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Chemical Engineering Journal 81 (2001) 179–186

Chemical Engineering Journal

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A modified Langmuir model for the prediction of the effects of ionic strength on the equilibrium characteristics of protein adsorption onto ion exchange/affinity adsorbents

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Received 25 June 1999; received in revised form 12 May 2000; accepted 22 May 2000

Abstract

An empirical model modified from the Langmuir isotherm model to account for the effects of ionic strength on the equilibrium characteristics of protein adsorption onto ion exchange/affinity adsorbents has been proposed and tested against experimental and literature data. The equilibrium isotherms for BSA adsorption onto a polystyrenic anion exchanger, Diaion HPA25, were established for five different NaCl concentrations at 25°C, pH 7.0. The apparent Langmuir parameters in the new model (q'_m and K'_d), which replace the Langmuir parameters (q_m and K_d) in the original Langmuir model, were determined by non-linear curve fitting. The proposed model has been shown to be applicable to various protein/adsorbent systems.

Keywords: Ion exchange; Protein adsorption; Ionic strength; Langmuir isotherm; Modeling

1. Introduction

The ability to characterize equilibrium accurately is important in all the chromatography processes including ion exchange chromatography and affinity chromatography. No prediction of column performance could be done without first measuring or estimating the adsorption isotherms of these components under the conditions of interest, and the assurance of the prediction is strongly dependent on the accuracy of these data.

Ionic strength has long been recognized as one of the most important factors affecting the equilibrium characteristics of the adsorption of proteins onto ion exchange/affinity adsorbents. Isotherm data regarding the influence of ionic strength on the protein adsorption have been obtained by studies of various investigators and have been reported in different sources over the years. Finette et al. [1] studied the effects of ionic strength and temperature on the equilibrium characteristics of proteins adsorbed to ion exchangers and affinity adsorbents and noted that ionic strength had much stronger effects than that of temperature. By studying the adsorption of bovine serum albumin (BSA) and other proteins on porous and non-porous anion exchangers, Janzen and Unger [2] concluded that the increased ionic strengths lead to lower binding strength (higher K_d) and lower binding capacity. Similar phenomena have been reported for various protein/adsorbent pairs by different authors [3–6]. However, to our knowledge, no systematic effort has been made to assemble those data into an informational model for the prediction of ionic strength on protein adsorption.

In this paper, we report an empirical model, modified from the Langmuir isotherm model, based on the analysis of literature data for the prediction of the effects of ionic strength on the isotherm of protein adsorption. The model is further tested against the experimental data obtained with BSA adsorption onto a polystyrene-based anion exchanger, Diaion HPA25.

2. Theory

2.1. Langmuir isotherm

Many different isotherm models, such as the linear, the Langmuir, the bi-Langmuir, the Fowler, the Freundlich and the Freundlich–Langmuir isotherms, have been proposed for the adsorption of solutes in a liquid solution onto a solid surface. Most of those models are essentially empirical although theoretical derivations have been accomplished in some cases. Among all those models, the Langmuir model is probably the most popular one due to its simplicity and

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1385-8947/01/$ – see front matter
PII: S1385-8947(00)00197-2

Nomenclature

C	equilibrium concentration of protein in the bulk solutions (mg/ml or M)
I	concentration of the small salt in the bulk solution (M)
K_d	dissociation coefficient of the protein–ligand complex (mg/ml or M)
K'_d	apparent dissociation coefficient of the protein–ligand complex (mg/ml or M)
q	equilibrium protein concentration in solid phase (mg/g or mol/g)
q_m	maximum protein binding capacity (mg/g, mg/ml, mol/g or M)
q'_m	apparent maximum protein binding capacity (mg/g, mg/ml, mol/g or M)

Greek symbols

η	empirical equation constant (M)
ψ	empirical equation constant (M)

its good agreement with experimental data. It is based on the following equation:

$$q = \frac{q_m C}{(C + K_d)} \tag{1}$$

where q and C are the equilibrium concentration of solute in solid and liquid phases, respectively. Constants q_m and K_d are Langmuir parameters. The constant q_m represents the maximum binding capacity and K_d is the dissociation coefficient of the solute–adsorbent complex, which represents the affinity between the solute and the adsorbents.

The Langmuir isotherm for the adsorption of solute from liquid solution was first expanded directly from the corresponding isotherm of gas–solid adsorption and was later derived thermodynamically [7], kinetically [8] and stoichiometrically [1]. All those derivations are based on a few common assumptions, namely, (1) all binding sites are equivalent, distinguishable and independent; (2) each binding site combines with only one solute molecule; and (3) a molecule adsorbed onto one binding site does not influence the adsorption of another molecule on a neighboring binding site. The Langmuir isotherm has been widely accepted as a practical method for integrating experimental data of protein adsorption onto ion exchange/affinity adsorbents. However, in the case of protein adsorption, some of the above assumptions are not necessarily realistic and the Langmuir model is more an empirical model than a rigorous theoretical one.

2.2. Effects of ionic strength on Langmuir parameters

The effect of ionic strength on the protein adsorption onto ion exchange/affinity adsorbents is a very complex phenomenon. It could be considered from the aspects as follows:

1. The salt counter ions compete against the protein ions for binding sites.
2. The salt co-ions shield the protein ions and the charged binding sites from each other.
3. The change of ionic strength changes the folding and configuration of protein molecules [9], resulting in variation of the hydrophobic interaction between the protein and the resin matrix.
4. The increase of ionic strength may cause the network of the resin to shrink, reducing the porosity of the resins and hence the availability of binding sites [10].

There is clearly a need to develop a model to incorporate the effects of ionic strength on the protein adsorption, but it is very difficult to develop a theoretical model for the prediction of such a complex effect. Instead, a semi-empirical model, modified from the Langmuir isotherm model, is proposed here for a bi-component (one protein–salt) system

$$q = \frac{(q_m/(1 + I/\psi))C}{C + (\eta/\eta - I)K_d} \tag{2}$$

where I is the concentration of the salt, q and C are the protein concentrations in the bulk solution and on the adsorbent, respectively; η and ψ are empirical constants and K_d and q_m are the 'true' Langmuir parameters which are independent of the salt concentration. They are determined either by experimental data from non-salt protein solution or by the methods described later.

Let

$$K'_d = \frac{\eta}{\eta - I} K_d \tag{3}$$

and

$$q'_m = \frac{q_m}{1 + I/\psi} \tag{4}$$

Eq. (2) is therefore

$$q = \frac{q'_m C}{(C + K'_d)} \tag{5}$$

Eq. (5) is an expression of Langmuir model of protein adsorption under the influence of salt ions. Constants K'_d and q'_m are apparent Langmuir parameters. The apparent Langmuir parameters can be obtained by integrating the experimental data at each individual salt concentration with the Langmuir isotherm by the semi-reciprocal plot, the Scatachard plot, or by non-linear curve fitting.

2.3. Apparent dissociation coefficients K'_d, dissociation coefficient K_d and constant η

The dissociation coefficient (K_d) is a measure of the affinity of the protein to the ion exchanger. As pointed out previously, it is independent of ionic strength. Equation constant η represents the dependence of the apparent dissociation coefficient on the ionic strength. The smaller the value of η, the more significant the dependence of the

apparent dissociation coefficient on the change of ionic strength. In the process of determining model parameters of a specified system, the apparent dissociation coefficient (K'_d) is determined directly from the experimental data. On the other hand, K'_d at different ionic strength could be calculated according to Eq. (3) provided that constants η and K_d have been determined previously. Eq. (3) describes the relationship between K'_d, K_d and I. When I=0, Eq. (3) gives K'_d=K_d. Hence, K_d could in theory be measured directly in a protein solution of apparently zero ionic strength.

However, in actual practice, it is not realistic to have such a condition since the stock protein will have some salts in it. It is therefore more favourable to obtain the dissociation coefficient K_d and the constant η by rearranging Eq. (3). This gives a linear equation as follows:

$$\frac{1}{K'_d} = \frac{1}{K_d} - \frac{\eta}{K_d} I \tag{6}$$

Graph of $1/K'_d$ versus I gives a straight line of an intercept of $1/K_d$ and a slope of $-\eta/K_d$.

2.4. Apparent maximum capacity q'_m, maximum capacity q_m and constant ψ

Eq. (4) describes the relationship between the apparent maximum capacity, maximum capacity and the salt concentration. The apparent maximum binding capacity, q'_m, is a function of salt concentration, I, for a specified protein/adsorbent system. When I=0, Eq. (4) gives q'_m=q_m. This confirms the argument that q_m is the maximum capacity at zero ionic strength and is independent of the concentration and the type of the salt. However, in a similar fashion to the determination of K_d, it is more accurate to measure the value of q_m by regressing the apparent Langmuir parameters obtained at various individual salt concentrations.

The equation constant ψ is a lumped constant which represents the effects of ionic strength on the binding capacity of the ion exchanger to the protein. It is an indication of the interaction between the protein and the salt ions. The smaller the value of ψ, the more dependent the apparent capacity is on the ionic strength. The values of q_m and ψ can also be determined by rearranging Eq. (4)

$$\frac{1}{q'_m} = \frac{1}{q_m} + \frac{1}{q_m \psi} I \tag{7}$$

Plotting $1/q'_m$ versus I gives a straight line with an intercept of $1/q_m$ and a slope of $1/q_m\psi$. In the process of modeling, the apparent maximum binding capacity at different ionic strength could be calculated according to Eq. (4) with given constants q_m and ψ.

3. Experimental

Bovine serum albumin (BSA), Anion exchange resin Diaion and other chemicals were bought from Sigma (St. Louis, MO). Equilibrium studies were conducted by weighing exactly 0.5 g wet ion exchange beads Diaion HPA25 into a 50 ml flask containing 25 ml of 2 mM phosphate buffer (pH 7.0) of appropriate concentrations of BSA and sodium chloride. The flask was then sealed by parafilm 'M' (American Can Company) and put on a shaker controlled at 25°C and 200 rpm. After adsorption for 36 h (previous experiments had shown that equilibrium was reached under this condition), the liquid phase was centrifuged at 10,000 rpm for 10 min. The protein concentration of the supernatant was analyzed by Lowry's method. Samples of high protein concentration were diluted to ensure that the protein concentration did not exceed the upper limitation of the Lowry's method.

4. Results and discussion

4.1. Isotherms of BSA adsorption onto Diaion HPA25

From the experimental data obtained from the batch adsorption experiments under different ionic strength at 25°C and pH 7.0, the adsorption isotherms were constructed and are shown in Fig. 1. The apparent maximum capacity (q'_m) and the apparent dissociation constant (K'_d) at each salt concentration were determined using non-linear curve fitting. Data were also analyzed using the semi-reciprocal plots (C/q versus C) and the Scatchard plots (q/C versus q) and these are discussed later. It should be noted that in the experimental data, the salt concentration refers to the sodium chloride added to the solution. The actual ionic strength will be slightly higher than that generated by the sodium chloride due to additional ions provided by the phosphate buffer and

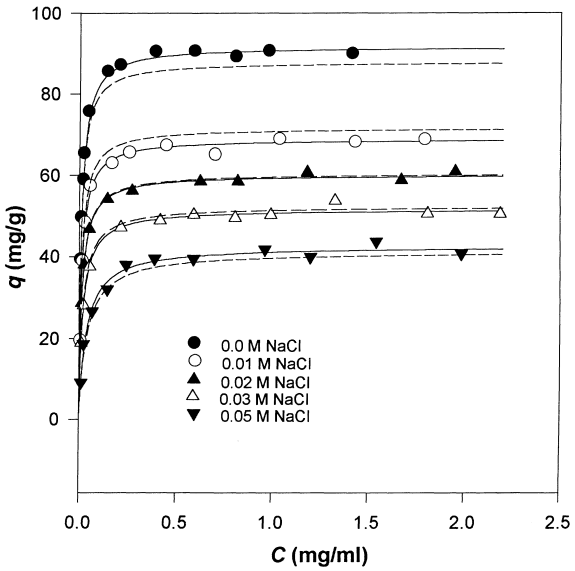

Fig. 1. Comparison between the experimental isotherms (symbol), best-fit Langmuir isotherms for given individual NaCl concentration (—) and the isotherms predicted by the modified Langmuir model (----).

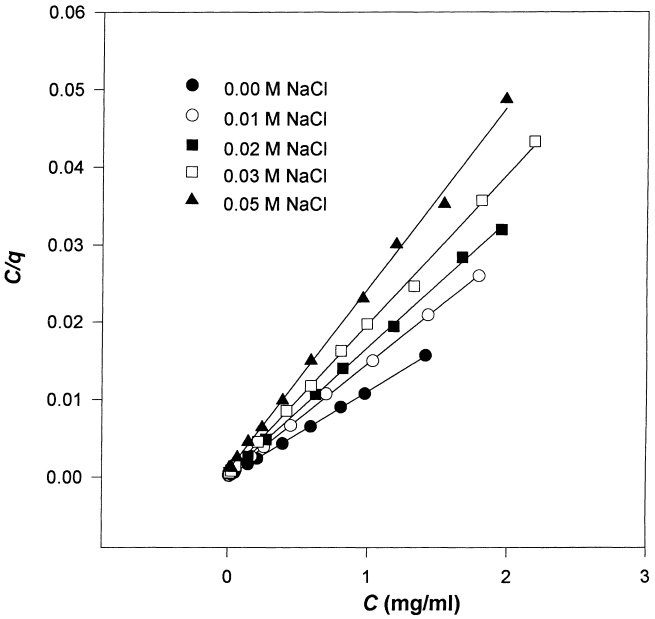

Fig. 2. Semi-reciprocal plot of BSA adsorption onto Diaion HPA25 at 25°C, pH 7.0 and different NaCl concentrations.

the fact that the stock protein is likely to have some salts in it. The 'background' effect of the ionic strength provided by the additional ions is, however, constant and the increase in ionic strength occurs as the NaCl concentration increases. In addition, given the low concentration of the buffer and the small amount of salts that may possibly be brought into the solution with the stock protein, it is reasonable to ignore the influence of the 'background' effect.

The semi-reciprocal plots (Fig. 2) and the Scatchard plots (Fig. 3) of equilibrium data indicate that the adsorption of BSA onto Diaion HPA25 obeys the Langmuir isotherm in the range of ionic strength investigated (0.0–0.05 M NaCl). The

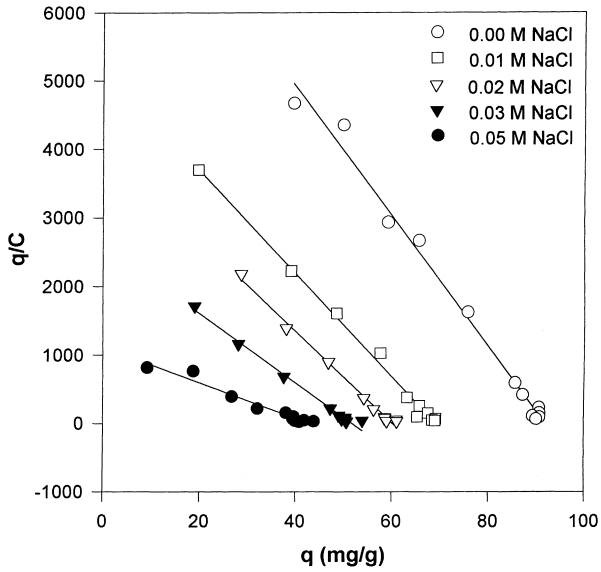

Fig. 3. Scatchard plot of BSA adsorption on Diaion HPA25 25°C, pH 7.0 and different NaCl concentrations.

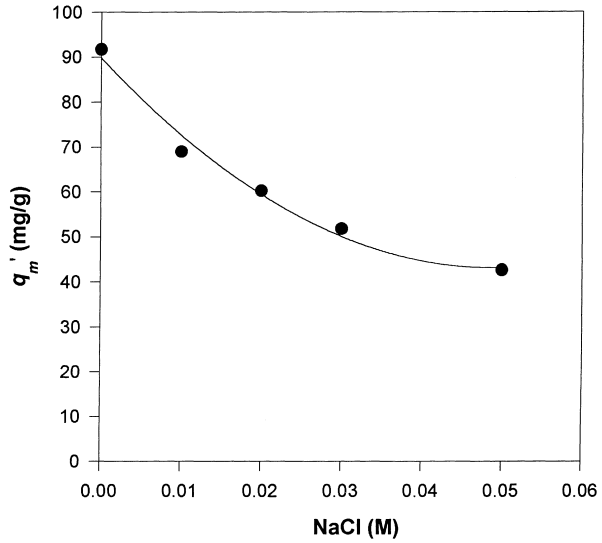

Fig. 4. Effects of ionic strength on the apparent maximum binding capacity (q'_m) of BSA adsorption onto Diaion HPA25 at 25°C and pH 7.0.

dependency of apparent Langmuir parameters on the ionic strength is shown in Figs. 4 and 5. As shown in Fig. 4, when the NaCl concentration of the solution increases from 0.0 to 0.05 M, the apparent maximum capacity decreases from 91.69 to 35.6 mg/g, a loss of about 61.2% of the capacity of Diaion HPA25. It is also evident from Fig. 5 that the increase of ionic strength causes dramatic increase of the apparent dissociation coefficient. When the sodium chloride is absent, the apparent dissociation coefficient is 0.00907 mg/ml. However, the apparent dissociation coefficient is enhanced to 0.0373 mg/ml when the NaCl concentration of the solution is 0.05 M, more than 3.5 times of that without NaCl.

The significant decrease of the binding capacity and affinity (increase of apparent dissociation coefficient, K'_d) result-

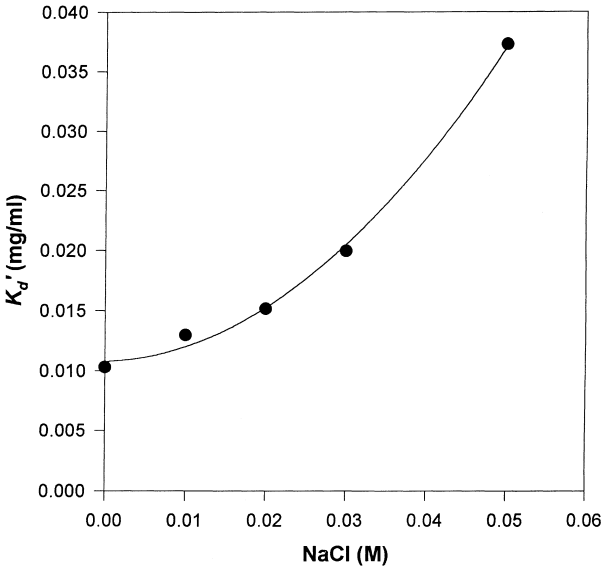

Fig. 5. Effects of ionic strength on the apparent dissociation coefficient (K'_d) of BSA adsorption onto Diaion HPA25 at 25°C and pH 7.0.

ing from the increase of ionic strength can be attributed to the interactions between salt ions and protein molecules and between salt ions and adsorbents. Those effects influence the electrostatic interactions and the Van Der Waals interactions between the protein molecules and the ion exchanger resins [11].

It is to be noted that the maximum binding capacity of BSA to Diaion HPA25 is 1.368×10^{-6} mol/g, which is only a very small fraction of the total capacity of the ion exchanger (2.4 meq/g). At least three factors may contribute to the failure of the stoichiometric match of the total capacity of the ion exchanger to the small counterions and the available capacity of it to the BSA molecules: (1) the molecular screening effects make a fraction of the binding sites located on the internal surface of the resins, which are readily available to small counterions, not accessible to the BSA molecules [12]; (2) the bulk BSA molecules, when bound to the binding sites, shield some neighboring sites and make them inaccessible to other protein molecules; (3) BSA is a multi-valence protein, and each protein ion is capable of occupying more than one binding sites.

4.2. Verification of the proposed model against experimental and literature data

Apparent Langmuir parameters of the adsorption of various proteins onto different ion exchanger/dye affinity adsorbents reported by different authors were obtained and are listed in Table 1. Those adsorbents include three cation exchangers, Sp Sepharose, Fractogel EMD and Fractogel TSK; two anion exchangers, Diaion HPA25 and TSK-DEAE 5PW (5PW); and one dye-affinity adsorbent, Cibacron Blue F3G-A immobilized onto LiChroprep DIOL (F3G). Five different proteins, BSA, human serum albumin (HSA), hen egg white lysozyme (HEWL), lysozyme and conalbumin, were investigated.

Huang and his coworkers [3] reported adsorption isotherms of albumin and conalbumin on anion exchanger TSK-DEAE 5PW in Tris-acetate/sodium acetate buffers (pH 8.6) of salt concentration 0, 0.025 and 0.05 M, respectively. The Langmuir isotherm was found to be appropriate for integrating the conalbumin adsorption data and the Binary Langmuir isotherm for the albumin adsorption. Hence only the conalbumin adsorption data are included in our analysis. It is to be noted that $\Sigma A/B$ is equivalent to q'_{m} and $1/B$ to K'_{d} in that paper. Hashim et al. [4] studied the effects of ionic strength on the the equilibrium behaviors of the adsorption of lysozyme on three strong cation exchangers, Sp Sepharose, Fractogel EMD and Fractogel TSK, in acetate/sodium acetate buffers (pH 5.0) of salt concentration of 0.07, 0.10 and 0.13 M. Langmuir model was used for the integration of all the experimental data and the apparent Langmuir parameters of all the three adsorbent/protein pairs are used in our analysis. Data from the work reported by

Table 1
Langmuir parameters from literature and experiments

Adsorbent	Protein	Buffer	Salt concentration (M)	q'_{m} (mg/g)	K'_{d} (mg/ml)
Sp Sepharose [4]	Lysozyme	Sodium acetate+acetate (pH 5.0)	0.07	111.6	0.009
			0.10	71.97	0.014
			0.13	62.22	0.026
Fractogel EMD [4]	Lysozyme	Sodium acetate+acetate (pH 5.0)	0.07	103.73	0.011
			0.10	67.29	0.014
			0.13	63.82	0.028
Fractogel TSK [4]	Lysozyme	Sodium acetate+acetate (pH 5.0)	0.07	38.38	0.012
			0.10	28.39	0.015
			0.13	25.38	0.030
Diaion HPA25 (this study)	BSA	2 mM Phosphate+NaCl (pH 7.0)	0.00	91.69	0.010
			0.01	68.94	0.013
			0.02	60.23	0.015
			0.03	51.72	0.020
			0.05	42.59	0.037
TSK-DEAE 5PW [3] (5PW)[a]	Conalbumin	Tris acetate+acetate+sodium acetate (pH 8.6)	0	30.8 mg/ml	0.247
			0.025	13.2 mg/ml	0.361
			0.050	4.8 mg/ml	2.381
Cibacron Blue F3G-A immobilized onto LiChroprep	HEWL	50 mM Tris–HCl+NaCl (pH 7.0)	0	16.16	0.46
			0.05	13.70	0.82
			0.10	11.51	0.94
			0.20	10.90	1.65
DIOL [1] (F3G)[a]	HSA	50 mM Tris–HCl+NaCl (pH 7.0)	0	5.09	0.31
			0.05	4.10	0.43
			0.10	3.75	0.72
			0.20	2.08	0.91

[a] Abbreviation used in the text and figures.

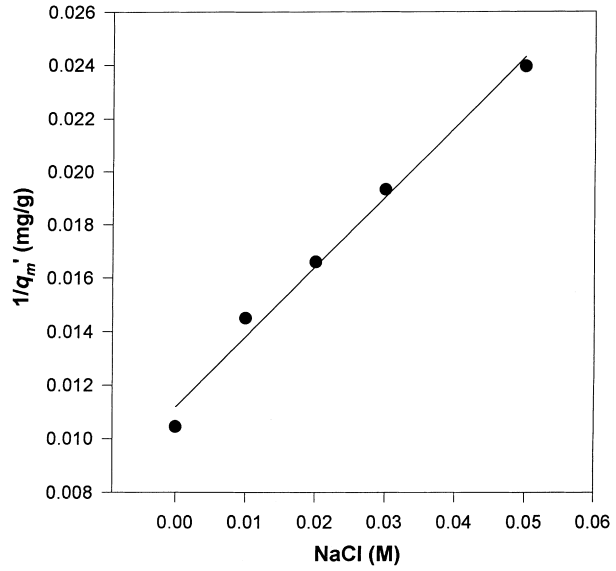

Fig. 6. Reciprocal of apparent maximum binding capacity ($1/q'_m$) for BSA adsorption as a function of NaCl concentration at 25°C and pH 7.0.

Finette et al. [1] were regarding the effects of ionic strength on the isotherm of the adsorption of HAS and HWEL on a dye-affinity adsorbent, Cibacron Blue F3G-A immobilized onto LiChroprep DIOL. Experiments were conducted in 50 mM Tris–HCl buffer (pH 7.0). The ionic strength of the buffers were adjusted with NaCl of four different concentrations: 0, 0.05, 0.10 and 0.20 M. Those seven adsorbent/protein pairs represent a wide range of adsorbents, proteins and buffers. The validity of the model was tested against these data, and is discussed below.

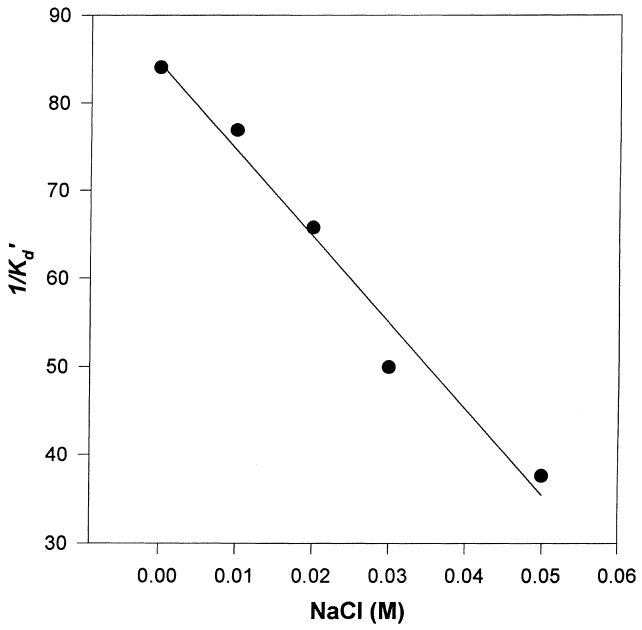

Fig. 7. Reciprocal of apparent dissociation coefficient ($1/K'_d$) as a function of NaCl concentration at 25°C and pH 7.0.

Table 2
Dissociation coefficient and constant η calculated according to the apparent Langmuir parameters as listed in Table 1

Ion exchanger	Protein	Salt	K_d		η (M)	r^2
			(mg/ml)	(M×10^7)		
DiaionHPA25	BSA	NaCl	0.00907	1.35	0.07194	0.997
5PW	Conalbumin	Sa[a]	0.237	35.37	0.0582	0.972
Sp Sepharose	Lysozyme	SA	0.0052	3.63	0.161	0.997
Fractogel EMD	Lysozyme	SA	0.00648	4.63	0.1766	0.978
Fractogel TSK	Lysozyme	SA	0.00694	4.85	0.173	0.971
F3G	HSA	NaCl	0.34	50.74	0.2789	0.855
F3G	HWEL	NaCl	0.53	370.62	0.267	0.839

[a] SA: sodium acetate.

The linear dependence of $1/q'_m$ and $1/K'_d$ on the NaCl concentration of our experimental data, as a characteristic of the model, is shown in Figs. 6 and 7, respectively. The r^2 values of the $1/K'_d$ versus I plot and the $1/q'_m$ versus I plot are about 0.997 and 0.990, respectively (Tables 2 and 3). Those high r^2 values are a clear indication of the validity of the model under the tested conditions.

Tables 2 and 3 show the r^2 values of the $1/K_d$ versus I correlation and the $1/q_m$ versus I correlation of all the seven adsorbent/protein pairs, respectively. As shown in Tables 2 and 3, the r^2 values for most cases are above 0.920. In the best cases, the r^2 values are 0.997 and 0.950 for Sp Sepharose/lysozyme pair and 0.997 and 0.990 for Diaion HPA25/BSA pair, respectively. Those results indicate that the model is applicable to a wide range of different ion exchangers and affinity adsorbents. However, keeping in mind the complexity of the protein adsorption process, it is not surprising to find that in some cases, the r^2 values are relatively low (0.839 in the lowest case of the $1/K_d$ versus I correlation for the lysozyme adsorption onto Fractogel EMD). In the cases of low linear correlation, nevertheless, the experimental data still follow the general tendency as predicted by the model. Namely, the apparent maximum binding capacity decrease with the increase of ionic strength, while the apparent dissociation coefficient increase with the increase of ionic strength.

Figs. 8 and 9 compare the experimentally obtained apparent Langmuir parameters of those seven adsorbent/protein pairs as listed in Table 1 against the corresponding predicted

Table 3
Maximum binding capacity and constant ψ calculated according to the apparent Langmuir parameters as listed in Table 1

Ion exchanger	Protein	Salt	q_m		ψ (M)	r^2
			(mg/g)	(mol/g×10^6)		
DiaionHPA25	BSA	NaCl	87.9	1.31	0.0466	0.990
5PW	Conalbumin	SA	56.82	0.848	0.0050	0.920
Sp Sepharose	Lysozyme	SA	909.1	63.57	0.00928	0.997
Fractogel EMD	Lysozyme	SA	303.0	21.19	0.0328	0.970
Fractogel TSK	Lysozyme	SA	88.49	6.19	0.0508	0.960
F3G	HSA	NaCl	5.79	0.086	0.1215	0.878
F3G	HWEL	NaCl	15.29	1.69	0.4395	0.928

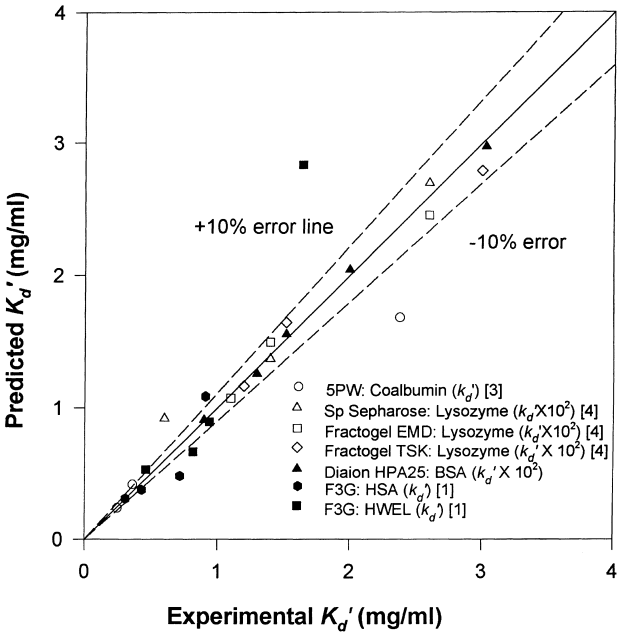

Fig. 8. Comparison of the predicted and the experimental data for the apparent coefficient (K_d').

data calculated according to the proposed model. To facilitate a clearer comparison, appropriate multiplication factors have been applied to some of the data as indicated in those two figures. As shown in the figures, the model predictions agree well with the experimental results, except for a few cases where some deviations were found. Most of the deviations were found in the prediction of K_d' values. As shown in Fig. 8, four cases out of the 25 points have an error larger than 10%. The other 21 points are either within or very close to the ±10% error lines. For the prediction of q_m' values, it is interesting to note that the only substantial deviation is in the case of conabumin adsorption onto 5PW (with no salt added) as shown in Fig. 9. The errors between the experimental and the predicted q_m' values of all the other 24 points are either within or very close to the ±10% error lines. This result shows that the proposed model could be used satisfactorily for correlating the apparent Langmuir parameters and the effects of ionic strength for a wide range of ion exchange/affinity adsorbents.

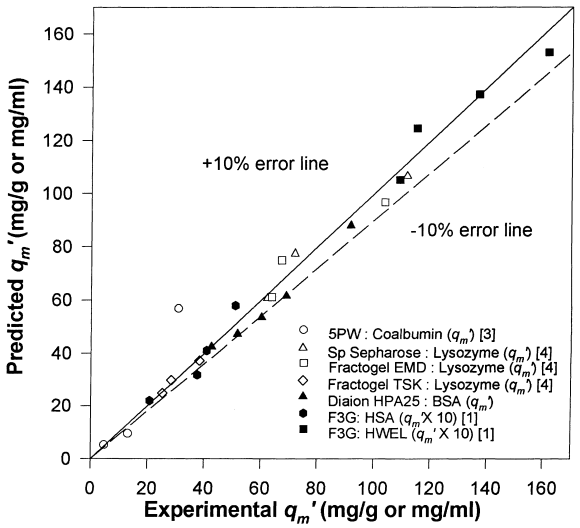

Fig. 9. Comparison of the predicted and the experimental data for the apparent maximum binding capacity (q_m').

Fig. 1 also compares the experimental isotherms of the BSA adsorption onto Diaion HPA25-BSA (symbols), the best-fit Langmuir isotherms under each individual NaCl concentration (solid lines) and the isotherms predicted by the new model (dashed lines) under NaCl concentrations of 0.0, 0.01, 0.02, 0.03 and 0.05 M, respectively. Both the Langmuir and the modified Langmuir isotherms match well with the experimental data. It is to be noted that in comparison with the best-fit Langmuir isotherms for each individual salt concentration, the new model does generate a somewhat larger deviation. However, this is reasonable considering that the new model has incorporated the salt concentration as an additional parameter. This allows the direct prediction of the effects of ionic strength on the adsorption behavior, which is an important feature that the original Langmuir model does not possess.

5. Conclusions

In conclusion, the proposed semi-empirical modification of the Langmuir model has been established as a good approach for predicting the effects of ionic strength on the equilibrium characteristics of protein adsorption onto ion exchange/affinity adsorbents. It is shown that the proposed model is applicable to various proteins/adsorbent systems. The accuracy of the predicted data generated by the model is satisfactory in terms of q_m', K_d' and the isotherms.

Acknowledgements

Financial support from the Natural Sciences and Engineering Research Council of Canada Strategic Grant is gratefully acknowledged.

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