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Specification of the Adsorption Model in Hydroxyapatite Chromatography. I. The Case of a Single Component System

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Abstract

Earlier, for the adsorption and desorption mechanism occurring in a hydroxyapatite column with gradient chromatography, a competition model was introduced. The purpose of the present work is to reconsider the model in view of the hypothesis that the adsorbed phase constitutes a grand canonical system; another chromatographic model is also proposed. In this paper the fundamental assumptions characterizing the grand canonical system are specified, and the adsorption isotherm occurring when there is only a single molecular component is calculated.

INTRODUCTION

A competition model was earlier introduced (*1*) for the adsorption and desorption mechanism occurring in a hydroxyapatite (HA) column with gradient chromatography carried out in an aqueous medium. The model states that adsorbing sites are arranged in some manner on the surfaces of the HA crystals packed in the column; sample molecules (like nucleic acid and protein with charged adsorption groups such as phosphate, carboxyl, ε-amino, and guanidinyl) and particular ions from the buffer constituting the gradient (e.g., phosphate, sodium, and potassium ions) compete for adsorption onto the sites (*1*, *2*). The sample molecules initially adsorbed at the inlet of the column are driven out of HA surfaces into solution or the mobile phase by competing ions whose concentration or molarity in solution increases with a lapse of time. A competing ion covers a single site when it is adsorbed whereas a sample molecule, in general, covers plural sites (*1*, *2*).

In earlier papers (*1*, *3–5*), on the basis of the competition model, the adsorption isotherms for the respective molecular species (called 1, 2, . . . , ρ) in the sample mixture were calculated as functions of (a) concentration or molarity of 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). The adsorption isotherms were then transformed to the fundamental chromatographic parameters $B'_{(\rho')}$, where $\rho' = 1, 2, \ldots, \rho$ (*1*, *3–5*).

On the other hand, in a series of papers, including Refs. *6–8*, a theory of linear gradient chromatography was developed in relationship with HA chromatography, and, in Ref. *9* the theory was rearranged, establishing a general theory of linear gradient chromatography. By combining the general theory of gradient chromatography with the competition model (i.e., by introducing the parameter $B'_{(\rho')}$ obtained above into the general theory of gradient chromatography), a concrete theoretical chromatogram can be calculated (*6–9*). Experimental verifications of the competition model are given in Refs. 10–12 for HA chromatography in relationship with the verification of the theory of gradient chromatography.

The competition model is presumably applicable to other chromatographies such as ion-exchange chromatography. In the introductory part of Ref. *2*, a fundamental difference between the ion-exchange and the HA chromatography is mentioned with descriptions of both the stereochemical structure and the geometrical arrangement of the adsorbing sites on the crystal surface of HA.

The purpose of this and the two following papers (*13*, *14*) consists in reconsidering the adsorption and desorption phenomena occurring in the column in view of the hypothesis that the adsorbed phase constitutes a grand canonical system. In this, the first paper, the fundamental assumptions characterizing the grand canonical system are specified, and the adsorption isotherm occurring when there is only a single molecular component is calculated. In the second paper (*13*), the argument is extended to the general case of a multicomponent system. 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. In the third paper (*14*), the competition model is derived by using this method, and the adsorption isotherms for the respective molecular species are calculated; these are transformed to the chromatographic parameters $B'_{(\rho')}$. The adsorption isotherms and the corresponding parameters $B'_{(\rho')}$ calculated above, of course, coincide with those obtained earlier (*1*, *3–5*). However, their physical meanings are much more specified in Ref. *14* than before.

Another chromatographic model in gradient elution is also proposed in Ref. *14*.

THEORETICAL

(A) Fundamental Assumptions for the Grand Canonical Adsorbed System

Assumption 1. On the surface of the adsorbent, adsorbing sites are arranged discretely with a coordination number z.*

Assumption 2. Calling the energy of a molecule in the adsorbed state the difference between the energy level of the molecule on the adsorbent surface and that of the molecule in solution, the total energy of the molecule in the adsorbed state is contributed to both the interaction energy of the molecule with adsorbing site(s) and the interaction energy with other molecules on the adsorbent surface. The motive force by which the adsorption of a molecule takes place arises from the energetical interaction of the molecule with adsorbing site(s) on the adsorbent surface.†

Assumption 3. The geometrical state of a molecule on the adsorbent surface can be represented in terms of location, orientation, and configuration of the molecule on the surface. Thus, for a molecule there are n_0 possible locations, where n_0 represents the total number of adsorbing sites on the adsorbent surface under consdieration. For a location there are z possible orientations (cf. Assumption 1), and for an orientation there are a number of possible adsorption configurations. The border effect on the adsorbent surface is negligible.

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*Possible values of z are 1, 2, 3, 4, and 6. For C and P adsorbing sites of HA, $z = 2$ and $z = 6$, respectively (see Ref. *2*).

†It is tacitly assumed that the total volume of the solution (i.e., the solution part of the system constituted of both adsorbed phase and solution) is constant, being independent of the adsorption and desorption phenomena; this is a reasonable assumption from a practical point of view.

Assumption 4. Given the total number n' of the molecules (the case of a single component system) or the total numbers $n'_{(1)}, n'_{(2)}, \ldots, n'_{(\rho)}$ of the molecules of component 1, 2, ..., ρ of the mixture (the case of a multicomponent system) on the adsorbent surface under consideration, only a single type of the adsorbed phase is realized at the same time. Within a phase the environment around any molecule is the same except for random fluctuations, and the surface of any molecule keeps in contact with the adsorbent surface, at least partially.

Assumption 5. Given the type of the adsorbed phase, the microscopical states on the adsorbent surface concerning adsorption configuration of the molecules are limited to a certain type. In other words, the realizable adsorption configurations of each molecule are limited to a certain number. If the type of the adsorbed phase varies, i.e., the average adsorption configuration of each molecule varies with a change in n' or $n'_{(1)}, n'_{(2)}, \ldots, n'_{(\rho)}$, however, the average number x' of adsorbing site(s) occupied by an adsorbed molecule (the case of a single component system) or the corresponding number $x'_{(\rho')}$ of an adsorbed molecule of component ρ′ of the mixture (where $\rho' = 1, 2, \ldots, \rho$; the case of a multicomponent system) is virtually constant.

Assumption 6. Given the type of the adsorbed phase, the microscopical states on the adsorbent surface concerning both location and orientation of the molecules are also limited to a certain type (cf. the argument in Appendix I of Ref. *3*). Within this limit, however, any microscopical state occurs in equal probability (Bragg-Williams approximation); the total number ω of the microscopical states (concerning both location and orientation) as well as the total mutual interaction energy U' among the molecules on the adsorbent surface can be represented as a function of the surface molecular density θ' ($= x'n'/n_0$; the case of a single component system) or the surface molecular densities $\theta'_{(1)}, \theta'_{(2)}, \ldots, \theta'_{(\rho)}$ (where $\theta'_{(\rho')} = x'_{(\rho')}n'_{(\rho')}/n_0$ with $\rho' = 1, 2, \ldots, \rho$; the case of a multicomponent system).

Assumption 7. To the contrary, if the functions $\omega(\theta')$ and $U'(\theta')$ are given, or the functions $\omega(\theta'_{(1)}, \theta'_{(2)}, \ldots, \theta'_{(\rho)})$ and $U'(\theta'_{(1)}, \theta'_{(2)}, \ldots, \theta'_{(\rho)})$ are given, the microscopical states concerning location, orientation, and configuration of the molecules on the adsorbent surface are limited to a certain type, and the type of the adsorbed phase is determined (self-consistency hypothesis).

(B) Adsorption Isotherm for a Single Component System

The partition function, Ξ, for the grand canonical single component adsorbed system fulfilling Assumptions 1-7 in Section (A) can be written as

$$\Xi = \sum_{n'=0}^{M} e^{n'\mu/kT} Z \tag{1}$$

where

$$Z = \omega(\theta') e^{-U'(\theta')/kT} \sum_{\{j\}_{\theta'}}^{[\dot{\nu}(\theta')]^{n'}} e^{-U_{\{j\}_{\theta'}}/kT} \tag{2}$$

and

$$\theta' = x'n'/n_0 \tag{3}$$

The physical meanings of the symbols involved in Eqs. (1)–(3) are:

n' = total number of the molecules on the adsorbent surface under consideration, varying between 0 and M.
M = upper limit of the n' value; this depends upon the type of the adsorbed phase.
μ = chemical potential.
k = Boltzmann constant.
T = absolute temperature.
x' = average number of adsorbing site(s) occupied by a molecule when it is adsorbed. Here, the physical meaning of "occupying" is not specified since x' only represents a constant. (In Ref. *14* it will be necessary to specify the physical meaning of the word.)
n_0 = total number of the adsorbing sites on the adsorbent surface.
θ' = surface molecular density on the adsorbent.
$\omega(\theta')$ = total number of the microscopical states concerning both location and orientation of the molecules on the adsorbent surface, occurring when the surface molecular density is θ'.
$U'(\theta')$ = total mutual interaction energy among the molecules on the adsorbent surface occurring when the surface molecular density is θ'.
$\{j\}_{\theta'}$ = a microscopical state concerning configuration of the molecules on the adsorbent surface, occurring when the surface molecular density is θ'. For details, see Eq. (9).

$[\dot{v}(\theta')]^{n'}$ = total number of the microscopical states concerning configuration of the molecules on the adsorbent surface, occurring when the surface molecular density is θ'. For details, see the explanation of Eq. (10).

$U_{\{j\}_{\theta'}}$ = total interaction energy of the molecules with adsorbing sites on the adsorbent surface occurring when a microscopical state $\{j\}_{\theta'}$ is realized.

$\omega(\theta')$ can be represented as

$$\omega(\theta') = \omega[n'] = \frac{1}{n'!}\prod_{n''=1}^{n'} \phi[n''] \tag{4}$$

where, if ω is considered to be a function of n' instead of θ', brackets are used instead of parentheses to insert the variable; similar expressions will also be applied to other parameters. In the right-hand side of Eq. (4), $\phi[n'']$ represents the increment in times of the total number of both location and orientation of the molecules on the adsorbent surface occurring when the n''th molecule is added, provided that $n'' - 1$ molecules existed on the adsorbent surface before the addition, and that the molecules are provisionally discernible from one another; $\phi[1]$ represents, however, the number of both location and orientation of a molecular on the adsorbent surface occurring in the absence of any other molecules. $\phi[1]$ can be written as

$$\phi[1] = n_0 z \tag{5}$$

in which z represents the coordination number of the adsorbing sites on the adsorbent surface.

Let us introduce the parameter

$$p(\theta'') = p[n''] = \phi[n'']/\phi[1] \tag{6}$$

By its definition, p can be assumed to fulfill the relationship

$$\left.\begin{aligned} &\lim_{\theta''\to 0} p(\theta'') \approx \lim_{n''\to 1} p[n''] = 1 \\ &\text{and} \\ &\lim_{\theta''\to\theta''_{\max}} p(\theta'') \approx \lim_{n''\to M+1} p[n''] = 0 \end{aligned}\right\} \tag{7}$$

and, in many instances, to decrease with an increase of θ'' or n''. In some instances it would also be reasonable to assume that the type of the adsorbed phase on the adsorbent surface is virtually independent of θ'' or n'' (cf. Assumptions 5 and 6 in Section A). Under this assumption the parameter $p(\theta'')$ or $p[n'']$ has a physical meaning of the probability that, when the n''th molecule is added at random to the adsorbent surface on which $n'' - 1$ molecules are already adsorbed, it is successfully adsorbed onto the surface without being sterically hindered by the already adsorbed molecules; although the n''th molecule is added at random to the adsorbent surface, the randomness occurs within limits that are compatible with the state of the molecule realizable in the adsorbed phase.

By using Eqs. (5) and (6), and again introducing the new parameter

$$y[n''] = \frac{p[n'']}{1 - \dfrac{n'' - 1}{M}} \tag{8}$$

Eq. (4) can be rewritten as

$$\begin{aligned} \omega(\theta') = \omega[n'] &= \frac{(n_0 z)^{n'}}{n'!} \prod_{n''=1}^{n'} p[n''] \\ &= \left(\frac{n_0 z}{M}\right)^{n'} \frac{M!}{n'!(M - n')!} \prod_{n''=1}^{n'} y[n''] \end{aligned} \tag{4'}$$

Below is argued the third term in the right-hand side of Eq. (2). Thus, in the term, $\{\mathbf{j}\}_{\theta'}$ is defined as

$$\{\mathbf{j}\}_{\theta'} \equiv \{j_1, j_2, \ldots, j_i, \ldots, j_{n'}\}_{\theta'} \tag{9}$$

in which i ($i = 1, 2, \ldots, n'$) represents the number provisionally given to each of the n' molecules on the adsorbent surface; j_i where

$$j_i = 1, 2, \ldots, \dot{\nu}(\theta') \tag{10}$$

represents the number given to each of the configurations which the ith molecule can take provided the surface molecular density on the adsorbent is θ'. The maximum value, $\dot{\nu}(\theta')$, of j_i or the total number of the possible configurations of a molecule is independent of i. This means that the total number of the microscopical states $\{\mathbf{j}\}_{\theta'}$ is equal to $[\dot{\nu}(\theta')]^{n'}$. It can be assumed that

$$\lim_{\theta'\to 0} \dot{\nu}(\theta') \approx \lim_{n'\to 1} \dot{\nu}[n'] = \nu \tag{11}$$

where ν is a constant greater than, or equal to, unity; in many instances, $\dot{\nu}$ would decrease with an increase of θ' or n' sterically hindered by geometrical interactions with other molecules.

The third term in the right-hand side of Eq. (2) can now be rewritten as

$$\begin{aligned}
\sum_{\{j\}_{\theta'}}^{[\dot{\nu}(\theta')]^{n'}} e^{-U_{\{j\}_{\theta'}}/kT} &= \sum_{\{j\}_{\theta'}}^{[\dot{\nu}(\theta')]^{n'}} \prod_{i=1}^{n'} e^{[\dot{E}<j_i>]_{\theta'}/kT} \\
&= \prod_{i=1}^{n'} \left[\sum_{j=1}^{\dot{\nu}(\theta')} e^{[\dot{E}<j>]_{\theta'}/kT} \right]_i \\
&= \left[\sum_{j=1}^{\dot{\nu}(\theta')} e^{[\dot{E}<j>]_{\theta'}/kT} \right]^{n'} \\
&= [\bar{\tau}(\theta') e^{\bar{E}(\theta')/kT}]^{n'}
\end{aligned} \tag{12}$$

with

$$\bar{E}(\theta') = \sum_{j=1}^{\dot{\nu}(\theta')} [\dot{g}<j>]_{\theta'} [\dot{E}<j>]_{\theta'} \tag{13}$$

$$\ln \bar{\tau}(\theta') = -\sum_{j=1}^{\dot{\nu}(\theta')} [\dot{g}<j>]_{\theta'} \ln [\dot{g}<j>]_{\theta'} \tag{14}$$

and

$$[\dot{g}<j>]_{\theta'} = \frac{e^{[\dot{E}<j>]_{\theta'}/kT}}{\sum_{j=1}^{\dot{\nu}(\theta')} e^{[\dot{E}<j>]_{\theta'}/kT}} \tag{15}$$

The physical meanings of some parameters involved in Eqs. (12)–(15) are:

$-[\dot{E}<j_i>]_{\theta'}$ or $-[\dot{E}<j>]_{\theta'}$ ($[\dot{E}<j_i>]_{\theta'}$, $[\dot{E}<j>]_{\theta'} > 0$) = interaction energy with adsorbing site(s) of the ith molecule in the j_ith configuration occurring when the surface molecular density on the adsorbent is θ'; unless necessary j_i is written simply as j.

$[\dot{g}<j>]_{\theta'}$ = probability that a molecule takes the jth adsorption configuration when the surface molecular density on the adsorbent is θ', showing a Boltzmann distribution.

$\bar{E}(\theta')$ = expectation value of $[\dot{E}<j>]_{\theta'}$.

$\ln \bar{\tau}(\theta')$ = expectation value of $\ln \{1/[\dot{g}<j>]_{\theta'}\}$.

By substituting both Eqs. (4′) and (12) into Eq. (2):

$$Z = \frac{M!}{n'!(M-n')!} \cdot \left(\frac{n_0 z}{M}\right)^{n'} \prod_{n''=1}^{n'} y[n'']e^{-U'(\theta')/kT}[\bar{\tau}(\theta')e^{\bar{E}(\theta')/kT}]^{n'}$$

$$= \frac{M!}{n'!(M-n')!} \left(\frac{n_0 z}{M} e^{\frac{1}{n'}\sum_{n''=1}^{n'} \ln y[n'']} e^{\frac{1}{n'}\int_0^{n'} \ln \tilde{\tau}(\theta'')dn''}\right.$$

$$\left. e^{-\frac{1}{kT}\frac{1}{n'}\int_0^{n'} \tilde{E}'(\theta'')dn''} e^{\frac{1}{kT}\frac{1}{n'}\int_0^{n'} \tilde{E}(\theta'')dn''}\right)^{n'} \tag{16}$$

is obtained where

$$\ln \tilde{\tau}(\theta'') = \frac{d[n'' \ln \bar{\tau}(\theta'')]}{dn''} \tag{17}$$

$$\tilde{E}'(\theta'') = \frac{dU'(\theta'')}{dn''} \tag{18}$$

and

$$\tilde{E}(\theta'') = \frac{d[n''\bar{E}(\theta'')]}{dn''} \tag{19}$$

The left-hand sides of Eqs. (17)–(19) are partial specific quantities representing the increments of the total quantities $n'' \ln \bar{\tau}(\theta'')$, $U'(\theta'')$, and $n''\bar{E}(\theta'')$ of othe adsorbed system occurring when a molecule is added.

In a grand canonical system, the probability of occurrence of the n' or θ' values, in general, shows a very sharp distribution around the expectation value n or θ. Therefore, in Eq. (16), the following substitutions can be executed:

$$\left.\begin{array}{l}
\dfrac{1}{n'}\displaystyle\sum_{n''=1}^{n'} \ln y[n''] \approx \ln y[n] \equiv \ln y(\theta) \\[2ex]
\dfrac{1}{n'}\displaystyle\int_0^{n'} \ln \tilde{\tau}(\theta'')dn'' \approx \ln \tilde{\tau}[n] \equiv \ln \tilde{\tau}(\theta) \\[2ex]
\dfrac{1}{n'}\displaystyle\int_0^{n'} \tilde{E}'(\theta'')dn'' \approx \tilde{E}'[n] \equiv \tilde{E}'(\theta) \\[2ex]
\text{and} \\[2ex]
\dfrac{1}{n'}\displaystyle\int_0^{n'} \tilde{E}(\theta'')dn'' \approx \tilde{E}[n] \equiv \tilde{E}(\theta)
\end{array}\right\} \qquad (20)$$

A justification for the substitutions of Eq. (20) is given in the Appendix. Rewriting Eq. (16) by using Eq. (20) in which $y(\theta)$ is represented in terms of $p(\theta)$. (This can be done by using Eq. 8 where the term $n'' - 1$ in the denominator can approximately be replaced by n'' which is now equal to n.) and substituting Eq. (16) into Eq. (1), we obtain for the final expression of Ξ

$$\begin{aligned}
\Xi &= \sum_{n'=0}^{M} \frac{M!}{n'!(M-n')!}\left[\frac{n_0 z}{M} e^{\mu/kT} \frac{p(\theta)}{1-\dfrac{n}{M}} \tilde{\tau}(\theta) e^{-\tilde{E}'(\theta)/kT} e^{\tilde{E}(\theta)/kT}\right]^{n'} \\
&= \left[1 + \frac{n_0 z}{M} e^{\mu/kT} \frac{p(\theta)}{1-\dfrac{n}{M}} \tilde{\tau}(\theta) e^{-\tilde{E}'(\theta)/kT} e^{\tilde{E}(\theta)/kT}\right]^{M} \qquad (21)
\end{aligned}$$

Since the probability that n' molecules are adsorbed on the adsorbent surface is equal to $e^{n'\mu/kT}Z/\Xi$, n can be represented by using Eq. (21) as

$$\begin{aligned}
n &= \frac{1}{\Xi}\sum_{n'=0}^{M} n' e^{n'\mu/kT} Z \\
&= kT \frac{\partial}{\partial \mu} \ln \Xi
\end{aligned}$$

$$= \frac{n_0 z e^{\mu/kT} \dfrac{p(\theta)}{1 - \dfrac{n}{M}} \tilde{\tau}(\theta) e^{-\tilde{E}'(\theta)/kT} e^{\tilde{\tilde{E}}(\theta)/kT}}{1 + \dfrac{n_0 z}{M} e^{\mu/kT} \dfrac{p(\theta)}{1 - \dfrac{n}{M}} \tilde{\tau}(\theta) e^{-\tilde{E}'(\theta)/kT} e^{\tilde{\tilde{E}}(\theta)/kT}} \tag{22}$$

Equation (22) can be rewritten after arrangement as

$$x'\lambda = \frac{\theta}{zp(\theta)\tilde{\tau}(\theta)} e^{\tilde{E}'(\theta)/kT} e^{-\tilde{\tilde{E}}(\theta)/kT} \tag{22'}$$

in which

$$\lambda = e^{\mu/kT} \tag{23}$$

represents the absolute activity; this can be assumed to be approximately proportional to the concentration of molecules in solution. Equation (22′) therefore represents the adsorption isotherm, i.e., the relationship between the molecular concentration in solution and the surface molecular density on the adsorbent.

(C) Convenient Expression of the Adsorption Isotherm, Eq. (22′)

In Eq. (22′), in contrast with $p(\theta)$ fulfilling Eq. (7), $\tilde{\tau}(\theta)$, $\tilde{E}'(\theta)$, and $\tilde{\tilde{E}}(\theta)$ can be assumed to fulfill the relationships

$$\lim_{\theta\to 0} \tilde{\tau}(\theta) = \lim_{\theta\to 0} \bar{\bar{\tau}}(\theta) = \tau \tag{24}$$

$$\lim_{\theta\to 0} \tilde{E}'(\theta) = \lim_{\theta\to 0} \frac{U'}{n} = 0 \tag{25}$$

and

$$\lim_{\theta\to 0} \tilde{\tilde{E}}(\theta) = \lim_{\theta\to 0} \bar{\bar{E}}(\theta) = E \tag{26}$$

where ln τ and E are positive constants representing the entropy factor per molecule and the absolute value of the interaction energy with adsorbing site(s) per molecule occurring provided the molecule is isolated from the other molecules on the adsorbent surface, respectively.

Let us introduce the parameters

$$p^*(\theta) = \frac{\tilde{\tau}(\theta)}{\tau} p(\theta) \tag{27}$$

and

$$E^*(\theta) = \tilde{E}'(\theta) - \tilde{E}(\theta) + E \tag{28}$$

In many instances, in relationship with the fact that $\dot{\nu}$ decreases with an increase of θ (see the explanation of Eq. 11), $\tilde{\tau}$ would decrease with an increase of θ. Therefore, corresponding to Eq. (7), the relationships

$$\left.\begin{aligned} &\lim_{\theta\to 0} p^*(\theta) = 1 \\ \text{and} \\ &\lim_{\theta\to\theta_{\max}} p^*(\theta) = 0 \end{aligned}\right\} \tag{29}$$

would hold, and $p^*(\theta)$ would decrease with an increase of θ. Especially if the adsorption configuration of a molecule is independent of θ, $\tilde{\tau}$ is always equal to τ, and p or p^* (Eq. 27) represents a probability (see the explanation of Eq. 6). Corresponding to Eq. (26), we have

$$\lim_{\theta\to 0} E^*(\theta) = 0 \tag{30}$$

It can be considered that $-E + E^*(\theta)$ [being equal to $-\tilde{E}(\theta) + \tilde{E}'(\theta)$; see Eq. 28] represents the total energy of an adsorbed molecule, or the difference between the energy level of the molecule on the adsorbent surface and that of the molecule in solution (cf. Assumption 2 in Section A). Therefore, $E^*(\theta)$ measures the deviation of the energy of the adsorbed molecule from $-E$, i.e., the energy occurring provided the molecule is isolated from the other molecules on the adsorbent surface. In this sence $E^*(\theta)$ can be called a mutual interaction energy per molecule. It can be consdiered that $p^*(\theta)$ represents the mutual geometrical interaction factor for a molecule with other molecules on the adsorbent surface.

Equation (22′) can now be rewritten as

$$x'\lambda = \Psi(\theta)e^{-E/kT} \tag{31}$$

in which

$$\Psi(\theta) = \frac{\theta e^{E^*(\theta)/kT}}{z\tau p^*(\theta)} \tag{32}$$

If θ is small, Eq. (32) reduces to

$$\Psi(\theta) \approx \theta/z\tau \tag{33}$$

Finally, if the molecule has a rigid structure and if functional adsorption groups are arranged on the molecular surface, E can be represented as

$$E = x\varepsilon \tag{34}$$

in which $-\varepsilon$ ($\varepsilon > 0$) is the adsorption energy of a functional group onto one of the sites of the adsorbent. x is the average number of functional groups per molecule that react with sites of the adsorbent provided the molecule is isolated. x and $\ln \tau$ can be written as

$$x = \sum_{j=1}^{\nu} g\langle j\rangle x\langle j\rangle \tag{35}$$

and

$$\ln \tau = -\sum_{j=1}^{\nu} g\langle j\rangle \ln g\langle j\rangle \tag{36}$$

where

$$g\langle j\rangle = \frac{e^{x\langle j\rangle\varepsilon/kT}}{\sum_{j=1}^{\nu} e^{x\langle j\rangle\varepsilon/kT}} \tag{37}$$

$x\langle j\rangle$ represents the number of functional groups per molecule that react with sites of the adsorbent when the molecule is taking the jth configuration*.

(D) Another Method for the Derivation of Eq. (22′)

Assuming *a priori* that the total number, n, of the molecules on the adsorbent surface is constant and that the adsorbed molecules constitute a canonical system, the Helmholtz free energy, F, of the system can be calculated. The chemical potential, μ, of the adsorbed system can be derived from the relationship $\mu = \partial F/\partial n$ whereas the chemical potential μ° in solution that is in equilibrium with the adsorbed system can be represented as $\mu^\circ = kT \ln \lambda$ in which λ is approximately proportional to the concentration of the molecules in solution. Equation (22′) can be derived from the equilibrium condition $\mu = \mu^\circ$.

DISCUSSION

See the Discussion Section in Part III of this series (*14*).

APPENDIX

Introducing the parameter:

$$\ln X[n''] \equiv \ln \frac{n_0 z}{M} + \frac{\mu}{kT} + \ln y[n''] + \ln \tilde{\tau}[n''] - \frac{\tilde{E}'[n'']}{kT} + \frac{\tilde{E}[n'']}{kT} \tag{a1}$$

Equation (1), into which Eq. (16) is substituted, can be written as

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*The protein molecule would fundamentally be represented by the rigid molecular model on the surface of which charged adsorption groups are arranged (Introduction Section). Actually, however, the protein molecule is adsorbed onto either the **a** (or **b**) crystal surface of HA by using negatively charged adsorption groups (carboxyl or, in some instances, phosphate groups) or the **c** crystal surface by using positively charged adsorption groups (ε-amino or guanidinyl groups); on the **a** (or **b**) and the **c** crystal surface, positively and negatively charged adsorbing sites (called C and P sites) are arranged, respectively (see introductory part of Ref. 2). It can, therefore, be deduced that the total energy, $-E$, of a molecule on a crystal surface is contributed to not only the adsorption energy, $-x\varepsilon$, itself, but also the repulsive interaction energy between oppositely charged functional groups of the molecule and adsorbing crystal sites. In this instance, more complicated equations than Eqs. (35)–(37) are required.

$$\Xi = \sum_{n'=0}^{M} \psi_1(n') \tag{a2}$$

where

$$\psi_1(n') = \frac{M!}{n'!(M-n')!} \prod_{n''=1}^{n'} X[n''] \tag{a3}$$

On the other hand, Eq. (21) (i.e., Eq. 1 into which Eq. 16 rewritten by using Eq. 20 is substituted) can be rewritten as

$$\Xi = \sum_{n'=0}^{M} \psi_2(n') \tag{a4}$$

where

$$\psi_2(n') = \frac{M!}{n'!(M-n')!} \{X[n]\}^{n'} \tag{a5}$$

Statistical mechanics, in general, concludes that, when $M \gg 1$, $\psi_1(n')$ shows a very sharp distribution around the expectation value, n, of n', which gives ψ_1 a maximum value; n can be represented as a solution of the equation

$$\frac{d \ln \psi_1(n')}{dn'} = 0 \tag{a6}$$

Thus, calculating the left-hand side of Eq. (a6) by using both Stirling's approximation and the relationship

$$\frac{d \sum_{n''=1}^{n'} \ln X[n'']}{dn'} = \ln X[n'] \tag{a7}$$

and writing n instead of n',

$$-\ln n + \ln (M-n) + \ln X[n] = 0 \tag{a8}$$

is obtained; n can be considered to be a solution of Eq. (a8).

When $M \gg 1$, $\psi_2(n')$ also shows a very sharp distribution around the expectation value, n^*, of n', which gives ψ_2 a maximum value; n^* can be represented as a solution of the equation

$$\frac{d \ln \psi_2(n')}{dn'} = 0 \tag{a9}$$

Thus, calculating the left-hand side of Eq. (a9) by using Stirling's approximation, and writing n^* instead of n',

$$-\ln n^* + \ln(M - n^*) + \ln X[n] = 0 \tag{a10}$$

is obtained; n^* can be considered to be a solution of Eq. (a10).

It is evident that the solution, n, of Eq. (a8) is equal to the solution, n^*, of Eq. (a10), justifying the substitutions of Eq. (20).

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