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Equilibria of biomolecules on ion-exchange adsorbents

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ABSTRACT

Equilibrium adsorption isotherms of bovine serum albumin on DEAE-Sepharose Fast Flow ion exchanger were studied with different salt concentrations and buffer strengths at varying pH using a batch method. An extended Langmuir–Freundlich isotherm equation was used to fit these data and other experimental data taken from the literature. It was found that the model correctly approximates the adsorption of various proteins and amino acids at different salt concentrations using a single set of parameters.

INTRODUCTION

Ion-exchange chromatography continues to play a major role in the recovery and purification of biomolecules in the biotechnology industry. Optimisation of chromatographic operations such as adsorption, selective elution and regeneration becomes increasingly important as systems are scaled up. Currently, considerable work is being done to characterize and model the fundamental adsorption processes occurring in bioseparation systems. Studies of adsorption isotherms have been applied as a method of characterizing the ion-exchange adsorption of biomolecules at different pH values and mobile phase ionic strengths. Measurements of protein equilibria tend to be correlated differently to those of amino acids. The equilibrium dissociation reactions of amino acids are well characterized, and have been incorporated into ion-exchange equilibria models using the mass action law. Carta *et al.* [1] examined the equilibrium uptake of amino acids by a strong cation exchanger and found an excellent fit of binary exchange data. Similar results were obtained by Yu *et al.* [2] and also by Sanders *et al.* [3].

Protein ion exchange operates on the same principle as amino acid adsorption; however, due to the extreme complexity of the interactions involved, a more empirical approach must be used. Huang and Horváth [4] used the Langmuir adsorption model for fitting equilibrium isotherms of proteins on cation exchangers. Huang *et al.* [5] found the Langmuir equation inadequate for some proteins due to the extremely steep initial slope of the adsorption isotherm. These isotherms were better approximated with a Langmuir equation which incorporated two types of non-cooperative independent adsorption sites. Whitley *et al.* [6] fit protein adsorption data using the Langmuir isotherm, but also used a model incorporating mass action parameters to allow for dependence on salt concentration.

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In this article, we incorporate salt concentration into an extended form of the Langmuir–Freundlich equation to predict the dependence of salt on ion-exchange adsorption isotherms for both amino acids and proteins.

THEORY

The simplest theoretical model for monolayer adsorption was proposed by Langmuir [7] to describe the adsorption of gases on surfaces. This convenient model (eqn. 1) is based on the assumption that the adsorbed molecule is held at localized sites and the energy of adsorption is constant over all sites. It is often used to describe adsorption phenomena in biological systems even though the parameters may lose some of their physical meaning. Experimental adsorption data are typically fitted for a particular mobile phase composition:

$$q = \frac{q_{max}\ b\ C}{1 + b\ C} \tag{1}$$

where q = adsorbed solute concentration (mol/unit adsorbent), q_{max} = maximum concentration of adsorbed solute (mol/unit adsorbent), b = Langmuir equilibrium constant and C = liquid phase concentration (mol/unit solution).

Another well known adsorption model is derived from the Langmuir equation but uses a distribution of adsorption energies for the surface sites. Using an exponential decay function to represent this distribution, Zeldowitsch [8] derived an approximate solution (eqn. 2) known as the Freundlich isotherm. For values of η (power term of Freundlich isotherm) greater than 1 the isotherm shape is favorable (convex), and for values of η less than 1 the isotherm shape is unfavorable (concave). When η equals 1 the isotherm is linear.

$$q = K\ C^{1/\eta} \tag{2}$$

where K = Freundlich equilibrium constant.

A limitation of Freundlich isotherm is that the amount adsorbed increases indefinitely with the concentration in solution. To solve this inadequacy the Langmuir and Freundlich isotherms may be combined (eqn. 3). Because this equation contains two fitting terms, it is much better for approximating adsorption of a heterogeneous nature.

$$q = \frac{q_{max}\ b\ C^{1/\eta}}{1 + b\ C^{1/\eta}} \tag{3}$$

The Langmuir isotherm can be readily extended to an n-component mixuture, and has been used to describe mixed gas adsorption by Markham and Benton [9]. For gaseous systems, the equation implies a competition for free homogeneous sites on the adsorbent surface.

$$q_i = \frac{q_{maxi}\ b_i\ C_i}{1 + \sum_{j=1}^{n} b_j\ C_j} \tag{4}$$

When the extended Langmuir model may proves inadequate in prediction of mixture equilibria, the equation can be modified by the introduction of a power law of the Freundlich form [10], also commonly known as a loading ratio:

$$q_i = \frac{q_{maxi}\ b_i\ C_i^{1/n_i}}{1 + \sum_{j=1}^{n} b_j\ C_j^{1/n_j}} \tag{5}$$

It should be noted that these equations are not thermodynamically consistent and cannot then be expected to apply over the entire concentration range. Nevertheless, the expressions have been shown to provide a reasonably good empirical correlation of binary equilibrium data,

EXPERIMENTAL

Materials

DEAE-Sepharose Fast Flow, a weak anion exchanger, was purchased from Sigma (St. Louis, MO, U.S.A.). This ion exchanger is a macroporous gel composed of agarose which has been used in large-scale applications because of its cost and good stability. Protein used in this study, bovine serum albumin (A-7906), was purchased from Sigma. The two buffers, tris(hydroxymethyl)aminomethane (Tris) adjusted with hydrochloric acid to the required pH and sodium acetate adjusted with acetic acid were also purchased from Sigma. Sodium chloride was purchased from BDH (Kilsyth, Australia).

Batch experiments

For measurements by the batch method, a series of protein solutions having different concentrations was prepared and placed in individual microcentrifuge tubes with the anion exchanger. The tubes were gently rotated end-over-end for 4 h. Afterwards, the slurry was centrifuged, and the protein concentration of the supernatant was measured by analytical high-performance liquid chromatography (HPLC) at 280 nm.

Equipment and HPLC analysis

For HPLC analysis of proteins a Backman System Gold chromatographic system (San Ramon, CA, U.S.A.), consisting of a Model 126 dual-pump programmable solvent module, a Model 167 scanning detector module-167 and a Model 210A sample injection valve, was used. Sample sizes of 20 μl were analysed using a 75 mm $\times$ 7.5 mm TSK-DEAE-5PW column from Toya Soda (Tanda, Japan) with 10 m*M* Tris–HCl buffer (pH 7.8), containing 50 m*M* sodium chloride as the mobile phase.

RESULTS AND DISCUSSION

Analysis

For our purposes, we treat the adsorption of a biomolecule in the presence of a salt as a binary equilibria system (eqn. 4). Experimental isotherm data and isotherm

data from the literature were analysed using a non-linear curve-fitting program in SigmaPlot 4.01 (Jandel Scientific). A single set of best-fit parameter values were found for adsorption data at different salt concentrations.

$$q_{bio} = \frac{q_{max}\ b_{bio}\ C_{bio}^{1/\eta_{bio}}}{1 + b_{bio}\ C_{bio}^{1/\eta_{bio}} + b_{salt}\ C_{salt}^{1/\eta_{salt}}} \tag{6}$$

The subscripts "bio" and "salt" stand for biomolecule and displacing salt molecule, respectively.

Alternatively, eqn. 6 may be rearranged (eqn. 7), to give a single component Langmuir–Freundlich isotherm.

$$q_{bio} = \frac{q_{max}\ b^*\ C_{bio}^{1/\eta_{bio}}}{1 + b^*\ C_{bio}^{1/\eta_{bio}}} \tag{7}$$

If the experimental data is analyzed using this more traditional method, the equilibrium constant b^* should show the following dependence on salt concentration:

$$b^* = \frac{b_{bio}}{1 + b_{salt}\ C_{salt}^{1/\eta_{salt}}} \tag{8}$$

Experimental isotherms

The equilibrium binding isotherms for bovine serum albumin (BSA) were measured at different salt concentration on DEAE-Sepharose Fast Flow. Isotherms

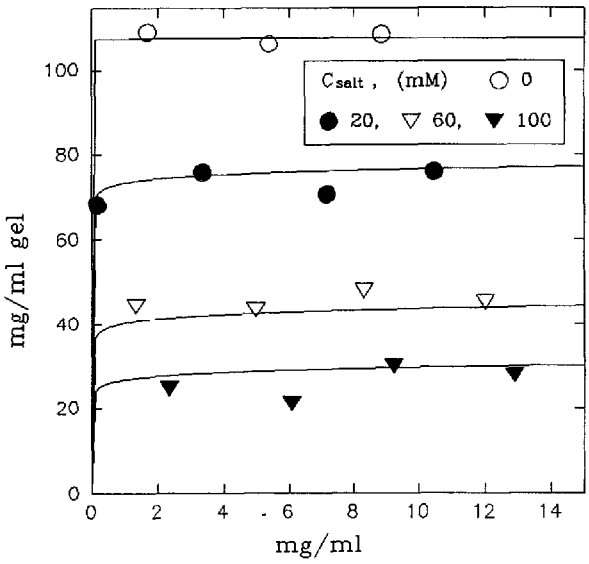

Fig. 1. Extended Langmuir–Freundlich isotherms for the uptake of BSA on the weak anion exchanger (DEAE-Sepharose Fast Flow), in Tris–HCl buffer (12 m*M*) at pH 9.1, at various sodium chloride concentrations.

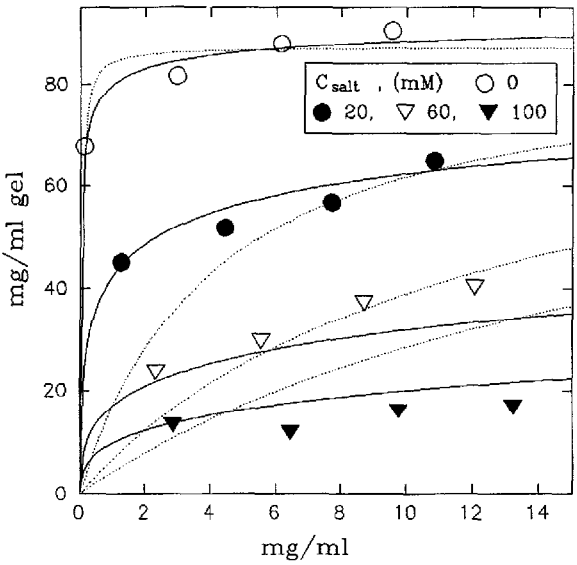

Fig. 2. Extended Langmuir–Freundlich isotherms for the uptake of BSA on the weak anion exchanger (DEAE-Sepharose Fast Flow), in sodium acetate–acetic acid buffer (12 m*M*) at pH 5.8, at various sodium chloride concentrations.

for different pH values and buffer strengths are shown in Figs. 1–3. At a pH of 9.1 and low buffer strength, BSA has very strong affinity for the anion exchanger. Data in Fig. 1 show very rectangular isotherms which decrease in total capacity with increasing salt

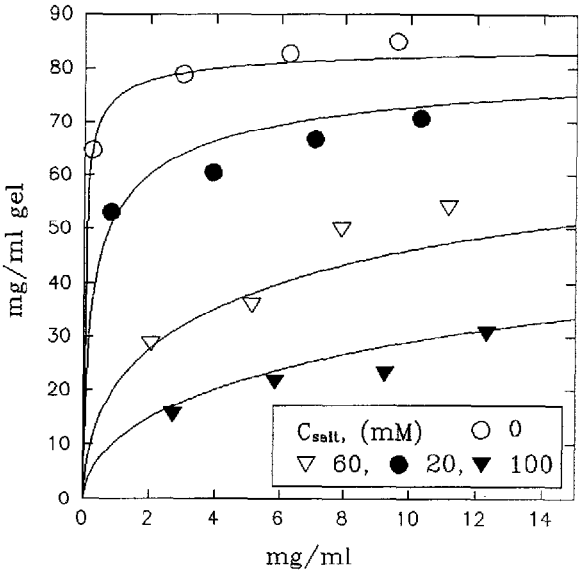

Fig. 3. Extended Langmuir–Freundlich isotherms for the uptake of BSA on DEAE-Sepharose Fast Flow, in Tris–HCl buffer (45 m*M*) at pH 8.1, at various sodium chloride concentrations.

TABLE I

PARAMETER VALUES FOR EXPERIMENTAL ISOTHERMS

Fig.	Protein	pH	Buffer (mM)	b_{bio} ($M^{-\eta_{bio}}$)	b_{salt} ($M^{-\eta_{bio}}$)	q_{max} (mg/ml)	η_{salt}	η_{bio}
1	BSA	9.1	12	$1.2 \cdot 10^8$	$8.1 \cdot 10^4$	107.8	0.86	16.6
2	BSA	5.8	12	$3.3 \cdot 10^5$	$9.0 \cdot 10^2$	95.8	0.76	2.70
2	BSA	5.8	12	$1.9 \cdot 10^6$	$5.9 \cdot 10^3$	87.2	1.00	1.00
3	BSA	8.1	45	$4.7 \cdot 10^5$	$2.3 \cdot 10^3$	85.1	0.60	1.78

concentration. When the pH is lowered to 5.8, approximately 1 pH unit above the isoelectric point of BSA, the protein affinity decreases and the isotherms become less rectangular.

Best-fit parameter values using the binary Langmuir–Freundlich isotherm (eqn. 6) are shown in Table I. For each case the model provides a good fit to all the experimental data using one set of parameters. Fig. 2 shows a comparison with the simple binary Langmuir isotherm were η_{bio} and η_{salt} both equal 1. It is not surprising to see that Freundlich parameters deviate from 1 and vary with pH. BSA is a large protein (mol. wt. 66 000) known to have a number of different binding sites. Also, weak ion-exchange adsorbents like DEAE contain a range of molecules having different ionization constants that could form a heterogeneous binding surface.

Isotherm data from Carta et al. [1]

Carta *et al.* [1] studied the equilibrium uptake of the amino acids phenylalanine

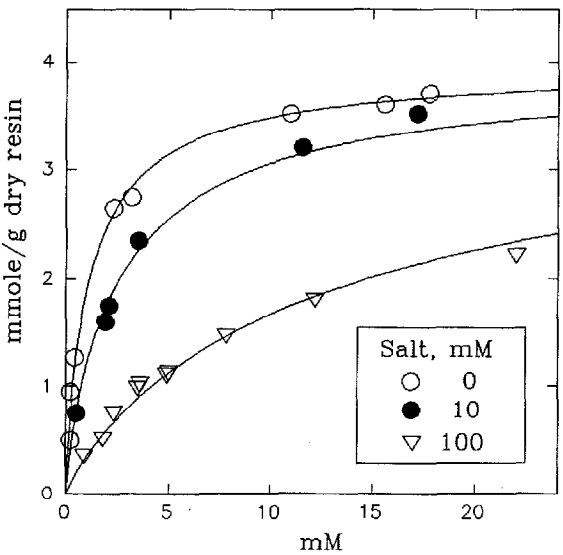

Fig. 4. Extended Langmuir isotherms for the uptake of phenylalanine on the strong cation exchanger (Amberlite 252), in no buffer between pH 1.61 and 2.90, at various chloride ion concentrations (Carta *et al.* [1]).

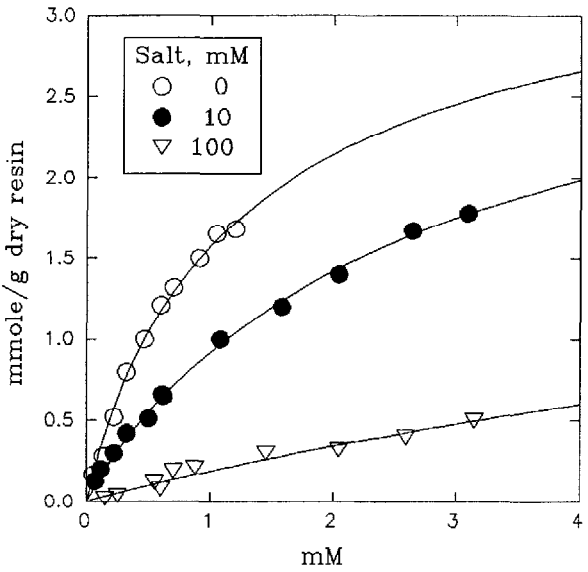

Fig. 5. Extended Langmuir isotherms for the uptake of tyrosine on the strong cation exchanger (Amberlite 252), in no buffer between pH 2.09 and 3.2, at various chloride ion concentrations (Carta *et al.* [1]).

and tyrosine by Amberlite 252, a strongly acidic, cation-exchange resin. Since amino acids were adsorbed in the absence of buffer, pH did not remain constant. Isotherms in Figs. 4 and 5 show equilibrium binding which is much weaker than that of BSA.

Best-fit parameter values using the extended Langmuir–Freundlich equation are shown in Table II. The model again provides a good fit to the experimental data for all concentrations of salt. For both amino acids the Freundlich parameters are nearly equal to 1. In this case the model reduces to a multicomponent Langmuir isotherm which may indicate adsorption of a homogeneous nature.

Isotherm data from Huang and Horváth [4]

Huang and Horváth [4] studied the equilibrium uptake of the proteins lysozyme (mol.wt. 14 400), α-chymotrypsinogen (25 000) and RNase A (13 700) on both strong and weak cation exchangers with silica support. All equilibrium binding isotherms were measured at pH 7.0 and are shown in Figs. 6–8. Using the extended Langmuir–Freundlich equation to find best fit parameters listed in Table III, the

TABLE II

PARAMETER VALUES FOR ISOTHERMS OF CARTA *et al.* [1]

Fig.	Amino acid	pH	b_{bio} $(M^{-\eta_{bio}})$	b_{salt} $(M^{-\eta_{salt}})$	q_{max} (mM/ml)	η_{salt}	η_{bio}
4	Phenylalanine	1.6 to 2.9	362	66	4.0	1.12	1.15
5	Tyrosine	2.1 to 3.2	464	134	3.6	0.99	1.08

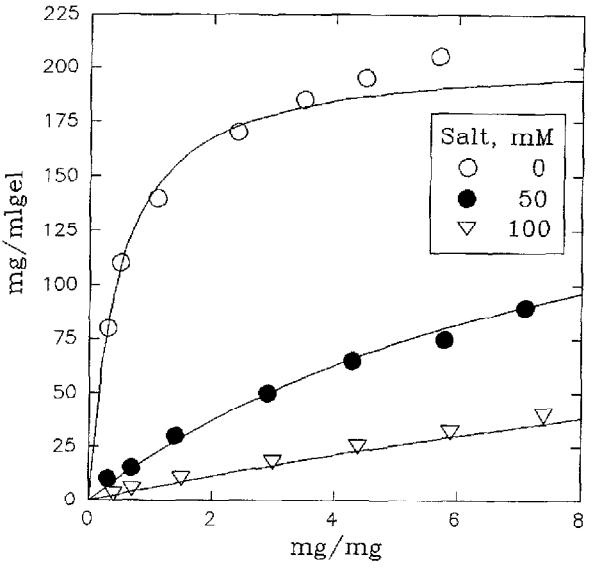

Fig. 6. Extended Langmuir–Freundlich isotherms for the uptake of α-chymotrypsinogen on the strong cation exchanger (SCX-300), in sodium phosphate buffer (25 m*M*) at pH 7.0, at various ammonium sulfate concentrations (Huang and Horváth [4]).

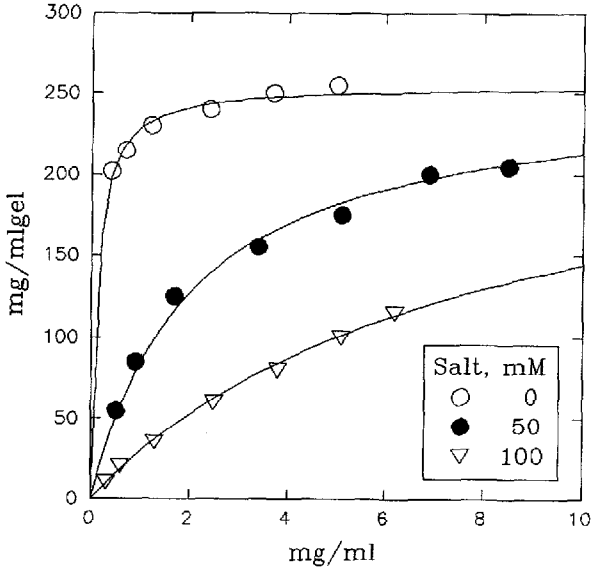

Fig. 7. Extended Langmuir–Freundlich isotherms for the uptake of lysozyme on the weak cation exchanger (WCX-300H), in sodium phosphate buffer (25 m*M*) at pH 7.0, at various ammonium sulfate concentrations (Huang and Horváth [4]).

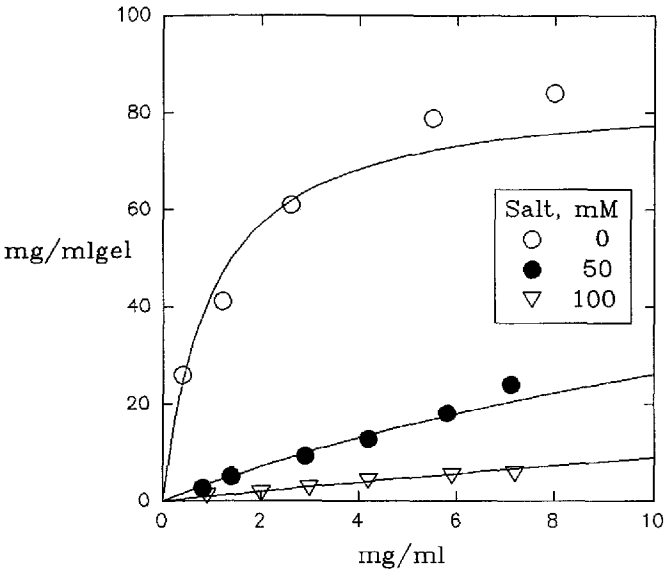

Fig. 8. Extended Langmuir–Freundlich isotherms for the uptake of RNase A on the weak cation exchanger (WCK-300H), in sodium phosphate buffer (25 mM) at pH 7.0, at various ammonium sulfate concentrations (Huang and Horváth [4]).

model again provides an excellent approximation of the data. It is also interesting to see that for all three proteins the Freundlich parameters η_{bio} and η_{salt} are approximately equal to 0.5 and 1.0, respectively. This may suggest that the binding sites of these proteins interact similarly with the ion exchange surface.

CONCLUSIONS

A simple multicomponent model has been shown to predict the dependence of salt concentration on the equilibrium adsorption of amino acids and proteins to an ion exchanger. This model could be easily incorporated into a fixed-bed model to predict the effect of different salt gradients on the elution of proteins using ion-exchange chromatography. Although empirical, the extended Langmuir–Freundlich isotherm

TABLE III

PARAMETER VALUES FOR ISOTHERMS OF HUANG AND HORVÁTH [4]

Fig.	Protein	pH	Buffer (mM)	b_{bio} ($M^{-\eta_{bio}}$)	b_{salt} ($M^{-\eta_{salt}}$)	q_{max} (mg/ml gel)	η_{salt}	η_{bio}
6	Lysozyme	7.0	25	$1.2 \cdot 10^5$	$6.7 \cdot 10^3$	255	0.49	1.06
7	α-Chymotrypsinogen	7.0	25	$5.7 \cdot 10^4$	$4.9 \cdot 10^3$	205	0.55	0.95
8	RNase A	7.0	25	$1.3 \cdot 10^4$	$2.6 \cdot 10^4$	85	0.44	0.81

provides a good fit to several different sets of equilibrium isotherm data. It has been proposed that Freundlich parameters deviating from 1 may indicate heterogeneous adsorption sites on the adsorbent surface and or the adsorbate. However, further studies are needed to test the applicability of this model and the validity of these theories.

ACKNOWLEDGEMENTS

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