Available Area Isotherm

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A new isotherm is presented for adsorption of proteins, the available area isotherm. This isotherm has a steric basis, unlike the (steric) mass action model. The shape of the available area isotherm is determined only by geometric exclusion. With the new isotherm, experimental results can be fitted equally well as with existing ones, such as the steric mass action model. However, it has several advantages. For fitting of single-protein isotherms one less parameter is needed, its theoretical basis is more realistic, it can be extended consistently to many components, it is applicable to adsorption by both ion-exchange and hydrophobic interaction, and it can easily be combined with equations describing the effect of pH and ionic strength to obtain a complete description of adsorption behavior. © 2004 American Institute of Chemical Engineers *AIChE J,* 50: 848–853, 2004

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Introduction

Understanding the adsorption behavior of proteins is important for the development of chromatography as a preparative separation method. The design of efficient chromatographic techniques, like simulated moving bed chromatography, requires thorough understanding.

An overview of adsorption isotherms of proteins in liquid chromatography is given by Bellot and Condoret (1993). The Langmuir isotherm is the most widely used isotherm for protein adsorption. The *steric* mass action model (Brooks and Cramer, 1992) is a model for adsorption by ion exchange. This model usually gives good agreement with experimental results (after fitting), and thus is considered to be the best model for protein adsorption.

The two isotherms mentioned above were developed for adsorption of molecules much smaller than proteins. Incorporation of the large size of proteins in the steric mass action model is somewhat artificial. In this article we propose a new isotherm that takes geometrical exclusion due to already adsorbed proteins into account: the available area isotherm. This isotherm is not restricted to adsorption due to ion exchange (like the steric mass action model), but it is a general model for adsorption of proteins. The new isotherm fits experiments equally well as the steric mass action isotherm. However, it allows a more fundamental understanding of protein adsorption and has a better thermodynamic basis.

Below we first derive the available area model and, to allow comparison, of the two other models mentioned. In the discussion these models are compared from various perspectives.

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Derivation of Isotherms

In this section the available area isotherm is derived. To allow comparison with other models, the Langmuir and the steric mass action isotherms are also derived. Finally, a way of applying the available area isotherm to ion-exchange equilibria is introduced.

The available area isotherm

The model considers the proteins as spheres (or disks). Each protein has its own radius; other geometrical parameters are not required. The surface of adsorption is flat with a homogeneous binding strength. The spheres are distributed randomly on this surface but are not allowed to overlap. Random distribution implies that the adsorbed proteins show negligible repulsive or attractive interactions. Figure 1 illustrates the model. The proteins cover a certain fraction of the surface, χ. New spheres cannot overlap those already on the surface, so they adsorb only if their center is at least one radius away from all neigh-

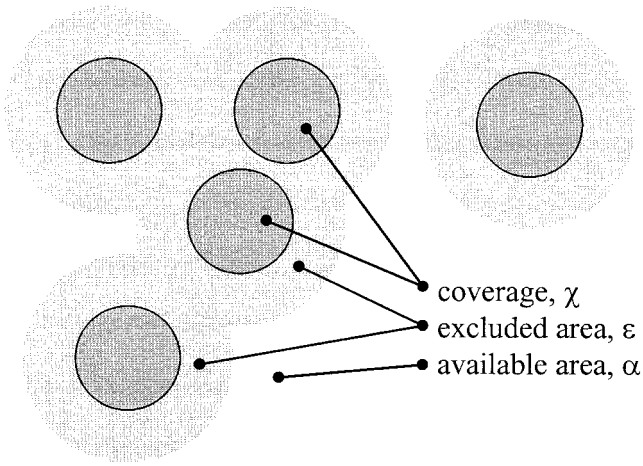

Figure 1. Coverage by adsorbed proteins and the excluded and available area for proteins in solution.

bors. This leads to a fraction ε of the surface being "excluded area." The area excluded by one protein for an equally sized protein is four times the area of the protein. When many proteins are adsorbed, the excluded areas show a considerable overlap. The space left is the "available area" fraction, α.

The derivation starts with the same assumption as in the Langmuir equation: at equilibrium the adsorption rate and the desorption rate are equal. As in the Langmuir equation the desorption rate is proportional to the surface coverage. The adsorption rate is proportional to the concentration in the liquid and, in contrast with the Langmuir equation, to the available area. We obtain

$$k_{ads} c \alpha = k_{des} \chi \Rightarrow c = K \frac{\chi}{\alpha} \tag{1}$$

where the k values are rate constants, c is the unadsorbed concentration, and K is an equilibrium constant. To complete the isotherm a relation between the coverage and the available area is needed. An exact expression can be derived only for low surface coverages; a general relation will be derived through numerical simulations.

In this article we consider only single-component isotherms; however, the concepts can also be applied to multicomponent isotherms.

Available area at low surface coverage

It is easy to calculate the area that one protein will exclude for the others. However, for higher concentrations, overlap between excluded areas will occur. At low surface coverage this overlap can be accounted for by using a model analogous to that for describing the concentration dependency of the steric exclusion of proteins from fibrous structures (Bosma and Wesselingh, 2000).

On a real surface proteins adsorb at random locations, provided they do not overlap. On the model surface the proteins are allowed to adsorb at *entirely* random locations: they are allowed to overlap. When a certain amount of protein is adsorbed, a certain area fraction χ is covered by proteins. The total covered area, counting double covered double and so forth, will be larger than χ, an entity we have designated $\chi_{overlap}$. We can then imagine the process of increasing the radius of the adsorbed proteins and decreasing the radius of the dissolved proteins, while keeping their sum constant. During this process the available area for (the center of) the dissolved protein will not change. The oversized proteins will now occupy a total area $\varepsilon_{overlap}$, which includes overlapping excluded areas. The relation between $\chi_{overlap}$ and $\varepsilon_{overlap}$ is simple

$$\varepsilon_{overlap} = 4\chi_{overlap} \tag{2}$$

Because of the geometric similarity the obtained $\varepsilon_{overlap}$ is related to the excluded area ε in the same way as $\chi_{overlap}$ and χ are related. When overlap is not allowed, the fraction of the area that is not covered decreases linearly with increasing coverage; when overlap is allowed it decreases exponentially with increasing coverage. The relations sought for are

$$1 - \chi = e^{-\chi_{overlap}} \tag{3}$$

and

$$1 - \varepsilon = e^{-\varepsilon_{overlap}} \tag{4}$$

The last equation also gives the available area fraction ($\alpha = 1 - \varepsilon$). Combining the equations gives the available area at low surface coverage

$$\alpha = (1 - \chi)^4 \tag{5}$$

Available area at high surface coverage

At high surface coverage the model used above breaks down. This problem can be studied by numerical simulation of the surface coverage. In these calculations a square surface area was used with a length between 10 and 100 times the protein radius. A calculation started with an empty surface. Then up to 10^9 attempts were made to adsorb a new protein at a randomly chosen position. An attempt was successful if the protein did not overlap already adsorbed proteins. At each boundary it was assumed that the surface continued on the other side of the square. During a calculation the number of unsuccessful attempts was recorded at each value of the surface coverage. After these attempts the surface was searched for small available areas left to obtain the maximum coverage. After a large number of calculations the available area at each value of the surface coverage was obtained by dividing the number of successful attempts by the total number of attempts at that surface coverage. The maximum coverage was also obtained.

The value of the maximum coverage χ_{max} is 0.5470. The standard deviation of the maximum coverage depends on the size of the surface. With the length of the square being 100 times the protein radius, it was 0.0032. The results for the available area are shown in Figure 2. An empirical modification of Eq. 5 can accurately describe these results

$$\alpha = \left(1 - \frac{\chi}{\chi_{\max}}\right)^{(4+5.5\chi^2)\chi_{\max}} \tag{6}$$

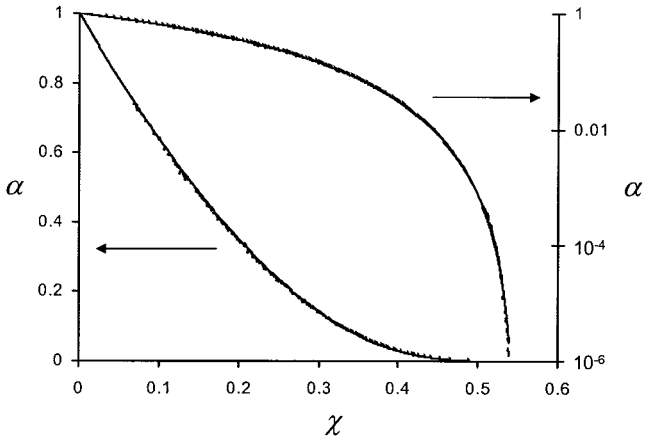

Figure 2. Available area for adsorption vs. surface coverage for a single protein: —, Eq. 6; - - -, numerical calculations.

For low surface coverages, Eqs. 5, 6, and the numerical simulations give the same results.

We conclude this section with an explicit equation for the available area isotherm. By combining Eq. 1 with Eq. 6 and replacing the absolute coverage χ by the relative coverage θ ($=\chi/\chi_{max}$), we find

$$c = K\theta(1 - \theta)^{-2.2-0.9\theta^2} \tag{7}$$

This equation is a little complicated. As shown in the discussion section, with the steric mass action model the goodness of fit is insensitive to the value of the power, so a simpler expression can be used

$$c = K\theta(1 - \theta)^{-2.7} \tag{8}$$

The power of -2.7 is an average of the power in Eq. 7. Equation 8 is similar to the steric mass action model; the difference is that the power is now a constant.

Langmuir isotherm

Here it is assumed that the adsorption surface contains a certain number of discrete adsorption sites. The isotherm is derived by assuming that the adsorption rate is proportional to both the unadsorbed concentration c and the fraction of free adsorption sites $1 - \theta$, and assuming that the desorption rate is proportional to the fraction of occupied adsorption sites θ. Setting both rates equal gives the equilibrium equation

$$k_{ads}c(1 - \theta) = k_{des}\theta \Rightarrow c = K\frac{\theta}{1 - \theta} \tag{9}$$

Here θ is the surface coverage defined by: $\theta = q/q_{max} = \chi/\chi_{max}$, where q and q_{max} are the adsorbed concentration and the adsorption capacity, respectively.

Steric mass action model

The mass action model describes the adsorption of small multivalent ions. Kopaciewicz et al. (1983) proposed to apply it also to protein adsorption, designating it the stoichiometric displacement model.

The mass action model considers protein adsorption as an exchange reaction of a protein and a number of small ions z, which we will designate the binding charge. This gives an equilibrium equation that can be written as

$$k_{ads}c(1 - \theta)^z = k_{des}\theta c_{ion}^z \Rightarrow c = Kc_{ion}^z\frac{\theta}{(1 - \theta)^z} \tag{10}$$

where z is the binding charge, c_{ion} is the small ion concentration in solution and $1 - \theta$ is proportional to the adsorbed small ion concentration that can be replaced by proteins. Ideally the protein adsorption capacity is equal to $1/z$ times the small ion capacity. However, this is not true: the small ion capacity is usually much higher. This problem is resolved by the *steric* mass action model. In this model a protein not only exchanges with z small ions but also shields a number of small ions (Brooks and Cramer, 1992). This number can differ for different proteins.

The steric mass action model does not change the results of calculations: the equilibrium is still described by Eq. 10.

Available area isotherm for ion exchange

The available area isotherm predicts the effect of the protein concentration on the relative changes in the slope at higher protein concentrations. Isotherms can be measured at various ionic strengths. To obtain an equilibrium expression, including the effect of ionic strength, the available area model has to be combined with a model for equilibrium at infinite dilution. This model will determine the initial slope of the isotherm. In the remainder of this section some possibilities for such a model are discussed. We will use one of these to compare this combined model with the steric mass action model.

An empirical theory for the effect of pH and ionic strength on the equilibrium coefficient is given by Bosma and Wesselingh (1998). More fundamental models, based on a solution of the Poisson Boltzmann equation, are proposed by Roth et al. (1996), Roush et al. (1994), Ståhlberg et al. (1991), and Jönsson and Ståhlberg (1999). These are models for the adsorption equilibrium at low surface coverage, which describe the initial slope of the isotherm. These models can be combined with the available area model, but not with the steric mass action model, because the steric mass action model already contains an empirical effect of ionic strength. Later in this article we use a combination of the available area model with a *local* mass action model, the simplest option to describe the effect of ionic strength on the initial slope.

We apply the mass action model locally, in the area of the adsorbing protein. The idea is that the ionic interactions between a protein and the surface occur at the area of contact. Whether other proteins are adsorbed nearby does not matter; we assume that they are too far off to affect the electrical forces. The *local* small ion concentration in the area of contact is equal to the small ion capacity of the ion exchanger q_{ion},

which is constant. The "available area with local mass action model" is given by

$$k_{ads}c\alpha q_{ion}^{z} = k_{des}\chi c_{ion}^{z} \Rightarrow c = K\left(\frac{c_{ion}}{q_{ion}}\right)^{z}\frac{\chi}{\alpha} \qquad (11)$$

Equation 11 is the same as Eq. 1 but with an equilibrium constant that depends on the ionic strength.

Discussion

This discussion compares the available area isotherm and the steric mass action model. The comparison is made on different levels. First, theoretical foundations are compared; this is a thermodynamic discussion. Then the similarities between the isotherms and their capability to fit experimental data are discussed.

Theoretical foundation of the isotherms

The mass action model is originally for small molecules such as metal ions. The steric mass action formalism attempts to make the mass action model theoretically acceptable when the adsorbing molecules have large size differences, such as for proteins and small ions. There are, however, three problems with the steric mass action model.

The first is with the definition of the surface fraction $(1 - \theta)$ of the adsorbed small molecules in Eq. 10. Three choices are possible:

(1) Using the adsorbed small ions that can be replaced by proteins

(2) Using the adsorbed small ions that are not shielded by adsorbed proteins

(3) Using all the adsorbed small ions

Usually the first choice is made without discussion. The second gives isotherms that differ only slightly from the first; it is never used. The third one is the best defined, and preferable from a thermodynamic perspective. However, it does not fit well in the steric mass action model.

A second problem is the twofold effect of the binding charge z, in the steric mass action model. The binding charge influences the shape of the isotherm: a higher binding charge makes an isotherm (when it is fitted to data points) initially steeper, then less steep, and finally steeper again. However, the binding charge also accounts for the effect of ionic strength on the initial slope. For ion exchange of small ions this relation is well accepted, but should it also be accepted for protein ion exchange? As mentioned, more fundamental models for protein ion exchange describe the interaction between a single protein and the ion exchanger surface with the Poisson Boltzmann equation. From the solutions of these models at varying ionic strengths an apparent binding charge can be calculated. But why should the shape of the isotherm be related to this apparent binding charge? In other words, the steric mass action model contains a relationship that more fundamental models do not contain.

A third problem appears when one tries to picture the mechanism of protein adsorption and relate this to the adsorption rate equation. According to the steric mass action model (Eq. 10), when the adsorbed amount of proteins increases, the adsorption rate decreases *because of a change in ionic interactions* (given that $1 - \theta$ represents the adsorbed small ion concentration). However, ionic interactions occur mainly in the area shielded by a protein because the Debye length is smaller than the protein size. The Debye length characterizes the distance over which ionic interactions occur, which is about 1 nm in a 0.1 molar 1/1 electrolyte. The size of a protein is about 3 nm. The ionic interactions between a protein and the adsorption surface should then be independent of the adsorbed protein concentration and the adsorption rate should decrease with increasing coverage *as a result of increasing steric hindrance*.

However, the available area model can also be criticized. First, spherical proteins and a flat adsorption surface are assumed. Proteins are not spherical and protein adsorption media are not flat; for example, Sepharose media are fibrous. However, we believe that our assumptions are reasonable: proteins are compact molecules and Sepharose fibers usually have diameters larger than those of proteins. Second, repulsive or attractive interactions between proteins or adsorption of proteins on already adsorbed proteins are neglected. However, the Langmuir isotherm and the steric mass action model also neglect these interactions. Furthermore, various types and degrees of interactions would have to be considered.

Similarities between the isotherms

It has been observed that the (steric) mass action model can well describe the effect of ionic strength on the adsorption equilibrium with parameters that do not depend on the ionic strength (Bosma and Wesselingh, 1998; Karst Lewus and Carta, 1999). Therefore we use the steric mass action model as a reference.

We tried to fit the Langmuir and the "available area with local mass action model" to the steric mass action model. For the Langmuir model (Figure 3) both the equilibrium constant and the adsorption capacity depend on the ionic strength. The figure also shows that the available area with local mass action isotherm has the same shape as that of the steric mass action

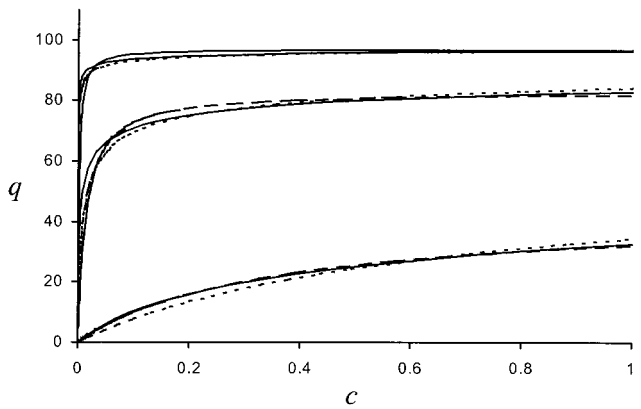

Figure 3. Comparison of the mass action isotherm (—) with the available area mass action isotherm (- - -) and the Langmuir isotherm (– – –).

The mass action isotherms were calculated with Eq. 10 with $q_{max} = 100$, $K = 10$, $z = 4$, and $c_{ion} = 0.5$, 0.1, and 0.02, respectively, from weak to strong binding. The available area isotherms were fitted to the mass action isotherms with Eq. 7 with $K = K'c_{ion}^{z}$, with $q_{max} = 100$, $K' = 10$, and $z = 3.2$. The Langmuir isotherms were fitted with Eq. 9 with ($q_{max} = 43$, $K = 0.34$), ($q_{max} = 83$, $K = 0.014$), and ($q_{max} = 97$, $K = 0.0018$), respectively, from weak to strong binding.

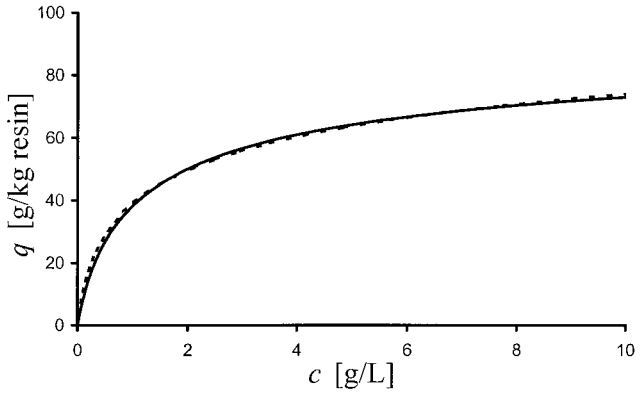

Figure 4. Mass action isotherms with $z = 2$ (—) and $z = 6$ (- - -) fitted to each other.

Parameters: ($K = 1$, $z = 2$, $q_{max} = 100$) and ($K = 1.1$, $z = 6$, $q_{max} = 183$).

isotherm. The low surface coverage limits found with the two isotherms are different ($z = 3.2$ vs. $z = 4$); this reflects that fact that, when data points at higher coverage are used for fitting, the two fitted isotherms have different limits at low coverage. With respect to the fitting of experimental data, the available area model and the steric mass action model perform about equally well.

In Figure 4 we fitted a steric mass action isotherm with $z = 6$ to an isotherm with $z = 2$. As can be observed, a good fit can be obtained. This shows how deceiving the statement "z determines the isotherm shape" is. It can be concluded that, for fitting of individual isotherms with the steric mass action model, the third fitting parameter z is superfluous.

There may be cases in which the steric mass action model has the advantage of being more flexible because of its third fitting parameter. For instance, a related group of isotherms with $z = 6$ (similar to the group of mass action isotherms in Figure 3) cannot be fitted very well to such a group with $z = 2$. Whether this is an advantage will become clear when the models are compared to such measured groups of isotherms. This is done in the next section.

Comparison with experiments

We compared the "fitting capability" of the steric mass action and the available area models by fitting them to measured isotherms reported in the literature. The isotherms we used are those measured by Raje and Pinto (1998), Li and Pinto (1995), Bowen and Pan (1997), Yoshida et al. (1994), James and Do (1991), and Huang and Horváth (1987).

We used three fitting methods:

(1) First, we compared all measured isotherms individually to both models. We fitted the adsorption capacity, the equilibrium constant, and, only for the steric mass action model, the binding charge. All measurements can be described well by both models. As a fitting parameter, the binding charge in the steric mass action model seems to be superfluous, given that good fits can be obtained at various values of this parameter (between $z = 2$ and $z = 8$ the minimum sum of squared errors varies less than 7%; see also Figure 4).

(2) Second, we made a comparison by fitting the equilibrium constant for each isotherm individually and fitting the adsorption capacity and, only for the steric mass action model, the binding charge for groups of isotherms. Within these groups only the ionic strength or the pH differed between the isotherms. We found that all measurements can still be well described by both models. In more than half of the groups the sum of squared errors of the steric mass action model was less than 2% lower than that of the available area model, even though the steric mass action model has one more fitting parameter.

(3) Third, we made a comparison by fitting all the parameters for groups of isotherms. In this case the available area isotherm was combined with the local mass action model, also giving this model three fitting parameters. Only the groups of measured isotherms with a varying ionic strength were used in the comparison. Again it was found that both models can well describe the measurements. The results for the fitted parameters are given in Table 1, along with results for the sums of squared errors. For case 1b, Figure 5 graphically shows the performance of the models. The differences between the models and the measurements are small.

It is striking in Table 1 that for all cases, except case 4a, the fitted value of the binding charge z scarcely depends on the model used. Because in the available area model z purely represents the effect of ionic strength, it appears that z in the steric mass action model also represents the effect of the ionic strength and, only for a small part, that of the shape of the isotherm.

Table 1. Results of Fitting the Models to Measured Data*

	Available Area Model				Steric Mass Action Model			
Source	K_0	q_{max}	z	e_{AA}	K_0	q_{max}	z	e_{AA}/e_{SMA}
1a	4.81×10^{-10}	120	4.46	4.77	5.85×10^{-3}	146	5.33	1.82
1b	4.43×10^{-10}	104	4.48	1.29	6.72×10^{-3}	130	4.67	1.69
2	7.57×10^{-14}	237	6.06	1.50	3.28	441	6.52	1.53
3	3.54×10^{-4}	250	1.87	0.032	2.94×10^{-2}	213	1.77	1.00
4a	7.04×10^{-6}	119	3.16	11.4	8.66×10^{-2}	115	6.52	1.54
4b	1.10×10^{-4}	124	2.70	3.72	0.309	119	3.19	1.20
4c	8.78×10^{-9}	104	4.30	1.52	4.41×10^{-2}	124	4.79	0.85
5a	1.80×10^{-4}	329	2.59	0.046	1.83	160	2.73	1.13
5b	4.53×10^{-6}	322	2.93	0.59	1.78	325	2.67	0.68
5c	3.53×10^{-4}	154	2.68	0.11	0.198	329	2.53	0.86

*For details see text; e refers to the sum of squared errors.
†The numbers in the first column refer to Raje and Pinto (1998), Figure 3 (1a) and Figure 4 (1b); Li and Pinto (1995) (2); Yoshida et al. (1994) at pH 6.9 (3); James and Do (1991), Figure 1 (4a), Figure 2 (4b), and Figure 3 (4c); and Huang and Horváth (19897), Figure 2 (5a), Figure 3 (5b), and Figure 4 (5c).

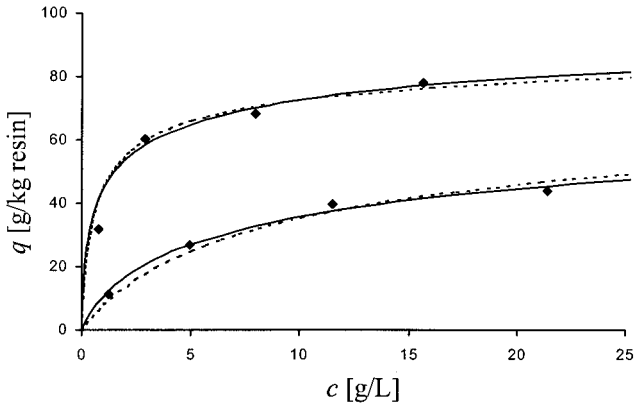

Figure 5. Representation of measured (Raje and Pinto, 1998; Figure 4) and fitted isotherms for the adsorption of lysozyme on a weak cation exchanger at pH 7.0 at two salt concentrations: —, steric mass action model; - - -, available area isotherm.

Overall we conclude that measured isotherms can be fitted well by both models and that the binding charge z in the steric mass action model is a superfluous parameter.

Conclusions

The available area isotherm has a different thermodynamic basis than that of existing isotherms. It takes the effect of the large size of proteins and the accompanying geometrical exclusion into account. The shape of the isotherm is determined by this effect and not by the charge of the proteins, as in the commonly used steric mass action model. For a single protein both isotherms are equally satisfactory. However, the new isotherm has several advantages:

- It has a better thermodynamic foundation.
- It can be combined with any model for the effect of pH or ionic strength on the initial slope, such as a more fundamental model based on the solution of the Poisson Boltzmann equation. In the steric mass action model the effect of ionic strength on the initial slope is already included.
- It is a general isotherm for large molecules, not only for ion exchange but also for adsorption attributed to hydrophobic interaction.
- For fitting of a single isotherm the available area model needs two parameters, whereas the steric mass action model needs a third parameter z, which is supposed to affect the isotherm shape, but is actually superfluous (see Figure 4).

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