneity in the carrier liquid. In other words, the apparent diffusion (or the reciprocal motion itself occurring between neighboring microcolumns) contributes negatively to the diffusion that is *directly* provoked by the first type of flow heterogeneity. Similarly, the longitudinal diffusion provoked in association with a vertical motion of molecules within a microcolumn contributes negatively to the diffusion that is *directly* provoked by the second type of flow heterogeneity in the carrier liquid (see Ref. *1*, Appendix I).

APPENDIX

The migration of solute molecules in a microcolumn, in which no longitudinal diffusion occurs, can be represented as

$$\mathrm{div}_{m_\lambda}\left[\frac{\overrightarrow{1 - B'(m_\lambda)}}{B'(m_\lambda)} C'\right] + \frac{\partial C'}{\partial s_\lambda} = 0 \tag{A1}$$

Equation (A1) has a general solution:

$$C' = \frac{B'(m_\lambda)}{1 - B'(m_\lambda)} \chi'\left[s_\lambda - \int \frac{B'(m_\lambda)}{1 - B'(m_\lambda)} dm_\lambda\right] \tag{A2}$$

If a molecular band with an infinitesimal width occurs, C' in Eq. (A2) should be represented as a delta function; this leads to both Eqs. (32) and (28′).

SYMBOLS

m mean concentration of the gradient element in the mobile phase within any infinitesimal vertical section of the column. In some instances, m especially 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. (To be precise, the terminology "concentration" should be replaced by "concentration or activity." For the sake of simplicity, similar abbreviations will be used for any similar concepts appearing in any part of this paper.)

m_0 concentration of the gradient element in the mobile phase within the first infinitesimal vertical section at the inlet of the column

m_{in} concentration of the gradient element at the beginning of the concentration gradient flowing initially into the first infinitesimal vertical section at the inlet of the column

m_λ concentration of the gradient element in the mobile phase within any infinitesimal vertical section of a microcolumn. In some instances, m_λ especially repre-

sents the concentration in the mobile phase within the last infinitesimal vertical section at the outlet of the microcolumn. m_λ also represents the concentration of the gradient element occurring within the vertical section of the microcolumn in which the infinitesimal band of sample molecules (of the chromatographic component under consideration) exists

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'. Cf. the legend of Fig. 1

s_λ parameter with a dimension of concentration concerning a microcolumn. In the second point of view on gradient chromatography, s represents "time" elapsing in the microcolumn, and, in the first point of view, the product of $g'/\delta\lambda$ and $L'_\lambda \cdot \delta\lambda$, or g' and L'_λ. In some instances when $L'_\lambda \cdot \delta\lambda$ represents the longitudinal position of the infinitesimal band of sample molecules (of the chromatographic component under consideration) occurring in the microcolumn, $(s_\lambda/g') \cdot \delta\lambda$ also represents the position of the band in the microcolumn [since $L'_\lambda \cdot \delta\lambda = (s_\lambda/g') \cdot \delta\lambda$; Eq. 33]

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 interstitial volumes involved between the column inlet and the longitudinal position under consideration (since $r/g' = L'$; Eq. 19)

r' parameter equivalent with r

$\dot{r}$ parameter with a dimension of concentration concerning a microcolumn. $(\dot{r}/g') \cdot \delta\lambda$ (with dimensions of volume) represents the longitudinal position of the infinitesimal band of sample molecules (of the chromatographic component under consideration) occurring in the microcolumn; this is expressed as the sum of interstitial volumes involved between the inlet of the microcolumn and the longitudinal position under consideration

L' longitudinal position on the column expressed as the sum of interstitial volumes involved between the column inlet and the longitudinal position under consideration. In some instances, L' especially represents

the total column "length," i.e., the total interstitial volumes involved in the column

L'' parameter equivalent with L'

L'_λ parameter with dimensions of volume concerning a microcolumn. $L'_\lambda \cdot \delta\lambda$ represents the longitudinal position on the microcolumn expressed as the sum of interstitial volumes involved between the inlet of the microcolumn and the longitudinal position under consideration. In some instances, $L'_\lambda \cdot \delta\lambda$ especially represents the total "length" of the microcolumn, i.e., the total interstitial volumes involved in the microcolumn. $L'_\lambda \cdot \delta\lambda$ also represents the longitudinal position of the infinitesimal band of sample molecules (of the chromatographic component under consideration) occurring in the microcolumn

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

V elution volume

$\delta\lambda$ ratio of the volume of the liquid that flows into (and out of) a microcolumn to the volume that flows into (and out of) the actual whole column. The microcolumn is defined in such a way that the volume of the liquid that flows into (and out of) any microcolumn is the same within any unit time interval (*1*). This means that the $\delta\lambda$ value for any microcolumn is equal to one another

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 expressed in units of volume. $g'/\delta\lambda$ represents the slope of the concentration gradient occurring in a microcolumn

Θ positive parameter with dimensions of volume measuring the longitudinal diffusion in the column

C density of sample molecules (of the chromatographic component under consideration) in the mobile phase in the column

C_0	initial density of sample molecules (of the chromatographic component under consideration) in the mobile phase in the column
C'	density of sample molecules (of the chromatographic component under consideration) in the mobile phase in a microcolumn
χ'	density of sample molecules (of the chromatographic component under consideration) in the stationary phase in a microcolumn
Ω	total density of sample molecules (of the chromatographic component under consideration) in the vertical section of the column; in this concept, the idea of the existences of the mobile and the stationary phase is not involved
Ω_0	initial total density of sample molecules (of the chromatographic component under consideration) in the vertical section of the column
F	parameter defined by Eq. (7)
f	common symbol representing C, Ω, and F
$B(s, m)$, $\tilde{B}(r)$, 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. B, which is not accompanied by parentheses, is used in the case when B is constant
$B'(m_\lambda)$	partition of sample molecules (of the chromatographic component under consideration) in the mobile phase in a vertical section of a microcolumn

REFERENCES

1. T. Kawasaki, *Sep. Sci. Technol., 22,* 121 (1987).
2. T. Kawasaki, *Ibid., 17,* 1397 (1982).
3. T. Kawasaki and S. Takahashi, *Ibid., 23,* 193 (1988).
4. T. Kawasaki, S. Takahashi, and K. Ikeda, *Eur. J. Biochem., 152,* 361 (1985).
5. T. Kawasaki, M. Niikura, S. Takahashi, and W. Kobayashi, *Biochem. Int., 13,* 969 (1986).
6. T. Kawasaki, *Sep. Sci. Technol., 16,* 885 (1981).
7. T. Kawasaki, *Ibid., 17,* 337 (1982).
8. T. Kawasaki, *Biopolymers, 9,* 277 (1970).
9. T. Kawasaki, *Ibid., 9,* 291 (1970).
10. T. Kawasaki, *J. Chromatogr., 93,* 313 (1974).
11. T. Kawasaki, *Ibid., 93,* 337 (1974).
12. T. Kawasaki, *Sep. Sci. Technol., 16,* 325 (1981).

Received by editor June 26, 1987