JOURNAL OF COLLOID AND INTERFACE SCIENCE **183,** 26–34 (1996)
ARTICLE NO. 0515

Adsorption of Proteins at Liquid/Fluid Interfaces

V. B. FAINERMAN,*,† R. MILLER,*,[1] AND R. WÜSTNECK‡

*Max-Planck-Institut für Kolloid- und Grenzflächenforschung, Rudower Chaussee 5, D-12489 Berlin-Adlershof, Germany; †Institute of Technical Ecology, Blvd. Shevchenko 25, 340017 Donetsk, Ukraine; and ‡University of Potsdam, Institute of Solid State Physics, Rudower Chaussee 5, D-12489 Berlin-Adlershof, Germany

Received November 16, 1995; accepted May 29, 1996

A thermodynamic treatment of protein adsorption layers is presented and an equation of state of the interfacial layer is derived. Only by assuming different models for the activity coefficients in the interfacial layer different are models derived considering ideal and nonideal entropy and enthalpy of mixing. As special cases, well-known equations from other authors are obtained which are frequently used for the description of surfactant and polymer adsorption. Concerning the configuration of adsorbed protein molecules, i.e., number of protein segments adsorbed, the composition of the adsorption layer is caused by a self-regulation process which is mainly influenced by the chain flexibility and determines the surface pressure. Only four basic parameters describe the adsorption equilibrium of the protein. A sufficiently good agreement between literature data and the model is obtained, although best-fit procedures do not exist so far and the isotherm parameters were adjusted only manually.

Key Words: **protein adsorption; adsorption isotherm; liquid interface; thermodynamic model; surface pressure isotherm.**

1. INTRODUCTION

The theoretical and practical importance of the adsorption of polyelectrolytes, particularly proteins, at fluid interfaces stimulated the development of different models under equilibrium and dynamic conditions and for surfaces under deformation, as demonstrated in recent reviews (1–5). Besides the statistical theories and scaling models of de Gennes, for example, there are many thermodynamic models. These models use Butler's equation (6) for the chemical potentials of the components in the bulk phase and at the interface as the starting point. Examples of such models are those of Joos (7), Ter-Minassian-Saraga (8), and Lucassen-Reynders (9), which describe many details of the adsorption behavior at interfaces.

Although the description of many systems was successful, the capacity of the theories has not been completely utilized. At first the Butler equation is considered to describe the different demand ω of adsorbed molecules at the interface. For surfactant solutions this can be expressed by the adsorption principle of Braun-Le-Chatelier (10–12). It states that when the adsorbed molecule may occupy different parts of the interface, then for small values of Π a maximum surface area ω is occupied, whereas minimum ω is achieved at large Π. Obviously this agrees with the well-known fact that the adsorption layer thickness increases with increasing protein concentration. Thermodynamic approaches cannot take into consideration the adsorption layer thickness, while in statistical and scaling theories it is a necessary prerequisite. On the other hand, nonideal behavior of mixing in the interfacial layer allows further possibilities for the present model.

The present paper gives a thermodynamic treatment of protein adsorption layers, i.e., deriving an equation of state of the interfacial layer, the adsorption isotherm, and the isotherm of interfacial tension. Different models are derived considering ideal and nonideal entropy and enthalpy of mixing at the interface. As special cases, equations from other authors are obtained which are well known and frequently used for the description of surfactant and polymer adsorption. Literature data are used to test the new theoretical model presented.

2. ADSORPTION LAYER MODELS

The equation for the chemical potentials μ_i for the ith component in the solution bulk (α) and at the interface (S) can be written (6, 9) as

$$\mu_i^{\mathrm{S}} = \mu_i^{0\mathrm{S}} + RT \ln f_i^{\mathrm{S}} x_i^{\mathrm{S}} - \sigma\omega_i \qquad [1]$$

$$\mu_i^{\alpha} = \mu_i^{0\alpha} + RT \ln f_i^{\alpha} x_i^{\alpha}, \qquad [2]$$

where $\mu_i^{0\mathrm{S}}$ and $\mu_i^{0\alpha}$ are the standard chemical potentials at the interface and in the bulk, σ is the surface tension, ω_i is the partial molar surface demand, f_i is the activity coefficient, R is the gas constant, T is the temperature, $x_i = N_i/\Sigma N_i$ are the molar ratios, and N_i are the numbers of moles of the component i.

Using the pure solvent as the standard state, i.e., $x_0^{\alpha} = 1$, and assuming infinite dilution for the dissolved components

[1] To whom correspondence should be addressed.

0021-9797/96 $18.00

($i \geqslant 1$), i.e., $x_i^\alpha \to 0$, from Eqs. [1] and [2] results (9, 11) in

$$\ln \frac{f_i^S x_i^S}{k_i f_i^\alpha x_i^\alpha} = -\frac{\Pi \omega_i}{RT}, \qquad [3]$$

where $k_1 = 1$ and $k_i = (x_i^S/x_i^\alpha)_{x_i^\alpha \to 0}$ at $i \geqslant 1$ are the distribution coefficients at infinite dilution, $\Pi = \sigma_0 - \sigma$ is the surface (or interfacial) pressure, and σ_0 is the surface tension of the pure solvent.

For simplicity, in the following we consider only the solvent and one dissolved component (protein). All equations can be equivalently generalized for other cases, such as protein mixtures or mixtures of a protein and a surfactant. It should be noted that proteins usually contain dissolved ions. When the concentration of the dissolved electrolyte sufficiently exceeds the concentration of the effective charge of the protein (free charge (13)), in conformity with Gibbs' equation, the contribution of the counterions to σ may be neglected.

When we use Eq. [3] for solutions of one protein, the index i refers to different possible configurations of the protein at the interface. The different configurations are characterized by different values of ω_i and k_i. Obviously, the values $(x_i^\alpha f_i^\alpha)_{i \geqslant 1} = x^\alpha f^\alpha$ are equal for all states.

The number of possible states of protein molecules at the interface n can be expressed by the maximum ω_{max} and the minimum $\omega_{min} = \omega_1$ of the partial molecular surface area of a protein molecule and the increment $\Delta\omega$, by which the value of the partial molecular surface area changes when the protein transfers from one state into another. The number of different states is given by

$$n = \frac{\omega_{max} - \omega_1}{\Delta\omega} + 1. \qquad [4]$$

The physical meaning of ω_{max} corresponds to the minimum adsorption layer thickness, ω_{min} to the maximum one. The value $\Delta\omega$ is determined by the flexibility of the protein chain. The flexibility can be estimated by using scaling theories (4, 13) while of course the relation $\Delta\omega \leqslant \omega_1$ holds.

2.1. Adsorption Equilibrium

For deriving the equation of state $\Pi(\Gamma)$, the adsorption isotherm $\Gamma(c)$, and the equation of surface tension $\Pi(c)$, it is necessary to determine the value ω_0 and express the molar ratio x_i^S by the adsorption Γ_i. Starting from a pair of equations [3] for a solution of one protein having only one adsorption state ($i = 0, 1$ corresponds to a simple surfactant solution), and taking $f_1^\alpha = f_i^S = 1$ and $k_1 f_1^\alpha x_1^\alpha = bc$, we obtain

$$x_0^S = \exp\left(-\frac{\Pi\omega_0}{RT}\right), \qquad [5]$$

$$x_1^S = bc \exp\left(-\frac{\Pi\omega_1}{RT}\right). \qquad [6]$$

Here, b is the equilibrium adsorption constant, and c the bulk concentration of the surface-active component. For the case $\omega_0 = \omega_1$, Eqs. [5] and [6] transform into the Szyszkowski equation

$$\Pi = -\frac{RT}{\omega_1} \ln(1 + bc). \qquad [7]$$

Lucassen-Reynders (14, 15) has shown that for realizing this condition it is necessary to chose the dividing surface such that the total adsorption of all components equals the saturation adsorption, i.e.,

$$\Gamma_0 + \Gamma_1 = 1/\omega_1. \qquad [8]$$

For a mixture of proteins or for different states ω_i of one protein the value ω_0 should be equal the average partial molar surface of all states ω_Σ. Therefore Eq. [8] transforms into

$$\sum_{i=0}^{n} \Gamma_i = 1/\omega_\Sigma. \qquad [9]$$

The position of the dividing surface, defined by Lucassen-Reynders through Eq. [9], is near the dividing surface of Gibbs, for which $\Gamma_{H_2O} = 0$. The former is shifted toward the solution by $\Delta = (\omega_{H_2O}/\omega_\Sigma)a_{H_2O}$. Here ω_{H_2O} and a_{H_2O} are the molar surface area of water and the diameter of a water molecule, respectively. With increasing saturation of the interface by protein molecules, the dividing surface of Lucassen-Reynders is further shifted toward the bulk by a value almost equal to the thickness of the adsorption layer. The selection of the dividing surface has a considerable advantage because it permits one to exclude the influence of the adsorption layer thickness on the parameters in Eq. (3); i.e., the values x_i^S are determined in the present model by $x_i^S = \Gamma_i\omega_\Sigma$. As Eq. [3] is invariable to the selection of ω_0 we may use $\omega_0 = \omega_{H_2O}$ as it is done in many approaches. In this case, however, it is necessary to include the thickness of the adsorption layer δ, and the value x_i^S is determined by the molar volumes of all components of the system. The corresponding equation for x_0^S was given, for example, in (16). In the model we assume ideality in the solution bulk; i.e., $f_0^\alpha = f^\alpha = 1$. First, the equation of an ideal interfacial layer is derived. In the following paragraph additional corrections allow extension of the model to nonideal layers.

2.2. The Ideal Interfacial Layer

The equation of state of the adsorption layer is derived from Eq. [3] for the case $i = 0$, and assuming $f_0^S = 1$,

$$\Pi = -\frac{RT}{\omega_\Sigma}\ln(1 - \Gamma_\Sigma\omega_\Sigma), \qquad [10]$$

where

$$\Gamma_\Sigma = \sum_{i=1}^{n}\Gamma_i \qquad [11]$$

is the total adsorption of the protein—the sum over all adsorption states. The average partial molar surface area in Eq. [10], ω_Σ, is determined in the same way as it was proposed by Lucassen-Reynders for surfactant mixtures (15), namely by calculating the weighted average using the interfacial concentration of the different states

$$\omega_\Sigma = \frac{\sum_{i=1}^{n}\omega_i\Gamma_i}{\Gamma_\Sigma}. \qquad [12]$$

The interfacial tension isotherm is derived from Eq. [3] by addition of all components and states at the interface (i.e., $i = 0$ to $i = n$) for $f_i^S = 1$ following the procedure of Joos for solutions of two surfactants (17),

$$c\sum_{i=1}^{n} b_i\exp\left(-\frac{\Pi\omega_i}{RT}\right) = 1 - \exp\left(-\frac{\Pi\omega_\Sigma}{RT}\right), \qquad [13]$$

where c is the bulk concentration of the protein, b_i are the adsorption constants of the i different states. The adsorption isotherm of the ith state is derived from Eq. [3] by using all pairs of equations ($i = 0$ and $i \geqslant 1$) at the same Π, which yields

$$b_i c = \frac{\Gamma_i\omega_\Sigma}{(1 - \Gamma_\Sigma\omega_\Sigma)^{\omega_i/\omega_\Sigma}}. \qquad [14]$$

A ratio of the adsorptions of different states are easily obtained from Eq. [3]:

$$\frac{\Gamma_i}{\Gamma_j} = \frac{b_i}{b_j}\exp\left[-\frac{\Pi(\omega_j - \omega_i)}{RT}\right]. \qquad [15]$$

Equation [15] is the generalization of the adsorption principle of Braun-Le-Chatelier (10) for the protein adsorption.

Equations [13]–[15] include the constant b_i for different states. As a first approach we can assume that b_i = const for all states. A physically reasonable assumption would also be

$$\frac{b_i}{b_1} = \left(\frac{\omega_i}{\omega_1}\right)^\alpha. \qquad [16]$$

Analogously to Eq. [16], for a homologous series of surfactants the constants b_i increase with chain length exponentially,

$$\frac{b_k}{b_1} = e^{\beta(k-1)}, \qquad [17]$$

where k and l are the numbers of CH_2-groups in the surfactant molecule and β is a constant.

The values of ω_i in the ith state can be written as

$$\omega_i = \omega_1 + \Delta\omega(i - 1). \qquad [18]$$

For simplicity we can express i and n by their preceding values: $i = i + \Delta i$, where $\Delta i = \Delta\omega/\omega_1$. Variable i takes values between 1 and n, with $n = \omega_{\max}/\omega_1$. In a general case i can be a fraction. Taking into account this substitution the relations [16] and [18] become

$$\omega_i = i\omega_1 \qquad [19]$$

and

$$b_i = b_1 i^\alpha. \qquad [20]$$

For $\alpha = 0$ we get $b_i = b_1 = \text{const}$, whereas for $\alpha > 0$ the values of b_i increase with increasing ω_1. Taking into account Eqs. [15], [19], and [20] the value of average partial molar surface area (cf. Eq. [12]) can be expressed by ω_1:

$$\omega_\Sigma = \omega_1\sum_{i=1}^{n} i^{(\alpha+1)}\exp\left(-\frac{i\Pi\omega_1}{RT}\right)\Bigg/ \sum_{i=1}^{n} i^\alpha\exp\left(-\frac{i\Pi\omega_1}{RT}\right). \qquad [21]$$

The total adsorption (Eq. [11]) can be expressed now by the adsorption in the state of minimum partial molar surface area, and the adsorption in the ith state (Eq. [15]) by the total adsorption:

$$\Gamma_\Sigma = \Gamma_1\sum_{i=1}^{n} i^\alpha\exp\left[-\frac{(i-1)\Pi\omega_1}{RT}\right], \qquad [22]$$

$$\Gamma_i = \Gamma_\Sigma i^\alpha\exp\left[-\frac{(i-1)\Pi\omega_1}{RT}\right]\Bigg/ \sum_{i=1}^{n} i^\alpha\exp\left[-\frac{(i-1)\Pi\omega_1}{RT}\right]. \qquad [23]$$

Taking into account Eqs. [16] and [18], the surface tension isotherm [13] and the adsorption isotherm [14] are rewritten

$$b_1 c = \left(1 - \exp\left(-\frac{\Pi\omega_\Sigma}{RT}\right)\right) \Big/ \sum_{i=1}^{n} i^\alpha \exp\left(-\frac{i\Pi\omega_1}{RT}\right) \quad [24]$$

and

$$b_i c = \frac{\Gamma_i \omega_\Sigma}{i^\alpha (1 - \Gamma_\Sigma \omega_\Sigma)^{i\omega_1/\omega_\Sigma}}. \quad [25]$$

The system of equations [10], [21], [23] to [25] completely describes an ideal adsorption layer of proteins. This system is self consistent; inserting Γ_i from [23] and $(1 - \Gamma_\Sigma\omega_\Sigma)$ from [10] into Eq. [25] yields Eq. [24].

From Eqs. [21] and [23] the partial adsorptions Γ_i, $i > 1$ become small for large Π while Γ_1 increases until $\omega_\Sigma \cong \omega_1 = \omega_{\min}$. At the same time the adsorption layer thickness becomes maximum

$$\delta_{\max} \cong \frac{V}{\omega_1}, \quad [26]$$

where V is the molar volume of the protein.

For $\Pi \to 0$ Eq. [21] can be written

$$\omega_{\Sigma 0} = \omega_\Sigma|_{\Pi\to 0} = \omega_1 \Big(\sum_{i=1}^{n} i^{\alpha+1} \Big/ \sum_{i=1}^{n} i^\alpha\Big). \quad [27]$$

On the other hand, from Eq. [27] and $\alpha = 1$ we can derive

$$\omega_{\Sigma 0} = \omega_1 \left(\frac{2n + 1}{3}\right). \quad [28]$$

Thus, at $\Pi \to 0$ the average partial molar surface area becomes approximately $(2/3)\omega_{\max}$. In this case the thickness of the adsorption layer is a minimum:

$$\delta_{\min} = \frac{3}{2}\frac{V}{\omega_{\max}}. \quad [29]$$

It should be noted here that the influence of the coefficient α is small; changing α from 0 to ∞, the prefactor in Eq. [29] changes from 2 to 1. In the composition of the adsorption layer the component with the maximum value ω_i is dominant,

$$\Gamma_{i0} = \Gamma_i|_{\Pi\to 0} = \Gamma_\Sigma \Big(i^\alpha \Big/ \sum_{i=1}^{n} i^\alpha\Big), \quad [30]$$

or in the case $\alpha = 1$,

$$\Gamma_{i0} = \Gamma_\Sigma \frac{2i}{n(n+1)}. \quad [31]$$

The adsorption in the case $i = n$ becomes approximately n times higher then in the case $i = 1$.

The analysis of the basic equations [10], [21], and [23] to [25] shows that all parameters ω_1, n, α and b_1 may be found from experimental data for $\Pi \to 0$ and $\Pi \to \Pi_{\max}$. Using a scaling model, it is also possible to estimate independently ω_1, n, and Δi (4, 13).

2.3. The Nonideal Interfacial Layer

For a nonideal interfacial layer the coefficient of activity $f_i^S \neq 1$. Nonideality may be caused by two factors:

- nonideal entropy of mixing of components in the interfacial layer caused by different partial molar areas;
- nonideal enthalpy of mixing governed by intermolecular interaction of the components in the adsorption layer.

The contribution from nonideal entropy of mixing to the interfacial pressure ($\Delta\Pi$) has been considered by Lucassen-Reynders (9) in the form

$$\Delta\Pi = \frac{RT}{\omega_0} \sum_j \left(1 - \frac{\omega_0}{\omega_j}\right)\omega_j \Gamma_j. \quad [32]$$

The index j refers to different solutes, the index 0 to the solvent. The same contribution to the surface pressure was found by Damaskin (18, 19).

On the basis of statistical calculations using different configurations of polymers, Singer (20) found a contribution to Π caused by nonideal entropy of mixing (in the form of Eq. [32]),

$$\Delta\Pi = \frac{RT}{\omega_0}\left(1 - \frac{\omega_0}{\omega}\right)\frac{z}{2}\ln\left(1 - \frac{2}{z}\omega\Gamma\right), \quad [33]$$

with z being a value approximately equal to the coordination number.

As $z > 2$ (for a flexible chain z takes values between 4 and 6 (21, 22)), even at complete interfacial coverage $\omega\Gamma \cong 1$ it is possible to limit the development of the logarithmic function after the first term in Eq. [33], which transforms into Eq. [32]. A more complicated form of $\Delta\Pi$ was derived by Frisch and Simha (23), who took into account a loop-formation. Graham and Phillips (22) have shown that for very long chains this complicated relationship transforms into Eq. [33].

Equation [32] as a correction of Eq. [10] becomes remarkable only for $\omega\Gamma \ll 1$. For $\omega_j \gg \omega_0$ both contributions

are more or less equal. For protein solutions this range is not very important, because experimental data show remarkable values of Π only for almost-saturated adsorption layers.

In our model, for which $\omega_0 = \omega_\Sigma$ holds, the contribution of nonideal mixing to the interfacial pressure is negligible (cf. the Appendix).

An equation of state for the interfacial layer and an adsorption isotherm for surfactant solutions taking into account intermolecular interaction was first derived by Frumkin (24) and later developed by others (18, 19). The theory of nonideal solutions, published, for instance, in (25, 26), takes into account the influence of all components on the activity coefficients of the other components. In the present approach we will not distinguish between the interactions of protein molecules in different states. The activity coefficient for water and protein is taken as (27, 28)

$$\ln f_0^S = -a\Gamma_\Sigma^2\omega_\Sigma^2, \qquad [34]$$

$$\ln f_{i\geq 1}^S = -a(1 - \Gamma_\Sigma\omega_\Sigma)^2, \qquad [35]$$

where a is a constant.

From Eqs. [3], [34], and [35] relationships for a nonideal adsorption layer can be derived,

the equation of state

$$\Pi = -\frac{RT}{\omega_\Sigma}[\ln(1 - \Gamma_\Sigma\omega_\Sigma) - a\Gamma_\Sigma^2\omega_\Sigma^2], \qquad [36]$$

the adsorption isotherm for i states

$$b_1 c = \Gamma_i\omega_\Sigma \exp\left[a\Gamma_\Sigma^2\omega_\Sigma^2\left(i\frac{\omega_1}{\omega_\Sigma} - 1\right) + 2a\Gamma_\Sigma\omega_\Sigma - a\right] \Big/ i^\alpha(1 - \Gamma_\Sigma\omega_\Sigma)^{i\omega_i/\omega_\Sigma}. \qquad [37]$$

Equations [21] to [23] are the same for ideal and nonideal adsorption layers. An almost quadratic dependence between the surface pressure and the protein adsorption yields also the scaling theory (4, 13, 16). For high electrolyte concentrations when the contribution of the solute ions and polymer chains to the osmotic pressure Π_{OS} (or the interfacial pressure Π) are of the same order of magnitude, $\Pi_{0S} \sim c^{9/4}$ (13), or $\Pi \sim \Gamma_\Sigma^{9/4}$ (16).

The influence of nonideality of the entropy on activity coefficient of the protein at the interface is taken into account in the coefficient α in Eqs. [21] to [23] and [37] (cf. the Appendix).

3. CALCULATIONS AND COMPARISON TO EXPERIMENTAL DATA

The Eqs. [21]–[23], [36], and [37] are used for some model calculations. First, Eq. [21] was used to find $\omega_\Sigma = \omega_\Sigma(\Pi)$. Then, from ω_Σ we calculated $\Gamma_\Sigma = \Gamma_\Sigma(\Pi)$ via Eq. [36], and $\Gamma_i = \Gamma_i(\Pi)$ via Eq. [23]. Finally we obtain $b_1 c = b_1 c(\Pi)$ from Eq. [37]. The molecular mass of the protein, the values of the maximum and minimum partial molar surfaces ω_Σ and ω_1, the increment $\Delta\omega$, and the constants α and a were varied.

The following results were obtained:

- In comparison to the case $\Delta\omega = \omega_1$, the decrease of the increment $\Delta\omega$ only weakly influences the dependencies Π and Γ_Σ from c.
- The influence of the constant α, which varies in the range $0\cdots 2$, is limited to small values of Π because of the dominant influence of the exponent in Eqs. [21]–[23].
- Values ω_{max}, ω_1 and the constants a and b govern the equilibrium adsorption of a protein at the water/air interface.

Calculations using the present model confirm many well-known relations (16, 21, 22, 29, 30):

- the remarkable increase of Π after a certain amount of adsorption Γ_m has been reached, which is in the range from 0.2 to 0.5 mg/m^2,
- a strong increase of adsorption and surface pressure dependent on the bulk concentration,
- a shift of Γ_m into the region of small adsorptions and an increase of maximum surface pressure Π in the case of increasing flexibility of the chain, i.e., with increasing ω_{max} and decreasing ω_1.

The differences between the adsorption of a protein in comparison to that of usual surfactants are caused by the evolution of the protein adsorption layer. Figure 1 shows the dependence of Γ_Σ and some Γ_i from the surface pressure Π for a protein with the molecular mass of M = 20,000, ω_{max} = 80 nm^2 (ω values refer to one molecule), $\omega_1 = \Delta\omega$ = 2 nm^2 (i.e., n = 40), a = 240, and α = 1. Obviously, the dependence $\Gamma_i = \Gamma_i(\Pi)$ shows an extreme character with an exception for i = 1. The higher the i (i.e., ω_i) the smaller is the Π value, at which the adsorption of the ith component shows a maximum. At high Π the state of minimum ω_i is dominant in the composition of the adsorption layer. It should be noticed that in the present case for better fitting of experimental data of proteins with almost similar M-values, $a > 0$ was assumed. The value of the constant a influences Γ_Σ for a given Π. The ratio Γ_i/Γ_Σ, however, is independent of a, as can be seen from Eq. [23].

Figures 2 and 3 show the experimental data of Graham and Phillips (27) for β-casein, which are compared with model calculations. A good agreement with the experimental data is obtained for the following values of the parameters in the model: ω_{max} = 60 to 100 nm^2, $\omega_1 = \Delta\omega$ = 5 to 10 nm^2, a = 200 to 400. The curves were calculated by assuming the following parameters: ω_{max} = 75 nm^2, $\omega_1 = \Delta\omega$ = 5 nm^2, α = 0, b_1 = 2000 liter/g, a = 400. The assumption α = 0

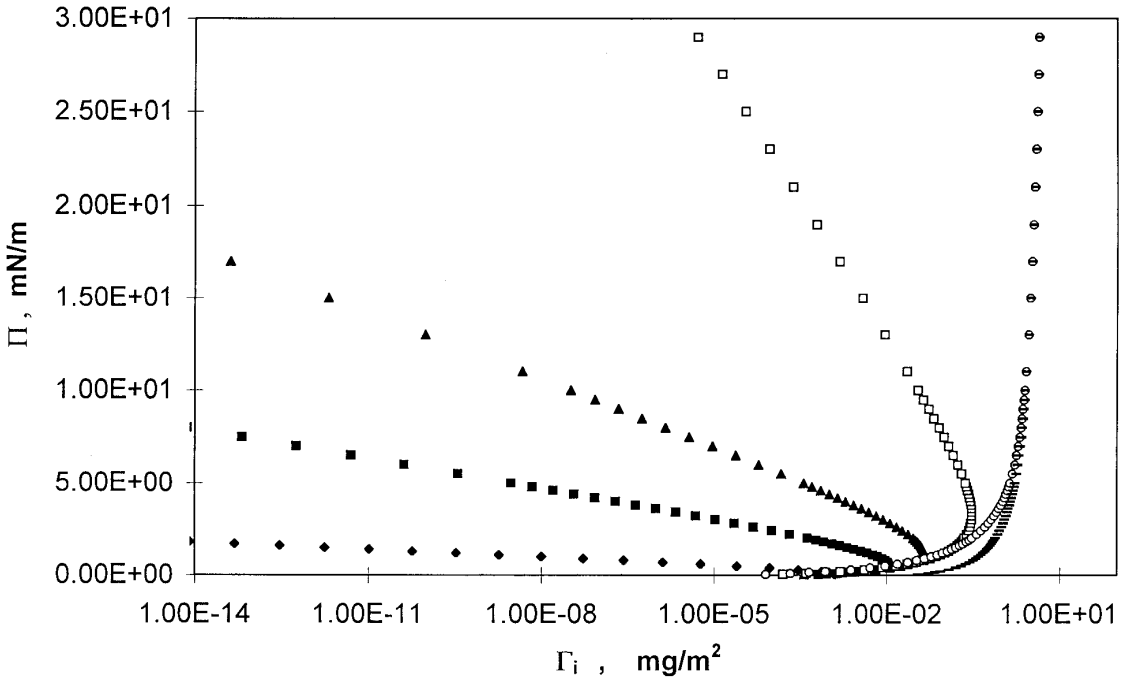

FIG. 1. Dependence of the sum of adsorption and of adsorption in the states 1, 2, 5, 10, and 40 on the surface pressure for a protein of the molecular mass $M = 20000$, $\omega_1 = 2$ nm^2, $n = 40$, $\alpha = 1$, and $a = 240$.

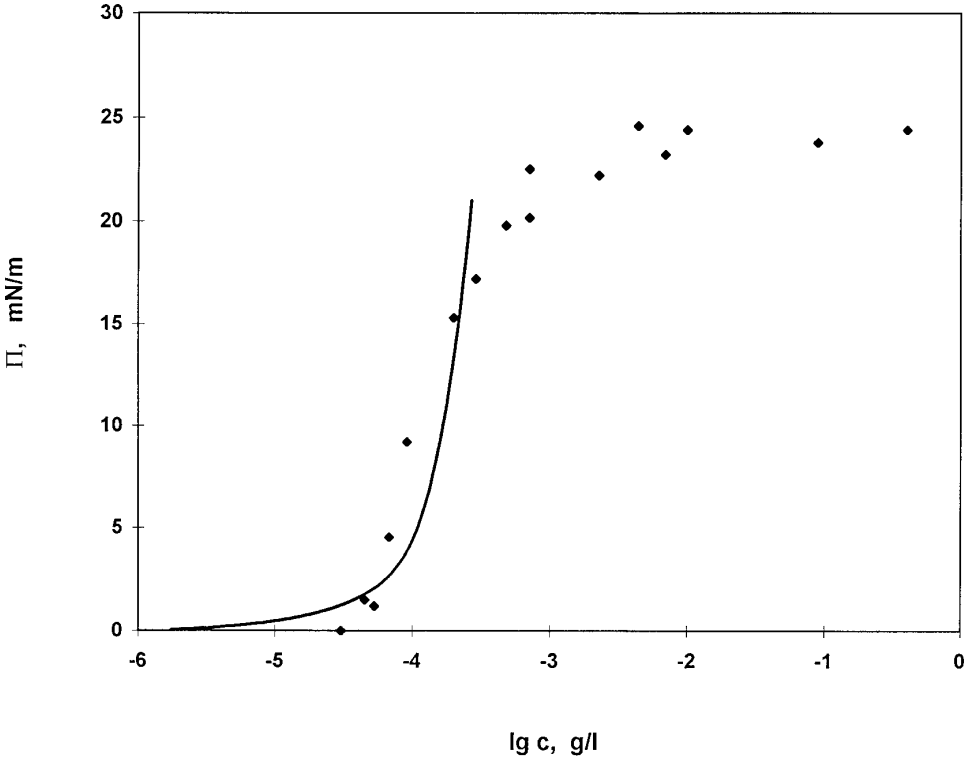

FIG. 2. Dependence of surface pressure on the concentration of a β-casein solution. Data taken from Graham and Phillips (27). Lines calculated using the present model, $\omega_1 = 5$ nm^2, $n = 15$, $\alpha = 0$, and $a = 400$, and $b_1 = 2000$ liter/g.

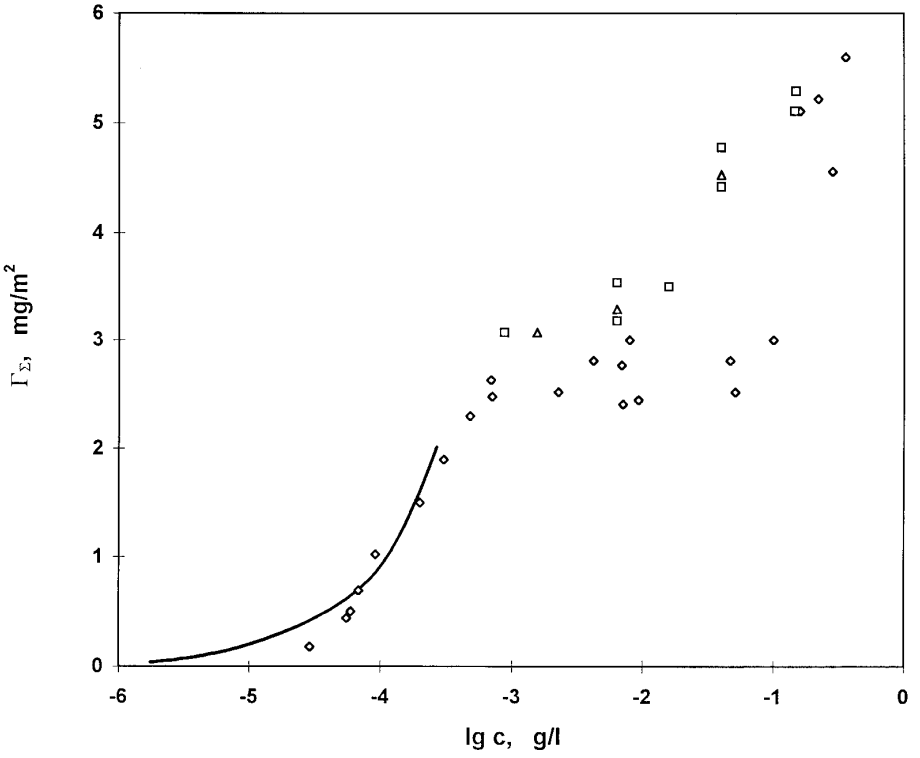

FIG. 3. Dependence surface concentration ($\diamond$, determined by radiotracer; $\square$, $\triangle$, determined by ellipsometry) on the concentration of a β-casein solution. Data taken from Graham and Phillips (27). Lines calculated using the same model and parameter values as for Fig. 2.

means that the activity of the protein molecule does not depend on the molecular interfacial state (cf. Eq. [20]).

The estimation of the maximum and minimum thickness of the adsorption layer of β-casein by using Eqs. [26] and [27] yields 8 to 10 nm and 0.5 to 1 nm, respectively. These values agree with those found by ellipsometry by Graham and Phillips (27) as well as with other data (16) where the thickness was calculated on the basis of a scaling theory. Furthermore, using the data $\omega_\Sigma = \omega_\Sigma(c)$ the actual values of the adsorption layer thickness were calculated by

$$\delta(c) = V/\omega_\Sigma(c). \qquad [38]$$

The data of $\delta(c)$ agree very well with the values given in (16, 21).

The following conclusions can be drawn when comparing the experimental and theoretical results depicted in Figs. 2 and 3:

• with one and the same set of parameters (ω_1, ω_{max}, b, and a) the three independent experiments of Graham and Phillips (27) obtained $\Pi = \Pi(c)$, $\Gamma_\Sigma = \Gamma_\Sigma(c)$, and $\delta = \delta(c)$ can be described by any possible combination of theoretical functions, for example $\Pi = \Pi(\Gamma_\Sigma)$, $\delta = \delta(\Gamma_\Sigma)$, etc.;

• the agreement between theory and the experimental data can possibly be improved significantly by using a fit-program, which does not exist so far for the presently derived model;

• in the theoretical model the concentration c used is the quantity which is in equilibrium with Γ_Σ, while in the experiments of Graham and Phillips (27) c denotes the initial bulk concentration c_0 in the Langmuir trough. Taking into consideration the adsorbed amount at the surface, we get $c = c_0 - \Gamma_\Sigma/h$; h is the depth of the solution in the trough, $h \approx 1$ cm. For the data at the lowest concentration the equilibrium concentration c is about 50% smaller than the initial concentration c_0, leading to a shift of the experimental point toward lower concentration, which is in favor of a better agreement with the theory.

4. IMPACT ON ADSORPTION KINETICS

The self-regulation of the surface concentration of different states of adsorbed protein molecules influences the adsorption kinetics and hence the dynamic surface tension of protein solutions. A new theoretical model of a dynamic protein adsorption layer will be discussed in a subsequent paper. Here, we only want to give some conclusions drawn from Eq. [23].

We can conclude that at low surface pressure, say $\Pi < 2$ mN/m or $c < 0.05$ g/liter, the dynamic adsorption layer is in quasi-equilibrium. This state of low adsorption layer

thickness (16, 21) will be reached mainly by a diffusion-controlled adsorption process (21, 29–33). A further adsorption of protein molecules from the bulk according to Eq. [23] is connected with a desorption of segments. At a surface pressure of say $\Pi > 3$ mN/m a transition from adsorption states of larger to smaller surface area demand will become step by step dominant (cf. Fig. 1). Exactly at this stage, as can be drawn from the experimental data in (8), the adsorption kinetics slows down with respect to a diffusion-controlled mechanism. We can assume that the replacement of desorbing segments by new adsorbing molecules is described by a first-order reaction and that the momentary deviation from equilibrium is small. If we consider Eq. [23] we get the relationship for the change of surface pressure

$$\frac{d\Pi}{dt} = k_0 \exp\left(-\frac{\Delta\omega\Pi}{RT}\right), \qquad [39]$$

where k_0 is a constant. This equation is well known and was derived empirically by MacRitchie (3, 34, 35). The value of $\Delta\omega$ is of the order of 0.5 to 2.5 nm^2 (8, 35). Due to Ter-Minassian-Saraga (8) these values are too small for a protein molecule. According to our model these values belong only to the differences in the adsorbed state and are physically completely sensible.

5. CONCLUSIONS

The equations of state of the adsorption layer, the isotherm of the surface tension, and adsorption isotherms for different states of protein configurations were derived for the interface liquid/gas. The composition of the adsorption layer regarding the configuration, i.e., number of protein segments adsorbed, is caused by a self-regulation process which is mainly influenced by the chain flexibility and determines the surface pressure. Only four basic parameters describe the adsorption equilibrium of the protein. A sufficient agreement between published experimental results and the model was observed.

The influence of the evolution of the protein adsorption layer on the kinetics of adsorption was discussed. As a result of the model yields the MacRitchie equation for the velocity of surface tension change.

APPENDIX

The activity coefficients of component i in the interfacial layer for nonideal entropy of mixing is given by (9)

$$\ln f_i^{\mathrm{S}} = 1 - (\omega_1/\omega_0)\left(\sum_{j=0}^{n} \Gamma_j\omega_j \Big/ \left(\frac{\omega_j}{\omega_0}\right)\right). \qquad [A1]$$

In our model we have $\omega_0 = \omega_\Sigma$ and $\Sigma_{j=0}^{n} \Gamma_j = 1/\omega_\Sigma$ and thus from [A1] we obtain for water (index 0) and the protein molecules (i different adsorption states)

$$\ln f_0^{\mathrm{S}} = 0, \qquad [A2]$$

$$\ln f_{i\geq 1}^{\mathrm{S}} = 1 - \omega_i/\omega_\Sigma = 1 - i\omega_1/\omega_\Sigma. \qquad [A3]$$

The resulting activity coefficients are equal to the product of the partial activity coefficients, calculated for nonideal enthalpy of mixing via Eqs. [34] and [35] or nonideal entropy of mixing via Eqs. [A2] and [A3]. Thus, the equation of state [36] remains valid when [A2] is used, while the application of Eq. [A3] produces an additional factor, $\exp(-i\omega_1/\omega_\Sigma)$, in Eq. [37]. The effect of this factor is the same as that of the coefficient α in Eqs. [21] to [23] and [37], a partial reorientation of protein molecules in benefit of a state $\omega_i > \omega_\Sigma$. An analogous result is obtained by choosing $\alpha = 0.2 \div 0.5$.

ACKNOWLEDGMENTS

The authors want to express their thankfulness to Prof. P. Joos from the University of Antwerp for many helpful discussions. The work was financially supported by the INTAS, project 93-2463, of the European Community and by the DFG, Wu 187/3.

REFERENCES

1. Takahashi, A., and Kawaguchi, M., *Adv. Polym. Sci.* **46,** 1 (1982).
2. Fleer, G. J., and Scheutjens, J. M. H. M., *Adv. Colloid Interface Sci.* **16,** 341 (1982).
3. MacRitchie, F., *Adv. Colloid Interface Sci.* **25,** 341 (1986).
4. de Gennes, P.-G., *Adv. Colloid Interface Sci.* **27,** 189 (1987).
5. Miller, R., *Trends Polym. Sci.* **2,** 47 (1991).
6. Butler, J. A. V., *Proc. Roy. Soc. Ser. A* **138,** 348 (1932).
7. Joos, P., *Biochim. Biophis. Acta* **375,** 1 (1975).
8. Ter-Minassian-Saraga, L., *J. Colloid Interface Sci.* **80,** 393 (1981).
9. Lucassen-Reynders, E. H., *Colloids Surf. A* **91,** 79 (1994).
10. Joos, P., and Serrien, G., *J. Colloid Interface Sci.* **145,** 291 (1991).
11. Fainerman, V. B., Makievski, A. V., and Joos, P., *Colloids Surf. A* **90,** 213 (1994).
12. Fainerman, V. B., Miller, R., and Makievski, A. V., *Langmuir* **11,** 3054 (1995).
13. Dobrynin, A. V., Colli, R. H., and Rubinstein, M., *Macromolecules* **28,** 1859 (1995).
14. Lucassen-Reynders, E. H., *J. Phys. Chem.* **70,** 1771 (1966).
15. Lucassen-Reynders, E. H., *J. Colloid Interface Sci.* **41,** 156 (1972); *J. Colloid Interface Sci.* **85,** 178 (1982).
16. Doullard, R., Daoud, M., Lefebvre, J., Miner, Ch., Lecanny, G., and Coutret, J., *J. Colloid Interface Sci.* **163,** 277 (1994).
17. Joos, P., *Bull. Soc. Chim. Belg.* **76,** 591 (1967).
18. Damaskin, B. B., *Electrochimija* **5,** 249 (1969).
19. Damaskin, B. B., Frumkin, A. N., and Borovaja, N. A., *Electrochimija* **8,** 807 (1972).
20. Singer, S. J., *J. Chem. Phys.* **16,** 872 (1948).
21. Benjamins, J., de Feijter, J. A., Evans, M. T. A., Graham, D. E., and Phillips, M. C., *Diss. Faraday Soc.* **59,** 218 (1978).
22. Graham, D. E., and Phillips, M. C., *J. Colloid Interface Sci.* **70,** 427 (1979).

23. Frisch, H. L., and Simha, R., *J. Chem. Phys.* **24,** 652 (1956); *J. Chem. Phys.* **27,** 702 (1957).
24. Frumkin, A. N., *Z. Phys. Chem.* **116,** 466 (1924).
25. Prigogine, I., ''The Molecular Theory of Solutions.'' North-Holland, Amsterdam, 1968.
26. Rowlinson, J. S., and Swinton, F. L., ''Liquids and Liquid Mixtures,'' 3rd ed. Butterworth, London, 1982.
27. Graham, D. E., and Phillips, M. C., *J. Colloid Interface Sci.* **76,** 415 (1979).
28. Xu, S., and Damodaran, S., *Langmuir* **10,** 472 (1994).
29. Ghosh, S., and Bull, H. B., *Biochemistry* **2,** 411 (1963).
30. Tornberg, E., *in* ''ACS Symposium Series, N92, Functionality and Protein Structure'' (Akila Pour-El, Ed.), p. 105, 1972.
31. Graham, D. E., and Phillips, M. C., *J. Colloid Interface Sci.* **70,** 403 (1979).
32. Kalischewski, K., and Schügerl, K., *Colloid Polym. Sci.* **257,** 1099 (1979).
33. de Feijter, J. A., and Benjamins, J., *in* ''Food Emulsions and Foams'' (E. Dickinson, Ed.), Special publication no. 58, p. 72. Royal Chem. Soc., London, 1987.
34. MacRichie, F., *Colloids Surf.* **41,** 25 (1989).
35. MacRichie, F., *Anal. Chim. Acta* **249,** 241 (1991).