

## Fractional statistical theory of adsorption applied to protein adsorption

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### ABSTRACT

Experimental adsorption isotherms of bovine serum albumin (BSA) adsorbed on sulfonated microspheres were described by means of two analytical models: the first is the well-known Langmuir–Freundlich model (LF), and the second, called fractional statistical theory of adsorption (FSTA), is a statistical thermodynamics model developed recently by Ramirez-Pastor et al. [Phys. Rev. Lett. 93 (2004) 186101]. The experimental data, obtained by Hu et al. [Biochem. Eng. J. 23 (2005) 259] for different concentrations of sulfonate group on the surface of the microspheres, were correlated by using a fitting algorithm based on least-squares statistics. The combination of LF and FSTA models, along with the choice of an adequate fitting procedure, allowed us to obtain several conclusions: (i) as previously reported in the literature, the maximum amount adsorbed increases as the amount of sulfonate group increases; (ii) the equilibrium constant does not appear as a sensitive parameter to the amount of sulfonate group on the surface of the microspheres; and (iii) the values of the fitting parameters obtained from FSTA may be indicative of a mismatch between the equilibrium separation of the intermolecular interaction and the distance between the adsorption sites. The exhaustive study presented here has shown that FSTA model is a good one considering the complexity of the physical situation, which is intended to be described and could be more useful in interpreting experimental data of adsorption of molecules with different sizes and shapes.

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### 1. Introduction

The protein adsorption onto polymer particles has been studied for decades because of its significant interest in a wide range of applications in biology, medicine, and biotechnology [1–12]. The particles used for that purpose are generally copolymerized, or their surfaces are modified chemically and/or biologically [10–14]. In particular, the use of microspheres has been studied from all viewpoints, and these can be used as functional tools by themselves or by coupling with biocompounds [13–15].

It is commonly known that the interactions between proteins and solid surfaces play important roles in the adsorption process. Major interactions involved in the adsorption can be classified as electrostatic, hydrophobic, hydrogen-bonding and van der Waals interactions [16]. Early, hydrophobic interaction was considered as the most important factor affecting protein adsorption, and an increase in the hydrophobicity would result in a higher level of adsorption [17]. The amount of adsorbed protein by this interaction force is maximum in the neighborhood of the isoelectric point of the protein, and the pH at maximum adsorption shifts to a more acidic region with an increase in ionic strength [18]. Other researchers have claimed that hydrogen-bonding interactions are

more important than hydrophobic interactions [19,20]. More recently, it was showed that the electrostatic contributions constitute a key factor among the interactions, so the adsorption is controlled by the surface charge [21–23].

A system of sulfonated and carboxylated polystyrene microspheres as support is a prototype to study protein adsorption, and numerous contributions have been devoted to this topic. Among them, Yoon et al. discussed the relationship between hydrophobic and hydrogen-bonding interactions using BSA (bovine serum albumin) as model protein in the adsorption process onto carboxylated microspheres [18,24–26]. The researchers demonstrated that the hydrophobic interaction predominated over hydrogen-bonding for the low carboxylated microspheres, while hydrogen-bonding was dominant for the high carboxylated ones.

Other papers studying the mechanism of adsorption of BSA onto sulfonated microspheres [15,27–32] have shown that an increase in the content of electrostatic groups results in a corresponding increase in the protein adsorbed. The maximal content of surface groups leads to a highest adsorption amount. On the basis of such results, the authors concluded that electrostatic interactions dominate the adsorption (at least in the highly modified surface). Although the conclusion drawn is ostensibly rational, it is also necessary to point out that the effect of strong hydrophobicity in the polystyrene core cannot be neglected even in the case of the highly modified surface.

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Despite these results, the complete description of the role of each interaction involved in the protein adsorption process is still an open problem, and the conclusions obtained from the analysis of experimental data are strongly dependent on the fitting model.

From a theoretical point of view, most current theories of protein adsorption are based on early work conducted by Freundlich [33] and Langmuir [34]. In the first case, an empirical nonlinear equation with two free parameters was proposed by Freundlich (and Küster) in 1894. In the second case, the model derived by Langmuir, and its generalizations [35] rely upon the crude assumption of spherically symmetric admolecules (each molecule of adsorbate occupies a single adsorption site). As a consequence of these limitations, there exists a lack of research concerning the probable mechanism of protein adsorption and the configuration of the protein in the adsorbed state.

In this context, the main purpose of the present work is to apply the fractional statistical theory of adsorption (FSTA) [36] to analyze the problem of protein adsorption and show that its treatment can be significantly oversimplified from this perspective. FSTA is based on a generalization of the formalism of quantum fractional statistics (QFS), proposed by Haldane [37] and Wu [38] as an extended form of Pauli's exclusion principle. The appealing feature of this theory is that a system of interacting particles confined in a finite region of the space can be characterized by an "statistical exclusion parameter"  $g$  accounting for the configuration of the molecule in the adsorbed state as a model parameter.

The paper is organized as follows: the basis of FSTA are presented in Section 2, along with the well-known Freundlich [33], Langmuir [34] and Langmuir–Freundlich [35] isotherms. In order to test the accuracy and applicability of the proposed model, Section 3 is devoted to the analysis of experimental data for bovine serum albumin (BSA) adsorbed on sulfonated microspheres [21]. The results of the study are compared with previous published data obtained by Langmuir–Freundlich analysis [21]. Finally, general conclusions are given in Section 4.

## 2. Theory

The problem of interpreting experimental data of adsorption by using different theoretical models has been considered repeatedly [34,39–42]. However, the number of adjustable parameters required for accurate information to be derived from such an analysis is still an open question.

The history of the problem leads us to Freundlich and Küster, who in 1894 published the first mathematical fit to an adsorption isotherm [33]. The Freundlich (or Küster) isotherm equation is a strictly empirical expression representing the isothermal variation of adsorption of a quantity of gas adsorbed by unit mass of solid adsorbent with pressure. Thus,

$$\theta = K_F p^{1/n_F}, \quad (1)$$

where the coverage  $\theta$  represents the amount adsorbed  $Q$ , in terms of the maximum amount adsorbed  $Q_{max}(\theta = Q/Q_{max})$ ;  $p$  is the pressure of gas and  $K_F$  and  $n_F$  are empirical constants whose values depend upon adsorbent and gas at particular temperature.

Later on, Langmuir [34] considered that the adsorbent is represented by a homogeneous lattice of adsorption sites and developed the following essential assumption: each molecule of adsorbate occupies one adsorption site of the surface. On the basis of the dynamic equilibrium between adsorbed and gas phases and discarding chemical dissociation, Langmuir derived the well-known equation for studying localized adsorption without adsorbate–adsorbate lateral interactions

$$\theta = \frac{K_e p}{1 + K_e p}, \quad (2)$$

where  $K_e$  is the equilibrium constant, which depends on the adsorption energy of the sites on the homogeneous surface. Langmuir isotherm has also been derived from rigorous statistical thermodynamics arguments [43].

In 1950, a simple generalization of the Langmuir and Freundlich isotherms was derived by Sips [35]. The resulting equation, known as Langmuir–Freundlich isotherm, can be written as:

$$\theta = \frac{K_{LF} p^{1/n_{LF}}}{1 + K_{LF} p^{1/n_{LF}}}, \quad (3)$$

where  $K_{LF}$  and  $n_{LF}$  are the Langmuir–Freundlich constants.

More recently, a new theory to describe adsorption of polyatomic adsorbates has been introduced [36]. FSTA is based on a generalization of the formalism of QFS, proposed by Haldane [37] and Wu [38] as an extended form of Pauli's exclusion principle. Haldane's statistics relies on the fact that, given  $(N - 1)$  identical particles confined in fixed volume, each of which having  $G$  accessible states at infinitely low density, the number of states available to a  $N$ th particle added to the volume is

$$d_N = G - \sum_{N'=1}^{N-1} g(N') = G - G_0(N), \quad (4)$$

being the number of states excluded per particle,  $g(N)$ , a measure of the statistical interactions. In addition, the number of configurations for a system of  $N$  particles and  $G$  states is:

$$W(N) = \frac{(d_N + N - 1)!}{[N!(d_N - 1)!]} = \frac{[G - G_0(N) + N - 1]!}{\{N![G - G_0(N) - 1]!\}}. \quad (5)$$

Clearly,  $g(N) = 0[G_0(N) = 0]$  and  $g(N) = 1[G_0(N) = N - 1]$  holds for bosons and fermions, respectively.

In Ref. [36], the QFS was extended as to describe classical systems like the adsorption of polyatomic molecules at the gas–solid interface. The arguments follow: one isolated molecule interacting with a regular solid surface confined in a fixed volume can be represented by an adsorption field having a total number  $G$  of local minima in the space of coordinates necessary to define the adsorption configuration (usually rendered by lattice sites in a discrete representation of the field).  $G$  was rationalized as being the number of equilibrium states available to a single molecule at infinitely low density. Depending on the ratio between the typical size of the molecule in the adsorbed configuration and the distance between neighboring minima, some states out of  $G$  are prevented from occupation upon adsorption of another molecule. Moreover, because of possible concurrent exclusion of states by two or more molecules, the number of states excluded per molecule,  $g(N)$ , depends in general on the number of molecules  $N$  within the volume. Accordingly, given  $(N - 1)$  identical molecules adsorbed on a surface of  $M$  sites, the number of states available to a  $N$ th one added to it can be obtained from Eq. (4) with  $g(N) \geq 0$ .

By assuming  $U_0$  being the adsorption energy per particle, the canonical partition function  $Q(N, M, T)$  can be written as [43]:

$$Q(N, M, T) = W(N) \exp(-\beta N U_0) q_i^N, \quad (6)$$

where  $\beta = 1/k_B T$ ,  $k_B$  are the Boltzmann constant, and  $q_i$  is the partition function from the internal degrees of freedom of a single molecule in the adsorbed state.

In the canonical ensemble the Helmholtz free energy  $F(N, M, T)$  relates to  $Q(N, M, T)$  through [43]

$$\beta F(N, M, T) = -\ln Q(N, M, T). \quad (7)$$

The remaining thermodynamic functions can be obtained from the general differential form [43]

$$dF = -SdT - \Pi dM + \mu dN, \quad (8)$$

where  $S$ ,  $\Pi$  and  $\mu$  designate the entropy, spreading pressure and chemical potential, respectively, which, by definition, are

$$S = - \left( \frac{\partial F}{\partial T} \right)_{M,N} \quad \Pi = - \left( \frac{\partial F}{\partial M} \right)_{T,N} \quad \mu = \left( \frac{\partial F}{\partial N} \right)_{T,M} \quad (9)$$

Thus, a general form for the chemical potential of noninteracting adsorbed polyatomics is obtained from Eq. (9) as:

$$\beta\mu = \ln \left\{ \frac{n[1 - \tilde{G}_0(n) + n] \tilde{G}_0^{(n-1)}}{[1 - \tilde{G}_0(n)] \tilde{G}_0} \right\} - \ln K_e, \quad (10)$$

where  $n = N/G$  is the density ( $n$  finite as  $N, G \rightarrow \infty$ ), which is proportional to the standard surface coverage  $\theta$ ,  $n = a\theta$ ,  $\theta$  being either the ratio  $N/N_m$  or the ratio  $v/v_m$ , where  $N(v)$  is the number of admolecules (adsorbed amount) at given  $\mu, T$  and  $N_m(v_m)$  is the one corresponding to monolayer completion. In addition,  $\tilde{G}_0(n) \equiv \lim_{N,G \rightarrow \infty} G_0(N)/G$ ,  $\tilde{G}_0 \equiv d\tilde{G}_0/dn$  and  $K_e = q_i \exp(-\beta U_0)$  is the equilibrium constant (as in Eq. (2)). Hereafter we examine the simplest approximation within FSTA, namely  $g = \text{constant}$ , which is rather robust as it will be shown below. Considering that  $\tilde{G}_0 = gn$  and  $\tilde{G}_0 = g$ , a particular isotherm function arises from Eq. (10)

$$K_e p = \frac{a\theta[1 - a\theta(g-1)]^{g-1}}{(1 - a\theta g)^g}, \quad (11)$$

where  $p \propto \exp(\beta\mu)$ . It can be demonstrated that Eq. (11) reduces to the rigorous isotherm of noninteracting chains ( $k$ -mers) adsorbed flat on a one-dimensional lattice [44] if  $g$  equals the number of chain units (size)  $k$ . This is already a simple example of the underlying relationship between the statistical exclusion parameter  $g$  and the spatial configuration of the admolecule. Other examples (out of a whole variety of adsorption configurations that the proposed formalism allows to deal with) are given in the following. Let us consider adparticles composed by  $k$  elementary units in which  $k'$  out of  $k$  units of the molecule are attached to surface sites and  $(k - k')$  units are detached and tilted away from them. For a lattice of  $M$  sites,  $\theta = k'N/M$ . Thus, for a molecule with  $k$  units, each of which occupying an adsorption site,  $k' = k$  and  $G = Mm$ , where  $m$  is the number of distinguishable configurations of the molecule per lattice site (at zero density) and depends on the lattice/molecule geometry. Then,  $1/a = k'm$ . For instance, straight  $k$ -mers adsorbed flat on sites of a square lattice would correspond to  $m = 2$ ,  $g = 2k$  and  $a = 1/(2k)$ . On the other hand,  $m = 1$ ,  $g = 1$  and  $a = 1$  represents the case of end-on (normal to the surface) adsorption of  $k$ -mers. Instead,  $m = 1$ ,  $g = k'$  and  $a = 1/k'$  represents an adsorption configuration in which  $k'$  units of the  $k$ -mer are attached to a one-dimensional lattice and  $(k - k')$  units at the ends are detached.

As discussed in the last paragraph, the parameters  $a$  and  $g$  in Eq. (11) have a precise physical meaning, can be obtained from adsorption experiments and are related directly to the spatial configuration of a polyatomic molecule in the adsorbed state. In the next section, Langmuir–Freundlich (Eq. (3)) and FSTA (Eq. (11)) equations will be used to fit experimental results of protein adsorption.

### 3. Comparison with experimental results of BSA adsorbed on sulfonated microspheres

In this section, analysis of experimental results has been carried out in order to determine the reaches and limitations of the theoretical models discussed here. The experimental data were taken from the work by Hu et al. [21]. In Ref. [21], a system of BSA adsorbed on sulfonated microspheres was studied. Poly (methyl methacrylate) (PMMA) microspheres were produced by dispersion polymerization. 2-acrylamido-2-methylpropane sulfoacid (AMPS, Sigma Chemical Co., USA) was used as functional monomer to

introduce the sulfonate groups on the surface of the microspheres. According to the different amount of AMPS, four types of microspheres were prepared. The corresponding concentrations of sulfonate group on the surface of the microspheres were 0 (microsphere 1),  $2.82 \times 10^{-2}$  (microsphere 2),  $3.55 \times 10^{-2}$  (microsphere 3) and  $4.18 \times 10^{-2}$  (microsphere 4) nmol/m<sup>2</sup>. Finally, for obtaining the adsorption isotherms, the modified PMMA microspheres (0.20 g) were incubated with 10 ml buffer solutions (at selected pH) containing different amounts of BSA (0.25–1 mg/ml) at 37 °C for 3 h. Interested readers are referred to Ref. [21] for a more complete description of the preparation of microspheres 1–4 and of the adsorption procedure.

Before starting the comparison, and in order to analyze adsorption from liquid solutions, it is convenient to write the theoretical isotherms given in Section 2 in a more appropriate form. In this framework, Eq. (3) adopts the form

$$Q = Q_{max} \frac{K_{LF} C^{1/n_{LF}}}{1 + K_{LF} C^{1/n_{LF}}}, \quad (12)$$

where ( $Q_{max}$ )  $Q$  represents the (maximum) amount of solute (BSA) adsorbed on the surface of the adsorbent (microspheres) and  $C$  is the concentration of the solute in the liquid.  $K_{LF}$  and  $n_{LF}$  are as in Eq. (3).

In the case of FSTA model (Eq. (11)), in addition to replacing pressure  $p$  by concentration  $C$  and surface coverage  $\theta$  by  $Q/Q_{max}$ , it is assumed that: since  $g = \text{constant}$ , if one molecule has  $m$  distinguishable ways of adsorbing per lattice site at zero density; then,  $g = mk'$  states are excluded when one molecule is adsorbed occupying  $k'$  sites on the lattice and  $a = 1/(mk') = 1/g$ . Thus, Eq. (11) can be rewritten as

$$K_e C = \frac{(Q/Q_{max})[g - (g-1)Q/Q_{max}]^{g-1}}{[g - g(Q/Q_{max})]^g}, \quad (13)$$

where  $K_e$  is as in Eq. (11).

As can be noticed, Eqs. (12) and (13) have the same number of adjustable parameters:  $Q_{max}$ ,  $K_{LF}$  and  $n_{LF}$ , Eq. (12); and  $Q_{max}$ ,  $K_e$  and  $g$ , Eq. (13). However, while  $K_{LF}$  and  $n_{LF}$  are empirical parameters,  $K_e$  and  $g$  carry meaningful quantitative information about adsorption energy and the spatial configuration of the admolecule, respectively.

In order to understand the basic phenomenology, we begin by revisiting the results of Hu et al. [21]. In Ref. [21], experimental adsorption isotherms obtained as described above were fitted by using the linearized form of the Langmuir–Freundlich isotherm:

$$\frac{1}{Q} = \frac{1}{Q_{max} K_{LF} C^{1/n_{LF}}} + \frac{1}{Q_{max}}. \quad (14)$$

In addition,  $n_{LF}$  was fixed at 0.5. In this scheme, each isotherm could be correlated by using  $Q_{max}$  and  $K_{LF}$  as adjustable parameters. The results obtained by Hu et al. [21] are collected in Table 1.<sup>1</sup>

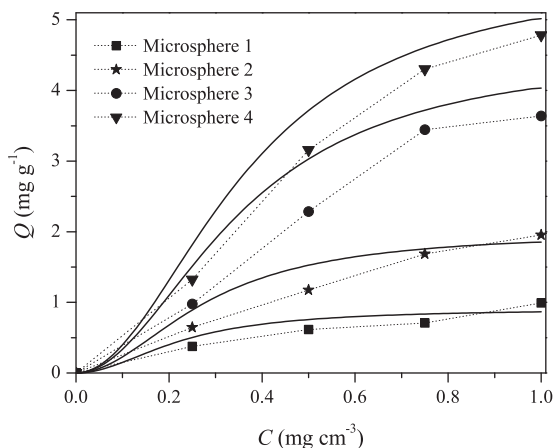
On the basis of the results in Table 1, Hu et al. [21] established that when the sulfonate groups on the surface of the microspheres increase, the  $Q_{max}$  value also increases. If hydrophobic interaction governs the process,  $Q_{max}$  values would decrease with increasing amount of hydrophilic groups. For that reason, the authors concluded that the electrostatic interaction governed the adsorption in this case. On the other hand,  $K_{LF}$  shows the opposite tendency to  $Q_{max}$ . Namely,  $K_{LF}$  decreases when the amount of sulfonate group increases. A high value of  $K_{LF}$  would indicate a faster adsorption to reach the equilibrium amount. Therefore, the authors concluded that the adsorption due to electrostatic interaction is higher than

<sup>1</sup> Table 1 in the present paper repeats the data in Table 2 of Ref. [21]. However, Table 1 has been included here to facilitate the reader with a self-contained presentation.

**Table 1**  
Fitting parameters corresponding to the study in Ref. [21].  $\Delta_Q$  is a measure of the deviation between experimental and theoretical data (see Eq. (17)).

Microsphere	$Q_{max}$ (mg/m <sup>2</sup> )	$K_{LF}$	$n_{LF}$	$\Delta_Q$ (%)
1	0.91	19.73	0.5 <sup>a</sup>	21.84
2	2.00	12.86	0.5 <sup>a</sup>	19.36
3	4.55	7.97	0.5 <sup>a</sup>	26.55
4	5.70	7.49	0.5 <sup>a</sup>	16.76

<sup>a</sup> The corresponding values were maintained to be constant during the fitting procedure.



**Fig. 1.** Adsorption isotherms of BSA on sulfonated microspheres at pH 4.3 and temperature 37 °C. Symbols correspond to experimental data from Ref. [21], and lines provide theoretical results from Eq. (12). The parameters used in the theoretical model are listed in Table 1.

that due to hydrophobic interaction, but the relative rate of adsorption is slower.

To visualize the quality of the fitting procedure in Ref. [21], experimental data were compared with theoretical adsorption isotherms from Eq. (3), using the values of the parameters reported in Table 1. The results are shown in Fig. 1, where symbols and lines represent experiment and theory, respectively. Clearly, the fitting is not satisfactory and, consequently, the statistical analysis does not support the conclusions reached by Hu et al. [21].

Then, as a first step to try to solve this problem, we decided to repeat the linear fit of the experimental data by using the linearized form of the Langmuir–Freundlich isotherm (Eq. (14)). For this purpose, the least-squares statistics were considered [45]. Thus, suppose that  $n$  data points  $(x_i, y_i, z_i; i = 1, \dots, n)$  are being fitted to a model that has  $L$  adjustable parameters  $(a_j; j = 1, \dots, L)$ . The model predicts a functional relationship between the measured independent and dependent variables,

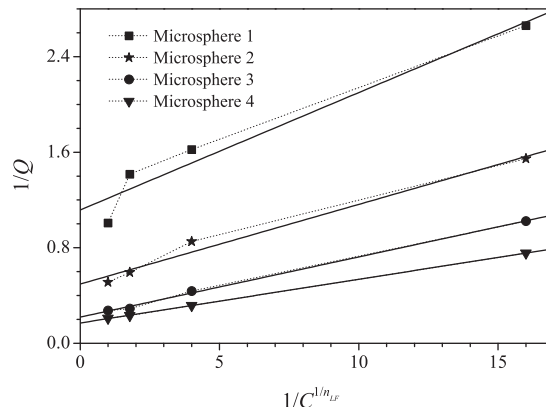
$$z(x, y) \equiv z(x, y; a_1, \dots, a_L) \tag{15}$$

where the dependence on the parameters is indicated explicitly on the right-hand side. What, exactly, do we want to minimize to get fitted values for the  $a_j$ 's? The first thing that comes to mind is the familiar least-squares fit, minimize over  $a_1, \dots, a_L$ :

$$\sum_{i=1}^{n_m} [z_i - z(x_i, y_i; a_1, \dots, a_L)]^2 \tag{16}$$

The parameters of the model are then adjusted to achieve a minimum in function (16), yielding best-fit parameters. The adjustment process is thus a problem in minimization in many dimensions.

The least-squares procedure was performed for each experimental isotherm. The results are shown in Fig. 2. In this case, Eq.

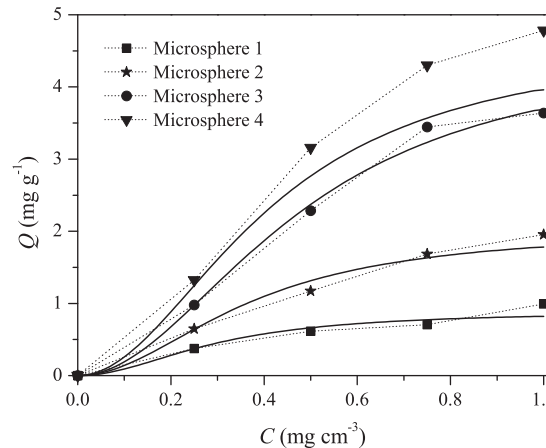


**Fig. 2.** Derivative of the adsorbed amount versus pressure ( $\ln p$ ) for the same set of data plotted in Fig. 1.

**Table 2**  
Fitting parameters corresponding to the data in Figs. 2 and 3.  $\Delta_Q$  is a measure of the deviation between experimental and theoretical data (see Eq. (17)).

Microsphere	$Q_{max}$ (mg/m <sup>2</sup> )	$K_{LF}$	$n_{LF}$	$\Delta_Q$ (%)
1	0.90	11.38	0.5 <sup>a</sup>	8.81
2	2.03	7.40	0.5 <sup>a</sup>	6.26
3	4.55	4.36	0.5 <sup>a</sup>	2.90
4	5.88	4.64	0.5 <sup>a</sup>	13.07

<sup>a</sup> The corresponding values were maintained to be constant during the fitting procedure.



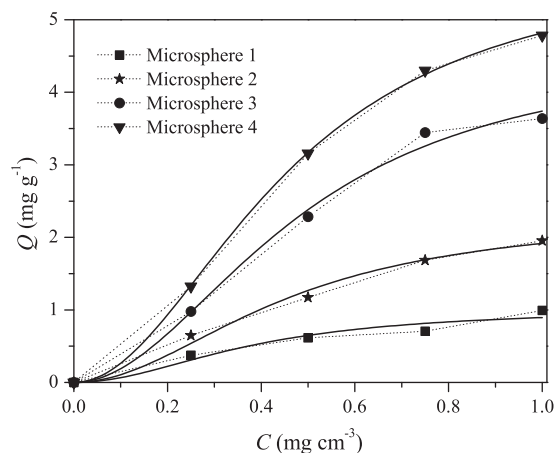
**Fig. 3.** Adsorption isotherms of BSA on sulfonated microspheres at pH 4.3 and temperature 37 °C. Symbols correspond to experimental data from Ref. [21], and lines provide theoretical results from Eq. (12). The parameters used in the theoretical model are listed in Table 2.

(14) corresponds to  $z(x, y) \equiv z(x, y; a_1, \dots, a_L)$ , being  $(Q_{max}K_{LF})^{-1}$  and  $Q_{max}^{-1}$  the adjustable parameters, which are calculated from the slope and the intercept, respectively. The fitting parameters are indicated in Table 2.

To compare with the results from Ref. [21], the procedure in Fig. 1 was repeated, but this time using the fitting parameters in Table 2. The results are shown in the Fig. 3. As can be observed, the settings are many better than those shown in the Fig. 1. However, it is important noteworthy that the linearity assumption is not valid for the complete range of values of  $C$ .

To solve the drawback of nonlinearity, adjustments can be made using Eq. (12) instead of using its linearized form. For that,





**Fig. 4.** Adsorption isotherms of BSA on sulfonated microspheres at pH 4.3 and temperature 37 °C. Symbols correspond to experimental data from Ref. [21], and lines provide theoretical results from Eq. (12). The parameters used in the theoretical model are listed in Table 3.

**Table 3**

Fitting parameters corresponding to the data in Fig. 4.  $\Delta_Q$  is a measure of the deviation between experimental and theoretical data (see Eq. (17)).

Microsphere	$Q_{max}$ (mg/m <sup>2</sup> )	$K_{LF}$	$n_{LF}$	$\Delta_Q$ (%)
1	1.02	6.91	0.5 <sup>a</sup>	11.84
2	2.31	4.86	0.5 <sup>a</sup>	6.77
3	4.62	4.25	0.5 <sup>a</sup>	3.24
4	5.83	4.68	0.5 <sup>a</sup>	0.86

<sup>a</sup> The corresponding values were maintained to be constant during the fitting procedure.

least-squares statistics were used but now Eq. (12) corresponds to  $z(x,y) \equiv z(x,y; a_1, \dots, a_L)$ , being  $K_{LF}$  and  $Q_{max}$  the adjustable parameters. The results are shown in the Fig. 4, and the fitting parameters are listed in Table 3.

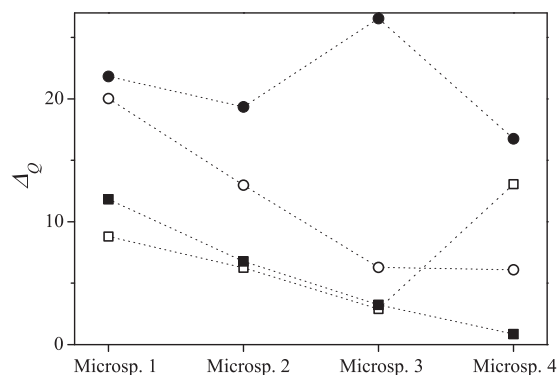
The differences between experimental and theoretical data can be very easily rationalized with the help of the percentage reduced coverage  $\Delta_Q$ , which is defined as:

$$\Delta_Q = \sum_{i=1}^n \left\{ 100 \cdot \left| \frac{Q_{theo}^i - Q_{exp}^i}{Q_{exp}^i} \right| \right\}, \quad (17)$$

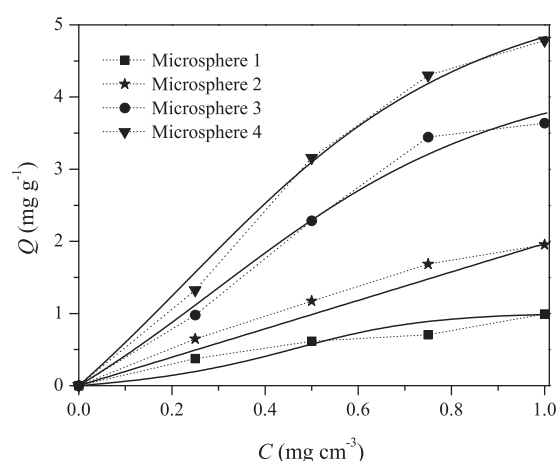
where  $Q_{exp}^i$  and  $Q_{theo}^i$  represent the experimental and theoretical values of the amount of solute adsorbed on the surface, respectively. Each pair of values ( $Q_{exp}^i, Q_{theo}^i$ ) is obtained at fixed concentration  $C$ .

The values of  $\Delta_Q$  obtained from the data in Figs. 1, 3 and 4 are listed in the fifth column of Tables 1–3, respectively, and are plotted in Fig. 5. Clearly, the best fitting is the one that uses Eq. (12) as fitting function. Then, the new parameters in Table 3 indicate that: (1)  $Q_{max}$  follows the functionality pointed out in Ref. [21]. Namely,  $Q_{max}$  increases as the amount of sulfonate groups increases; and (2)  $K_{LF}$  does not show a defined tendency with the amount of sulfonate groups on the surface of the microspheres.

Finally, Eq. (13) was used as fitting function. In order to analyze the behavior of  $K_e$ , and given the robustness of the values of  $Q_{max}$  obtained from the different fitting procedures, the maximum amount of solute adsorbed on the surface was set using the results in Table 3, being  $K_e$  and  $g$  the adjustable parameters. The results are presented in Fig. 6 and Table 4. In addition, the corresponding values of  $\Delta_Q$  were included in Fig. 5. The fitting is satisfactory and, as in the case discussed above,  $K_e$  does not appear as a sensitive parameter to the concentration of the sulfonate groups. On the other hand, a small growing of exclusion parameter was observed



**Fig. 5.**  $\Delta_Q$  (Eq. (17)) corresponding to the data in Fig. 1, solid circles; Fig. 3, open squares; Fig. 4, solid squares and Fig. 6, open circles.



**Fig. 6.** Adsorption isotherms of BSA on sulfonated microspheres at pH 4.3 and temperature 37 °C. Symbols correspond to experimental data from Ref. [21], and lines provide theoretical results from Eq. (13). The parameters used in the theoretical model are listed in Table 4.

**Table 4**

Fitting parameters corresponding to the data in Fig. 6.  $\Delta_Q$  is a measure of the deviation between experimental and theoretical data (see Eq. (17)).

Microsphere	$Q_{max}$ (mg/m <sup>2</sup> )	$K_e$	$g$	$\Delta_Q$ (%)
1	1.02 <sup>a</sup>	2.57	0.19	20.03
2	2.31 <sup>a</sup>	2.51	0.35	12.99
3	4.62 <sup>a</sup>	2.36	0.36	6.29
4	5.83 <sup>a</sup>	2.53	0.38	6.09

<sup>a</sup> The corresponding values were maintained to be constant during the fitting procedure.

as  $Q_{max}$  increased. The values of  $g$  (minor than unity) may be indicative that energetically BSA does not exclude other sites when adsorbed on the surface.

The detailed and rigorous study presented in this section (i) corroborates the results obtained by Hu et al. [21] with respect to the values of  $Q_{max}$  and (ii) indicates also that the behavior observed for  $K_{LF}$  in Ref. [21] is a direct result of an inappropriate fitting strategy.

#### 4. Conclusions

The adsorption isotherms of BSA onto sulfonated microspheres, published in the literature by Hu et al. [21] and measured for different concentrations of sulfonate group on the surface of the

microspheres, have been analyzed through two statistical models. The first one is the well-known LF model. The second, FSTA, is a statistical thermodynamics model, which incorporates the configuration of the molecule in the adsorbed state as a model parameter. LF and FSTA have the same number of adjustable parameters. However, while LF equation is written in terms of empirical parameters, the quantities involved in the FSTA equation carry meaningful quantitative information about adsorption energy and the spatial configuration of the admolecule.

A nonlinear regression technique was used in fitting the experimental data, where the isotherms were fitted with two free parameters. This procedure has been shown to be an improvement over the one presented in Ref. [21].

The combination of LF and FSTA models, along with an adequate fitting procedure, revealed that: (i)  $Q_{max}$  follows the functionality pointed out in Ref. [21]. Namely,  $Q_{max}$  increases as the amount of sulfonate groups increases; and (ii)  $K_e$  (or  $K_{LF}$ ) does not appear as a sensitive parameter to the amount of sulfonate groups on the surface of the microspheres.

The above findings, mainly the nondependence of  $K_e$  (or  $K_{LF}$ ) with the amount of sulfonate groups, lead to different conclusions from those drawn by Hu et al. [21] with respect to the interactions governing the adsorption process. In fact, according to Roach et al. [46], BSA is adsorbed more rapidly to  $\text{CH}_3$ -terminated surface than onto the OH-terminated surface, which can be due to hydrophobic interactions. So,  $K_e$  (or  $K_{LF}$ ) should not be affected by the presence of electrostatic charges on the surface, although BSA could be orientated in some extension by such groups to produce a higher protein adsorbed amount ( $Q_{max}$ ) onto hydrophobic surface portions. Such conclusions agree with the protein adsorption behavior observed due to changes in pH and ionic strength. Near to the BSA isoelectric point, the adsorption reaches a maximum, and a higher and lower pH results in the significant decrease in the adsorption amount [21]. Regarding such results, it is evident that the electrostatic interaction does not govern the adsorption process. Other interactions such as hydrophobic or hydrogen-bonding has been reported to produce more or less adsorption [1,18,24,25,29,46].

The exhaustive study presented here has shown that the FSTA model is a good one considering the complexity of the physical situation which is intended to be described and could be very useful in interpreting experimental data. However, further comprehensive analysis of experimental isotherms through the proposed formalism appears necessary to discern its applicability, reliability, and accuracy.

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