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A Theory of Gradient Hydroxyapatite Chromatography: A Specification of the Intermediate Abstract Flux

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Abstract

On the partial basis fo the theory for the intermediate abstract flux, the chromatogram for gradient hydroxyapatite chromatography can be calculated for a mixture of molecules with the same dimensions and the same shape, taking into account both repulsive molecular interactions occurring on the hydroxyapatite serfaces and longitudinal diffusion in the column. In the present paper the general relationship between the intermediate abstract flux and the ideal chromatogram occurring in the absence of longitudinal diffusion is specified. The earlier theories for the intermediate abstract flux are reconsidered from the new point of view.

INTRODUCTION

The adsorption and desorption phenomena in a hydroxyapatite (HA) column can be represented by using a model where adsorption sites are arranged in some manner on the surfaces of HA. Sample molecules with adsorption groups and particular ions from the buffer compete for these crystal sites (*1*). Gradient chromatography is carried out by applying a linear molarity gradient of competing ions. This migrates from the top to the bottom of the column at the same time with the migration of bands of the sample molecules. Since the migration rate of sample molecules is smaller than, or at most equal to, the migration rate of the molarity gradient, it is apparent that the molecules migrate upward along the molarity gradient.

Earlier (*1–6*) a theory of linear gradient chromatography was developed for the simplest case of small sample loads when mutual interactions among

sample molecules are negligible and when a narrow band of the molecules is formed initially at the top of the column. It can be assumed that longitudinal diffusion of both sample molecules and competing ions occurs in the column essentially due to heterogeneity in the flow rate. This is provoked by heterogeneity in interspaces among HA crystals packed in the column (*1*). The chromatographic process is virtually a quasi-static process. Thermodynamic equilibria are locally attained in the column within any small time interval (for details, see Ref. *1*). Since competing ions are locally distributed within a column section due to diffusion (see above), it is necessary to specify the meaning of the molarity gradient of the ions. This can be defined as the gradient obtained by connecting mean molarities, m, occurring within respective column sections. The gradient is linear with linear gradient chromatography since the diffusion effect is canceled out among different column sections (for details, see Refs. *1* and *3*).

In principle, it is impossible for a gradient chromatographic process to be described on the basis of a continuity equation for the actual flux of sample molecules occurring in the column (*3*). It is necessary to consider an abstract flux with density, $C_{(\rho')}$, of molecules existing in the interstitial liquid in the column which migrates upward along the molarity gradient (*3*). However, the density $C_{(\rho')}$ is different from the density $\Omega_{(\rho')}$ of the actual flux which is concerned with molecules in the interstices, including the crystal surfaces, in the column. Therefore, the abstract flux is fundamentally different from the apparent flux that migrates upward along the molarity gradient (see above; for details, see Ref. *3*). In the absence of the mutual molecular interactions, the abstract flow equation can be solved under a given initial boundary condition, and the theoretical chromatogram can be calculated (*1–6*). Because of mathematical difficulty, however, it is practically difficult to treat, on the basis of the abstract flux, the general case when mutual molecular interactions occur on the HA surfaces. The interactions occurring in the interstitial liquid in the column are generally negligible since the molecular concentration ($C_{(\rho')}$) in solution is low.

The mathematical structures of both actual and abstract fluxes are mutually derivable from the structures of one another (Ref. *1*, Appendix II; Ref. *3*). In the process of the derivation, an intermediate abstract flux (i.a.f.) appears (see Eq. A13 in Ref. *1*, Appendix II; or Eq. 20 in Ref. *3*). This flux, with density, $\chi_{(\rho')}$ of sample molecules adsorbed on the crystal surface of HA, migrates along the gradient made by the quantity s (see Eq. 2), and it is only concerned with molecules adsorbed on the HA surface. The i.a.f. does not involve the diffusion term since diffusion does not occur on the HA surface. In contrast to the actual gradient chromatographic process with molecular diffusion that can be described only on the basis of the abstract flux (see

above), the idealized chromatographic process occurring in the absence of diffusion can be described on the basis of either the abstract flux or the i.a.f. In Appendix II in Ref. *1* the ideal chromatogram was calculated on the basis of the i.a.f. for the case of no molecular interactions. In Ref. *7* the calculation was performed for the mixture of molecules with the same dimensions and the same shape, taking into account the repulsive interactions among sample molecules adsorbed on the HA surface.

The theory for i.a.f. is important. Thus, in Ref. *8*, combining this theory with the general theory with small sample loads (*1–6*), a method was developed by which the chromatogram can approximately be calculated for a mixture of molecules with the same dimensions and the same shape, taking into account both repulsive molecular interactions occurring on the HA surfaces and longitudinal diffusion in the interstices in the column. In Ref. *9* this method was applied to the experimental analysis of collagen chromatograms.

The calculation of the ideal chromatogram as based on i.a.f. (see above) consists in (a) solving the continuity equation, or the simultaneous continuity equations if there are molecular interactions, for this flux under a suitable intitial boundary conditions, and (b) calculating the chromatogram from the solution. However, both the type of the initial boundary condition used in Step (a) and the method for the calculation applied in Step (b) are fundamentally different between the case of no molecular interactions (Appendix II in Ref. *1*) and the case of molecular interactions (*7*). The purpose of the present paper is to specify the general relationship between i.a.f. and the ideal chromatogram occurring in the absence of molecular diffusion. It is confirmed that the ideal chromatogram can be calculated on the basis of the same principle in both the absence and the presence of repulsive molecular interactions.

THEORETICAL

(A) General Relationship between the Intermediate Abstract Flux and the Ideal Chromatogram Occurring in the Absence of Longitudinal Diffusion in the Column

For a mixture of molecular components (1, 2, ..., ρ) with the same dimensions and the same shape, the simultaneous continuity equations for i.a.f. occurring in the presence of mutual molecular interactions on the HA surface can be written (Ref. *1*, Appendix II; Refs. *3, 7,* and *8*) as

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

where

$$s=gL \tag{2}$$

$$\chi=\sum_{\rho''=1}^{\rho}\chi_{(\rho'')} \tag{3}$$

$$\frac{B_{(\rho')}}{1-B_{(\rho')}}=\frac{dr_{(\rho')}(m)}{dm}H(\chi) \tag{4}$$

$$r_{(\rho')}(m)=\frac{1}{q_{(\rho')}}\int_{m_{\text{in}}}^{m}(\varphi' m+1)^{x'}\,dm$$

$$=\frac{1}{\varphi' q_{(\rho')}(x'+1)}[(\varphi' m+1)^{x'+1}-(\varphi' m_{\text{in}}+1)^{x'+1}] \tag{5}$$

and

$$\frac{dr_{(\rho')}(m)}{dm}=\frac{1}{q_{(\rho')}}(\varphi' m+1)^{x'} \tag{6}$$

The physical meanings of the symbols involved in Eqs. (1)–(6) are:

m = molarity of competing ions, constituting a linear gradient in the column.

m_{in} = initial molarity of competing ions at the beginning of the molarity gradient introduced at the top of the column.

L = distance from the top of any longitudinal position in the column. In some instances, L represents the total length of the column.

g = positive constant representing the slope of the molarity gradient of competing ions in the column. This is expressed as the increase in ion molarity per unit length of the column, measured from the bottom to the top. [Therefore, s (Eq. 2) has a dimension of molarity, representing the difference in ion molar-

ity between the top and the bottom of the column when L represents the total column length.]

$\chi_{(\rho')}$ = molecular density of component ρ' on the crystal surface of HA, defined as unity provided the crystal surface is saturated only with component ρ'. [Therefore, χ (Eq. 3) represents the density for all components 1, 2, . . . , ρ, being equal to unity when the crystal surface is saturated with them.]

$B_{(\rho')}(m, \chi)$ = partition of component ρ' in the interstitial liquid in the column, or the ratio of the amount of molecules of component ρ' in solution to the total amount occurring in a longitudinal position in the column. The partition is determined when both m and χ are given (see Eq. 4).

$H(\chi)$ = function of χ representing the mutual molecular interactions occurring on the crystal surface of HA. When χ tends to zero, then H tends to unity. With repulsive interactions, H increases monotonically with an increase in χ. For details, see Remark (1) below.

$q_{(\rho')}$ = positive constant representing a molecular property of component ρ'. For detail, see Remark (2) below.

x' = average number (in the equilibrium state) of sites of HA on which the adsorption of competing ions is impossible due to the presence of an adsorbed molecule. x' therefore represents the effective dimensions of the molecule, assumed to be the same for any component in the mixture.

φ' = positive constant representing the property of competing ions.

Some comment on the function $r_{(\rho')}$ (Eq. 5) is mentioned in Remark (3). Equation (1) can be rewritten by using Eq. (4) as

$$\frac{\partial[H(\chi)\chi_{(\rho')}]}{\partial s} + \frac{\partial \chi_{(\rho')}}{\partial r_{(\rho')}} = 0 \qquad (\rho' = 1, 2, \ldots, \rho) \qquad (1')$$

In this section the argument is made on the basis of Eq. (1) whereas Eq. (1') will be used in Sections (C) and (D).

The i.a.f. has the fundamental property that it occurs independently of molecules existing in the interstitial liquid in the column. This is the reason why i.a.f. does not involve the diffusion term (see Introduction Section and Ref. *1*, Appendix II). Due to this property of i.a.f., it can, in general, be stated that the chromatogram, $f_{(\rho'),s}$, for component ρ' occurring when s is given (i.e., when both length L of the column and slope g of the molarity gradient are given; see Eq. 2) should be represented as a function of m as

$$f_{(\rho'),s}(m) = -\left[\frac{\partial \chi_{(\rho')}}{\partial m}\right]_s \tag{7}$$

fulfilling a conservation condition

$$\int_{m_{in}}^{\infty} f_{(\rho'),s}(m)\, dm = x^*_{(\rho')} \tag{8}$$

where $\chi^*_{(\rho')}$ represents the total amount of component ρ' that has initially been loaded on the column (cf. Ref. 7). We consider below the case when molecules are "retained" on the column initially, i.e., before the application of the molarity gradient of the ions. In this instance $\chi^*_{(\rho')}$ is virtually equal to the amount of molecules that are intially adsorbed on the crystal surfaces of HA in the column (see below; cf. Remark 4).

In this stage of the argument, however, we have no knowledge about how the quantity $\chi^*_{(\rho')}$ should be represented. A proof is given below that the quantity should be expressed in such a unit that the $\chi^*_{(\rho')}$ value be numerically equal to the mean initial density of component ρ' on the crystal surfaces of the total column, viz., the proportion on the crystal surfaces in the column that are initially occupied by component ρ'. $\chi^*_{(\rho')}$ is equal to unity provided all the crystal surfaces are saturated only with component ρ'. This means that the quantity $\chi^*_{(\rho')}$ has both extensive and intensive properties.

Proof: At the top ($L = 0$) of the column where the relationship $s = 0$ is fulfilled (see Eq. 2), the inflow of molecules does not occur when once the sample load has been finished. This means that, after the sample load, a condition is always fulfilled that when $s = 0$, then

$$\chi_{(\rho')} = 0 \qquad (\rho' = 1, 2, \ldots, \rho) \tag{9}$$

On the other hand, provided the slope g of the molarity gradient has an infinitesimal value δg, the value (written as δs) of the parameter s (Eq. 2) at the bottom L of the column should also be infinitesimal, and it can be represented as

$$\delta s = L\, \delta g \tag{10}$$

δs has a physical meaning of the molarity difference of competing ions between the top and the bottom of the column, being constant (with respect to time) with linear gradient chromatography. In this instance the first term on the left-hand side of Eq. (1) becomes

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

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

Equation (1) can therefore be rewritten as

$$-\frac{d\chi_{(\rho')}}{d(m/\delta s)}=C_{(\rho')}\qquad(\rho'=1,2,\ldots,\rho)$$

where

$$C_{(\rho')}=\frac{B_{(\rho')}}{1-B_{(\rho')}}\chi_{(\rho')}\tag{12}$$

On the other hand, calling L' the total interstitial volumes in the column, α the interstitial volume per unit length of the column, and V the elution volume, we have

$$L'=\alpha L\tag{13}$$

and

$$\frac{dm}{dV}=\frac{\delta g}{\alpha}\tag{14}$$

and from Eqs. (10), (13), and (14),

$$d\left(\frac{m}{\delta s}\right)=d\left(\frac{V}{L'}\right)\tag{15}$$

can be derived. The left-hand side of Eq. (15) shows an increase in ion molarity measured in unit of the difference, δs, in ion molarity between the

top and bottom of the column. This is equal to the increase in elution volume measured in units of the total interstitial volumes involved in the column (the right-hand side of the equation). This means that the left-hand side of Eq. (15) or the denominator on the left-hand side of Eq. (11) can be defined independently of the value of δs, and it can have a physical meaning even when δs is an infinitesimal quantity.

In Eq. (11) $\chi_{(\rho')}$ has a meaning of the average molecular density of component ρ' on the HA surfaces in the column or the total amount of component ρ' on the crystal surfaces in the column expressed in such a unit that the amount is numerically equal to the molecular density. Moreover, with molecules that are initially "retained" on the column, the amount of molecules existing in the interstitial liquid in the column is negligibly small in comparison with the amount of molecules that are present on the crystal surfaces, and, as long as Eq. (11) is fulfilled, the same situation continues during the whole process of chromatography (cf. Remark 4). This means that $\chi_{(\rho')}$ virtually represents the total amount of component ρ' in the column. The left-hand side of Eq. (11) represents the decrease in the total amount of component ρ' in the column per unit increase in ion molarity measured in units of δs. The right-hand side of Eq. (11) representes the amount of component ρ' eluted at the same time out of the column. Therefore, denoting by $\chi^*_{(\rho')}$ the initial average molecular density of component ρ' on the HA surfaces in the column, a conservation condition,

$$\int_{m=m_{\text{in}}}^{m=\infty} C_{(\rho')}\, d\left(\frac{m}{\delta s}\right) = \chi^*_{(\rho')} \tag{16}$$

should be fulfilled. On the other hand, since the chromatogram $f_{(\rho'),\delta s}$ represents the elution of component ρ' out of the column occurring per unit increase in ion molarity, it is evident that $f_{(\rho'),\delta s}$ is related to $C_{(\rho')}$ by the relationship

$$f_{(\rho'),\delta s} = \frac{C_{(\rho')}}{\delta s} \tag{17}$$

By substituting Eq. (17) into Eq. (16), Eq. (8) can be derived. This means that $\chi^*_{(\rho')}$ introduced into Eq. (16) is identical with $\chi^*_{(\rho')}$ introduced into Eq. (8).

Remark (1). In general, it is reasonable to assume that the mutually superimposed state of molecules does not occur on the HA surface at least when chromatography is proceeding (*8*), and $H(\chi)$ can be represented as

$$H(\chi) = \frac{e^{(\tilde{E}/kT)\psi(\chi)}}{p(\chi)} \qquad \text{(a)}$$

where the numerator and the demominator on the right-hand side are concerned with energetic and geometrical interactions, respectively. Thus $\tilde{E}$ represents the interaction energy per molecule provided the maximum possible contact with other molecules is made. $\psi(\chi)$ is the function of χ that increases monotonically with an increase in χ, being equal to zero and unity when χ is zero and unity, respectively. $p(\chi)$ is the probability that, when a new molecule is added at random to the HA surface, a proportion χ of which is already occupied by molecules, it is not superimposed on the already adsorbed molecules. The final chromatogram depends only slightly upon the shape of the function $p(\chi)$, and it is a good approximation to assume that $p(\chi) \approx 1 - \chi$ (cf. Refs. *7* and *8*).

Remark (2). $q_{(\rho')}$ can be written as

$$q_{(\rho')} = \beta\tau_{(\rho')}e^{x_{(\rho')}\varepsilon/kT} \qquad \text{(b)}$$

where β is the positive constant related to the property of the column, $-\varepsilon\,(\varepsilon > 0)$ is the adsorption energy of a functional group of the molecule on one of sites of HA, $x_{(\rho')}$ is the average number (in the equilibrium state) of functional groups per molecule of component ρ' that react with sites of HA, and $\tau_{(\rho')}$ is the number of effective geometrical conformation(s) of a component ρ' on the HA surface (in the equilibrium state) (cf. Refs. *1* and *8*).

Remark (3). With small sample loads χ is virtually equal to zero, and Eq. (a) reduces to

$$H(\chi) = 1 \qquad \text{(c)}$$

The relationships

$$\frac{dr_{(\rho')}}{dm} = \frac{B_{(\rho')}}{1 - B_{(\rho')}} \qquad \text{(d)}$$

and

$$r_{(\rho')} = \int_{m_{\text{in}}}^{m} \frac{B_{(\rho')}}{1 - B_{(\rho')}}\, dm \qquad \text{(e)}$$

are fulfilled (cf. Eqs. 4–6). In Ref. *1*, $r_{(\rho')}$ was defined by Eq. (e) for the case

of small sample loads (see Eq. 11 in Ref. *1*). For the general case of molecular interactions, however, $r_{(\rho')}$ can be defined by Eq. (5).

Remark (*4*). Practically only the case when molecules are initially "retained" on the column is important since, unless this is the situation, it is unnecessary to apply the molarity gradient. For "retained" molecules the $B_{(\rho')}$ value is small when $m = m_{in}$, and almost all molecules in the column are adsorbed on the HA surfaces since this is the reason why the molecules are "retained." However, under the experimental condition where s is small, molecules are generally eluted out of the column in molarities that are close to m_{in}. Therefore, during the elution process the $B_{(\rho')}$ value is kept close to zero. In order for almost all molecules to be eluted out of the column, an extremely large volume of the solvent in comparison with the total interstitial volumes of the column is necessary. This means that, from a practical point of view, the molecular elution does not occur under the experimental condition where s is small and where the column has a finite length.

(B) Initial Boundary Condition of Eq. (1) or (1')

Equation (11), or the solution of Eq. (11) obtained under the conservation condition of Eq. (16), gives a boundary condition to Eq. (1) or (1'). However, this type of boundary condition is that which can be attained by gradually changing the structure of the experimental equipment (in this instance the structure means the slope g of the molarity gradient), and it cannot be applied for the purpose of solving Eq. (1) or (1'). For this purpose it is necessary to give the initial boundary condition occurring at time zero. In this instant, $m = m_{in}$ at the top of the column. It will be understood, however, that the chromatogram becomes independent of the type of initial boundary condition if g approaches zero. In this extreme case the chromatogram *as a function of* m always becomes a sharp peak appearing at $m = m_{in}$ (although it appears over a large volume of the solvent). This situation is related to the fact that the mathematical form of the initial boundary condition (obtained when a band with an infinitesimal width is initially formed at the top of the column) resembles in part the form of the boundary condition obtained when g approaches zero (see below).

Thus let us consider the case when a band of the molecules (with the components $1, 2, \ldots, \rho$) is initially formed within an infinitesimal width δL at the top of the column (cf. Remark 1 below). In i.a.f. the initial distribution of the molecules is concentrated within an infinitesimal range $[0, \delta s]$ where δs is defined as

$$\delta s = g\ \delta L \tag{18}$$

Since at the top ($L = 0$) of the column (where the relationship $s = 0$ is fulfilled; see Eq. 2), the inflow of molecules does not occur after the sample load has been finished, then Eqs. (9), (11), and (16) hold. The solution of Eq. (11) obtained under the condition of Eq. (16) appears to constitute (part of) the initial boundary condition of Eq. (1) or (1'). In this instance, however, Eq. (15) is absent, and the procedure of directly solving Eq. (11) (in which the infinitesimal parameter δs is involved) should be avoided. Instead of this procedure, let us create a hypothesis to connect an actual column to the top of the ideal column in which longitudinal diffusion does not occur. We assume that the actual column has a critical length ΔL (cf. Remark 2 below), and that the sample load is done within ΔL. We denote by $L = 0$ the top of the total compound column (identical with the top of the actual column) and by $L = \Delta L$ the boundary between the actual and the ideal column. Instead of the initial boundary condition we consider the boundary condition of Eq. (1) or (1') to occur at position $L = \Delta L$ of the compound column. We finally gradually deform the actual part of the compound column to the ideal structure of no longitudinal diffusion. By this procedure the ΔL value tends to infinitesimal (cf. Remark 2 below), and the total column becomes identical with the original ideal part.

Defining Δs as

$$\Delta s = g\ \Delta L \tag{19}$$

the boundary condition occurring at the boundary between the actual and the ideal part of the compound column can be represented in such a way that when both $L = \Delta L$ (i.e., $s = \Delta s$) and $m > m_{\text{in}}$ (i.e., $r_{(\rho')} > 0$; see Eq. 5), then

$$-\frac{d\chi_{(\rho')}}{d(m/\Delta s)} = C_{(\rho')} \qquad (\rho' = 1, 2, \ldots, \rho) \tag{20}$$

and that when both $m = m_{\text{in}}$ (i.e., $r_{(\rho')} = 0$) and $L > \Delta L$ (i.e., $s > \Delta s$), then

$$\chi_{(\rho')} = 0 \qquad (\rho' = 1, 2, \ldots, \rho) \tag{21}$$

Expecially for Eq. (1'), Eq. (20) can be rewritten as

$$-\frac{d\chi_{(\rho')}}{dr_{(\rho')}} = \frac{1}{\Delta s} H(\chi)\chi_{(\rho')} \qquad (\rho' = 1, 2, \ldots, \rho) \tag{20'}$$

For Eq. (20) or (20') a conservation condition

$$\int_{m=m_{\text{in}}}^{m=\infty} C_{(\rho')}\, d\left(\frac{m}{\Delta s}\right) = \frac{1}{\Delta s}\int_0^{\infty} H(\chi)\chi_{(\rho')}\, dr_{(\rho')} = \chi^*_{(\rho')} \qquad (22)$$

is fulfilled. In parallel with the fact that Eq. (8) can be derived from Eq. (16) (Section A), Eq. (8) can also be derived from Eq. (22).

Remark (1). Actually the width in the initial molecular band at the column top cannot be infinitesimal except the case of an infinitesimal sample load. However, it is common practice with gradient chromatography that molecules are eluted out of the column over a considerably large volume of the solution (see Introduction Section in Ref. *1*). Therefore, assumption of the infinitesimal initial band is a good approximation for the final result of the calculation of the chromatogram. Moreover, with repulsive molecular interactions the width in the final chromatogram still increases.

Remark (2). The critical length ΔL can be defined as

$$\Delta L = \frac{4\hat{D}}{|v_0|} \qquad (f)$$

where $\hat{D}$ (with dimensions of length^2/time) is the diffusion coefficient for thermal Brownian diffusion plus diffusion due to the second type of flow heterogeneity (briefly, B-dif plus STFH-dif; see Refs. *5, 6,* and *10*), and $|v_0|$ (with a dimension of length/time) is the mean flow rate of the solvent in the column. Due to its mechanism, it can, in general, be assumed that, within the width ΔL at the column top, the first type of flow heterogeneity is negligible, and only the B-dif plus STFH-dif is important (*5, 6, 10*). In the ideal state of no longitudinal diffusion, $\hat{D}$ tends to zero, so that ΔL tends to zero (see Eq. f).

(C) The Case of No Molecular Interactions with Small Sample Loads

In this instance $H(\chi)$ becomes

$$H(\chi) = 1 \qquad (23)$$

(see Remark 3 in Section A), and Eqs. (1′), (20′), and (22) reduce to

$$\frac{\partial \chi_{(\rho')}}{\partial s} + \frac{\partial \chi_{(\rho')}}{\partial r_{(\rho')}} = 0 \qquad (24)$$

$$-\frac{d\chi_{(\rho')}}{dr_{(\rho')}} = \frac{1}{\Delta s}\chi_{(\rho')} \tag{25}$$

and

$$\frac{1}{\Delta s}\int_0^\infty \chi_{(\rho')}\, dr_{(\rho')} = \chi^*_{(\rho')} \tag{26}$$

respectively; Eqs. (24)–(26) hold independently of the coexistence of all the other components than the component ρ' in the mixture. Under Eq. (26), Eq. (25) has a solution fulfilling

$$\frac{r_{(\rho')}}{\Delta s} = \ln\frac{\chi^*_{(\rho')}}{\chi_{(\rho')}} \tag{27}$$

On the other hand, the general solution of Eq. (24) can be written as

$$\chi_{(\rho')} = \Phi(r_{(\rho')} - s) \tag{28}$$

and, under the boundary condition given by both Eq. (27) (originally derived from Eq. 20) and Eq. (21), Eq. (28) has a form represented as

$$\left.\begin{aligned} &\frac{r_{(\rho')} - s + \Delta s}{\Delta s} = \ln\frac{\chi^*_{(\rho')}}{\chi_{(\rho')}} && (\text{for } r_{(\rho')} \geq s - \Delta s) \\ &\text{and} \\ &\chi_{(\rho')} = 0 && (\text{for } r_{(\rho')} < s - \Delta s) \end{aligned}\right\} \tag{29}$$

By substituting Eq. (29) into Eq. (7):

$$\left.\begin{aligned} &f_{(\rho'),s}(m) \equiv -\frac{\partial\chi_{(\rho')}}{\partial m} = \frac{1}{\Delta s}\chi_{(\rho')}\frac{dr_{(\rho')}}{dm} && (\text{for } r_{(\rho')} \geq s - \Delta s) \\ &\text{and} \\ &f_{(\rho'),s}(m) \equiv -\frac{\partial\chi_{(\rho')}}{\partial m} = 0 && (\text{for } r_{(\rho')} < s - \Delta s) \end{aligned}\right\} \tag{30}$$

are obtained. It should be noted (a) that $\chi_{(\rho')}$ fulfills Eq. (26) while $f_{(\rho'),s}$ fulfilling Eq. (8), and (b) that, when $r_{(\rho')} \geq s - \Delta s$, then $\chi_{(\rho')}$ is a continuous

function of $r_{(\rho')}$ that increases monotonically with a decrese of $r_{(\rho')}$. (This can be confirmed directly from the first equality in Eq. 29. However, the same conclusion can also be derived from the form of Eq. 25. Cf. the corresponding argument for Eq. 42.) This means that, when $\Delta s \to +0$, Eq. (30) reduces to

$$f_{(\rho'),s}(m) = \chi^*_{(\rho')}\, \delta(r_{(\rho')} - s)\, \frac{dr_{(\rho')}}{dm} \tag{31}$$

(D) The General Case of Molecular Interactions

Step 1. Corresponding to Eqs. (24), (25), and (26), we have Eqs. (1′), (20′), and (22). If an equation for component ρ'' in the simultaneous equations, Eq. (20′), is divided by another for component ρ', then

$$\frac{d \ln \chi_{(\rho'')}}{d \ln \chi_{(\rho')}} = \frac{q_{(\rho')}}{q_{(\rho'')}} \tag{32}$$

is obtained where Eq. (6) has been used. Taking into account the fact that when $r_{(\rho')} = r_{(\rho'')} = 0$, then $\chi_{(\rho')} = \chi^*_{(\rho')}$ and $\chi_{(\rho'')} = \chi^*_{(\rho'')}$, Eq. (32) can be integrated to give

$$\chi_{(\rho'')} = \chi^*_{(\rho'')} \left[\frac{\chi_{(\rho')}}{\chi^*_{(\rho')}} \right]^{q_{(\rho')}/q_{(\rho'')}} \tag{33}$$

By substituting Eq. (33) into Eq. (3):

$$\chi = \sum_{\rho''=1}^{\rho} \chi^*_{(\rho'')} \left[\frac{\chi_{(\rho')}}{\chi^*_{(\rho')}} \right]^{q_{(\rho')}/q_{(\rho'')}} \tag{34}$$

is derived, which is the expression of χ as a function of the molecular density, $\chi_{(\rho')}$, of only one component ρ' of the mixture. Due to Eq. (34) it is possible to write

$$H(\chi)\chi_{(\rho')} = Y_{(\rho')}(\chi_{(\rho')}) \tag{35}$$

and Eqs. (1′), (20′), and (22) can be rewritten as

$$\frac{\partial Y_{(\rho')}(\chi_{(\rho')})}{\partial s} + \frac{\partial \chi_{(\rho')}}{\partial r_{(\rho')}} = 0 \tag{36}$$

$$-\frac{d\chi_{(\rho')}}{dr_{(\rho')}} = \frac{1}{\Delta s} Y_{(\rho')}(\chi_{(\rho')}) \tag{37}$$

and

$$\frac{1}{\Delta s}\int_0^{\infty} Y_{(\rho')}(\chi_{(\rho')})\, dr_{(\rho')} = \chi^*_{(\rho')} \tag{38}$$

respectively; Eqs. (36)–(38) hold independently of the coexistence of all the other components than component ρ' in the mixture.

Step 2. Under Eq. (38), Eq. (37) has a solution fulfilling

$$\frac{r_{(\rho')}}{\Delta s} = \int_{\chi_{(\rho')}}^{\chi^*_{(\rho')}} \frac{1}{Y_{(\rho')}(\chi_{(\rho')})}\, d\chi_{(\rho')} \tag{39}$$

On the other hand, the general solution of Eq. (36) can be written as

$$\chi_{(\rho')} = \Phi\left\{r_{(\rho')} - \left[\frac{dY_{(\rho')}(\chi_{(\rho')})}{d\chi_{(\rho')}}\right]^{-1} s\right\} \tag{40}$$

Under the boundary condition given by both Eq. (39) (originally derived from Eq. 20) and Eq. (21), Eq. (40) has a form represented as

$$\left.\begin{aligned} &\frac{r_{(\rho')} - \left[\frac{dY_{(\rho')}(\chi_{(\rho')})}{d\chi_{(\rho')}}\right]^{-1} s + \Delta s}{\Delta s} = \int_{\chi_{(\rho')}}^{\chi^*_{(\rho')}} \frac{1}{Y_{(\rho')}(\chi_{(\rho')})}\, d\chi_{(\rho')} \\ &\left(\text{for } r_{(\rho')} \geq \left[\frac{dY_{(\rho')}}{d\chi_{(\rho')}}\right]^{-1} s - \Delta s\right) \end{aligned}\right\} \tag{41}$$

and

$$\chi_{(\rho')} = 0 \qquad \left(\text{for } r_{(\rho')} < \left[\frac{cY_{(\rho')}}{d\chi_{(\rho')}}\right]^{-1} s - \Delta s\right)$$

from which

$$\left.\begin{aligned}
&\frac{Y_{(\rho')}(\chi_{(\rho')})}{\Delta s} \\
&= -\frac{\dfrac{\partial \chi_{(\rho')}}{\partial m}}{\dfrac{dr_{(\rho')}}{dm} + s\left[\dfrac{dY_{(\rho')}(\chi_{(\rho')})}{d\chi_{(\rho')}}\right]^{-2} \dfrac{d^2 Y_{(\rho')}(\chi_{(\rho')})}{d\chi_{(\rho')}^2} \dfrac{\partial \chi_{(\rho')}}{\partial m}} \\
&\qquad \left(\text{for } r_{(\rho')} \geq \left[\frac{dY_{(\rho')}}{d\chi_{(\rho')}}\right]^{-1} s - \Delta s\right)
\end{aligned}\right\} \tag{42}$$

and

$$\frac{Y_{(\rho')}(\chi_{(\rho')})}{\Delta s} = 0 \quad \left(\text{for } r_{(\rho')} < \left[\frac{dY_{(\rho')}}{d\chi_{(\rho')}}\right]^{-1} s - \Delta s\right)$$

are derived; the second equality in Eq. (42) holds because when $\chi_{(\rho')}$ tends to zero, then $Y_{(\rho')}(\chi_{(\rho')})$ tends to $\chi_{(\rho')}$ i.e., to zero (cf. Eqs. a, 34, and 35). It should be noted (a) that $Y_{(\rho')}(\chi_{(\rho')})$ fulfills Eq. (38), and (b) that, when $r_{(\rho')} \geq [dY_{(\rho')}/d\chi_{(\rho')}]^{-1}s - \Delta s$, then $Y_{(\rho')}$ increases both continuously and monotonically with a decrease of $r_{(\rho')}$. [This conclusion can be derived from both the form of Eq. (37) and the fact that $Y_{(\rho')}$ increases not only continuously but also monotonically with an increase of $\chi_{(\rho')}$ (cf. Eqs. a, 34, and 35).] This means that, when $\Delta s \to +0$, Eq. (42) reduces to two equations:

$$\begin{aligned}
f_{(\rho'),s}(m) &\equiv -\frac{\partial \chi_{(\rho')}}{\partial m} \\
&= \frac{1}{s}\left[\frac{dY_{(\rho')}(\chi_{(\rho')})}{d\chi_{(\rho')}}\right]^2 \left[\frac{d^2 Y_{(\rho')}(\chi_{(\rho')})}{d\chi_{(\rho')}^2}\right]^{-1} \frac{dr_{(\rho')}}{dm}
\end{aligned} \tag{43}$$

and

$$r_{(\rho')} = \left[\frac{dY_{(\rho')}(\chi_{(\rho')})}{d\chi_{(\rho')}}\right]^{-1} s \tag{44}$$

where Eq. (43) has been derived by equating to zero the denominator on the right-hand side of the first equality in Eq. (42). The argument above can be justified by the fact that, if Eq. (44) is partially differentiated with respect to m, Eq. (43) is spontaneously generated. Since $r_{(\rho')}$ is a function of m (Eq. 5),

both Eqs. (43) and (44) define $f_{(\rho'),s}$ as a function of m by using $\chi_{(\rho')}$ as an intermediate parameter.

The set of Eqs. (43) and (44) corresponds to Eq. (31). In fact, Eq. (31) symbolically shows that, in the absence of molecular interactions, a sharp chromatogram, $f_{(\rho'),s}$, is obtained at molarity m of competing ions fulfilling the relationship

$$r_{(\rho')}(m) = s \tag{45}$$

On the other hand, in the presence of molecular interactions, a chromatogram $f_{(\rho'),s}$ with a finite width (Eq. 43) appears over m values fulfilling Eq. (44) instead of Eq. (45). When $\chi_{(\rho')}$ tends to 0 or when the molecular interactions are reduced, then $Y_{(\rho')}$, $dY_{(\rho')}/d\chi_{(\rho')}$, and $d^2Y_{(\rho')}/d\chi_{(\rho')}{}^2$ tend to $\chi_{(\rho')}$, 1, and 0, respectively, and Eq. (44) coincides with Eq. (45). At the same time, the value of $f_{(\rho'),s}$ in Eq. (43) approaches infinity, fulfilling Eq. (38) or (8). This means that the set of Eqs. (43) and (44) converges to Eq. (31) at the limit of no molecular interactions.

DISCUSSION

(A) Other Methods for the Calculation of Chromatograms; The Case of No Molecular Interactions

Equation (28) can be rewritten as

$$r_{(\rho')} - s = \Phi^{-1}(\chi_{(\rho')}) \tag{28'}$$

We are considering the case when a band of molecules with an infinitesimal width is formed initially (i.e., when $m = m_{in}$ or $r_{(\rho')} = 0$) at the top ($L = 0$ or $s = 0$) of the column. It is important to note that this initial condition is characterized by the fact that it is fulfilled independently of the initial value, $\chi^*_{(\rho')}$, of $\chi_{(\rho')}$. In order for this situation to occur, it is necessary that

$$\Phi^{-1}(\chi_{(\rho')}) = 0 \tag{46}$$

By substituting Eq. (46) into Eq. (28'), Eq. (45) is obtained.

Equation (45), which has been derived by using this method, can be considered to represent a situation that the probability for $\chi_{(\rho')}$ to have a value different from zero when $r_{(\rho')}$ takes a value between $r_{(\rho')}$ and $r_{(\rho')} + dr_{(\rho')}$ (provided s is given) is $\delta(r_{(\rho')} - s)\, dr_{(\rho')}$ [to be precise

$$\int_{r_{(\rho')}}^{r_{(\rho')}+dr_{(\rho')}} \delta(r_{(\rho')} - s)\, dr_{(\rho')}$$

since δ is the delta function]. This means that the probability for $\chi_{(\rho')}$ to have a value different from zero when m takes a value between m and $m + dm$ should be $\delta(r_{(\rho')} - s)(dr_{(\rho')}/dm)\, dm$. This situation can now be *interpreted* to represent the actual state where molecules exist in the interstial liquid in the column in which the molarity of competing ions is between m and $m + dm$, since m has a physical meaning of the ion molarity in the interstitial liquid. Therefore, if we consider s that corresponds to the bottom, L, of the column (see Eq. 2), then $\delta(r_{(\rho')} - s)(dr_{(\rho')}/dm)\, dm$ should represent the probability that the molecules of component ρ' be eluted between the ion molarity m and $m + dm$ out of the column. The chromatogram $f_{(\rho'),s}$ should, therefore, be represented by Eq. (31).

(B) The General Case of Molecular Interactions

Equation (40) can be rewritten as

$$r_{(\rho')} - \left[\frac{dY_{(\rho')}(\chi_{(\rho')})}{d\chi_{(\rho')}}\right]^{-1} s = \Phi^{-1}(\chi_{(\rho')}) \qquad (40')$$

The argument made for the case of no molecular interactions can be extended to the case of molecular interactions, and Eq. (46) can be considered to be fulfilled. By substituting Eq. (46) into Eq. (40'), Eq. (44) is derived, and by partially differentiating Eq. (44) with respect to m, Eq. (43) is obtained.

(C) Relationships with Earlier Methods

The method used in Section (A) for the case of no molecular interactions was applied in Appendix II of Ref. *1* with a modification. Thus, instead of Eqs. (46) and (28'), the initial boundary condition

$$[\chi_{(\rho')}]_{r_{(\rho')}=0} = \delta(s) \qquad (47)$$

(cf. Eq. A21 in Ref. *1*, Appendix II) and Eq. (28) were considered, respectively. Under the condition of Eq. (47), Eq. (28) has a form represented as

$$\chi_{(\rho')} = \delta(r_{(\rho')} - s) \tag{48}$$

(cf. Eq. A22 in Ref. *1*, Appendix II), which corresponds to Eq. (45). It should be emphasized that Eq. (48) shows only symbolically the fact that the $\chi_{(\rho')}$ value is different from zero only within an infinitesimal molarity range around molarity m fulfilling Eq. (45). This means that the chromatogram, $f_{(\rho'),s}$, cannot be derived by directly applying Eq. (48) to Eq. (7). Instead of this procedure it is necessary to introduce an interpretation given in the last half of Section (A); as a result the chromatogram (Eq. 31) can be derived (see Section A).

A method similar to that used in Section (B) for the case of molecular interactions was applied in Ref. *7*. In Ref. *7*, however, Eq. (44) (i.e., Eq. 33 in Ref. *7*) was derived in relationship with the fact that s tends to zero when g tends to zero (cf. Eq. 2) instead of the fact that s tends to zero when L tends to zero. As argued in Theoretical Section (B), Eq. (44) should be derived on the basis of the fact that s tends to zero when L tends to zero.

Both Eq. (39) (originally derived from Eq. 20) and Eq. (21) constitute a boundary condition to Eq. (36) with a general solution given by Eq. (40). It should be noted, however, that the form (Eq. 46) of the function Φ in Eq. (40) cannot be derived by directly applying the limit of Eq. (39), i.e.,

$$\frac{r_{(\rho')}}{\lim_{\Delta s \to 0} \Delta s} = \int_{\chi_{(\rho')}}^{\chi^*(\rho')} \frac{1}{Y_{(\rho')}(\chi_{(\rho')})} \, d\chi_{(\rho')} \tag{49}$$

to Eq. (40). This is due to the fact that, at the limit of $s \to +0$, Eq. (44) (which has been derived by applying Eq. 46 to Eq. 40'; see Section B) does not coincide with Eq. (40); viz., the factor $[dY_{(\rho')}/d\chi_{(\rho')}]^{-1}$ involved in Eq. (44) is different from the factor

$$\int_{\chi_{(\rho')}}^{\chi^*(\rho')} Y_{(\rho')}^{-1} \, d\chi_{(\rho')}$$

involved in Eq. (49). The contradiction has occurred because the limit of $\Delta s \to +0$ (Eq. 49) was *a priori* considered without any reasoning (cf. the argument in Theoretical Section B related to Eq. 15). A sophistical argument for this problem was made in Appendix I of Ref. *7*.

In the Theoretical Section it has been shown that the chromatograms that were obtained in both Ref. *1*, Appendix II, and Ref. *7* by using different

methods can be calculated on the basis of the general principle of Eq. (7), independently of the occurrence of the mutual molecular interactions.

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