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Tsutomu Kawasaki[a]
[a] CHROMATOGRAPHIC RESEARCH LABORATORY KOKEN BIOSCIENCE INSTITUTE, TOKYO, JAPAN

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A Fundamental Structure of the General Theory of Overload Quasi-Static Linear Gradient Chromatography

TSUTOMU KAWASAKI

CHROMATOGRAPHIC RESEARCH LABORATORY
KOKEN BIOSCIENCE INSTITUTE
3-5-18 SHIMO-OCHIAI, SHINJUKU-KU, TOKYO 161, JAPAN

Abstract

The earlier theory of overload linear gradient chromatography developed over 15 years ago by using an ideal molecular model with infinite dimensions is rearranged and improved in order for it to be compatible with the new concepts that are involved in the general theory of gradient chromatography developed recently. By applying the ideal molecular model, the structure of the overload chromatographic theory is considerably reduced, emphasizing the fundamental structure. Chromatograms with a shape close to a right-angled triangle with tailing are often experienced under overload condition. On the basis of the present theory, this type of chromatogram can be explained in terms of the occurrence of a quasi-crystalline phase of the adsorbed molecules that are repulsively interacting with one another on the adsorbent surfaces in the column.

INTRODUCTION

Earlier (*1–3*), a general theory of quasi-static linear gradient chromatography was developed; this is schematically outlined in the Ref. *3* Appendix. The theory is unique in that it involves several novel concepts: "two points of view on gradient chromatography," "abstract flux," "universal differential equation for any chromatography," etc. The scope of this theory is limited, however, to the simplest case when the mutual interaction among sample molecules is negligible. Therefore, the theory is valid under small sample load conditions only.

With preparative chromatography, large amounts of sample molecules are often loaded per unit cross-sectional area of the column, realizing a so-called overload chromatographic condition. Under this condition, mutual molecular interactions occurring in the stationary phase can be assumed to play an important role; the mutual interaction occurring in the mobile phase is negligible since the molecular concentration in solution is usually low. The approximate theory of quasi-static linear gradient chromatography in which account is taken of the mutual molecular interaction occurring in the stationary phase was developed in Refs. *4–6*. The main part (*4*, *6*) of this theory is limited to the case when all the molecules have the same dimensions and the same shape, however. The relationship between the small load theory (*1–3*) and the overload theory (*4–6*) is argued in detail in Ref. *3*.

The study of overload gradient chromatography goes back to 15 years ago when a theory began by applying an ideal molecular model with infinite dimensions (*7–10*); the effect of longitudinal diffusion in the column is neglected in this theory. The purpose of the present work is to rearrange and improve the initial overload theory (*7–10*) in order for it to be compatible with the new concepts that are involved in both the recent theory of gradient chromatography (*1–6*) and the recently specified adsorption and desorption model (*11–13*). By applying the ideal molecular model with infinite dimensions, the structure of the overload chromatographic theory is considerably reduced, emphasizing the fundamental structure. The present work will therefore promote a better understanding of the overload theory developed in Refs. *4–6*.

In the chromatographic theory (*1–10*), a parameter $B'_{(\rho')}$ plays a fundamental role; this represents the partition of molecules (of the chromatographic component ρ' under consideration) in the mobile phase in an elementary volume in the column, or the ratio of the amount of molecules in the mobile phase to the total amount in that elementary volume. It can be considered (*11–13*) that $B'_{(\rho')}$ is a function of (a) molarity, m, of the gradient element in the interstice in the elementary volume under consideration, and (b) molecular densities $\theta_{(1)}, \theta_{(2)}, \ldots, \theta_{(\rho)}$ on the adsorbent surface for all the components $1, 2, \ldots, \rho$ of the sample mixture, including the component ρ' under consideration; with small sample loads, $B'_{(\rho')}$ is a function of only (a).

Over 18 years ago, in order to explain the dependence of $B'_{(\rho')}$ upon m with hydroxyapatite (HA) chromatography carried out in an aqueous system, a competition model was introduced (*14*), and this model was applied in studies in Refs. *1–10*. The model states that adsorbing sites are arranged in some manner on the surfaces of the packed particles (HA crystals) in the column; sample molecules (with adsorption groups) and the gradient element (i.e., particular ions from the buffer constituting the molarity gradient) compete for adsorption onto the sites. A competing ion covers a single site when it is adsorbed whereas a sample molecule, in general, covers plural sites (*11–14*). The competition model will be applicable to not only HA chromatography but also ion-exchange chromatography. In Ref. *13* another qualitative model was proposed to explain the dependence of $B'_{(\rho')}$ upon m with both reversed-phase chromatography and HA chromatography carried out in an organic solvent system. In the present work, the competition model is applied. The conclusion attained in the present work will be valid, at least partially, if the competition model is replaced by another, however.

Experimental verifications of the theory under small load (*1–3*) and overload (*4–6*) conditions are given for HA chromatography in Refs. *15* and *16* and in Ref. *17*, respectively.

THEORETICAL

(A) Static Part: Partition $B'_{(\rho')}$ for Ideal Molecules with Infinite Dimensions

On the basis of the competition model (Introduction Section), partition $B'_{(\rho')}$ can be represented by Eq. (26) in Ref. *13*, which can be rewritten as

$$B'_{(\rho')} = \frac{1}{1 + Y_{(\rho)}^{-x'(\rho)}} \qquad (\rho' = 1, 2, \ldots, \rho) \tag{1}$$

where

$$Y_{(\rho')} = e^{-\frac{E_{(\rho')} + kT \ln \tau_{(\rho')}}{x'_{(\rho')}kT} + \frac{E^*_{(\rho')}(\theta)}{x'_{(\rho')}kT} - \frac{\ln p^*_{(\rho')}(\theta)}{x'_{(\rho')}} - \frac{\ln \beta}{x'_{(\rho')}}} (\varphi m + 1) \tag{2}$$

$$\theta = (\theta_{(1)}, \theta_{(2)}, \ldots, \theta_{(\rho)}) \tag{3}$$

and

$$\theta_{(\rho')} = \frac{x'_{(\rho')} n_{(\rho')}}{n_0} \tag{4}$$

The physical meanings of the symbols involved in Eqs. (1)–(4) are:

ρ' = a molecular component of the sample mixture, representing one of the components 1, 2, . . . , ρ.
$x'_{(\rho')}$ = number of adsorbing sites on the adsorbent surface on which the adsorption of competing ions is impossible due to the presence of an adsorbed ρ' molecule. Therefore, $x'_{(\rho')}$ is the parameter measuring dimensions of a ρ' molecule.
$E_{(\rho')}$ = absolute value of the interaction energy with adsorbing site(s) per molecule of component ρ' occurring provided the molecule is isolated on the adsorbent surface.
$\tau_{(\rho')}$ = configurational entropy factor per molecule of component ρ' occurring provided the molecule is isolated on the adsorbent surface. (For details, see Refs. 11 and 12.)
n_0 = total number of adsorbing sites on the adsorbent surface in the elementary volume of the column.
$n_{(\rho')}$ = total number of the molecules of component ρ' that are adsorbed on the adsorbent surface in the elementary volume of the column.
$\theta_{(\rho')}$ = molecular density for component ρ' on the adsorbent surface.
$E^*_{(\rho')}(\boldsymbol{\theta})$ = mutual interaction energy per molecule of component ρ' on the adsorbent surface; $E^*_{(\rho')} > 0$, $E^*_{(\rho')} = 0$, and $E^*_{(\rho')} < 0$ represent the cases with repulsive, no, and attractive interactions, respectively. (For details, see Refs. 11 and 12.)
$p^*_{(\rho')}(\boldsymbol{\theta})$ = geometrical interaction factor for a molecule of component ρ' on the adsorbent surface. $p^*_{(\rho')}(\boldsymbol{\theta})$ takes finite positive values, tending to 1 and 0 when $\Sigma_{\rho''=1}^{\rho}\theta_{(\rho'')}$ tends to 0 and the maximum value, respectively. (For details, see Refs. 11 and 12.)
β = positive constant that is proportional to the ratio of n_0 to the interstitial volume of the column element. (For details, see Ref. 13.)
φ = positive constant representing the property of competing ions. (For details, see Ref. 13.)
m = molarity of competing ions, constituting a linear gradient in the total column.

In the case of ideal molecules of infinite dimensions with properties:

$$x'_{(\rho')} \approx \infty \tag{5}$$

$$E_{(\rho')} + kT \ln \tau_{(\rho')} = O(x'_{(\rho')}) \tag{6}$$

and

$$E^*_{(\rho')}(\theta) = O(x'_{(\rho')}) \tag{7}$$

Equation (2) reduces to

$$Y_{(\rho')} \approx e^{-\frac{E_{(\rho')} + kT \ln \tau_{(\rho')}}{x'_{(\rho')}kT} + \frac{E^*_{(\rho')}(\boldsymbol{\theta})}{x'_{(\rho')}kT} - \frac{\ln p^*_{(\rho')}(\boldsymbol{\theta})}{x'_{(\rho')}}} (\varphi m + 1) \qquad \times (\varphi m + 1) \tag{8}$$

(B) Some Approximations and Preliminary Considerations

In order to simplify the argument, we introduce the following approximations:

(a) Both $E^*_{(\rho')}$ and $p^*_{(\rho')}$ are functions of $\Sigma^{\rho}_{\rho''=1}\theta_{(\rho'')}$.
(b) The maximum possible value, $[\Sigma^{\rho}_{\rho''=1}\theta_{(\rho'')}]_{\max}$, of $\Sigma^{\rho}_{\rho''=1}\theta_{(\rho'')}$ is independent of the ratio among $\theta_{(1)}, \theta_{(2)}, \ldots, \theta_{(\rho)}$.
(c) The ratio between $E^*_{(\rho')}$ and $x'_{(\rho')}$ (i.e., $E^*_{(\rho')}/x'_{(\rho')}$) is independent of the ρ' value.
(d) $x'_{(\rho')}$ is equal to the number of adsorbing sites on the adsorbent surface on which the adsorption of *any type of sample molecule* is impossible due to the presence of an adsorbed ρ' molecule.

Under these approximations, writing

$$\chi_{(\rho')} = \frac{\theta_{(\rho')}}{\left[\sum_{\rho''=1}^{\rho} \theta_{(\rho'')}\right]_{\max}} \tag{9}$$

and

$$\chi = \sum_{\rho'=1}^{\rho} \chi_{(\rho')} \tag{10}$$

we have

$$0 < \chi < 1 \tag{11}$$

Further, writing

$$\hat{\eta} \cdot \zeta(\chi) = \frac{E^*_{(\rho')}(\chi)}{x'_{(\rho')}} \tag{12}$$

and

$$\eta_{(\rho')} = \frac{E_{(\rho')} + kT \ln \tau_{(\rho')}}{x'_{(\rho')}} \tag{13}$$

Eq. (8) can be represented as

$$Y_{(\rho')} \approx e^{-\frac{\eta_{(\rho')} - \hat{\eta} \cdot \zeta(\chi)}{kT} - \frac{\ln p^*_{(\rho')}(\chi)}{x'_{(\rho')}}} (\varphi m + 1) \tag{14}$$

In the above, the function $\zeta(\chi)$ is characterized by the relationships

$$\lim_{\chi \to +0} \zeta(\chi) = 0 \tag{15}$$

and

$$\lim_{\chi \to 1-0} \zeta(\chi) = 1 \tag{16}$$

and, in many instances, it can be assumed that ζ increases monotonically with an increase of χ; this assumption will be used in all the arguments below.

$\hat{\eta}$ is a constant, and, if repulsive (attractive) interactions occur among sample molecules on the adsorbent surface, $\hat{\eta} > 0$ ($\hat{\eta} < 0$); in the absence of the energetical interactions, $\hat{\eta} = 0$.

The function $p^*_{(\rho')}(\chi)$, which takes only positive values, is characterized by the relationships

$$\lim_{\chi \to +0} p^*_{(\rho')}(\chi) = 1 \tag{17}$$

and

$$\lim_{\chi \to 1-0} p^*_{(\rho')}(\chi) = 0 \tag{18}$$

[cf. the explanation of $p^*_{(\rho')}(\theta)$ in Section A].

Let $m^0_{(\rho')}$ be the m value occurring when both $Y_{(\rho')} = 1$ and $\chi \to +0$ in Eq. (14). By using both Eqs. (15) and (17), $m^0_{(\rho')}$ can be represented as

$$m^0_{(\rho')} = \frac{e^{\eta_{(\rho')}/kT} - 1}{\varphi} \tag{19}$$

Finally, let $\hat{m}_{(\rho')}$ be the m value occurring when both $Y_{(\rho')} = 1$ and $\chi \to 1 - 0$ in Eq. (14). $\hat{m}_{(\rho')}$ can be represented as

$$\hat{m}_{(\rho')} = \frac{e^{(\eta_{(\rho')} - \hat{\eta})/kT} - 1}{\varphi} \tag{20}$$

Equation (20) has been derived by using both Eqs. (16) and (18), taking into account the consideration that $\lim_{\chi \to 1-0} \ln p^*_{(\rho')}(\chi) = -\infty$ but that the term $[\ln p^*_{(\rho')}(\chi)]/x'_{(\rho')}$ in Eq. (14) continues to take an infinitesimal value until χ just attains unity since $x'_{(\rho')} = \infty$ (Eq. 5). [It should be noted that, due to its physical meaning, $\hat{m}_{(\rho')} \geqslant 0$ whereas, in Eq. 20, $\hat{m}_{(\rho')} < 0$ if $\eta_{(\rho')} < \hat{\eta}$. This means that, in the case of repulsive interactions when $\hat{\eta} > 0$, the situation where both relationships $Y_{(\rho')} = 1$ and $\chi \to 1 - 0$ are fulfilled is realized only if $\eta_{(\rho')} \geqslant \hat{\eta}$ (> 0).]

(C) Priority of the Adsorption among Different Molecular Species

Let $\chi'_{(\rho')}(m)$ be the adsorption capacity of the adsorbent realized *in the presence (on the adsorbent surface) of molecular species* ρ' when the molarity, m, of competing ions is given, i.e., the maximum possible value of χ that can be realized in the presence (on the adsorbent surface) of species ρ'

when m is given. We also define $\Omega_{(\rho')}$ as the total amount of species ρ' in the elementary volume of the column expressed in such a way that $\Omega_{(\rho')} = 1$ when the amount is equal to that which just saturates the total surfaces of the adsorbent in the column element (i.e., the amount that just realizes the state of $\chi = 1$). We discuss below the priority of the adsorption among different molecular species occurring in different cases when m is given.

[I] The case when $\chi'_{(1)}(m) = \chi'_{(2)}(m) = \cdots = \chi'_{(\rho)}(m)$ and $[E_{(\rho')} + kT \ln \tau_{(\rho')}]/[E_{(\rho'+1)} + kT \ln \tau_{(\rho'+1)}] > 1 (\rho' = 1, 2, \ldots, \rho - 1)$: If $\Sigma_{\rho''=1}^{\rho} \Omega_{(\rho'')} \leqslant \chi'_{(1)}(m) = \chi'_{(2)}(m) = \cdots = \chi'_{(\rho)}(m)$, then, due to the physical meaning of $\chi'_{(\rho'')}(m)$ $(\rho'' = 1, 2, \ldots, \rho)$, all the molecules are adsorbed. If $\Sigma_{\rho''=1}^{\rho} \Omega_{(\rho'')} > \chi'_{(1)}(m) = \chi'_{(2)}(m) = \cdots = \chi'_{(\rho)}(m)$, then the adsorption occurs according to priority in the order of species $1, 2, \ldots, \rho$. Thus, due to the assumption, $E_{(\rho')} + kT \ln \tau_{(\rho')} - (E_{(\rho'+1)} + kT \ln \tau_{(\rho'+1)}) \approx \infty$ (cf. Eqs. 5 and 6); this means that, out of the total amount $\Sigma_{\rho''=1}^{\rho} \Omega_{(\rho'')}$ of the molecules, $\Sigma_{\rho''=1}^{\rho'-1} \Omega_{(\rho'')} + \kappa \cdot \Omega_{(\rho')}$ $[= \chi'_{(1)}(m) = \chi'_{(2)}(m) = \cdots = \chi'_{(\rho')}(m); 0 \leqslant \kappa \leqslant 1]$ are adsorbed and that the rest of the molecules $[(1 - \kappa) \cdot \Omega_{(\rho')} + \Sigma_{\rho''=\rho'+1}^{\rho} \Omega_{(\rho'')}]$ is in solution where $\rho' = 1, 2, \ldots, \rho$ and the sums $\Sigma_{\rho''=1}^{0} \Omega_{(\rho'')}$ and $\Sigma_{\rho''=\rho+1}^{\rho} \Omega_{(\rho'')}$ are both defined to be zero. Figure 1 represents schematically the aspect of preferential adsorptions among five molecular species occurring when $\Sigma_{\rho''=1}^{5} \Omega_{(\rho'')} > \chi'_{(1)}(m) = \chi'_{(2)}(m) = \chi'_{(3)}(m) = \chi'_{(4)}(m) = \chi'_{(5)}(m)$, $\rho' = 3$ and $0 < \kappa < 1$. It can be seen in Fig. 1 that species 1 and 2 and some part of species 3 are preferentially adsorbed; the other part of species 3 and species 4 and 5 are in solution.

[II] The case when $\chi'_{(1)}(m) > \chi'_{(2)}(m) > \cdots > \chi'_{(\rho)}(m)$: If $\Sigma_{\rho''=1}^{\rho} \Omega_{(\rho'')} < \chi'_{(\rho)}(m)$, then, due to the physical meaning of $\chi'_{(\rho'')}(m)$ $(\rho'' = 1, 2, \ldots, \rho)$, all the molecules are adsorbed. If $\Sigma_{\rho''=1}^{\rho} \Omega_{(\rho'')} > \chi'_{(\rho)}(m)$, then the adsorption can be considered to occur according to priority in the order of species $1, 2, \ldots, \rho$. Thus, if $\chi'_{(\rho'-1)}(m) \geqslant \Sigma_{\rho''=1}^{\rho} \Omega_{(\rho'')} > \chi'_{(\rho')}(m)$ [where $\rho' = 1, 2, \ldots, \rho$, and $\chi'_{(0)}(m)$ is defined to be ∞], then, out of the total amount $\Sigma_{\rho''=1}^{\rho} \Omega_{(\rho'')}$ of the molecules, $\Sigma_{\rho''=1}^{\rho'-1} \Omega_{(\rho'')} + \Sigma_{\rho''=\rho'}^{\rho^*-1} \Omega_{(\rho'')} + \kappa \cdot \Omega_{(\rho^*)}$ $[= \chi'_{(\rho')}(m); \rho^* = \rho', \rho' + 1, \ldots, \rho$ and $0 \leqslant \kappa \leqslant 1]$ would be adsorbed, and the rest of the molecules $[(1 - \kappa) \cdot \Omega_{(\rho^*)} + \Sigma_{\rho''=\rho^*+1}^{\rho} \Omega_{(\rho'')}]$ would be in solution where the sums $\Sigma_{\rho''=1}^{0} \Omega_{(\rho'')}$, $\Sigma_{\rho''=\rho'}^{\rho'-1} \Omega_{(\rho'')}$, and $\Sigma_{\rho''=\rho+1}^{\rho} \Omega_{(\rho'')}$ are defined to be zero. Figure 2 represents schematically the aspect of preferential adsorptions among five molecular species occurring when $\chi'_{(1)}(m) > \chi'_{(2)}(m) \geqslant \Sigma_{\rho''=1}^{5} \Omega_{(\rho'')} > \chi'_{(3)}(m) > \chi'_{(4)}(m) > \chi'_{(5)}(m)$ (i.e., when $\rho' = 3$) and when several ρ^* and κ values are given. Figure 2 can be compared with Fig. 1.

[III] The general case when $\chi'_{(1)}(m) \geqslant \chi'_{(2)}(m) \geqslant \cdots \geqslant \chi'_{(\rho)}(m)$: If $\chi'_{(\rho')}(m) = \chi'_{(\rho'+1)}(m)$, it is possible to number the molecular species in such a way that the relationship $[E_{(\rho')} + kT \ln \tau_{(\rho')}]/[E_{(\rho'+1)} + kT \ln \tau_{(\rho'+1)}] > 1$ is

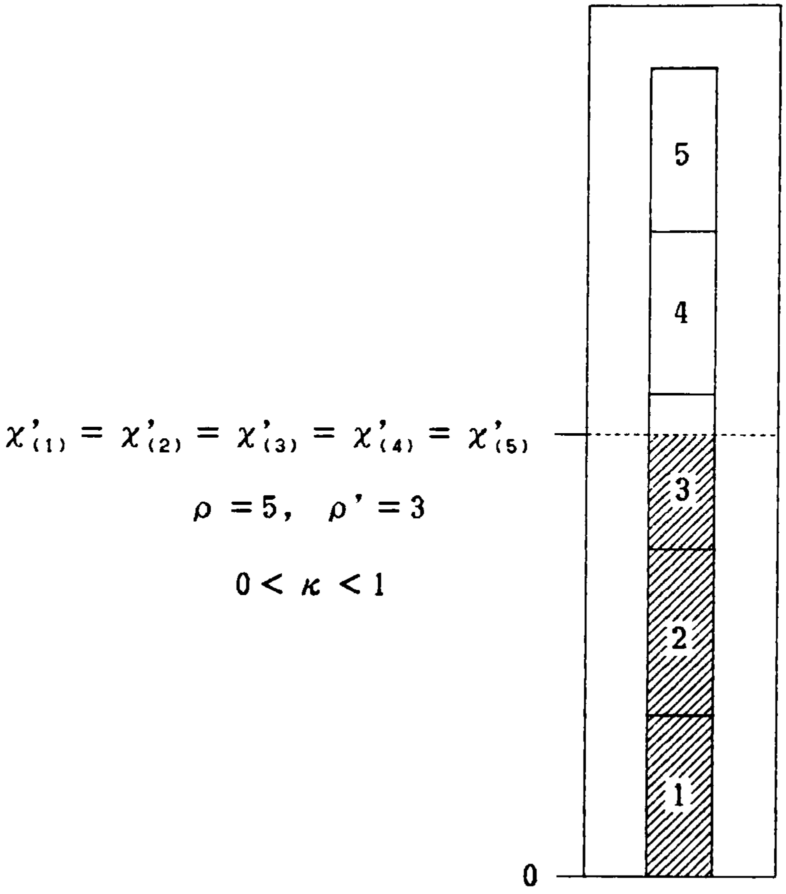

FIG. 1. Schematic representation of the aspect of preferential adsorptions among five molecular species occurring when $\Sigma_{\rho''=1}^{5}\Omega_{(\rho'')} > \chi'_{(1)}(m) = \chi'_{(2)}(m) = \chi'_{(3)}(m) = \chi'_{(4)}(m) = \chi'_{(5)}(m)$, $\rho' = 3$ and $0 < \kappa < 1$. The histogram shows a pile ($\Sigma_{\rho''=1}^{5}\Omega_{(\rho'')}$) of the molecules of the five species, and the part of the histogram drawn with oblique lines shows the molecules that are adsorbed on the adsorbent surface.

fulfilled. By applying this numbering method, it can be concluded on the basis of the arguments made in both paragraphs [I] and [II] that if $\Sigma_{\rho''=1}^{\rho}\Omega_{(\rho'')} < \chi'_{(\rho)}(m)$, then all the molecules are adsorbed; if $\Sigma_{\rho''=1}^{\rho}\Omega_{(\rho'')} > \chi'_{(\rho)}(m)$, then the adsorption occurs according to priority in the order of species 1, 2, . . . , ρ.

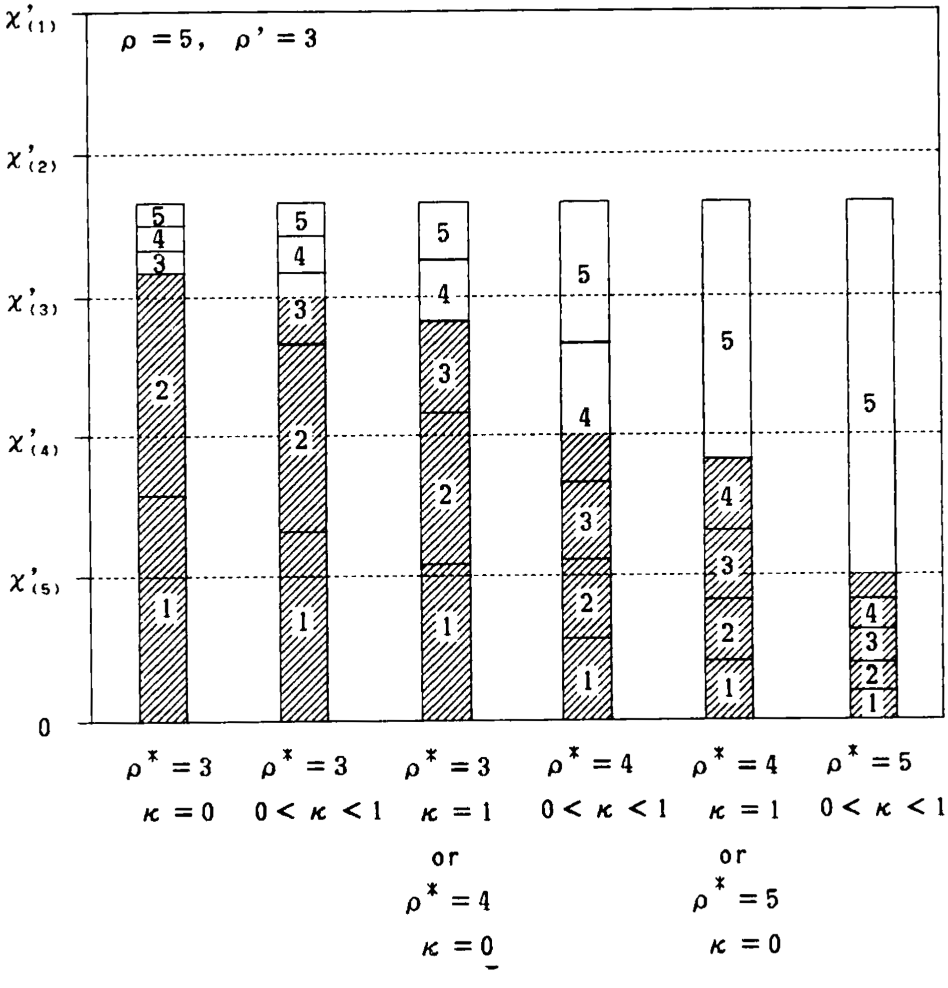

FIG. 2. Schematic representation of the aspect of preferential adsorptions among five molecular species occurring when $\chi'_{(1)}(m) > \chi'_{(2)}(m) > \Sigma^{5}_{\rho''=1}\Omega_{(\rho'')} > \chi'_{(3)}(m) > \chi'_{(4)}(m) > \chi'_{(5)}(m)$ (i.e., when $\rho' = 3$) and when several ρ^* and κ values are given. A histogram shows a pile $(\Sigma^{5}_{\rho''=1}\Omega_{(\rho'')})$ of the molecules of the five species, and the part of the histograms drawn with oblique lines shows the molecules that are adsorbed on the adsorbent surface.

(D) Representations of the Adsorption Capacity $\chi'_{(\rho')}(m)$

The adsorption capacity $\chi'_{(\rho')}(m)$ that has been introduced in Section (C) can be represented for the respective cases of repulsive ($\hat{\eta} > 0$), no ($\hat{\eta} = 0$), and attractive ($\hat{\eta} < 0$) molecular interactions as follows.

[I] The case of repulsive ($\hat{\eta} > 0$) interactions:

$$\left.\begin{aligned} \chi'_{(\rho')}(m) &= 1 \qquad (0 \leqslant m < \hat{m}_{(\rho')}) \\ \chi'_{(\rho')}(m) &= \zeta^{-1}\left[\frac{\eta_{(\rho')} - kT\ln(\varphi m + 1)}{\hat{\eta}}\right] \\ &= \zeta^{-1}\left[\ln\frac{\varphi m^0_{(\rho')} + 1}{\varphi m + 1} \Big/ \ln\frac{\varphi m^0_{(\rho')} + 1}{\varphi \hat{m}_{(\rho')} + 1}\right] \qquad (\hat{m}_{(\rho')} \leqslant m < m^0_{(\rho')}) \\ \chi'_{(\rho')}(m) &= 0 \qquad (m \geqslant m^0_{(\rho')}) \end{aligned}\right\} \tag{21}$$

where ζ^{-1} represents the inverse function of ζ.

Proof. When $\hat{\eta} > 0$, then $\hat{m}_{(\rho')} < m^0_{(\rho')}$ (Eqs. 19 and 20). Let us first give a proof of the first equation in Eq. (21). Thus, when $0 \leqslant m < \hat{m}_{(\rho')}$, if $\chi < 1$, then it can be concluded from Eq. (14) that $Y_{(\rho')} < 1$ since $[\ln p^*_{(\rho')}(\chi)]/x'_{(\rho')} \approx 0$ (cf. the explanation of Eq. 20). This means that $B'_{(\rho')} = 0$ (Eq. 1; cf. Eq. 5), or that all the ρ' molecules are adsorbed. It follows from this that the state of $\chi = 1$ is attainable in the presence of molecular species ρ', and the first equation in Eq. (21) is derived. Let us give a proof of the second equation in Eq. (21). Thus, it can be concluded from Eq. (14) that, when both $\hat{m}_{(\rho')} < m < m^0_{(\rho')}$ and $Y_{(\rho')} = 1$, then $\chi < 1$; this means that $[\ln p^*_{(\rho')}(\chi)]/x'_{(\rho')} \approx 0$ (cf. the argument in Section B) and that the relationship:

$$e^{-\frac{\eta_{(\rho')} - \hat{\eta}\cdot\zeta(\chi)}{kT}}(\varphi m + 1) = 1 \tag{22}$$

is realized. $\chi'_{(\rho')}(m)$ in the second equation in Eq. (21) represents χ fulfilling Eq. (22). Therefore, if $\chi'_{(\rho')}(m)$ in the second equation in Eq. (21) is substituted into χ in Eq. (14), then $Y_{(\rho')} = 1$ is obtained. If, in Eq. (14), the χ value is increased from $\chi'_{(\rho')}(m)$ while fixing the m value, then $Y_{(\rho')}$ increases from unity, and we have $B'_{(\rho')} = 1$ (Eq. 1; cf. Eq. 5). To the contrary, if, in Eq. (14), the χ value is decreased from $\chi'_{(\rho')}(m)$ while fixing the m value, then $Y_{(\rho')}$ decreases from unity, and we have $B'_{(\rho')} = 0$ (Eq. 1; cf. Eq. 5). This means that, if χ increases from $\chi'_{(\rho')}(m)$, the desorption of ρ'

molecules occurs (since $B'_{(\rho')} = 1$). If χ decreases from $\chi'_{(\rho')}(m)$ due to the desorption, however, the adsorption of ρ' molecules begins (since $B'_{(\rho')} = 0$). It can therefore be concluded that $\chi'_{(\rho')}(m)$ in the second equation in Eq. (21), in fact, has the physical meaning of the adsorption capacity when $\acute{m}_{(\rho')} < m < m^0_{(\rho')}$. On the basis of the second equation in Eq. (21), it can be shown that, when $m \to \acute{m}_{(\rho')} + 0$, then $\chi'_{(\rho')}(m) \to 1 - 0$; this means that $\chi'_{(\rho')}(m)$ given by the second equation in Eq. (21) is continuous with $\chi'_{(\rho')}(m)$ given by the first equation in Eq. (21), from which it can be concluded that $\chi'_{(\rho')}(m)$ in the second equation in Eq. (21) has the physical meaning of the adsorption capacity even when $\acute{m}_{(\rho')} \leqslant m < m^0_{(\rho')}$. It is easy to derive the right-hand side term from the intermediate term in the second equation in Eq. (21) by using both Eqs. (19) and (20). Finally, let us give a proof of the third equation in Eq. (21). Thus, it can be concluded from Eq. (14) that, when $m > m^0_{(\rho')}$, then $Y_{(\rho')} > 1$; this means that $B'_{(\rho')} = 1$ (Eq. 1; cf. Eq. 5) and that the third equation in Eq. (21) is fulfilled. On the basis of the second equation in Eq. (21), it can be shown that, when $m \to m^0_{(\rho')} - 0$, then $\chi'_{(\rho')}(m) \to +0$; this means that $\chi'_{(\rho')}(m)$ given by the second equation in Eq. (21) is continuous with $\chi'_{(\rho')}(m)$ given by the third equation in Eq. (21). It can therefore be concluded that the third equation in Eq. (21) is fulfilled even when $m \geqslant m^0_{(\rho')}$. *Q.E.D.*

[II] The case of no ($\acute{\eta} = 0$) interactions:

$$\begin{aligned} &\chi'_{(\rho')}(m) = 1 \qquad (0 \leqslant m < m^0_{(\rho')}) \\ &\chi'_{(\rho')}(m) = 0 \qquad (m \geqslant m^0_{(\rho')}) \end{aligned} \tag{23}$$

Proof. When $\acute{\eta} = 0$, then $\acute{m}_{(\rho')} = m^0_{(\rho')}$ (Eqs. 19 and 20). It can therefore be considered that the case of no interactions can be represented as an extreme case of repulsive interactions occurring when the $\acute{m}_{(\rho')}$ value approaches the $m^0_{(\rho')}$ value. This consideration leads to Eq. (23). Equation (23) can also be derived as an extreme case of attractive interactions. *Q.E.D.*

[III] The case of attractive ($\acute{\eta} < 0$) interactions:

$$\begin{aligned} \chi'_{(\rho')}(m) = 1 \qquad & 0 \leqslant m < m^0_{(\rho')}; \text{ or } m^0_{(\rho')} \leqslant m \leqslant \acute{m}_{(\rho')} \text{ and } \sum_{\rho''=1}^{\dot{\rho}} \Omega_{(\rho'')} \\ & \geqslant \zeta^{-1} \text{ for at least one of all possible } \dot{\rho} \text{ values)} \\ \chi'_{(\rho')}(m) = 0 \qquad & (m^0_{(\rho')} \leqslant m \leqslant \acute{m}_{(\rho')} \text{ and } \sum_{\rho''=1}^{\dot{\rho}} \Omega_{(\rho'')} < \zeta^{-1} \text{ for the} \end{aligned} \tag{24}$$

maximum possible $\dot{\rho}$ value; or $m > \hat{m}_{(\rho')}$)

where ζ^{-1} is an abbreviation for

$$\zeta^{-1}\left[\frac{\eta_{(\rho')} - kT\ln(\varphi m + 1)}{\hat{\eta}}\right]$$

or

$$\zeta^{-1}\left[\ln\frac{\varphi m^{0}_{(\rho')} + 1}{\varphi m + 1}\Big/\ln\frac{\varphi m^{0}_{(\rho')} + 1}{\varphi \hat{m}_{(\rho')} + 1}\right]$$

(cf. Eq. 21), and $\dot{\rho}$ represents molecular species fulfilling both relationships $\dot{\rho} > \rho'$ and $m < \hat{m}_{(\dot{\rho})}$; the molecular adsorption occurs according to priority in the order of $1, 2, \ldots, \rho$.

Proof. When $\hat{\eta} < 0$, then $m^{0}_{(\rho')} < \hat{m}_{(\rho')}$ (Eqs. 19 and 20). On the basis of the argument similar to that made for the case of repulsive interactions when $0 \leqslant m < \hat{m}_{(\rho')}$ (see paragraph [I]), it can be concluded that, when $0 \leqslant m < m^{0}_{(\rho')}$, then $\chi'_{(\rho')}(m) = 1$. Similarly, on the basis of the argument similar to that made for the case of repulsive interactions when $m > m^{0}_{(\rho')}$ (see paragraph [I]), it can be concluded that, when $m > \hat{m}_{(\rho')}$, then $\chi'_{(\rho')}(m) = 0$. When $m^{0}_{(\rho')} \leqslant m \leqslant \hat{m}_{(\rho')}$, by substituting ζ^{-1} into χ in Eq. (14), $Y_{(\rho')} = 1$ is obtained (cf. the argument in paragraph [I] for the case of repulsive interactions when $\hat{m}_{(\rho')} \leqslant m < m^{0}_{(\rho')}$). In contrast to the case of repulsive interactions, however, if, in Eq. (14), the χ value is increased from ζ^{-1} while fixing the m value, then $Y_{(\rho')}$ decreases from unity, and we have $B'_{(\rho')} = 0$ (Eq. 1; cf. Eq. 5). If the χ value is decreased from ζ^{-1} while fixing the m value, then $Y_{(\rho')}$ increases from unity, and we have $B'_{(\rho')} = 1$ (Eq. 1; cf. Eq. 5). It can therefore be concluded that, if χ exceeds the ζ^{-1} value, then the adsorption of ρ' molecules continues until χ attains the maximum value of unity; this means that $\chi'_{(\rho')}(m) = 1$. Unless χ exceeds the ζ^{-1} value, the adsorption of ρ' molecules does not occur at all; this means that $\chi'_{(\rho')}(m) = 0$. By defining molecular species $1, 2, \ldots, \rho$ so that the priority of the adsorption be in this order, species $\dot{\rho}$ ($\dot{\rho} = \rho', \rho' + 1, \rho' + 2, \ldots$) generally exist which fulfill the relationship $m < \hat{m}_{(\dot{\rho})}$ at a given m; the given m fulfills the relationship of $m^{0}_{(\rho')} < m < \hat{m}_{(\rho')}$ (cf. Fig A3 in Appendix I). This means that, if the relationship $\Sigma^{\dot{\rho}}_{\rho''=1}\Omega_{(\rho'')} > \zeta^{-1}$ is fulfilled for at least one of all possible $\dot{\rho}$ values, then $\chi'_{(\rho')}(m) = 1$. If the relationship $\Sigma^{\dot{\rho}}_{\rho''=1}\Omega_{(\rho'')} < \zeta^{-1}$ is fulfilled for all possible $\dot{\rho}$ values, then $\chi'_{(\rho')}(m) = 0$; in order for the relationship of $\Sigma^{\dot{\rho}}_{\rho''=1}\Omega_{(\rho'')} < \zeta^{-1}$ to be fulfilled for all possible $\dot{\rho}$ values, it is sufficient that the same relationship be fulfilled for the maximum possible $\dot{\rho}$ value. *Q.E.D.*

(E) Relative and Approximate Expressions of $\chi'_{(\rho')}(m)$

Let us introduce relative parameters:

$$y = \frac{m}{m^0_{(1)}} \tag{25}$$

$$y^0_{(\rho')} = \frac{m^0_{(\rho')}}{m^0_{(1)}} \tag{26}$$

$$\hat{y}_{(\rho')} = \frac{\hat{m}_{(\rho')}}{m^0_{(1)}} \tag{27}$$

$$w_{(\rho')} = \frac{\eta_{(\rho')}}{\eta_{(1)}} \tag{28}$$

and

$$\Xi = \frac{\hat{\eta}}{\eta_{(1)}} \tag{29}$$

By using both Eqs. (19) and (20), $y^0_{(\rho')}$ and $\hat{y}_{(\rho')}$ can be represented as

$$y^0_{(\rho')} = \frac{e^{w_{(\rho')}\cdot\eta_{(1)}/kT} - 1}{e^{\eta_{(1)}/kT} - 1} = \frac{(\varphi m^0_{(1)} + 1)^{w_{(\rho')}} - 1}{\varphi m^0_{(1)}} \approx w_{(\rho')} \tag{30}$$

and

$$\hat{y}_{(\rho')} = \frac{e^{(w_{(\rho')} - \Xi)\cdot\eta_{(1)}/kT} - 1}{e^{\eta_{(1)}/kT} - 1} = \frac{(\varphi m^0_{(1)} + 1)^{w_{(\rho')} - \Xi} - 1}{\varphi m^0_{(1)}} \approx w_{(\rho')} - \Xi \tag{31}$$

respectively.

We write below the relative expressions $\chi'_{(\rho')}(y)$ of $\chi'_{(\rho')}(m)$ for the respective cases of repulsive, no, and attractive molecular interactions; the approximate expression of $\chi'_{(\rho')}(y)$ (occurring when the extreme right-hand sides of both Eqs. 30 and 31 are realized) is also shown (see the second equation in Eq. 32):

[I] The case of repulsive ($\hat{\eta} > 0$ or $\Xi > 0$) interactions:

$$\left.\begin{aligned}
&\chi'_{(\rho')}(y) = 1 \qquad (0 \leqslant y < \hat{y}_{(\rho')}) \\
&\chi'_{(\rho')}(y) = \zeta^{-1}\left\{\frac{w_{(\rho')} - \dfrac{\ln[(e^{\eta_{(1)}/kT} - 1)\cdot y + 1]}{\eta_{(1)}/kT}}{\Xi}\right\} \\
&\qquad = \zeta^{-1}\left\{\frac{w_{(\rho')} - \dfrac{\ln(\varphi m^0_{(1)}y + 1)}{\ln(\varphi m^0_{(1)} + 1)}}{\Xi}\right\} \\
&\qquad \approx \zeta^{-1}\left[\frac{w_{(\rho')} - y}{\Xi}\right] \qquad (\hat{y}_{(\rho')} \leqslant y < y^0_{(\rho')}) \\
&\chi'_{(\rho')}(y) = 0 \qquad (y \geqslant y^0_{(\rho')})
\end{aligned}\right\} \tag{32}$$

[II] The case of no ($\hat{\eta} = 0$ or $\Xi = 0$) interactions:

$$\left.\begin{aligned}
&\chi'_{(\rho')}(y) = 1 \qquad (0 \leqslant y < y^0_{(\rho')}) \\
&\chi'_{(\rho')}(y) = 0 \qquad (y \geqslant y^0_{(\rho')})
\end{aligned}\right. \tag{33}$$

[III] The case of attractive ($\hat{\eta} < 0$ or $\Xi < 0$) interactions:

$$\begin{aligned}
&\chi'_{(\rho')}(y) = 1 \qquad \Big(0 \leqslant y < y^0_{(\rho')};\ \text{or } y^0_{(\rho')} \leqslant y \leqslant \hat{y}_{(\rho')} \text{ and } \sum_{\rho''=1}^{\dot{\rho}} \Omega_{(\rho'')} \geqslant \zeta^{-1} \\
&\qquad\qquad \text{for at least one of all possible } \dot{\rho} \text{ values}\Big) \\
&\chi'_{(\rho)}(y) = 0 \qquad \Big(y^0_{(\rho')} \leqslant y \leqslant \hat{y}_{(\rho')} \text{ and } \sum_{\rho''=1}^{\dot{\rho}} \Omega_{(\rho'')} < \zeta^{-1} \text{ for the maximum} \\
&\qquad\qquad \text{possible } \dot{\rho} \text{ value; or } y > \hat{y}_{(\rho')}\Big)
\end{aligned} \tag{34}$$

where $\dot{\rho}$ represents molecular species fulfilling both relationships $\dot{\rho} \geqslant \rho'$ and $y \leqslant \hat{y}_{(\dot{\rho})}$.

In Appendix I, the approximate $\chi'_{(\rho')}(y)$'s in Eqs. (32)–(34) are diagrammatically represented assuming that the function $\zeta(\chi)$ (Eq. 12) can be written as $\zeta(\chi) = \sqrt{\chi}$ (for this assumption, see Discussion Section A).

(F) Representations of $B'_{(\rho')}$ in Terms of $\chi'_{(\rho')}(m)$

Provided the molecular adsorption occurs according to priority in the order of 1, 2, ..., ρ, the partition $B'_{(\rho')}$ (where $\rho' = 1, 2, \ldots, \rho$) can be represented in terms of $\chi'_{(\rho')}$ as

$$\left.\begin{array}{ll} B'_{(\rho')} = 0 & \left[\sum\limits_{\rho''=1}^{\rho'} \chi_{(\rho'')} < \chi'_{(\rho')}(m)\right] \\[2ex] B'_{(\rho')} = \gamma'_{(\rho')} & \left[\sum\limits_{\rho''=1}^{\rho'} \chi_{(\rho'')} = \chi'_{(\rho')}(m)\right] \\[2ex] B'_{(\rho')} = 1 & \left[\sum\limits_{\rho''=1}^{\rho'} \chi_{(\rho'')} > \chi'_{(\rho')}(m)\right] \end{array}\right\} \tag{35}$$

in which

$$0 < \gamma'_{(\rho')} < 1 \tag{36}$$

$B'_{(\rho')}$ can also be represented as

$$\left.\begin{array}{ll} B'_{(\rho')} = 0 & (\gamma_{(\rho')} < 0) \\ B'_{(\rho')} = \gamma_{(\rho')} & (0 < \gamma_{(\rho')} < 1) \\ B'_{(\rho')} = 1 & (\gamma_{(\rho')} > 1) \end{array}\right\} \tag{37}$$

in which

$$\gamma_{(\rho')} = \frac{\sum\limits_{\rho''=1}^{\rho'} \Omega_{(\rho'')} - \chi'_{(\rho')}(m)}{\Omega_{(\rho')}} \tag{38}$$

In Appendix II, $B'_{(\rho')}$'s for both single and three component systems are illustrated as functions of y for the three cases of repulsive, no, and attractive molecular interactions. For the calculation, the approximate expressions of Eqs. (32)-(34) (cf. Eqs. 30 and 31) and Eqs. (37) and (38) have been used, where it is assumed that $\zeta(\chi) = \sqrt{\chi}$ (cf. Discussion

Section A). In the case of a single component system, the subscript (ρ′) is deleted from any parameter.

(G) Dynamic Part: Fundamental Differential Equation in the Absence of Longitudinal Diffusion in the Column and the Calculation of the Chromatogram on the Basis of the Equation

For a mixture of components, 1, 2, . . . , ρ, the chromatographic behavior of the molecules both in the presence of mutual molecular interactions on the adsorbent surface and in the absence of longitudinal diffusion in the column can be represented by using simultaneous partial differential equations (Eq. 1 in Ref. *6*) as

$$\frac{\partial\left[\dfrac{B_{(\rho')}(m,\chi)}{1 - B_{(\rho')}(m,\chi)} \cdot \chi_{(\rho')}\right]}{\partial s} + \frac{\partial \chi_{(\rho')}}{\partial m} = 0 \qquad (\rho' = 1, ,2, \ldots, \rho) \qquad (39)$$

where

$$B_{(\rho')}(m,\chi) = B'_{(\rho')}(m,\chi) \qquad (40)$$

and

$$s = g' \cdot L' = g \cdot L \qquad (41)$$

In Eq. (39), $B_{(\rho')}(m,\chi)$ represents the partition of sample molecules (of the chromatographic component ρ′ under consideration) in the mobile phase *in the vertical section* of the column; this is equal to the partition $B'_{(\rho')}(m,\chi)$ occurring *in an elementary volume* that belongs in the vertical section under consideration provided there is no longitudinal diffusion in the column (see Eq. 40). (For the reason why $B'_{(\rho')}$ and $B_{(\rho')}$ can be represented as functions of m and χ, see Section B.) In Eq. (41), g' and g represent the slope of the molarity gradient of competing ions in the column expressed as the increase in molarity per unit interstitial volume and unit length of the column, measured from the outlet to the inlet, respectively; L' and L represent the total interstitial volumes and the total length of the column, respectively. (In some instances, L' and L represent the distance from the inlet of the column of any longitudinal position of the column, expressed in units of volume and length, respectively.) s (Eq. 41), therefore, has a dimension of molarity.

The chromatogram, $f_{(\rho'),s}$, for a component ρ' occurring when s is given (i.e., when both the slope g of the gradient and the length L of the column are given) can be represented as a function of m by using a solution, $\chi_{(\rho')}$, of Eq. (39) as

$$f_{(\rho'),s}(m) = -\left[\frac{\partial \chi_{(\rho')}}{\partial m}\right]_s \tag{42}$$

fulfilling a conservation condition:

$$\int_{m_{\mathrm{in}}}^{\infty} f_{(\rho'),s}(m)\, dm = \chi^*_{(\rho')} \tag{43}$$

where m_{in} represents the molarity of competing ions at the beginning of the molarity gradient introduced at the inlet of the column; $\chi^*_{(\rho')}$ represents the total amount of component ρ' that has initially been loaded on the column, expressed as the proportion of the adsorption surfaces in the total column that are initially occupied by the molecules of component ρ'. (For details, see Refs. *3* and *6*.)

With ideal molecules fulfilling Eqs. (5)–(7), however, $\chi_{(\rho')}$ is uniquely determined by $\chi'_{(\rho')}(m)$ (Sections C and D), and $\chi'_{(\rho')}(m)$ is independent of s. This means that $\chi'_{(\rho')}(m)$ or $\chi_{(\rho')}$ does not directly depend upon g' or g (Eq. 41), and that the chromatographic behavior of the molecules can be represented by using simultaneous ordinary differential equations that can be derived as an extreme form of Eq. (39) occurring when g' or g tends to infinitesimal:

$$-\frac{d\chi_{(\rho')}}{d(m/\delta s)} = \frac{B_{(\rho')}(m,\chi)}{1 - B_{(\rho')}(m,\chi)} \cdot \chi_{(\rho')} \qquad (\rho' = 1, 2, \ldots, \rho) \tag{44}$$

where

$$\delta s = \lim_{g' \to +0} (g' \cdot L') = \lim_{g \to +0} (g \cdot L) \tag{45}$$

δs (Eq. 45) has a physical meaning of the molarity difference of competing ions between the inlet and the outlet of the total column, being constant (with respect to time) with linear gradient chromatography. $d(m/\delta s)$ (Eq. 44) therefore represents the increase in ion molarity measured in unit of δs. $B_{(\rho')}(m,\chi)$ in Eq. (44) now represents the partition of ρ' molecules in the mobile phase *in the total column*.

Proof of Eq. (44). The proof is involved in the derivation process of Eq. (11) in Ref. *6*. Thus, at the inlet ($L = 0$) of the column where the relationship $s = 0$ is fulfilled (See Eq. 41), the inflow of molecules does not occur when once the sample load is finished and a condition is fulfilled that when $s = 0$, then $\chi_{(\rho')} = 0$ (where $\rho' = 1, 2, \ldots, \rho$). This means that when g' or g tends to infinitesimal, the first term on the left-hand side of Eq. (39) becomes

$$\frac{\partial\left[\frac{B_{(\rho')}}{1 - B_{(\rho')}} \cdot \chi_{(\rho')}\right]}{\partial s} = \frac{1}{\delta s} \cdot \left\{\left[\frac{B_{(\rho')}}{1 - B_{(\rho')}} \cdot \chi_{(\rho')}\right]_{m,s=\delta s}\right.$$

$$\left. - \left[\frac{B_{(\rho')}}{1 - B_{(\rho')}} \cdot \chi_{(\rho')}\right]_{m,s=0}\right\} = \frac{1}{\delta s} \cdot \left[\frac{B_{(\rho')}}{1 - B_{(\rho')}} \cdot \chi_{(\rho')}\right]_{m,s=\delta s} \tag{46}$$

By substituting Eq. (46) into Eq. (39), Eq. (44) can be derived. *Q.E.D.*

Equation (44) can be solved under the initial condition that, when $m = m_{\text{in}}$, then

$$\chi_{(\rho')} = \chi^{*}_{(\rho')} \qquad (\rho' = 1, 2, \ldots, \rho) \tag{47}$$

where it is assumed that the initial molecular density on the adsorbent surfaces in the column is homogeneous or that it decreases monotonically from the inlet to the outlet of the column (for the exceptional case of attractive molecular interactions, see Section H). It is also assumed that the relationship

$$m_{\text{in}} < \frac{e^{[\eta_{(\rho')} - \hat{\eta} \cdot \zeta(\Sigma^{\rho}_{\rho''=1} \chi^{*}_{(\rho'')})]/kT} - 1}{\varphi} \qquad (\rho' = 1, 2, \ldots, \rho) \tag{48}$$

is fulfilled; Eq. (48) gives a necessary condition for Eq. (47) to be actually realized (cf. Eq. 20). The chromatogram $f_{(\rho')}$, which is independent of s (i.e., of both g and L), can be represented as a function of m by using a solution, $\chi_{(\rho')}$, of Eq. (44) as

$$f_{(\rho')}(m) = -\frac{d\chi_{(\rho')}}{dm} \tag{49}$$

fulfilling a conservation condition:

$$\int_{m_{\rm in}}^{\infty} f_{(\rho')}(m)\, dm = \chi^*_{(\rho')} \tag{50}$$

(H) Theoretical Chromatograms in the Case of a Single Component System

We write below the mathematical expressions of the theoretical chromatograms with a single component system obtained on the basis of Eqs. (44), (47), and (49) for the cases of repulsive, no, and attractive molecular interactions, where the proofs of the expressions are added. With a single component system, Eq. (44) represents a single equation, and the subscript (ρ′) is deleted from all the mathematical formulas.

[I] The case of repulsive ($\hat{\eta} > 0$) interactions:

$$\left.\begin{aligned} & f = 0 \qquad \left(m_{\rm in} < m < \frac{e^{[\eta - \hat{\eta}\cdot\zeta(\chi^*)]/kT} - 1}{\varphi}\right) \\ & f = -\frac{d}{dm}\,\zeta^{-1}\left[\frac{\eta - kT\ln(\varphi m + 1)}{\hat{\eta}}\right] \\ & \quad = -\frac{d}{dm}\,\zeta^{-1}\left[\ln\frac{\varphi m^0 + 1}{\varphi m + 1}\Big/\ln\frac{\varphi m^0 + 1}{\varphi \hat{m} + 1}\right] \qquad \left(\frac{e^{[\eta - \hat{\eta}\cdot\zeta(\chi^*)]/kT} - 1}{\varphi}\right. \\ & \quad \left. < m < m^0\right) \\ & f = 0 \qquad (m > m^0) \end{aligned}\right\} \tag{51}$$

Proof. When $m < (e^{[\eta-\hat{\eta}\cdot\zeta(\chi^*)]/kT} - 1)/\varphi$, then $\chi^* < \chi'$. This is because $(e^{[\eta-\hat{\eta}\cdot\zeta(\chi^*)]/kT} - 1)/\varphi$ represents the m value occurring when $\chi' = \chi^*$, and, if m decreases from this value, χ' increases (the case when $\chi^* < 1$) or χ' continues to take a constant value of unity (the case when $\chi^* = 1 - 0$). This means that $B' = 0$ (Eq. 35). It follows from this that $B = 0$ (Eq. 40), that $d\chi = 0$ (Eq. 44), that $\chi = \chi^*$ (Eq. 47), and that $f = 0$ (Eq. 49), thus demonstrating the first equation in Eq. (51). When $(e^{[\eta-\hat{\eta}\cdot\zeta(\chi^*)]/kT} - 1)/\varphi < m < m^0$, then $\chi^* > \chi'$. Therefore, from the physical meaning of χ', $\chi = \chi'(m) = \zeta^{-1}\{[\eta - kT\ln(\varphi m + 1)]/\hat{\eta}\}$ (see the second equation in Eq. 21). This, in fact, is the solution to Eq. (44) fulfilling the initial condition given by Eq. (47) since, when $m \rightarrow (e^{[\eta-\hat{\eta}\cdot\zeta(\chi^*)]/kT} - 1)/\varphi$ then $\chi \rightarrow \chi^*$. [It should be recalled that when $m_{\rm in} < m < (e^{[\eta-\hat{\eta}\cdot\zeta(\chi^*)]/kT} - 1)/\varphi$, then $\chi = \chi^*$; see above.

Here, it should be noted that the parameter $B_{(\rho')}$ or B fulfilling Eq. (44) is spontaneously determined if the function $\chi_{(\rho')}(m)$ or $\chi(m)$ fulfilling the initial condition given by Eq. (47) is determined by means of any consideration. In fact, $B_{(\rho')}$ or B is a function of $\chi_{(\rho')}$ or χ, and the decrease of $\chi_{(\rho')}$ occurring with an increase of m or $m/\delta s$ (left-hand side of Eq. 44) should represent the amount of ρ' molecules existing in solution in the total column (right-hand side of Eq. 44). This means that the function $\chi_{(\rho')}(m)$ or $\chi(m)$ as such is the solution to Eq. 44.] By substituting the expression of χ obtained above into Eq. (49), the second equation in Eq. (51) can be derived. Further, as far as χ that has just been obtained is concerned, when $m \rightarrow m^0$, then $\chi \rightarrow 0$. This means that, when $m > m^0$, then the relationship $\chi = 0$ holds in general, and that $f = 0$ (Eq. 49), thus demonstrating the third equation in Eq. (51). *Q.E.D.*

[II] The case of no ($\hat{\eta} = 0$) interactions:

$$f = \chi^* \cdot \delta(m - m^0) \tag{52}$$

in which δ represents the delta function.

Proof. Using the argument similar to that used in the proof of Eq. (51), it can be concluded that when $m < m^0$, then $\chi^* < \chi'$, and that $\chi = \chi^*$. When $m > m^0$, it is evident from the physical meaning of χ' that $\chi = \chi' = 0$ (see the second equation in Eq. 23). Therefore, by using the Heaviside function $H(x)$ [defined as $H(x) = 0$ when $x < 0$ and as $H(x) = 1$ when $x > 0$; $H(x)$ has a property that $dH(x)/dx = \delta(x)$], χ can be represented as $\chi = \chi^* \cdot [1 - H(m - m^0)]$, and we have $-d\chi/dm = \chi^* \cdot \delta(m - m^0)$. Therefore, by substituting this expression of $-d\chi/dm$ into Eq. (49), Eq. (52) can be derived. [For the fact that the function $\chi(m)$ as such can be considered to be a solution to Eq. 44, cf. the proof of Eq. 51.] *Q.E.D.*

[III] The case of attractive ($\hat{\eta} < 0$) interactions:

$$f = \chi^* \cdot \delta(m - \hat{m}) \tag{53}$$

Proof. The case of attractive interactions is different from both cases of repulsive and no interactions in that χ' depends upon Ω, i.e., χ', in general, increases with an increase of Ω (Eq. 24). As a result, the initial condition given by Eq. (47) cannot be applied directly, and the chromatographic behavior of the molecules is determined by the maximal value of χ' or χ that is locally realized in the vicinity of the column inlet at the initial stage of chromatography. The situation can be compared with the cases of repulsive and no interactions where the chromatographic behavior of the molecules is determined by the mean χ'

value occurring in the interior of the total column. Thus, usually, the sample molecules dissolved in the initial buffer with molarity m_{in} are loaded on the column, and the vicinity of the column inlet is saturated with molecules forming the initial band in which the state of $\Omega = \chi = 1$ is realized. When $m_{\text{in}} < m < \hat{m}$, the adsorption capacity χ' in the interior of the initial band conserves the maximal value of unity since $\Omega = 1 > \zeta^{-1}$ (see the first equation in Eq. 24; it should be noted that the maximum possible value of ζ^{-1} is unity), and the state of $\chi = \chi' = 1$ continues to be realized. When $m > \hat{m}$, then $\chi' = 0$ (see the second equation in Eq. 24), and it can be concluded from the physical meaning of χ' that $\chi = \chi' = 0$. Now, following the argument similar to that used in the proof of Eq. (52), Eq. (53) can be proved; in Eq. (53), χ^*, of course, represents the initial mean value of χ occurring in the interior of the total column. *Q.E.D.*

Conclusion. In the absence of the energetical molecular interactions ($\hat{\eta} = 0$), a chromatographic peak with an infinitesimal width occurs at molarity m^0 of the gradient; both the position and the shape of the peak are independent of the sample load (see Eq. 52). In the case of attractive interactions ($\hat{\eta} < 0$) also, a chromatographic peak with an infinitesimal width occurs, both the position and the shape of which are independent of the sample load (see Eq. 53). The elution molarity, $\hat{m}$, of the peak is higher than the molarity, m^0, occurring in the absence of the energetical interactions, however. In the case of repulsive interactions ($\hat{\eta} > 0$), the elution profile is fundamentally different. Thus, a chromatographic peak has a finite width comprized in the molarity range between $(e^{[\eta-\hat{\eta}\cdot\zeta(\chi^*)]/kT} - 1)/\varphi$ and m^0 [$= (e^{\eta/kT} - 1)/\varphi$; see Eq. 19]. ζ tends to zero when χ^* tends to zero (Eq. 15); this means that the molarity at which the chromatographic peak begins approaches the molarity at which the peak finishes with a decrease of χ^*, i.e., with either the increase in the column length or the decrease in the amount of the molecules loaded (cf. Figs. A10 and A11 in Appendix III). It can be concluded that actual chromatographic features that are experienced in many instances resemble the theoretical prediction obtained in the case of repulsive interactions; a similar conclusion can be attained even taking into account the longitudinal diffusion effect in the column. Hence, only the case of repulsive interactions will hereafter be treated for the multicomponent system (Sections I–K).

(I) Theoretical Chromatogram in the Case of a Multicomponent System with Repulsive Molecular Interactions

For each component ρ' ($\rho' = 1, 2, \ldots, \rho$) of the sample mixture, the theoretical chromatogram can be represented as

$$\left.\begin{aligned}
&f_{(\rho')} = 0 \qquad \left(m_{\text{in}} \leqslant m < \frac{e^{[\eta_{(\rho')}-\hat{\eta}\cdot\zeta(\Sigma_{\rho''=1}^{\rho'}\chi^*_{(\rho'')})]/kT} - 1}{\varphi}\right)\\
&f_{(\rho')} = -\frac{d}{dm}\zeta^{-1}\left[\frac{\eta_{(\rho')} - kT\ln(\varphi m + 1)}{\hat{\eta}}\right]\\
&\quad = -\frac{d}{dm}\zeta^{-1}\left[\ln\frac{\varphi m^0_{(\rho')} + 1}{\varphi m + 1}\Big/\ln\frac{\varphi m^0_{(\rho')} + 1}{\varphi \hat{m}_{(\rho')} + 1}\right]\\
&\left(\frac{e^{[\eta_{(\rho')}-\hat{\eta}\cdot\zeta(\Sigma_{\rho''=1}^{\rho'}\chi^*_{(\rho'')})]/kT} - 1}{\varphi} \leqslant m < \frac{e^{[\eta_{(\rho')}-\hat{\eta}\cdot\zeta(\Sigma_{\rho''=1}^{\rho'-1}\chi^*_{(\rho'')})]/kT} - 1}{\varphi}\right)\\
&f_{(\rho')} = 0 \qquad \left(m \geqslant \frac{e^{[\eta_{(\rho')}-\hat{\eta}\cdot\zeta(\Sigma_{\rho''=1}^{\rho'-1}\chi^*_{(\rho'')})]/kT} - 1}{\varphi}\right)
\end{aligned}\right\} \quad (54)$$

where the sum $\Sigma_{\rho''=1}^{0}\chi^*_{(\rho'')}$ is defined to be zero.

Proof. When $m < (e^{[\eta_{(\rho')}-\hat{\eta}\cdot\zeta(\Sigma_{\rho''=1}^{\rho'}\chi^*_{(\rho'')})]/kT} - 1)/\varphi$, then $\Sigma_{\rho''=1}^{\rho'}\chi^*_{(\rho'')} < \chi'_{(\rho')}$. This is because $(e^{[\eta_{(\rho')}-\hat{\eta}\cdot\zeta(\Sigma_{\rho''=1}^{\rho'}\chi^*_{(\rho'')})]/kT} - 1)/\varphi$ represents the m value occurring when $\chi'_{(\rho')} = \Sigma_{\rho''=1}^{\rho'}\chi^*_{(\rho'')}$, and, if m decreases from this value, $\chi'_{(\rho')}$ increases (the case when $\Sigma_{\rho''=1}^{\rho'}\chi^*_{(\rho'')} < 1$) or $\chi'_{(\rho')}$ continues to take a constant value of unity (the case when $\Sigma_{\rho''=1}^{\rho'}\chi^*_{(\rho'')} = 1 - 0$). This means that $B'_{(\rho')} = 0$ (Eq. 35), from which it can be derived that $d\chi_{(\rho')} = 0$, that $\chi_{(\rho')} = \chi^*_{(\rho')}$ and that $f_{(\rho')} = 0$, thus demonstrating the first equation in Eq. (54) (cf. the proof of the first equation in Eq. 51). When $(e^{[\eta_{(\rho')}-\hat{\eta}\cdot\zeta(\Sigma_{\rho''=1}^{\rho'}\chi^*_{(\rho'')})]/kT} - 1)/\varphi \leqslant m < (e^{[\eta_{(\rho')}-\hat{\eta}\cdot\zeta(\Sigma_{\rho''=1}^{\rho'-1}\chi^*_{(\rho'')})]/kT} - 1)/\varphi$, then

$$\sum_{\rho''=1}^{\rho'-1}\chi^*_{(\rho'')} < \chi'_{(\rho')} \leqslant \sum_{\rho''=1}^{\rho'}\chi^*_{(\rho'')} \qquad (55)$$

where the first inequality has been derived from the consideration similar to that used in the proof of the first equation in Eq. (54). Therefore, from the physical meaning of $\chi'_{(\rho')}$, we have

$$\sum_{\rho''=1}^{\rho'}\chi_{(\rho'')} = \chi'_{(\rho')}(m) = \zeta^{-1}\left[\frac{\eta_{(\rho')} - kT\ln(\varphi m + 1)}{\hat{\eta}}\right] \qquad (56)$$

since $\hat{m}_{(\rho')} \leqslant (e^{[\eta_{(\rho')}-\hat{\eta}\cdot\zeta(\Sigma_{\rho''=1}^{\rho'}\chi^*_{(\rho'')})]/kT} - 1)/\varphi < (e^{[\eta_{(\rho')}-\hat{\eta}\cdot\zeta(\Sigma_{\rho''=1}^{\rho'-1}\chi^*_{(\rho'')})]/kT} - 1)/\varphi < m^0_{(\rho')}$ (see the second equation in Eq. 21). Equation (56) can be rewritten as

$$\chi_{(\rho')} = \zeta^{-1}\left[\frac{\eta_{(\rho')} - kT\ln(\varphi m + 1)}{\hat{\eta}}\right] - \sum_{\rho''=1}^{\rho'-1}\chi_{(\rho'')} \qquad (56')$$

where the sum $\Sigma_{\rho''=1}^{0}\chi_{(\rho'')}$ is defined to be zero. On the other hand, the relationship

$$\sum_{\rho''=1}^{\rho'-1} \chi_{(\rho'')} = \sum_{\rho''=1}^{\rho'-1} \chi_{(\rho'')}^{*} \tag{57}$$

is fulfilled. In fact, writing one of 1, 2, . . . , $\rho' - 1$ as ρ''' and by using both the left-hand side inequality in Eq. (55) and the general inequalities

$$\chi'_{(1)} > \chi'_{(2)} > \cdots > \chi'_{(\rho)} \tag{58}$$

(see Section C), then

$$\sum_{\rho''=1}^{\rho'''} \chi_{(\rho'')}^{*} < \sum_{\rho''=1}^{\rho'-1} \chi_{(\rho'')}^{*} < \chi'_{(\rho')} < \chi'_{(\rho''')} \tag{59}$$

can be derived. The relationship between the extreme left-hand side and the extreme right-hand side of Eq. (59) is similar to the relationship $\Sigma_{\rho''=1}^{\rho'}\chi_{(\rho'')}^{*} < \chi'_{(\rho')}$, which has been used to derive the relationship of $\chi_{(\rho')} = \chi_{(\rho')}^{*}$ in the process of the proof of the first equation in Eq. (54). This means that $\chi_{(\rho''')} = \chi_{(\rho''')}^{*}$ and that Eq. (57) is fulfilled. Therefore, by substituting Eq. (57) into Eq. (56′),

$$\chi_{(\rho')} = \zeta^{-1}\left[\frac{\eta_{(\rho')} - kT\ln(\varphi m + 1)}{\hat{\eta}}\right] - \sum_{\rho''=1}^{\rho'-1} \chi_{(\rho'')}^{*} \tag{60}$$

is obtained. Equation (60) is a solution to Eq. (44) fulfilling the initial condition given by Eq. (47) since, when $m \to (e^{[\eta_{(\rho')} - \hat{\eta}\cdot\zeta(\Sigma_{\rho''=1}^{\rho'-1}\chi_{(\rho'')}^{*})]/kT} - 1)/\varphi$, then $\chi_{(\rho')} \to \chi_{(\rho')}^{*}$. [It should be recalled that when $m_{\text{in}} < m < (e^{[\eta_{(\rho')} - \hat{\eta}\cdot\zeta(\Sigma_{\rho''=1}^{\rho'-1}\chi_{(\rho'')}^{*})]/kT} - 1)/\varphi$, then $\chi_{(\rho')} = \chi_{(\rho')}^{*}$. For the fact that Eq. 60 represents the solution to Eq. 44, cf. the proof of Eq. 51.] By substituting Eq. (60) into Eq. (49), the second equation in Eq. (54) can be derived. Further, as far as $\chi_{(\rho')}$ given by Eq. (60) is concerned, when $m \to (e^{[\eta_{(\rho')} - \hat{\eta}\cdot\zeta(\Sigma_{\rho''=1}^{\rho'-1}\chi_{(\rho'')}^{*})]/kT} - 1)/\varphi$, then $\chi_{(\rho')} \to 0$. This means that, when $m > (e^{[\eta_{(\rho')} - \hat{\eta}\cdot\zeta(\Sigma_{\rho''=1}^{\rho'-1}\chi_{(\rho'')}^{*})]/kT} - 1)/\varphi$, then the relationship $\chi_{(\rho')} = 0$ holds in general, and that $f_{(\rho')} = 0$ (Eq. 49), thus demonstrating the third equation in Eq. (54). *Q.E.D.*

Remark. The total chromatogram for a given mixture can be represented as a sum of the chromatograms for the respective components 1, 2, . . . , ρ (Eq. 54) fulfilling the relationship

$$\eta_{(1)} > \eta_{(2)} > \cdots > \eta_{(\rho)} \tag{61}$$

Equation (61) can be derived in connection with the relationship given by Eq. (58). On the basis of both Eqs. (54) and (61), it can be understood that the total chromatogram is composed of two types of interval: (a)

$$[(e^{[\eta_{(\rho')} - \hat{\eta}\cdot\zeta(\Sigma_{\rho''=1}^{\rho'}\chi^*_{(\rho'')})]/kT} - 1)/\varphi,$$

$$(e^{[\eta_{(\rho')} - \hat{\eta}\cdot\zeta(\Sigma_{\rho''=1}^{\rho'-1}\chi^*_{(\rho'')})]/kT} - 1)/\varphi]$$

and (b)

$$[(e^{[\eta_{(\rho'+1)} - \hat{\eta}\cdot\zeta(\Sigma_{\rho''=1}^{\rho'}\chi^*_{(\rho'')})]/kT} - 1)/\varphi,$$

$$(e^{[\eta_{(\rho')} - \hat{\eta}\cdot\zeta(\Sigma_{\rho''=1}^{\rho'}\chi^*_{(\rho'')})]/kT} - 1)/\varphi]$$

where $\rho' = 1, 2, \ldots, \rho$, and the sum $\Sigma_{\rho''=1}^{0}\chi^*_{(\rho'')}$ is defined to be zero;

$$(e^{[\eta_{(\rho+1)} - \hat{\eta}\cdot\zeta(\Sigma_{\rho''=1}^{\rho}\chi^*_{(\rho'')})]/kT} - 1)/\varphi$$

and

$$(e^{[\eta_{(0)} - \hat{\eta}\cdot\zeta(\Sigma_{\rho''=1}^{0}\chi^*_{(\rho'')})]/kT} - 1)/\varphi$$

are defined to be $-\infty$ and ∞, respectively. In interval (a), $f_{(\rho')}$, in general, takes positive values whereas, in interval (b), $f_{(\rho')}$ is always equal to 0. Further, since $\chi^*_{(1)}, \chi^*_{(2)}, \ldots, \chi^*_{(\rho)}$ actually have finite values, interval (a), in general, has a finite width. As far as interval (b) (except those formed outside the total chromatogram) is concerned, however, it has a finite width only when $\eta_{(\rho')} > \eta_{(\rho'+1)}$; when $\eta_{(\rho')} = \eta_{(\rho'+1)}$, the width of interval (b) is infinitesimal. In this instance the chromatogram formed by component ρ' is identical with that formed by both component ρ' and $\rho' + 1$ provided that the $\chi^*_{(\rho')}$ value with the former chromatogram is equal to the $\chi^*_{(\rho')} + \chi^*_{(\rho'+1)}$ value with the latter one. This situation is diagrammatically shown in Figs. A10 and A12 or Figs. A11 and A13 in Appendix III.

(J) The Case When the Distribution of the Molecular Components Is Continuous

When the distribution of the molecular components is continuous, let us currently represent $\eta_{(1)}, \eta_{(2)}, \ldots, \eta_{(\rho)}$ by using a variable η. We call $F(\eta)$

the normalized distribution function of η fulfilling the relationship

$$\int_{-\infty}^{\infty} F(\eta)d\eta = 1 \tag{62}$$

and χ^* the total molecular density, $\Sigma_{\rho''=1}^{\rho}\chi^*_{(\rho'')}$, on the adsorbent. By using these symbols, the total chromatogram $f(m)$ $[\equiv \Sigma_{\rho''=1}^{\rho} f_{(\rho'')}(m)]$ can be represented as

$$f(m) = \chi^* \cdot F(\eta) \cdot \frac{d\eta}{dm} \tag{63}$$

in which

$$m = \frac{e^{\{\eta - \hat{\eta} \cdot \zeta[\chi^* \cdot \int_{\eta}^{\infty} F(\eta)d\eta]\}/kT} - 1}{\varphi} \tag{64}$$

Proof. On the basis of the argument made in Section I, it can be understood that the one-to-one correspondence holds between the two distributions of $f(m)$ and $F(\eta)$. Therefore, taking into account both Eqs. (50) and (62), Eq. (63) can be derived. It is now sufficient to give a proof of Eq. (64). Thus, when the distribution of the molecular component is continuous with a finite χ^* value, the widths of both intervals (a) and (b) (see Remark in Section I) are infinitesimal, and we have relationships

$$\sum_{\rho''=1}^{\rho'} \chi^*_{(\rho'')} \approx \sum_{\rho''=1}^{\rho'-1} \chi^*_{(\rho'')} \approx \chi^* \cdot \int_{\eta}^{\infty} F(\eta)\, d\eta \tag{65}$$

from which Eq. (64) can be derived. *Q.E.D.*

Provided

$$F(\eta) = \frac{1}{\chi^*} \cdot \sum_{\rho''=1}^{\rho} [\chi^*_{(\rho'')} \cdot \delta(\eta - \eta_{(\rho'')})] \tag{66}$$

both Eqs. (63) and (64) lead to Eq. (54).

Proof. By using an infinitesimal positive constant ε, Eq. (66) can be represented as

$$F(\eta) = \frac{\chi^*_{(\rho')}}{\chi^*} \cdot \frac{1}{\varepsilon} \qquad (\eta_{(\rho')} - \varepsilon < \eta < \eta_{(\rho')};\ \rho' = 1, 2, \ldots, \rho) \tag{67}$$

$$F(\eta) = 0 \qquad \text{(in the other range of } \eta) \tag{67}$$

Therefore, when

$$\eta_{(\rho')} - \varepsilon < \eta < \eta_{(\rho')} \tag{68}$$

Eq. (64) can be rewritten as

$$m = \frac{e^{\{\eta - \hat{\eta}\cdot\zeta[\Sigma_{\beta''=1}^{\rho'-1}\chi^*_{(\rho'')} + (\eta_{(\rho')}-\eta)\cdot\chi^*_{(\rho')}/\varepsilon]\}/kT} - 1}{\varphi} \tag{69}$$

from which

$$\frac{d\eta}{dm} = -\frac{\varepsilon}{\chi^*_{(\rho')}} \cdot \frac{d}{dm}\zeta^{-1}\left[\frac{\eta - kT\ln(\varphi m + 1)}{\hat{\eta}}\right] \tag{70}$$

can be derived. By substituting both the first equation in Eq. (67) and Eq. (70) into Eq. (63),

$$f(m) = -\frac{d}{dm}\zeta^{-1}\left[\frac{\eta - kT\ln(\varphi m + 1)}{\hat{\eta}}\right] \tag{71}$$

is obtained. On the other hand, the molarity range fulfilling Eq. (68) can be estimated by using Eq. (69):

$$\frac{e^{[\eta_{(\rho')} - \varepsilon - \hat{\eta}\cdot\zeta(\Sigma_{\beta''=1}^{\rho'}\chi^*_{(\rho'')})]/kT} - 1}{\varphi} < m < \frac{e^{[\eta_{(\rho')} - \hat{\eta}\cdot\zeta(\Sigma_{\beta''=1}^{\rho'-1}\chi^*_{(\rho'')})]/kT} - 1}{\varphi} \tag{72}$$

At the limit of ε → 0, Eq. (72) reduces to

$$\frac{e^{[\eta_{(\rho')} - \hat{\eta}\cdot\zeta(\Sigma_{\beta''=1}^{\rho'}\chi^*_{(\rho'')})]/kT} - 1}{\varphi} \leq m \leq \frac{e^{[\eta_{(\rho')} - \hat{\eta}\cdot\zeta(\Sigma_{\beta''=1}^{\rho'-1}\chi^*_{(\rho'')})]/kT} - 1}{\varphi} \tag{73}$$

and η in Eq. (71) can be rewritten as $\eta_{(\rho')}$ (cf. Eq. 68). It is also possible to write $f(m)$ in Eq. (71) as $f_{(\rho')}$, and Eq. (71) and the molarity range given by Eq. (73) coincide with the second equation in Eq. (54) and the molarity range in which this equation holds, respectively. [In the right-hand side inequality limiting the molarity range in which the second equation in Eq. 54 holds, the equality is not involved in contrast to the right-hand side inequality in Eq. 73 to which an equality is added. It is possible, however, to add an equality to the former inequality since $f_{(\rho')}$ in Eq. 54 (as a function of m) is continuous at $m = (e^{[\eta_{(\rho')}-\hat{\eta}\cdot\zeta(\Sigma_{\rho''=1}^{\rho'-1}\chi^{*}_{(\rho'')})]/kT} - 1)/\varphi$.] Unless Eq. (73) or (68) is fulfilled, then $F(\eta) = 0$ (the second equation in Eq. 67), and, from Eq. (63),

$$f(m) = 0 \tag{74}$$

is derived. Therefore, writing $f_{(\rho')}$ instead of $f(m)$ in Eq. (74), the first and the third equations in Eq. (54) are obtained. *Q.E.D.*

(K) Relative and Approximate Expressions of $f_{(\rho')}$ and $f(m)$

By using relative parameters introduced in Section E, Eq. (54) can be rewritten as

$$\left.\begin{aligned}
&f_{(\rho')} = 0 \qquad \left(y_{\text{in}} \leqslant y < \frac{(\varphi m^0_{(1)} + 1)^{w_{(\rho')}-\Xi\cdot\zeta(\Sigma_{\rho''=1}^{\rho'}\chi^{*}_{(\rho'')})} - 1}{\varphi m^0_{(1)}}\right)\\
&f_{(\rho')} = -\frac{d}{dy}\,\zeta^{-1}\left[\frac{w_{(\rho')} - \ln(\varphi m^0_{(1)}y + 1)/\ln(\varphi m^0_{(1)} + 1)}{\Xi}\right]\\
&\quad\left(\frac{(\varphi m^0_{(1)} + 1)^{w_{(\rho')}-\Xi\cdot\zeta(\Sigma_{\rho''=1}^{\rho'}\chi^{*}_{(\rho'')})} - 1}{\varphi m^0_{(1)}}\right.\\
&\quad\left.\leqslant y < \frac{(\varphi m^0_{(1)} + 1)^{w_{(\rho')}-\Xi\cdot\zeta(\Sigma_{\rho''=1}^{\rho'-1}\chi^{*}_{(\rho'')})} - 1}{\varphi m^0_{(1)}}\right)\\
&f_{(\rho')} = 0 \qquad \left(y \geqslant \frac{(\varphi m^0_{(1)} + 1)^{w_{(\rho')}-\Xi\cdot\zeta(\Sigma_{\rho''=1}^{\rho'-1}\chi^{*}_{(\rho'')})} - 1}{\varphi m^0_{(1)}}\right)
\end{aligned}\right\} \tag{75}$$

where

$$y_{\text{in}} = \frac{m_{\text{in}}}{m^0_{(1)}} \tag{76}$$

Equation (75) can approximately be represented as

$$f_{(\rho')} = 0 \qquad \left[y_{\text{in}} \leqslant y < w_{(\rho')} - \Xi\cdot\zeta\left(\sum_{\rho''=1}^{\rho'}\chi^{*}_{(\rho'')}\right)\right] \tag{77}$$

$$\left.\begin{aligned} f_{(\rho')} &= -\frac{d}{dy}\zeta^{-1}\left[\frac{w_{(\rho')}-y}{\Xi}\right] \qquad \left[w_{(\rho')} - \Xi\cdot\zeta\left(\sum_{\rho''=1}^{\rho'}\chi^*_{(\rho'')}\right)\right. \\ &\qquad \left. < y < w_{(\rho')} - \Xi\cdot\zeta\left(\sum_{\rho''=1}^{\rho'-1}\chi^*_{(\rho'')}\right)\right] \\ f_{(\rho')} &= 0 \qquad \left[y \geqslant w_{(\rho')} - \Xi\cdot\zeta\left(\sum_{\rho''=1}^{\rho'-1}\chi^*_{(\rho'')}\right)\right] \end{aligned}\right\} \tag{77}$$

Equations (63) and (64) can relatively be written as

$$f(y) = \chi^*\cdot F(w)\cdot\frac{dw}{dy} \tag{78}$$

and

$$y = \frac{(\varphi m^0_{(1)} + 1)^{w-\Xi\cdot\zeta(\chi^*\cdot\int_w^\infty F(w)dw)} - 1}{\varphi m^0_{(1)}} \tag{79}$$

respectively, where w is the current expression of $w_{(1)}, w_{(2)}, \ldots, w_{(\rho)}$. Equation (79) can approximately be represented as

$$y = w - \Xi\cdot\zeta\left(\chi^*\cdot\int_w^\infty F(w)\,dw\right) \tag{80}$$

In Appendix III, approximate $f_{(\rho')}$'s calculated by using Eq. (77) under two assumptions of $\zeta(\chi) = \chi$ and $\zeta(\chi) = \sqrt{\chi}$ are diagrammatically represented. $f(m)$'s for several types of continuous distribution, $F(w)$, calculated by using both Eqs. (78) and (80) under assumption of $\zeta(\chi) = \sqrt{\chi}$ are also depicted in Appendix III. (For details, see the Discussion Section.)

DISCUSSION

(A) The State of the Molecules on the Adsorbent Surface and Two Possible Forms of Function $\zeta(\chi)$

At least with gradient chromatography of the competitive type it can, in general, be assumed that the mutually superimposed state of molecules is hardly realizable on the adsorbent surfaces in the column when they are adsorbed. With this chromatography the adsorption energy per molecule is generally large enough for the value of the parameter $B'_{(p')}$ or $B_{(p')}$ to be virtually equal to zero when the molarity of competing ions is equal to the initial value m_{in}. As a result, the molecule is almost completely retained on the column before the gradient begins. Under this situation, provided the molecule under consideration is partially superimposed on another molecule on the adsorbent surface, the loss of the interaction energy of the former molecule with the adsorbent surface occurring due to the steric hindrance provoked by the latter molecule should also be large. It can, in general, be concluded (see below) that, provided the total adsorption energy per molecule is large enough for it to be retained on the column before the gradient is applied, the loss of the adsorption energy per molecule occurring due to the superposition on another molecule is much more important than the gain of entropy occurring due to the increase in the number of the adsorption configuration; this increase occurs by allowing the superimposed state. As a result, the mutually superimposed state of molecules is hardly realizable. Thus, in Appendix I of Ref. 7, the above was quantitatively examined on the basis of a simple statistical mechanical consideration by using elongated model molecules that are much longer than the interdistances among the neighboring adsorbing sites on the adsorbent surface; the molecules are more or less rigid but sufficiently flexible to attach themselves on the adsorbent surface when they have to pass over other molecules that have already been adsorbed. It was concluded (Ref. 7, Appendix I) that the superposition state can almost never be realized with these molecules. Since these molecules would represent the type that can most easily be superimposed on one another, it can, in general, be assumed that the mutually superimposed state of molecules is hardly realizable on the adsorbent surfaces in the column.

As far as molecules with an asymmetrical shape (such as those represented by a rod) are concerned, it can, in general, be assumed that the molecules are arranged in parallel with one another on the adsorbent surface avoiding the mutually superimposed state, provided that the molecular density on the adsorbent surface is high enough.* With this adsorption manner, the energetic interaction among molecules on the

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*It is tacitly assumed that a molecule is adsorbed on the adsorbent surface by using a side of the rod; the probability that the rod is adsorbed in such a way that the main axis of the rod is perpendicular to the adsorbent surface is negligibly small.

adsorbent surface would occur mainly through the side of the rod (which occupies the major part of the total surfaces of the rod), and two models can be proposed for the adsorbed phase of the molecules which is realized on the adsorbent surface. In the first model (called the amorphous phase model), the molecules are situated at random on the adsorbent surface and maintain parallel orientation with one another. In the second model (called the quasi-crystalline phase model), the positions of the molecules (arranged parallel with one another) are restricted to one another due to the energetic interaction among them through the side of the rod. [In the argument in Ref. *7*, Appendix I, for the possibility of the occurrence of the mutually superimposed state of the molecules on the adsorbent surface (see above), account is not taken of the quasi-crystalline phase model, and only the amorphous phase model is considered; this is because, in this argument, the effect of the energetic molecular interaction is neglected in both the superposition and nonsuperposition states of the molecules. It can be assumed, however, that the superposition state is unrealizable from a practical point of view even in the presence of energetic molecular interactions since the possibility of the occurrence of the superposition state is extremely small in the absence of the energetic molecular interaction; see Ref. *7*, Appendix I.]

In general, the mutual interaction energy $E^*_{(\rho')}$ or ζ (see Eq. 12) would be approximately proportional to the molecular density occurring in the direction perpendicular to the main axis of a *given* molecule. With the amorphous phase model, this density can be estimated to be χ on the basis of a Bragg-Williams approximation for the distribution of the molecules on the adsorbent surface, or we have

$$\zeta(\chi) \approx \chi \tag{81}$$

With the quasi-crystalline phase model, the molecular density in the perpendicular direction of a rod can be estimated to be of the order of $\sqrt{\chi}$ since the *axial* positions of the molecules are not determined at random; the rearrangement of the random positions of the molecules occurs on the adsorbent surface due to the energetic molecular interaction (see above). Hence, we have

$$\zeta(\chi) \approx \sqrt{\chi} \qquad (82)^*$$

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*In Refs *7-10*, Eq. (82) is simply assumed without justification. Detailed arguments on Eq. (82) will be made in a subsequent publication (T. Kawasaki and M. Niikura, "Overload Quasi-Static Linear Gradient Chromatography: Theory versus Hydoxyapatite High-Performance Liquid Chromatography," *Sep. Sci. Technol.*, In Press).

Both amorphous and quasi-crystalline phases can be considered even in the case of molecules with a symmetrical shape (such as those represented by a sphere). For these molecules, Eq. (81) seems to be realizable independent of the type of the adsorbed phase of the molecules since the energetic interaction can be assumed to occur in any direction around a given molecule. In the special case when the arrangement of the adsorption sites on the adsorbent surface is highly asymmetric (with a coordination number, z, equal to 1 or 2; cf. Assumption 1 in Ref. *11*, Theoretical Section A), Eq. (82) might be realizable with the quasi-crystalline phase, however.

(B) The Shape of the Theoretical Chromatogram and the Relationship with the Experiment

Typical chromatograms calculated by using Eq. (77) or both Eqs. (78) and (80) on the basis of both the amorphous (Eq. 81) and the quasi-crystalline phase (Eq. 82) model are illustrated in Appendix III. Thus, Figs. A10 and A11 depict chromatograms for single component systems obtained by using Eqs. (81) and (82), giving a rectangle and a right-angled triangle diagram, respectively.

Figures A12 and A13 are chromatograms for two component systems with the identical $\eta_{(p')}$ or $w_{(p')}$ value obtained by using Eqs. (81) and (82), respectively. It can be seen that the total shapes of the two component chromatograms (Figs. A12 and A13) are identical with those of the single component systmes (Figs. A10 and A11); the χ^* value with the single component chromatograms is equal to the $\chi^*_{(1)} + \chi^*_{(2)}$ value with the two component chromatograms, provided Ξ is constant (for details, see the explanation of the figures in Appendix III). In the case of a more than two component system with $w_{(1)} = w_{(2)} = \cdots = w_{(p)}$ in which Eq. (81) is fulfilled, rectangles that keep in contact with one another continue on the left-hand side of the two component chromatogram (Fig. A12), completing as a whole a rectangular chromatogram. In the case of the corresponding more than two component system in which Eq. (82) is fulfilled instead of Eq. (81), rectangles are replaced by trapezoids (cf. Fig. A13), completing as a whole a total chromatogram of right-angled triangle.

Figures A14 and A15 illustrate chromatograms for two component systems with different $\eta_{(p')}$ or $w_{(p')}$ values obtained by using Eqs. (81) and (82), respectively. It can be seen that, provided the chromatograms concerning the respective components of the mixtures approach one another to keep in contact, the total shapes of the two component chromatograms (Figs. A14 and A15) will be identical with those of the single component systems (Figs. A10 and A11); the χ^* value with the single component chromatograms is equal to the $\chi^*_{(1)} + \chi^*_{(2)}$ value with the two component chromatograms, provided Ξ is constant (for details, see the explanation of the figures in Appendix III). In the case of a more than two component system with $w_{(1)} > w_{(2)} > \cdots > w_{(p)}$ in which Eq. (81) is fulfilled, rectangles that are separated from one another are present on the left-hand side of the two component chromatogram (Fig. A14), completing as a whole a rectangular total chromatogram provided the respective rectangles approach one another to keep in contact. In the case of the corresponding more than two component system in which Eq. (82) is fulfilled instead of Eq. (81), rectangles are replaced by trapezoids (cf. Fig. A15), completing as a whole a total chromatogram of a right-angled triangle provided the respective trapezoids and a triangle approach one another to keep in contact.

Figure A16 illustrates several types of continuous distribution, $F(w)$, of molecular species with different w values. Parts(a)–(e) of Fig. A17 show the results of the calculation of the chromatograms for the distributions shown in Parts(a)–(e) of Fig. A16, respectively; the calculation has been done only for the case when Eq. (82) is fulfilled. When $\chi^* \to 0$ or under condition of infinitesimal sample load, all the chromatograms in Fig. A17 tend toward the patterns shown in Fig. A16.

It can now be understood that when Eq. (81) holds, a rectangular chromatogram is obtained in the case of a signle component system (Fig. A10), and, with multicomponent systems, the shape of the total chromatogram tends to become a rectangle as a whole due to repulsive interactions among molecules (Figs. A12 and A14). When Eq. (82) holds, a chromatogram of a right-angled triangle is obtained in the case of a signle component system (Fig. A11), and, with multicomponent systems, the shape of the total chromatogram tends to become a right-angled triangle as a whole (Figs. A13, A15, and A17). Chromatograms with a shape close to a right-angled triangle are often experienced under overload condition with gradient chromatography; this implies that it is Eq. (82) rather than Eq. (81) that is realized in many actual instances. Of course, this is a deduction based upon the ideal molecular model of infinite dimensions (with properties given by Eqs. 5–7); it is necessary to reexamine both Eqs. (81) and (82) in detail by using molecular models with finite dimensions.

APPENDIX I: DIAGRAMMATICAL REPRESENTATIONS OF APPROXIMATE $\chi'_{(\rho')}(y)$

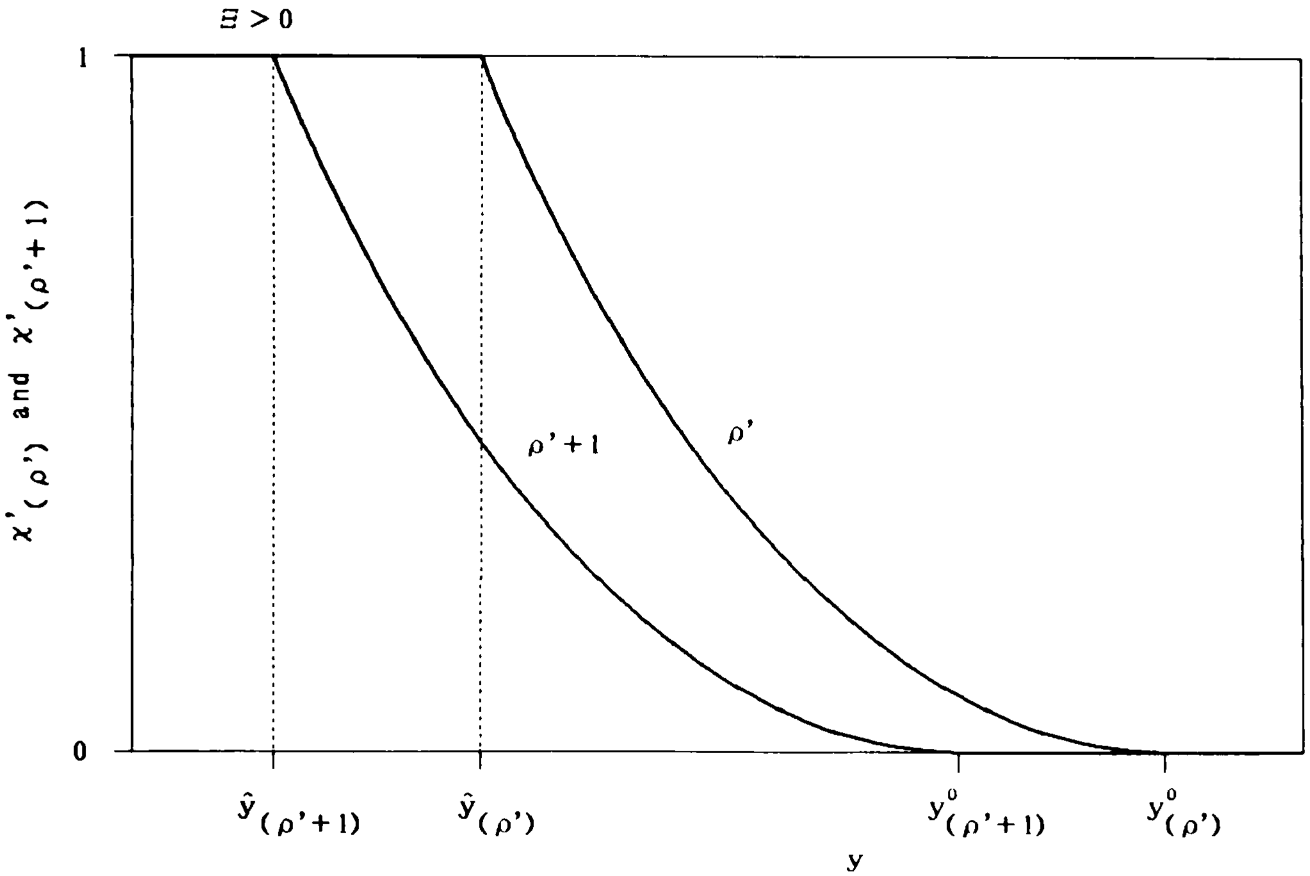

FIG. A1. Approximate $\chi'_{(\rho')}$ and $\chi'_{(\rho'+1)}$ as a function of y when repulsive ($\Xi > 0$) interactions are present and when $w_{(\rho')} > w_{(\rho'+1)}$. These have been drawn on the basis of Eq. (32), taking into account both Eqs. (30) and (31); it is assumed that $\zeta(\chi) = \sqrt{\chi}$. (Prototypes of this figure can be found in Refs. 7 and 8.)

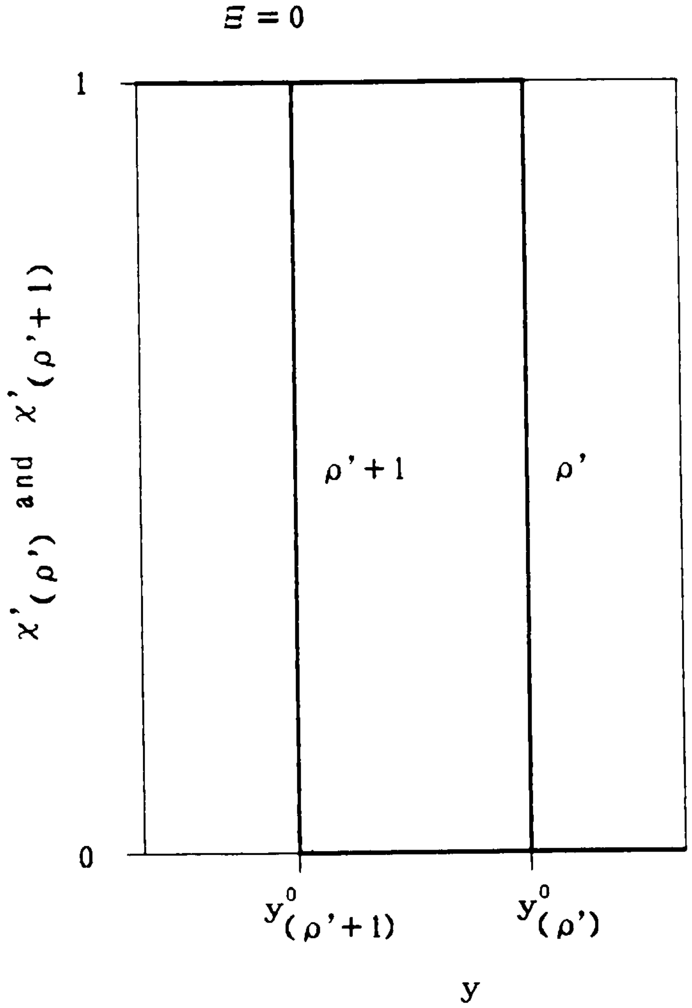

FIG. A2. Approximate $\chi'_{(\rho')}$ and $\chi'_{(\rho'+1)}$ as functions of y when the energetical interactions are absent ($\Xi = 0$) and when $w_{(\rho')} > w_{(\rho'+1)}$. These have been drawn on the basis of Eq. (33), taking into account both Eqs. (30) and (31). (Prototypes of this figure can be found in Refs. 7 and 8.)

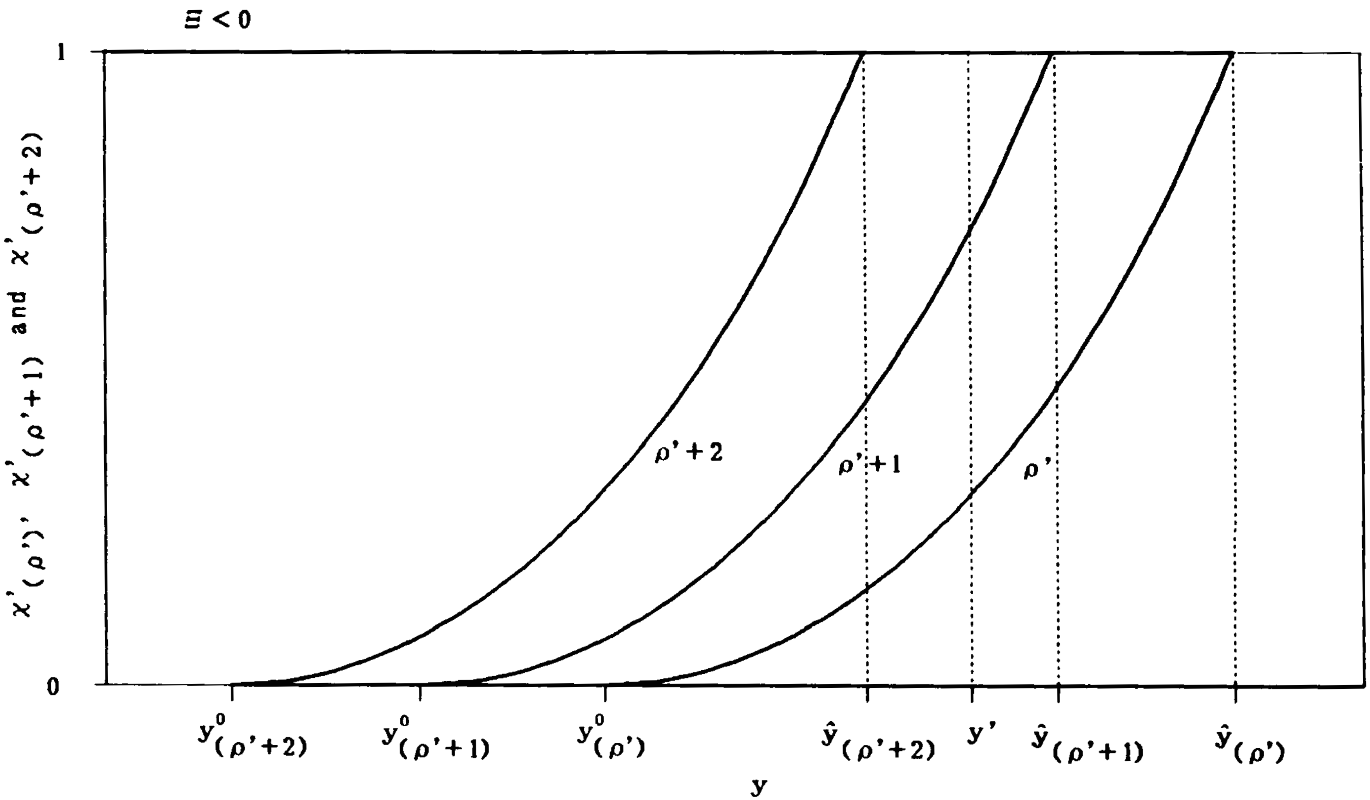

FIG. A3. Approximate $\chi'_{(\rho')}$, $\chi'_{(\rho'+1)}$, and $\chi'_{(\rho'+2)}$ as functions of y when attractive ($\Xi < 0$) interactions are present and when $w_{(\rho')} > w_{(\rho'+1)} > w_{(\rho'+2)}$. These have been drawn on the basis of Eq. (34), taking into account both Eqs. (30) and (31); it is assumed that $\zeta(\chi) = \sqrt{\chi}$. The theoretical curves should be interpreted on the basis of Eq. (34). For instance, when $y = y'$ ($y^0_{(\rho')} < y' < \hat{y}_{(\rho')}$), then $\dot{\rho}$ fulfilling both relationships $\dot{\rho} > \rho'$ and $y < \hat{y}_{(\dot{\rho})}$ represents ρ' and $\rho' + 1$. This means that if $\Sigma^{\rho'}_{\rho''=1}\Omega_{(\rho'')} > \zeta^{-1}$ or, at least, if $\Sigma^{\rho'+1}_{\rho''=1}\Omega_{(\rho'')} > \zeta^{-1}$, then $\chi'_{(\rho')}(y) = 1$. If $\Sigma^{\rho'+1}_{\rho''=1}\Omega_{(\rho'')} < \zeta^{-1}$, then $\chi'_{(\rho')}(y) = 0$. (Prototypes of this figure can be found in Refs. 7 and 8.)

APPENDIX II: DIAGRAMMATICAL REPRESENTATIONS OF APPROXIMATE B' AND $B'_{(\rho')}$

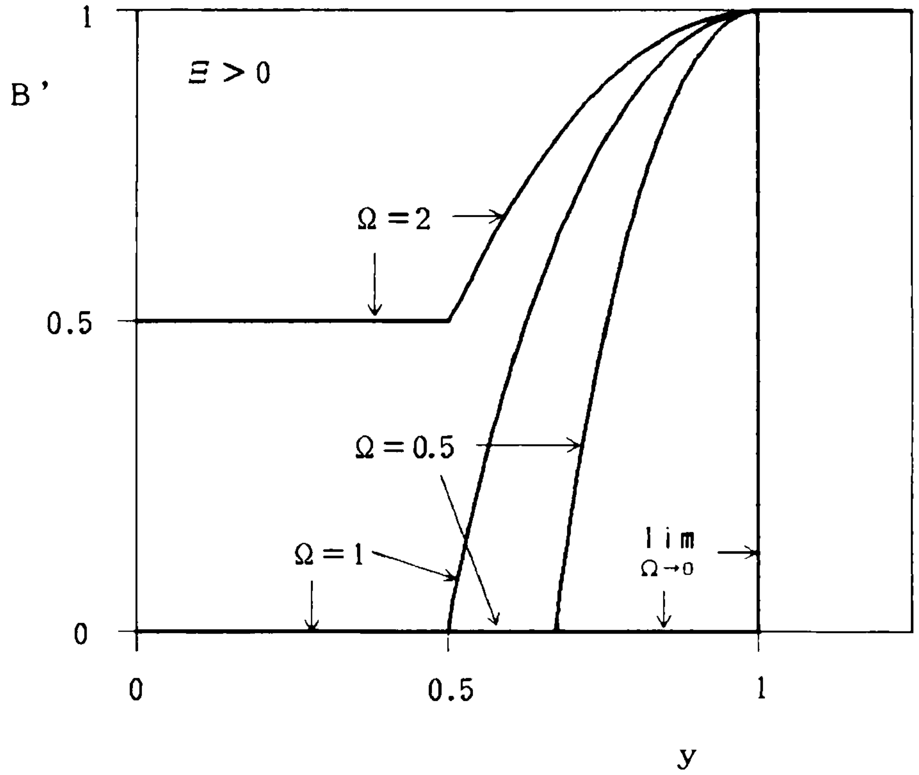

FIG. A4. Approximate B' as a function of y for several values of Ω when repulsive molecular interactions are present in a single component system. For the calculation, the approximate expression of Eq. (32) (cf. Eqs. 30 and 31) and Eqs. (37) and (38) have been used, assuming that $\zeta(\chi) = \sqrt{\chi}$, that $w = 1$, and that $\Xi = 0.5$. (For collagen molecules, it can be estimated that $\Xi \approx 0.2$; see Ref. *17*.) (Reproduced with modifications from Ref. *7*.)

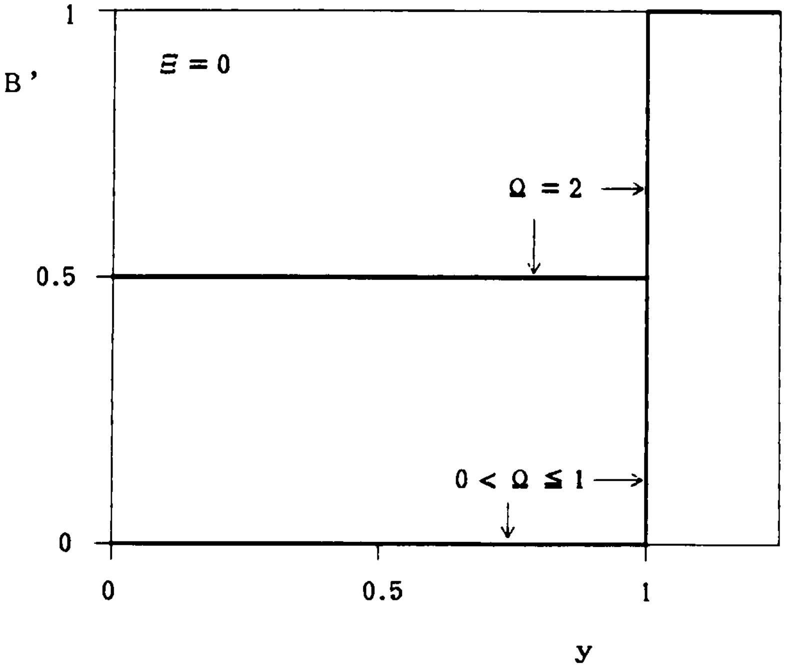

FIG. A5. As Fig. A4 when the energetical molecular interactions are absent in a single component system. This has been calculated by using the approximate expression of Eq. (33) (cf. Eqs. 30 and 31) and Eqs. (37) and (38), assuming that $w = 1$. (Reproduced with modifications from Ref. 7.)

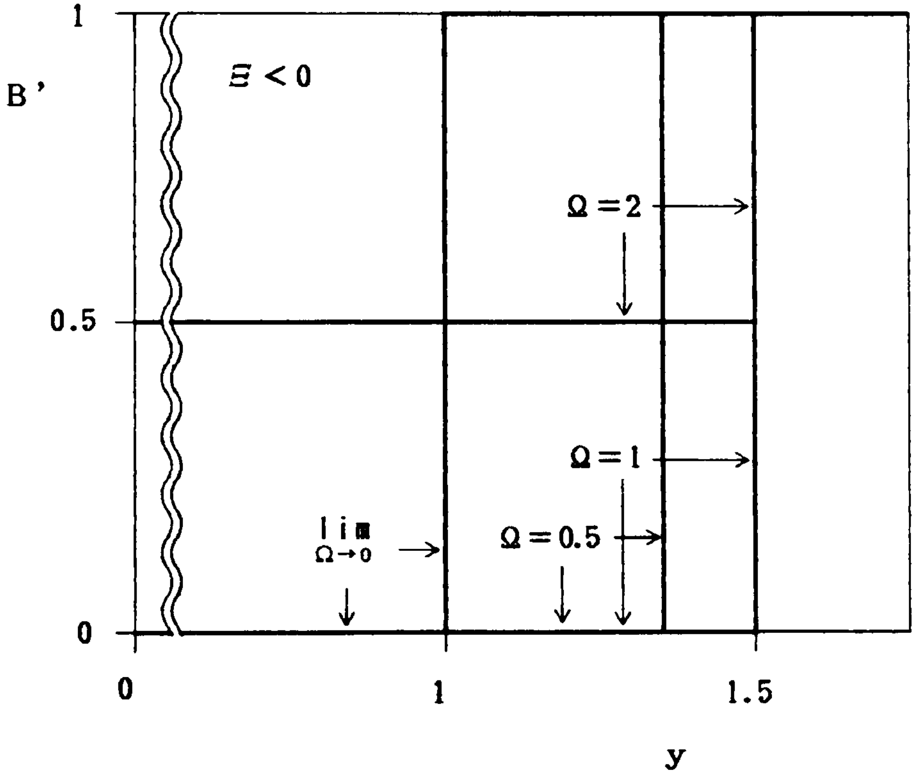

FIG. A6. As Fig. A4 when attractive molecular interactions are present in a single component system. For the calculation, the approximate expression of Eq. (34) (cf. Eqs. 30 and 31) and Eqs. (37) and (38) have been used, assuming that $\zeta(\chi) = \sqrt{\chi}$, that $w = 1$, and that $\Xi = -0.5$. (Reproduced with modifications from Ref. 7.)

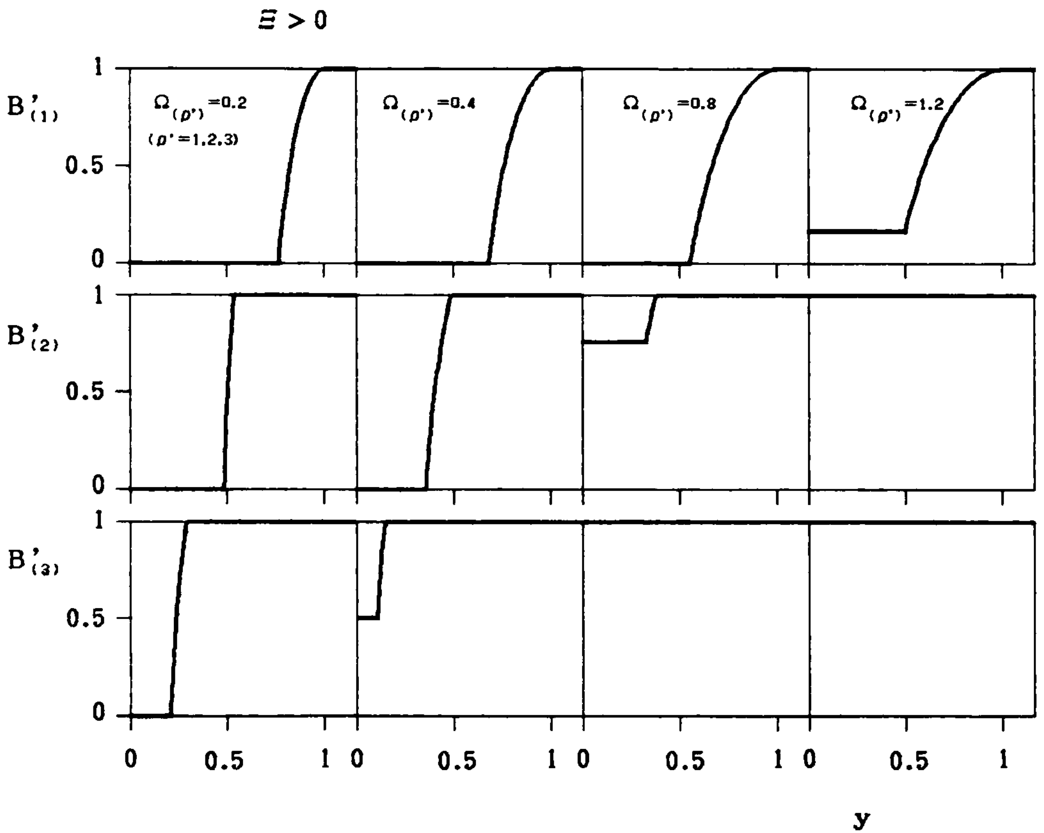

FIG. A7. $\Xi > 0$. Approximate $B'_{(1)}$, $B'_{(2)}$, and $B'_{(3)}$ as functions of y for several values of $\Omega_{(1)}$, $\Omega_{(2)}$, and $\Omega_{(3)}$ (fulfilling the relationship $\Omega_{(1)} = \Omega_{(2)} = \Omega_{(3)}$) when repulsive molecular interactions are present in a three component system. For the calculation, the approximate expression of Eq. (32) (cf. Eqs. 30 and 31) and Eqs. (37) and (38) have been used, assuming: $\zeta(\chi) = \sqrt{\chi}$, $w_{(1)} = 1$, $w_{(2)} = 0.8$, $w_{(3)} = 0.6$, and $\Xi = 0.5$. (Reproduced with modifications from Ref. *8*.)

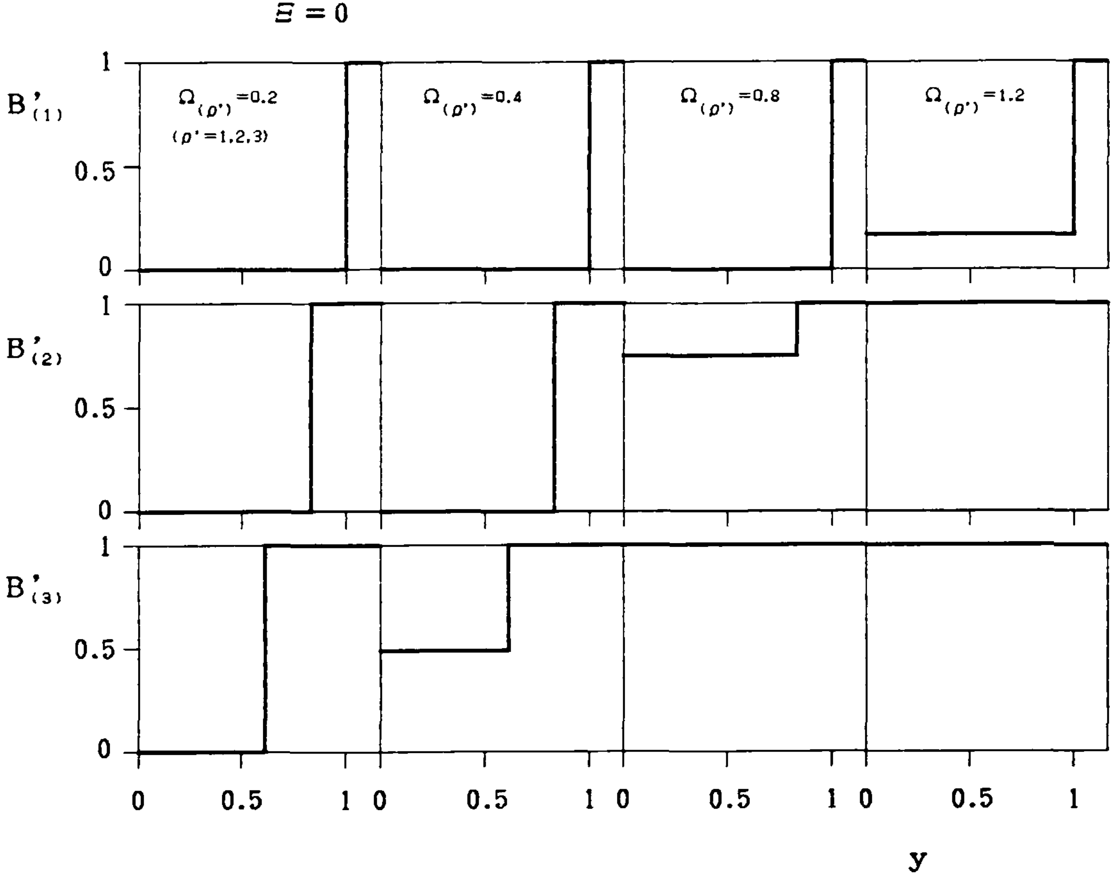

FIG. A8. $\Xi = 0$. As Fig. A7 when the energetical molecular interactions are absent in a three component system. For the calculation, the approximate expression of Eq. (33) (cf. Eqs. 30 and 31) and Eqs. (37) and (38) have been used, assuming: $w_{(1)} = 1$, $w_{(2)} = 0.8$, and $w_{(3)} = 0.6$. The three diagrams occurring when $\Omega_{(1)} = \Omega_{(2)} = \Omega_{(3)} = 0.2$ also represent those occurring when the total amount of the sample is infinitesimal and when Ξ has any value. (Reproduced with modifications from Ref. *8*.)

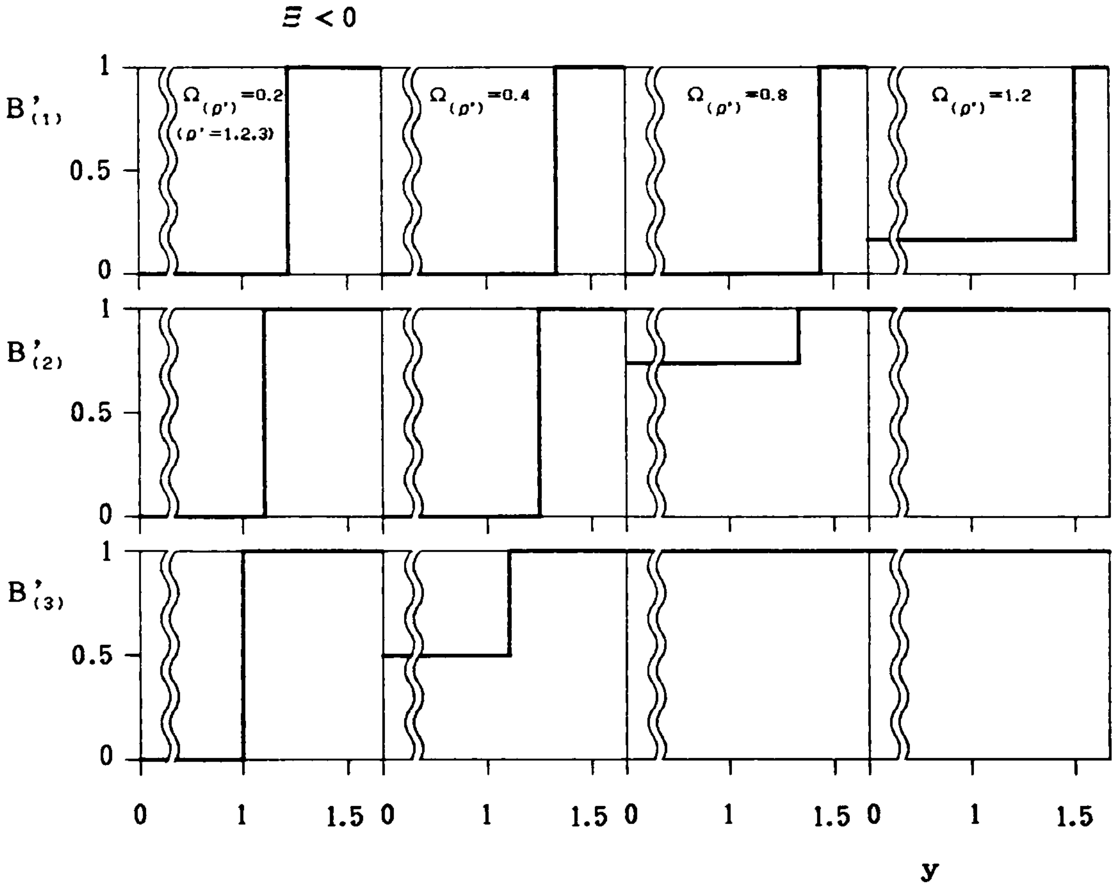

FIG. A9. $\Xi < 0$. As Fig. A7 when attractive molecular interactions are present in a three component system. For the calculation, the approximate expression of Eq. (34) (cf. Eqs. 30 and 31) and Eqs. (37) and (38) have been used, assuming: $\zeta(\chi) = \sqrt{\chi}$, $w_{(1)} = 1$, $w_{(2)} = 0.8$, $w_{(3)} = 0.6$, and $\Xi = -0.5$ (Reproduced with modifications from Ref. *8*.)

APPENDIX III: DIAGRAMMATICAL REPRESENTATIONS OF APPROXIMATE CHROMATOGRAM $f_{(p')}(y)$ or $f(y)$ WHEN REPULSIVE MOLECULAR INTERACTIONS ARE PRESENT

(cf. Discussion Section)

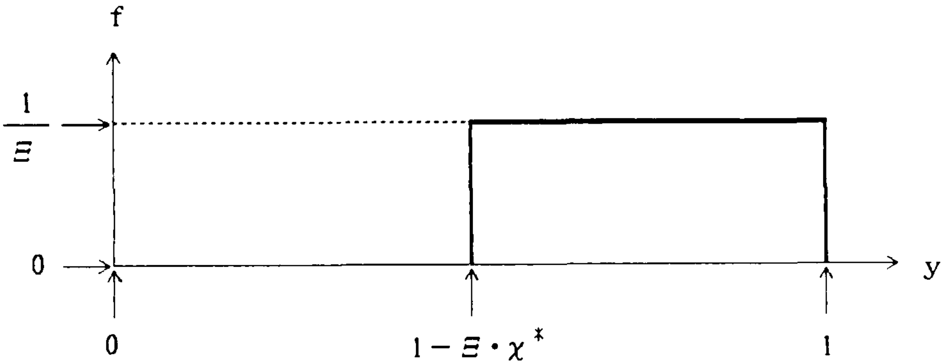

FIG. A10. Approximate f as a function of y for a single component system when $\zeta(\chi) = \chi$. This has been calculated by using Eq. (77) assuming: $\Xi \cdot \chi^* = 0.5$ and $w = 1$.

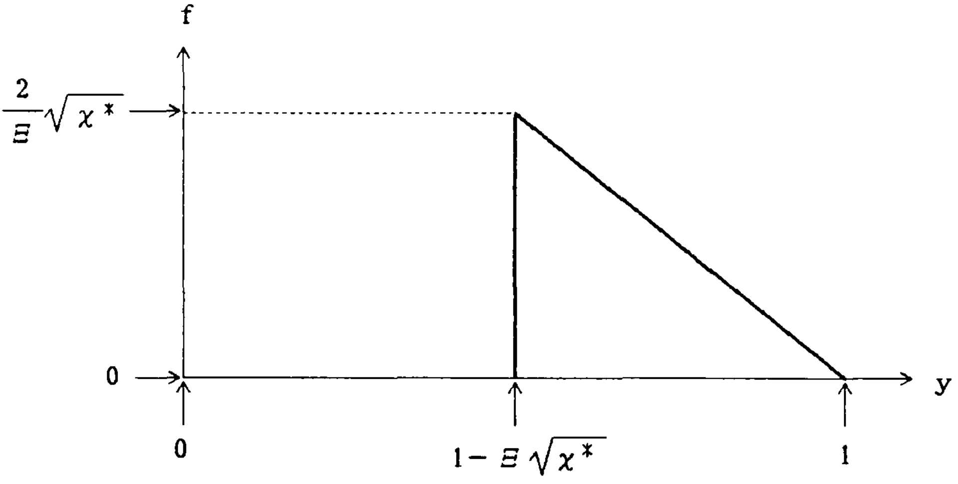

FIG. A11. As Fig. A10 when $\zeta(\chi) = \sqrt{\chi}$, where it has been assumed: $\Xi\sqrt{\chi^*} = 0.5$ and $w = 1$. (Reproduced from Ref. *10*.)

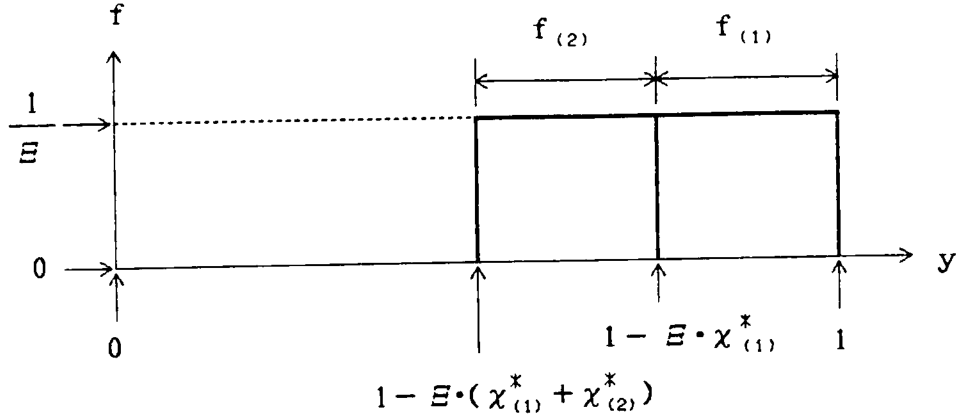

FIG. A12. Approximate $f_{(\rho')}$ or f as a function of y for a two component system with the identical $\eta_{(\rho')}$ or $w_{(\rho')}$ value when $\zeta(\chi) = \chi$. This has been calculated by using Eq. (77) assuming: $\Xi \cdot (\chi^*_{(1)} + \chi^*_{(2)}) = 0.5$, $\chi^*_{(1)} = \chi^*_{(2)}$, and $w_{(1)} = w_{(2)} = 1$. It can be seen that the shape of the total chromatogram (being a rectangle) is identical with that of the chromatogram for a single component system [Fig. A10; cf. "Remark" in Theoretical Section (I)]. In the case of a more than two component system with $w_{(1)} = w_{(2)} = \cdots = w_{(\rho)}$, rectangles that keep in contact with one another continue on the left-hand side of the two component chromatogram, completing as a whole a rectangular total chromatogram.

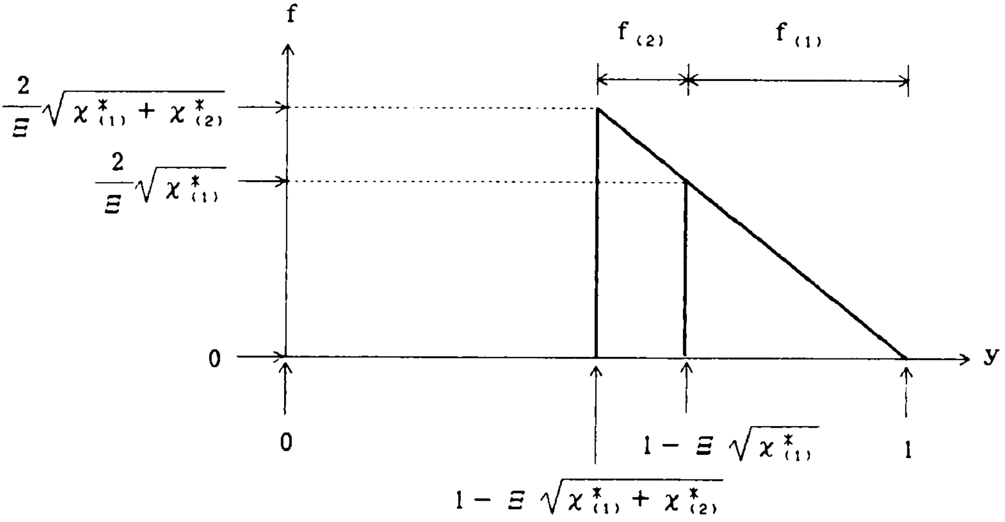

FIG. A13. As Fig. A12 when $\zeta(\chi) = \sqrt{\chi}$, where it has been assumed: $\Xi\sqrt{\chi^*_{(1)} + \chi^*_{(2)}} = 0.5$, $\chi^*_{(1)} = \chi^*_{(2)}$, and $w_{(1)} = w_{(2)} = 1$. It can be seen that the shape of the total chromatogram (being a right-angled triangle) is identical with that of the chromatogram for a single component system [Fig. A11; cf. "Remark" in Theoretical Section (I)]. In the case of a more than two component system with $w_{(1)} = w_{(2)} = \cdots = w_{(\rho)}$, trapezoids that keep in contact with one another continue on the left-hand side of the two component chromatogram, completing as a whole a total chromatogram of right-angled triangle. (Reproduced from Ref. *10*.)

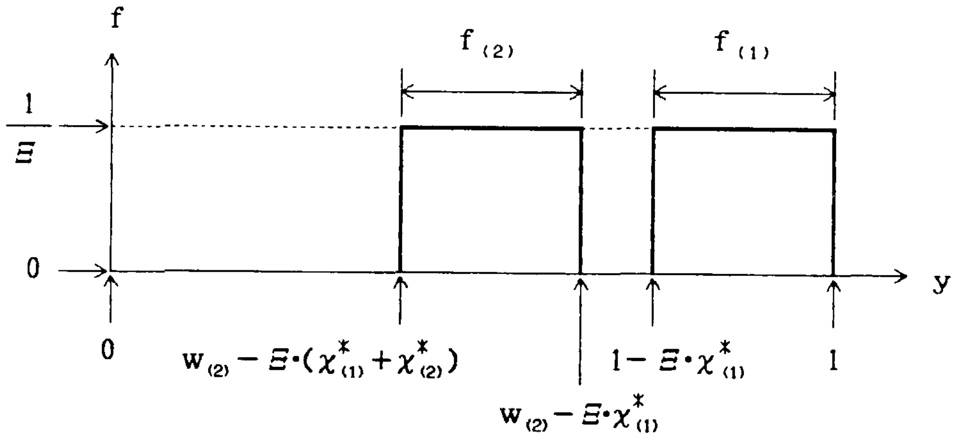

FIG. A14. Approximate $f_{(\rho')}$ or f as a function of y for a two component system with different $\eta_{(\rho')}$ or $w_{(\rho')}$ values when $\zeta(\chi) = \chi$. This has been calculated by using Eq. (77) assuming: $\Xi \cdot (\chi^*_{(1)} + \chi^*_{(2)}) = 0.5$, $\chi^*_{(1)} = \chi^*_{(2)}$, $w_{(1)} = 1$, and $w_{(2)} = 0.9$. It can be seen that, provided the chromatograms concerning the respective components of the mixture approach one another to keep in contact, the shaep of the total chromatogram (being a rectangle) will be identical with that of the chromatogram for a single component system (Fig. A10). In the case of a more than two component system with $w_{(1)} > w_{(2)} > \cdots > w_{(p)}$, rectangles that are separated from one another are present on the left-hand side of the two component chromatogram, completing as a whole a rectangular total chromatogram provided the respective rectangles approach one another to keep in contact.

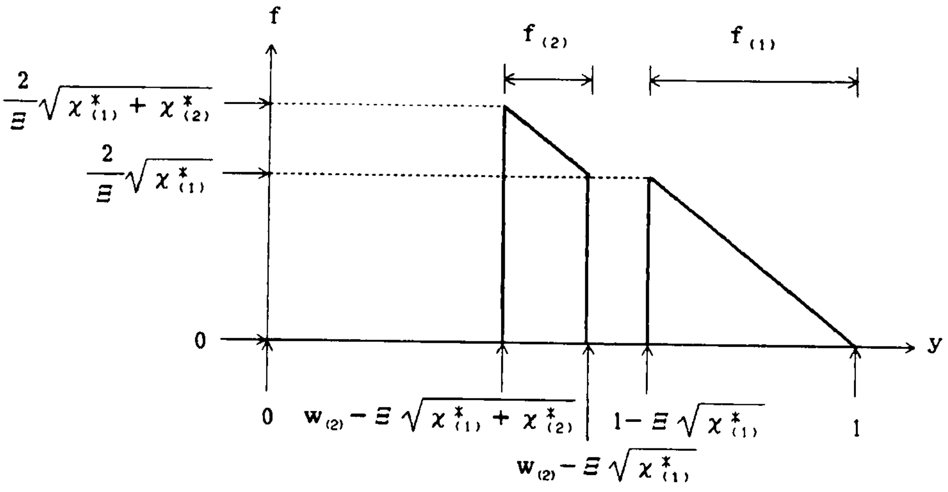

FIG. A15. As Fig. A14 when $\zeta(\chi) = \sqrt{\chi}$, where it has been assumed: $\Xi\sqrt{\chi^*_{(1)} + \chi^*_{(2)}} = 0.5$, $\chi^*_{(1)} = \chi^*_{(2)}$, $w_{(1)} = 1$, and $w_{(2)} = 0.9$. It can be seen that, provided the chromatograms concerning the respective components of the mixture approach one another to keep in contact, the shape of the total chromatogram (being a right-angled triangle) will be identical with that of the chromatogram for a single component system (Fig. A11). In the case of a more than two component system with $w_{(1)} > w_{(2)} > \cdots > w_{(p)}$, trapezoids that are separated from one another are present on the left-hand side of the two component chromatogram, completing as a whole a total chromatogram of right-angled triangle provided the respective trapezoids and a triangle approach one another to keep in contact. (Reproduced from Ref. *10*.)

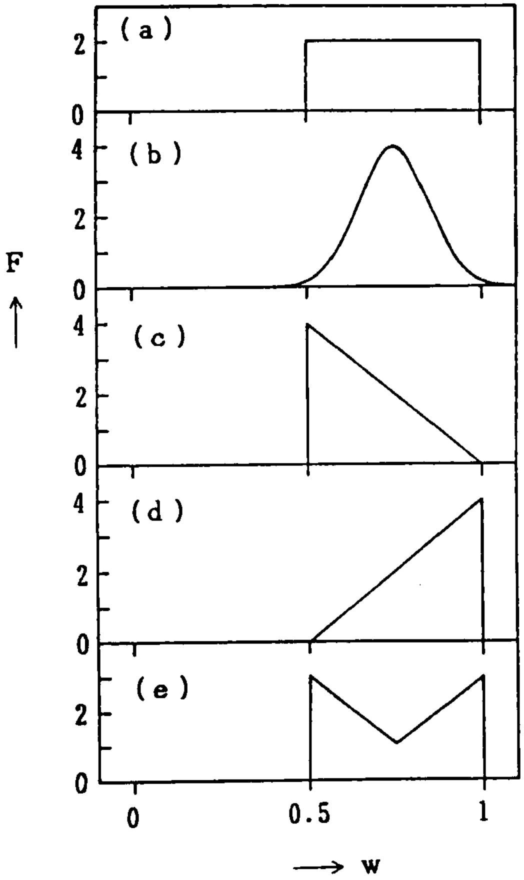

FIG. A16. Several types of continuous distributions, $F(w)$, of molecular species with different w values. (Reproduced from Ref. *10*.)

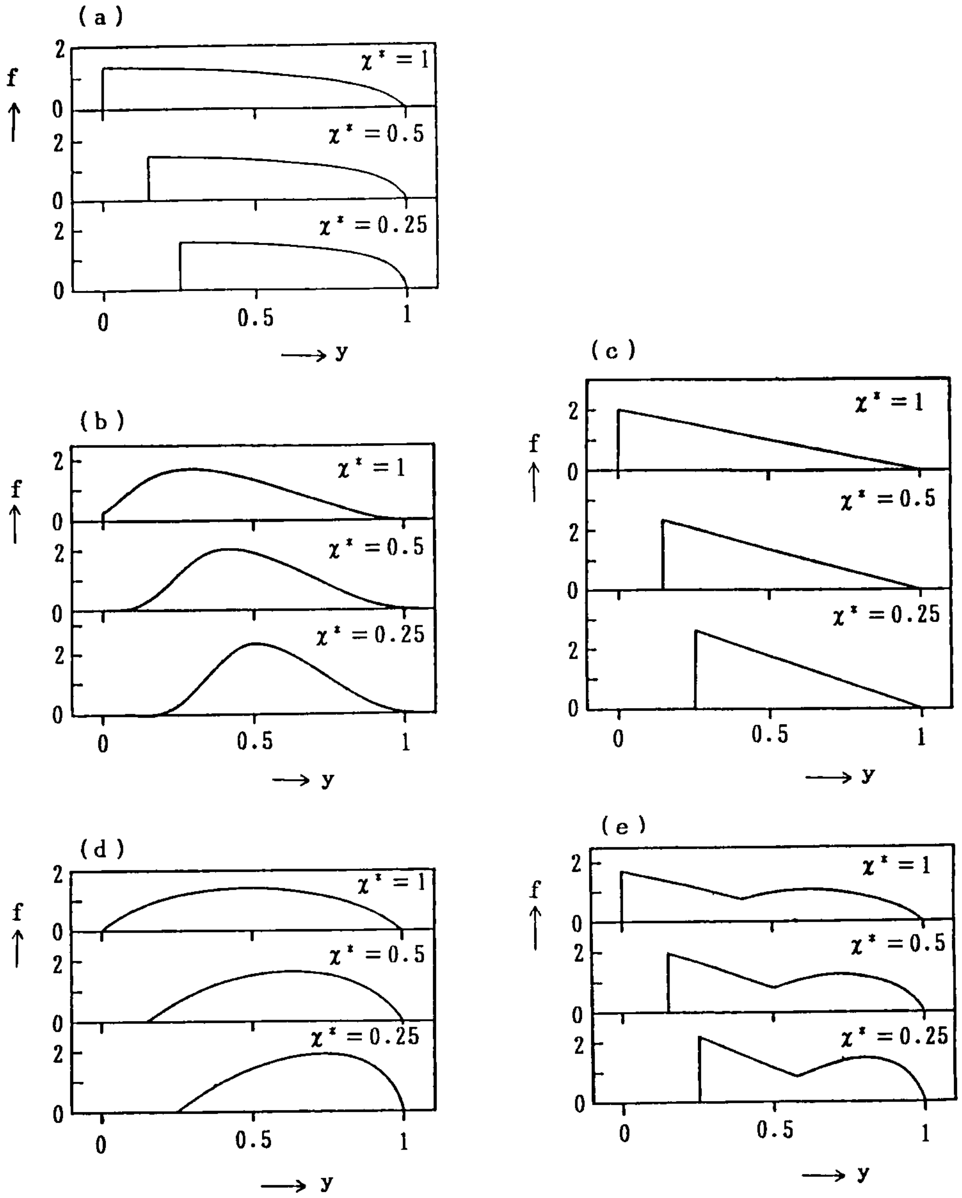

FIG. A17. Parts (a)–(e) represent chromatograms with $\Xi = 0.5$ and different χ^* values for the mixtures as shown in Fig. A16 (a)–(e), respectively. These have been calculated by using both Eqs. (78) and (80) for the case when $\zeta(\chi) = \sqrt{\chi}$; it is assumed that $y_{\text{in}} = 0$. When $\chi^* \rightarrow 0$ or under condition of infinitesimal sample load, all the chromatograms tend toward the patterns shown in Fig. A16. (Reproduced with modifications from Ref. *10*.)

REFERENCES

1. T. Kawasaki, *Sep. Sci. Technol., 22,* 121 (1987).
2. T. Kawasaki, *Ibid., 23,* 451 (1988).
3. T. Kawasaki, *Ibid., 23,* 2365 (1988).
4. T. Kawasaki, *J. Chromatogr., 161,* 15 (1978).
5. T. Kawasaki, *Sep. Sci. Technol., 17,* 575 (1982).
6. T. Kawasaki, *Ibid., 17,* 1397 (1982).
7. T. Kawasaki, *J. Chromatogr., 82,* 167 (1973).
8. T. Kawasaki, *Ibid., 82,* 191 (1973).
9. T. Kawasaki, *Ibid., 82,* 241 (1973).
10. T. Kawasaki, *Ibid., 93,* 257 (1974).
11. T. Kawasaki, *Sep. Sci. Technol., 23,* 601 (1988).
12. T. Kawasaki, *Ibid., 23,* 617 (1988).
13. T. Kawasaki, *Ibid., 23,* 1105 (1988).
14. T. Kawasaki, *Biopolymers, 9,* 277 (1970).
15. T. Kawasaki, *Sep. Sci. Technol., 16,* 439 (1981).
16. T. Kawasaki and S. Takahashi, *Ibid., 23,* 193 (1988).
17. T. Kawasaki, *Ibid., 17,* 407 (1982).

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