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

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To cite this Article Kawasaki, Tsutomu(1988) 'Specification of the Adsorption Model in Hydroxyapatite Chromatography. III. Competition Model in Gradient Chromatography and Another Relevant Model', Separation Science and Technology, 23: 10, 1105 — 1117

To link to this Article: DOI: 10.1080/01496398808058442

URL: http://dx.doi.org/10.1080/01496398808058442

SEPARATION SCIENCE AND TECHNOLOGY, 23(10 & 11), pp. 1105-1117, 1988

Specification of the Adsorption Model in Hydroxyapatite Chromatography. III. Competition Model in Gradient Chromatography and Another Relevant Model

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Abstract

If a component of the multicomponents adsorbed system argued in the preceding paper is assigned to the ions from the buffer solvent and the other components to the respective molecular species in the sample mixture, the competition model in gradient chromatography can be derived. In relationship with the competition model, another chromatographic model is also proposed. Whereas the competition model is applicable to the usual hydroxyapatite chromatography carried out in an aqueous medium, the new model is probably valid in a special case of hydroxyapatite chromatography carried out in the presence of an organic solvent and, at least in some cases, of reversed phase chromatography.

INTRODUCTION

The competition model in gradient chromatography [see the Introduction section of the first paper (*1*) of this series] can be represented as a special case of the grand canonical multicomponents adsorbed system argued in the second paper (*2*). Thus, if a component of the system is assigned to the ions from the buffer, each of which is assumed to be adsorbable onto a single site on the adsorbent surface, and the other components to the respective molecular species in the sample mixture, the system would represent the competition model itself. The adsorption isotherms for the respective molecular species (called 1, 2, . . . , ρ) can be calculated as functions of (a) concentration or molarity of the ions (called competing ions) in solution, (b) density on the adsorbent surface for the molecular species under consideration, and (c) densities on the adsorbent surface for the other molecular species in the sample mixture; with small sample loads, the adsorption isotherms are functions of only (a) and (b). If the adsorption isotherms are transformed to the corresponding fundamental chromatographic parameters $B'_{(\rho')}$ (where $\rho' = 1, 2, \ldots, \rho$), the theoretical chromatogram with competitive gradient elution can be calculated (*3–12*). In Theoretical section (A) the fundamental assumptions for the competition model are summarized.

In relationship with the competition model, another chromatographic model in gradient elution is proposed in which account is taken of mutual interactions among sample molecules occurring on the adsorbent surfaces in the column. Whereas the competition model is applicable to usual hydroxyapatite (HA) chromatography carried out in an aqueous medium, the new model is probably valid as a special case of HA chromatography carried out in the presence of an organic solvent and at least in some cases of reversed phase chromatography.

THEORETICAL

(A) Fundamental Assumptions for the Competition Chromatographic Model as Represented as a Special Case of the Grand Canonical Multicomponents Adsorbed System

Assumption 1. The absolute activity for a molecular species in the sample mixture is proportional to its concentration in solution; the proportionality constant is independent of (a) the type of the molecular species, (b) the presence of the other molecular species in the sample mixture, and (c) the concentration (or the molarity) of competing ions in solution. (Since, in chromatography, the molecular concentration of the sample mixture in solution is usually low, the assumption is reasonable, at least except for the independence of the proportionality constant from the concentration of competing ions in solution. The case where the proportionality constant depends upon the composition of the carrier liquid will be argued in the Discussion Section.)

Assumption 2. The absolute activity of competing ions as well as the ion concentration in solution is hardly influenced by the presence of sample molecules. [This is a reasonable assumption since, with gradient chromatography, the ion concentration in solution, i.e., the mobile phase in the column, is usually high enough, except at the beginning of the gradient, for almost all ions in a column section to be in the mobile phase; the ion concentration in the mobile phase is hardly influenced by adsorption and desorption phenomena of sample molecules. The relationship (approximately a linear relationship) between the absolute activity and the concentration in solution of competing ions is also hardly influenced by the presence of sample molecules since the molecular concentration in solution is usually low.]

Assumption 3. Both the energetical and the geometrical interaction among the sample molecules on the adsorbent surface are hardly influenced by the change in concentration of competing ions in solution.

Assumption 4. A competing ion covers a single site on the adsorbent surface when it is adsorbed; the adsorption of another ion onto the neighboring site is not sterically hindered by the already adsorbed ion.

(B) Adsorption Isotherms for the Respective Molecular Species in the Sample Mixture as Functions of Concentration or Molarity of Competing Ions in Solution

Denoting by 0 the competing ion and by 1, 2, ..., ρ the respective molecular species in the sample mixture, and referring to Eq. (34) of a previous paper (*2*), the adsorption isotherms for all the components, including the ion, of the mixture can be written as

$$\begin{cases} \lambda_{(0)} = \Psi_{(0)}(\theta_{(0)}, \boldsymbol{\theta}) e^{-E_{(0)}/kT} & (1) \\ x'_{(\rho')}\lambda_{(\rho')} = \Psi_{(\rho')}(\theta_{(0)}, \boldsymbol{\theta}) e^{-E_{(\rho')}/kT} \qquad (\rho' = 1, 2, \ldots, \rho) & (2) \end{cases}$$

where

$$\Psi_{(\rho')}(\theta_{(0)}, \boldsymbol{\theta}) = \frac{\theta_{(\rho')} e^{E^*_{(\rho')}(\theta_{(0)}, \theta)/kT}}{z\tau_{(\rho')} p^*_{(\rho')}(\theta_{(0)}, \boldsymbol{\theta})}$$

$$= \frac{\theta_{(\rho')}}{p_{(\rho')}(\theta_{(0)}, \boldsymbol{\theta})} \frac{e^{E^*_{(\rho')}(\theta_{(0)}, \boldsymbol{\theta})/kT}}{z\tilde{\tau}_{(\rho')}(\theta_{(0)}, \boldsymbol{\theta})}$$

$$(\rho' = 0, ,1, 2, \ldots, \rho; \text{ cf. Eqs. 30 and 35 of Ref. } 2) \qquad (3)$$

$$\boldsymbol{\theta} = (\theta_{(1)}, \theta_{(2)}, \ldots, \theta_{(\rho)}) \qquad (4)$$

$$\theta_{(\rho')} = \frac{x'_{(\rho')}n_{(\rho')}}{n_0} \qquad (\rho' = 0, 1, 2, \ldots, \rho) \tag{5}$$

$$p^*_{(\rho')}(\theta_{(0)}, \boldsymbol{\theta}) = \frac{\tilde{\tau}_{(\rho')}(\theta_{(0)}, \boldsymbol{\theta})}{\tau_{(\rho')}}p_{(\rho')}(\theta_{(0)}, \boldsymbol{\theta}) \qquad (\rho' = 0, 1, 2, \ldots, \rho;\ \text{cf. Eq. 30 of Ref. } \mathit{2}) \tag{6}$$

and

$$p_{(0)}(\theta_{(0)}, \boldsymbol{\theta}) = 1 - \theta_{(0)} - \sum_{\rho'=1}^{\rho} \theta_{(\rho')} \tag{7}$$

The physical meanings of the symbols involved in Eqs. (1)–(7) are:

n_0 = total number of the adsorbing sites on the adsorbent surface.

$n_{(\rho')}$ = total number of the competing ions ($\rho' = 0$) or the molecules of species ρ' ($\rho' = 1, 2, \ldots, \rho$) in the sample mixture that are adsorbed on the adsorbent surface under consideration.

$x'_{(\rho')}$ = average number of adsorbing sites that are occupied by a single competing ion ($\rho' = 0$) or a single molecule of species ρ' ($\rho' = 1, 2, \ldots, \rho$) in the sample mixture when it is adsorbed. Due to Assumption 4 in Section A, $x'_{(0)}$ is equal to unity, and we here specify $x'_{(\rho')}$ for $\rho' = 1, 2, \ldots, \rho$ in such a way that it represents the average number of sites on which the adsorption of competing ions is impossible due to the presence of an adsorbed ρ' molecule.

$\theta_{(\rho')}$ = surface density on the adsorbent for the competing ions ($\rho' = 0$) or for molecular species ρ' ($\rho' = 1, 2, \ldots, \rho$) in the sample mixture.

$\lambda_{(\rho')}$ = absolute activity for the competing ions ($\rho' = 0$) or for molecular species ρ' ($\rho' = 1, 2, \ldots, \rho$) in the sample mixture.

$E_{(\rho')}$ = absolute value of the interaction energy with adsorbing site(s) per competing ion ($\rho' = 0$) or per molecule of species ρ' ($\rho' = 1, 2, \ldots, \rho$) in the sample mixture occurring provided the ion or the molecule is isolated on the adsorbent surface.

k = Boltzmann constant.

T = absolute temperature.

$E^*_{(\rho')}(\theta_{(0)}, \boldsymbol{\theta})$ = mutual interaction energy per competing ion ($\rho' = 0$) or per molecule of species ρ' ($\rho' = 1, 2, \ldots, \rho$) in the sample mixture as defined in Theoretical Section B of Ref. *2.*

z = coordination number of the adsorbing sites on the adsorbent surface.

$\ln \tau_{(\rho')}$ = entropy factor per competing ion ($\rho' = 0$) or per molecule of species ρ' ($\rho' = 1, 2, \ldots, \rho$) in the sample mixture occurring provided the ion or the molecule is isolated on the adsorbent surface. For detail, see Theoretical Section B of Ref. *2.*

$\ln \tilde{\tau}_{(\rho')}(\theta_{(0)}, \boldsymbol{\theta})$ = entropy factor per competing ion ($\rho' = 0$) or per molecule of species ρ' ($\rho' = 1, 2, \ldots, \rho$) in the sample mixture. Cf. Eq. (20) of Ref. *2.*

$p_{(\rho')}(\theta_{(0)}, \boldsymbol{\theta})$ = factor related to both location and orientation on the adsorbent surface for the competing ions ($\rho' = 0$) or for molecular species ρ' ($\rho' = 1, 2, \ldots, \rho$) in the sample mixture (cf. Eq. 8 of Ref. *2*). Due to both Assumption 4 in Section A and the definition of $x'_{(0)}$ or $\theta_{(0)}$ given above, $p_{(0)}(\theta_{(0)}, \boldsymbol{\theta})$ can be represented by Eq. (7), endowed with a physical meaning of the probability that, when a competing ion is added at random to the adsorbent surface [on which the surface dnesities for the competing ions ($\rho' = 0$) and for molecular species $1, 2, \ldots, \rho$ in the sample mixture are $\theta_{(0)}, \theta_{(1)}, \theta_{(2)}, \ldots, \theta_{(\rho)}$, respectively], it is successfully adsorbed onto the adsorbent surface without being sterically hindered by the already adsorbed substances. Cf. the argument for Eq. (8) of Ref. *2* or Eq. (6) of Ref. *1.*

Due to Assumption 2 in Section A, it is possible to write

$$E^*_{(0)}(\theta_{(0)}, \boldsymbol{\theta}) \approx E^*_{(0)}(\theta_{(0)}, 0, \ldots, 0) \equiv E^*_{(0)}(\theta_{(0)}) \tag{8}$$

and

$$\tilde{\tau}_{(0)}(\theta_{(0)}, \boldsymbol{\theta}) \approx \tilde{\tau}_{(0)}(\theta_{(0)}, 0, \ldots, 0) \equiv \tilde{\tau}_{(0)}(\theta_{(0)}) \tag{9}$$

Further, due to Assumption 3 in Section (A), the approximations

$$E^*_{(\rho')}(\theta_{(0)}, \boldsymbol{\theta}) \approx E^*_{(\rho')}(\bar{\boldsymbol{\theta}}_{(0)}, \theta) \equiv E^*_{(\rho')}(\theta) \qquad (\rho' = 1, 2, \ldots, \rho) \tag{10}$$

and

$$\tilde{\tau}_{(\rho')}(\theta_{(0)}, \boldsymbol{\theta}) \approx \tilde{\tau}_{(\rho')}(\bar{\theta}_{(0)}, \boldsymbol{\theta}) \equiv \tilde{\tau}_{(\rho')}(\boldsymbol{\theta}) \qquad (\rho' = 1, 2, \ldots, \rho) \tag{11}$$

hold, where $\bar{\theta}_{(0)}$ represents the average value of $\theta_{(0)}$ realized in the course of the experiment; it can be considered that, in the extremely right-hand sides of Eqs. (10) and (11), $\bar{\theta}_{(0)}$ is involved as a parameter. By using Eqs. (7)–(11), Eq. (3) can be rewritten as

$$\Psi_{(0)}(\theta_{(0)}, \boldsymbol{\theta}) = \frac{\theta_{(0)}}{1 - \theta_{(0)} - \sum_{\rho'=1}^{\rho} \theta_{(\rho')}} \, \frac{e^{E^*_{(0)}(\theta_{(0)})/kT}}{z\tilde{\tau}_{(0)}(\theta_{(0)})} \tag{12}$$

and

$$\Psi_{(\rho')}(\theta_{(\rho')}, \boldsymbol{\theta}) = \frac{\theta_{(\rho')}}{p_{(\rho')}(\theta_{(0)}, \boldsymbol{\theta})} \, \frac{e^{E^*_{(\rho')}(\boldsymbol{\theta})/kT}}{z\tilde{\tau}_{(\rho')}(\boldsymbol{\theta})} \qquad (\rho' = 1, 2, \ldots, \rho) \tag{13}$$

Let us introduce the new function

$$\Phi_{(\rho')}(\boldsymbol{\theta}) = \frac{\theta_{(\rho')} e^{E^*_{(\rho')}(\boldsymbol{\theta})/kT}}{z\tau_{(\rho')} p^*_{(\rho')}(\boldsymbol{\theta})}$$

$$= \frac{\theta_{(\rho')}}{p_{(\rho')}(\boldsymbol{\theta})} \, \frac{e^{E^*_{(\rho')}(\boldsymbol{\theta})/kT}}{z\tilde{\tau}_{(\rho')}(\boldsymbol{\theta})} \qquad (\rho' = 1, 2, \ldots, \rho) \tag{14}$$

in which

$$p^*_{(\rho')}(\boldsymbol{\theta}) = \frac{\tilde{\tau}_{(\rho')}(\boldsymbol{\theta})}{\tau_{(\rho')}} p_{(\rho')}(\boldsymbol{\theta}) \qquad (\rho' = 1, 2, \ldots, \rho) \tag{15}$$

and $p_{(\rho')}(\boldsymbol{\theta})$ represents the factor related to both location and orientation on the adsorbent surface for molecular species ρ' in the sample mixture occurring provided that the competing ions are absent but that the adsorption configuration of all the sample molecules on the adsorbent surface is the same as the actual configuration realized in the presence of the ions (cf. Eqs. 8 and 30 of Ref. 2). In the Appendix, a proof is given that the relationship

$$\frac{p_{(\rho')}(\theta_{(0)}, \boldsymbol{\theta})}{p_{(\rho')}(\boldsymbol{\theta})} = \left[\frac{1 - \theta_{(0)} - \sum_{\rho''=1}^{\rho} \theta_{(\rho'')}}{1 - \sum_{\rho''=1}^{\rho} \theta_{(\rho'')}}\right]^{x'_{(\rho')}} \qquad (\rho' = 1, 2, \ldots, \rho) \tag{16}$$

is fulfilled, from which it follows that

$$\Psi_{(\rho')}(\theta_{(0)}, \boldsymbol{\theta}) = \left[\frac{1 - \sum_{\rho''=1}^{\rho} \theta_{(\rho'')}}{1 - \theta_{(0)} - \sum_{\rho''=1}^{\rho} \theta_{(\rho'')}}\right]^{x'_{(\rho')}} \Phi_{(\rho')}(\boldsymbol{\theta}) \qquad (\rho' = 1, 2, \ldots, \rho) \tag{17}$$

By using both Eqs. (12) and (17), and introducing the parameter

$$\Lambda = \lambda_{(0)} z \tilde{\mathfrak{t}}_{(0)}(\theta_{(0)}) e^{-\overset{*}{E}_{(0)}(\theta_{(0)})/kT} e^{E_{(0)}/kT} \tag{18}$$

Eqs. (1) and (2) can be rewritten as

$$\left\{\begin{aligned} &\Lambda = \frac{\theta_{(0)}}{1 - \theta_{(0)} - \sum_{\rho''=1}^{\rho} \theta_{(\rho'')}} && (19) \\ &x'_{(\rho')}\lambda_{(\rho')} = \Phi_{(\rho')}(\boldsymbol{\theta}) e^{-E_{(\rho')}/kT} \left[\frac{1 - \sum_{\rho''=1}^{\rho} \theta_{(\rho'')}}{1 - \theta_{(0)} - \sum_{\rho''=1}^{\rho} \theta_{(\rho'')}}\right]^{x'_{(\rho')}} && \end{aligned}\right.$$

$$(\rho' = 1, 2, \ldots, \rho) \tag{20}$$

In Eq. (18) it can be assumed that $\lambda_{(0)}$ is approximately proportional to the molarity, m, of competing ions in solution, and both $\tilde{\mathfrak{t}}_{(0)}(\theta_{(0)})$ and $e^{-\overset{*}{E}_{(0)}(\theta_{(0)})/kT}$ depend presumably only slightly upon $\theta_{(0)}$. This means that Λ is approximately proportional to m; the parameter

$$\varphi = \Lambda/m \tag{21}$$

is approximately constant.

By eliminating $\theta_{(0)}$ between Eqs. (19) and (20):

$$x'_{(\rho')}\lambda_{(\rho')} = \Phi_{(\rho')}(\boldsymbol{\theta})e^{-E_{(\rho')}/kT}(\Lambda + 1)^{x'_{(\rho')}}$$
$$= \Phi_{(\rho')}(\boldsymbol{\theta})e^{-E_{(\rho')}/kT}(\varphi m + 1)^{x'_{(\rho')}} \qquad (\rho' = 1, 2, \ldots, \rho) \tag{22}$$

is obtained. It can be considered that Eq. (22) is simultaneous equations for $\theta_{(1)}, \theta_{(2)}, \ldots, \theta_{(\rho)}$; these represent thte adsorption isotherms for the respective molecular species 1, 2, ..., ρ in the sample mixture as functions of (a) molarity m of competing ions in solution, (b) density $\theta_{(\rho')}$ on the adsorbent surface for the molecular species ρ' under consideration, and (c) densities $\theta_{(1)}, \theta_{(2)}, \ldots, \theta_{(\rho'-1)}, \theta_{(\rho'+1)}, \ldots, \theta_{(\rho)}$ for the other molecular species in the sample mixture. With small sample loads when $\Sigma_{\rho''=1}^{\rho} \theta_{(\rho'')}$ is small, Eq. (14) reduces to

$$\Phi_{(\rho')}(\theta) \approx \Phi_{(\rho')}(0, \ldots, 0, \theta_{(\rho')}, 0, \ldots, 0)$$
$$\approx \frac{\theta_{(\rho')}}{z\tau_{(\rho')}} \qquad (\rho' = 1, 2, \ldots, \rho) \tag{23}$$

and the adsorption isotherms are functions of only (a) and (b); the adsorption and desorption of the molecules of each species in the sample mixture occur independently.

(C) Fundamental Chromatographic Parameter $B'_{(\rho')}$

In order to study the evolution of sample molecules on the column, it is necessary to transform the adsorption isotherms (Eq. 22) to the corresponding fundamental chromatographic parameters $B'_{(\rho')}$ (where $\rho' = 1, 2, \ldots, \rho$); $B'_{(\rho')}$ represents the partition of ρ' molecules in solution or the mobile phase in the column (cf. Refs. *3–12*). $B'_{(\rho')}$ can be written as

$$B'_{(\rho')} = \frac{c_{(\rho')}V}{N_{(\rho')}} \qquad (\rho' = 1, 2, \ldots, \rho) \tag{24}$$

in which $N_{(\rho')}$ is the total number of the ρ' molecules in the system consisting of both the adsorbed phase and the solution (i.e., the mobile phase); the system corresponds to an elementary interstitial volume, including adsorbing surfaces of packed particles, in the column. V

represents the volume of the solution part of the system, i.e., the elementary interstitial volume itself in the column, and $c_{(\rho')}$ is the molecular concentration expressed as number/volume for species ρ' in V. Therefore, $c_{(\rho')}V$ represents the total number of ρ' molecules in the solution part of the system. Since, by its definition, $N_{(\rho')}$ is equal to the sum of $n_{(\rho')}$ ($= n_0\theta_{(\rho')}/x'_{(\rho')}$; see Eq. 5) and $c_{(\rho')}V$, and since $c_{(\rho')}$ is related to the absolute activity $\lambda_{(\rho')}$ by the relationship

$$\Gamma = \lambda_{(\rho')}/c_{(\rho')} \tag{25}$$

in which Γ is constant (Assumption 1 in Section A), then Eq. (24) can be rewritten as

$$B'_{(\rho')} = \frac{1}{1 + \Gamma \dfrac{n_0}{V} \dfrac{\theta_{(\rho')}}{x'_{(\rho')}\lambda_{(\rho')}}} \qquad (\rho' = 1, 2, \ldots, \rho) \tag{24'}$$

The adsorption isotherm for molecular species ρ' can be transformed to the partition $B'_{(\rho')}$ of the ρ' molecules in solution if Eq. (22) is substituted into Eq. (24'). Thus, we obtain after arrangement

$$B'_{(\rho')} = \frac{1}{1 + p^*_{(\rho')}(\boldsymbol{\theta})e^{-E^*_{(\rho')}(\boldsymbol{\theta})/kT}q_{(\rho')}(\varphi m + 1)^{-x'_{(\rho')}}} \qquad (\rho' = 1, 2, \ldots, \rho) \tag{26}$$

in which

$$q_{(\rho')} = \beta\tau_{(\rho')}e^{E_{(\rho')}/kT} \tag{27}$$

with

$$\beta = \Gamma z \frac{n_0}{V} \tag{28}$$

With small sample loads when $\Sigma^{\rho}_{\rho'=1} N_{(\rho')}$ or $\Sigma^{\rho}_{\rho'=1} \theta_{(\rho')}$ is small, Eq. (26) reduces to

$$B'_{(\rho')} = \frac{1}{1 + q_{(\rho')}(\varphi m + 1)^{-x'_{(\rho')}}} \qquad (\rho' = 1, 2, \ldots, \rho) \tag{29}$$

and the partition in solution of the molecules of each species in the sample mixture occurs independently in the column.

DISCUSSION

Both the adsorption isotherms (Eq. 22) and the corresponding chromatographic parameters $B'_{(\rho')}$ (Eq. 26 or 29) derived in the present work coincide with those obtained earlier (for instance, see Eq. 23 of Paper *4* and Eq. 22 of Paper *5* for the adsorption isotherms, and Eq. 37 of Paper *4*, Eq. 47 of Paper *5*, and Eq. 44 of Paper *11* for the parameters $B'_{(\rho')}$; the symbols B, $B_{(\rho')}$, or B' are used instead of $B'_{(\rho')}$ in the earlier papers). It should be underlined, however, that the present derivation method is different from that applied earlier (*4*, *5*), and that the present method is based upon well-defined fundamental assumptions [Assumptions 1–7 in Theoretical Section A of the first paper (*1*) of this series and Assumptions 1–4 in Theoretical Section A of this paper]. As a result, the physical meanings of the parameters involved in the expression of $B'_{(\rho')}$ (Eq. 26 or 29) are much more specific than before; this means that the competition model itself is much more specific.

HA chromatography is usually carried out in an aqueous medium (*6*, *8*, *12–16*), and for this chromatography the validity of the competition model was confirmed experimentally (*6*, *8*, *12*) in relationship with the verification of the general theory of gradient chromatography (*7*, *9–11*). (Cf. Refs. *13–15* for some qualitative bases of the model.) Thus, it can be deduced that sample molecules (like nucleic acid and protein with charged adsorption groups such as phosphate, carboxyl, ε-amino and guanidinyl groups) and particular ions from the buffer constituting the gradient (e.g., phosphate, sodium, and potassium ions) compete for adsorption onto adsorbing sites that are arranged in some manner on the surfaces of the HA particles packed in the column; the sample molecules initially adsorbed at the inlet of the column are driven out of the HA surfaces into solution or the mobile phase by competing ions (for details, see the introductory part of Ref. *16*). Substances without charges are unadsorbable onto the HA surfaces, and they are not retained on the column (*13–15*).

Recently it was shown (*17*, *18*), however, that even substances without charges [e.g., saponins (*17*), sugars, etc. (*18*)] can be retained on the HA column in the presence of very high concentrations (e.g., 70–90%) of acetonitrile in the aqueous medium. Isocratically, or by gradually reducing the concentration of acetonitrile in the carrier liquid (in other words, by gradually increasing the concentration of water), the substances can be chromatographed and eluted out of the column (*17*, *18*). At least two models can be proposed for the mechanism of this type of chromatography. Thus, the first model is a competition model. In this instance, however, it is water molecules that compete with sample molecules for adsorption onto the surfaces of HA particles packed in the column; the sample molecules initially adsorbed at the inlet of the column are driven out of the HA surfaces into the mobile phase by competing water molecules. Here, it is previously assumed that, by nature, both sample and water molecules are adsorbable onto the HA surface; in the medium in which water molecules occupy a major proportion, the uncharged sample molecules are not adsorbed onto the crystal surface since it is water molecules that are predominantly adsorbed. [With the usual competitive chromatography of charged macromolecules carried out in the aqueous medium (see above), it can be assumed that both macromolecules and competing ions are adsorbed much more strongly than water molecules onto the HA surface; the effect of the water adsorption is negligible.]

Another model states that the chemical potential $\mu_{(\rho')}$ or the absolute activity $\lambda_{(\rho')}$ of the sample molecules increases with an increase in the acetonitrile concentration or with a decrease in the water concentration in the carrier liquid; this would bring about the adsorption of the sample molecules onto the HA surface.

It should be emphasized that both models can be involved in the expression of Eq. (26) or (29), if, to the parameter m, the physical meaning of the water concentration in the carrier liquid is given. Thus, provided that only the latter new model is realized, it can be assumed that $\varphi = 0$ and that $\lambda_{(\rho')}$ increases with an increase in the acetonitrile concentration or with a decrease in water concentration, m, in the carrier liquid (see above); this would bring about increases in Γ (Eq. 25), β (Eq. 28), and $q_{(\rho')}$ (Eq. 27), and a decrease in $B'_{(\rho')}$ (Eq. 26 or 29), realizing the molecular adsorption. With gradient chroamtography carried out by gradually increasing water concentration m, the sample molecules initially adsorbed at the inlet of the column (at which $m \approx 0$ and $B'_{(\rho')} \approx 0$) would be desorbed into the mobile phase when m increases and $B'_{(\rho')}$ approaches unity.

Provided only the former competition model is realized, Γ (Eq. 25), β (Eq. 28), and $q_{(\rho')}$ (Eq. 27) are constant (Assumption 1 in Theoretical Section A), and $B'_{(\rho')}$ would increase to approach unity when m increases (see Eq. 26 or 29).

It should be noted that, in both chromatographic models, the interaction mechanism among the sample molecules occurring on the adsorbent surface is assumed to be common, being described by the factor $p^*_{(\rho')}(\boldsymbol{\theta})e^{-E^*_{(\rho')}(\boldsymbol{\theta})/kT}$ in the denominator of the right-hand side of Eq. (26).

The new chromatographic model is probably also valid in some cases of reversed phase chromatography.

APPENDIX

Proof of Eq. (16): Referring to Eq. (5′) of an earlier paper (*2*), we can write

$$\omega[n_{(0)}, \mathbf{n}] = \frac{(n_0 Z)^{\sum_{\rho'=0}^{\rho} n_{(\rho')}}}{\prod_{\rho'=0}^{\rho} n_{(\rho')}!} \prod_{l=1}^{\sum_{\rho'=0}^{\rho} n_{(\rho')}} \{p_{(\rho'')}[n'_{(0)}, \mathbf{n}']\}_l \qquad \text{(a1)}$$

and

$$\omega[\mathbf{n}] = \frac{(n_0 Z)^{\sum_{\rho'=1}^{\rho} n_{(\rho')}}}{\prod_{\rho'=1}^{\rho} n_{(\rho')}!} \prod_{l=1}^{\sum_{\rho'=1}^{\rho} n_{(\rho')}} \{p_{(\rho'')}[\mathbf{n}']\}_l \qquad \text{(a2)}$$

where

$$\mathbf{n} = (n_{(1)}, n_{(2)}, \ldots, n_{(\rho)}) \qquad \text{(a3)}$$

By using both Stirling's approximation and Eq. (a9) in Appendix II of Ref. *2* (in which $y_{(\rho')}$ is substituted by $p_{(\rho')}$), we can derive from Eqs. (a1) and (a2)

$$e^{\partial \ln \omega[n_{(0)}, \mathbf{n}]/\partial n_{(\rho')}} = p_{(\rho')}(\theta_{(0)}, \boldsymbol{\theta}) \frac{x'_{(\rho')} Z}{\theta_{(\rho')}} \qquad (\rho' = 0, 1, 2, \ldots, \rho) \qquad \text{(a4)}$$

and

$$e^{\partial \ln \omega[\mathbf{n}]/\partial n_{(\rho')}} = p_{(\rho')}(\boldsymbol{\theta}) \frac{x'_{(\rho')} Z}{\theta_{(\rho')}} \qquad (\rho' = 1, 2, \ldots, \rho) \qquad \text{(a5)}$$

respectively, from which

$$\frac{p_{(\rho')}(\theta_{(0)}, \boldsymbol{\theta})}{p_{(\rho')}(\boldsymbol{\theta})} = e^{\partial \ln\{\omega[n_{(0)}, \mathbf{n}]/\omega[\mathbf{n}]\}/\partial n_{(\rho')}} \qquad (\rho' = 1, 2, \ldots, \rho) \qquad \text{(a6)}$$

is obtained. On the other hand, due to both Assumption 4 in Section A and the definition of $x'_{(\rho')}$ or $\theta_{(\rho')}$ (see the explanation of Eqs. 1-7), we evidently have

$$\omega[n_{(0)}, \mathbf{n}] = \omega[\mathbf{n}] \frac{\left(n_0 - \sum_{\rho'=1}^{\rho} x'_{(\rho')} n_{(\rho')}\right)!}{n_{(0)}! \left(n_0 - n_{(0)} - \sum_{\rho'=1}^{\rho} x'_{(\rho')} n_{(\rho')}\right)!} \tag{a7}$$

from which, by using Stirling's approximation,

$$\frac{\partial \ln \{\omega[n_{(0)}, \mathbf{n}]/\omega[\mathbf{n}]\}}{\partial n_{(\rho')}} = \ln \left[\frac{1 - \theta_{(0)} - \sum_{\rho''=1}^{\rho} \theta_{(\rho'')}}{1 - \sum_{\rho''=1}^{\rho} \theta_{(\rho'')}} \right]^{x'_{(\rho')}} \qquad (\rho' = 1, 2, \ldots, \rho) \tag{a8}$$

is obtained. Finally, from both Eqs. (a6) and (a8), Eq. (16) is derived.

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Received by editor March 18, 1987