Biotechnology and Bioprocess Engineering **2006**, 11: 61-66

Protein Adsorption on Ion Exchange Resin: Estimation of Equilibrium Isotherm Parameters from Batch Kinetic Data

K. H. Chu[1]* and M. A. Hashim[2]

[1] Department of Chemical and Process Engineering, University of Canterbury, Private Bag 4800, Christchurch, New Zealand
[2] Department of Chemical Engineering, University of Malaya, 50603 Kuala Lumpur, Malaysia

Abstract The simple Langmuir isotherm is frequently employed to describe the equilibrium behavior of protein adsorption on a wide variety of adsorbents. The two adjustable parameters of the Langmuir isotherm – the saturation capacity, or q_m, and the dissociation constant, K_d – are usually estimated by fitting the isotherm equation to the equilibrium data acquired from batch equilibration experiments. In this study, we have evaluated the possibility of estimating q_m and K_d for the adsorption of bovine serum albumin to a cation exchanger using batch kinetic data. A rate model predicated on the kinetic form of the Langmuir isotherm, with three adjustable parameters (q_m, K_d, and a rate constant), was fitted to a single kinetic profile. The value of q_m determined as the result of this approach was quantitatively consistent with the q_m value derived from the traditional batch equilibrium data. However, the K_d value could not be retrieved from the kinetic profile, as the model fit proved insensitive to this parameter. Sensitivity analysis provided significant insight into the identifiability of the three model parameters.

Keywords: adsorption equilibrium, batch kinetics, Langmuir isotherm, parameter estimation, protein

INTRODUCTION

Bioproducts such as proteins are normally obtained from fermentation processes. The extraction of these products from the fermentation broth invariably involves some form of chromatography [1]. In particular, extensive research has been conducted regarding the use of ion exchange resins for the separation and purification of proteins. Currently, a substantial number of cation and anion exchangers are commercially available for protein purification, and these are typically prepared via the coupling of charged ligands to mechanically rigid porous beads [2-7]. Production-scale ion exchange chromatography is normally conducted with fixed-bed columns. However, the evaluation of the performance of an ion exchange adsorbent is more frequently conducted in a batch mode than in a fixed-bed configuration, as batch processes are simpler to model. For example, the experimental data generated from batch measurements can be readily quantified in terms of equilibrium and transport parameters, which can then be incorporated within a fixed-bed process model for design and optimization studies. It is, therefore, not surprising that batch-contacting procedures remain the method of choice for many researchers, although dynamic techniques, including frontal analysis, have been proposed as alternative methods for the characterization of protein adsorption.

A sizeable body of work exists in the protein adsorption literature with regard to the evaluation of equilibrium and mass transport parameters using the batch-contacting approach [8-12]. This study is focused on the modeling of protein adsorption equilibrium, which can be quantitatively described in terms of one or more isotherm models. Although the binding of proteins to ion exchange resins is a complex phenomenon [13,14], simple mathematical models – most notably the Langmuir isotherm – are often employed in descriptions of adsorption equilibrium behavior. The Langmuir model assumes that the adsorption process occurs on a surface which is composed of a fixed number of binding sites of equal energy, one molecule being adsorbed per binding site until a monolayer coverage is achieved [15]. Although these assumptions are frequently violated in actual protein adsorption, the Langmuir isotherm, which features two adjustable parameters: the saturation capacity, or q_m, and the dissociation constant, K_d, provides a satisfactory correlation of equilibrium data in most situations. This may be attributable to the fact that a mathematical model with two adjustable parameters is sufficient to account for the hyperbolic isotherm often encountered in cases of protein adsorption. The Langmuir isotherm, therefore, performs an important function in the domain of protein adsorption, and the estimation of its two parameters from experimental data is a matter of substantial practical interest.

***Corresponding author**
Tel: +64-3-364 2217 Fax: +64-3-364 2063
e-mail: khim.chu@canterbury.ac.nz

In most published studies, q_m and K_d were estimated by fitting the Langmuir expression (or its linearized forms) to equilibrium data generated via batch equilibration. The batch equilibration approach generally requires extensive manual manipulation, and tends to be quite time-consuming. In this study, we attempted to assess the possibility of identifying the two Langmuir parameters for protein adsorption to a cation exchanger, by matching the adsorption kinetic data obtained from a batch experiment to a rate model, predicated on the kinetic form of the Langmuir isotherm. This approach could provide a more rapid alternative to the conventional parameter estimation approach based on the equilibrium data obtained via batch equilibration. In addition, the proposed approach is more convenient for initial screening of adsorbents, which requires the rapid measurement of equilibrium parameters. Whereas batch kinetic measurements have been used fairly extensively in the estimation of mass transport parameters, they have rarely been used to estimate the equilibrium parameters. To the best of our knowledge, it appears that only one study has attempted to identify the Langmuir isotherm parameters from the batch adsorption kinetics [16].

THEORY

Consider a batch vessel, which initially contains only a protein solution, at a concentration of c_i. At time zero, fresh ion exchange particles are added to the vessel. The differential mass balance for the vessel is given by:

$$v\frac{dq}{dt} = -V\frac{dc}{dt} \tag{1}$$

where q is the ion exchange phase protein concentration, c is the liquid phase protein concentration, v is the ion exchange adsorbent volume, V is the liquid volume, and t is the time. The adsorption of the protein to the ion exchanger is assumed to be monovalent and homogeneous, according to the following reversible reaction:

$$P + A \leftarrow PA$$

where P represents the protein molecule, A represents an adsorption site on the ion exchanger, and PA is the protein-adsorbent complex. The rate of protein adsorption for the above reaction scheme can be expressed as:

$$\frac{dq}{dt} = k_1 c(q_m - q) - k_2 q \tag{2}$$

where k_1 is the forward interaction rate constant, k_2 is the reverse interaction rate constant, and q_m is the saturation capacity of the ion exchanger. At equilibrium, Eq. (2) results in the familiar Langmuir isotherm model:

$$q_e = \frac{q_m c_e}{K_d + c_e} \tag{3}$$

where the subscript e denotes an equilibrium value, and K_d is the dissociation constant, provided by the following equation:

$$K_d = \frac{k_2}{k_1} \tag{4}$$

Substituting Eq. (4) into Eq. (2) gives

$$\frac{dq}{dt} = k_1 c(q_m - q) - k_1 K_d q \tag{5}$$

The integration of Eqs. (1) and (5) with the appropriate initial conditions yields the following analytical solution [17]:

$$\frac{c}{c_i} = 1 - \frac{1}{c_i}\frac{v}{V}\frac{(b+a)\left[1-\exp\left(-2a\frac{v}{V}k_1 t\right)\right]}{\left[\frac{(b+a)}{(b-a)} - \exp\left(-2a\frac{v}{V}k_1 t\right)\right]} \tag{6}$$

in which a and b are defined as:

$$a = \sqrt{b^2 - c_i q_m \frac{V}{v}} \tag{6a}$$

$$b = 0.5\left(c_i\frac{V}{v} + q_m + K_d\frac{V}{v}\right) \tag{6b}$$

Eq. (6) is the solution of the rate model predicated on the kinetic form of the Langmuir isotherm, from which the concentration-time profile for a given batch adsorption system can be calculated. When the experimental conditions are specified (c_i, v, and V), Eq. (6) can be fitted to the batch concentration-time data, in order to identify the three adjustable parameters (k_1, K_d, and q_m) which appear in a nonlinear fashion within the equation. A nonlinear regression method may be used to facilitate this.

MATERIALS AND METHODS

Materials

Bovine serum albumin (BSA) was purchased from Sigma. Acetic acid and sodium acetate were obtained from Fluka. 0.1 M acetate buffer was prepared via the mixing of the required amounts of the chemicals with deionized water. The pH of the buffer solution was maintained at a value of 5. The strong cation exchanger, Frac-

togel EMD SO_3^- 650 (M), was purchased from Merck.

Batch Adsorption Kinetics

At time zero, a suspension of Fractogel EMD resin was added to a vessel containing a BSA solution at a pH of 5. The vessel was incubated and agitated in a water bath maintained at 25°C. A pump (Model 250, Perkin Elmer) was employed to circulate the protein solution in a recycle loop. The cation exchange particles were excluded from the recycle loop using a 10 μm HPLC pump inlet filter. The concentration of BSA in solution was continuously monitored with a UV/VIS spectrophotometer, which had been equipped with a flow cell (Lambda 3B, Perkin Elmer). Absorbance versus time data were recorded and converted into concentration versus time data, using a PC. The experimental conditions selected for study are given in Table 1.

Batch Adsorption Equilibrium

A measured quantity of the cation exchanger was added to each of a series of centrifuge tubes containing BSA solutions of different concentrations. The tubes were rotated end-over-end for 24 h at 25°C. After equilibration, the slurry was centrifuged, and the protein concentration of the supernatant was determined by UV absorbance, as described above. The amount of protein adsorbed onto the cation exchanger was then calculated via mass balance.

Parameter Estimation

The best-fit values of the three adjustable parameters (k_1, K_d, and q_m) in Eq. (6) were estimated by minimizing the error between the experimental transient adsorption data and model calculations. The minimization algorithm was predicated on a combination of the Gauss-Newton and Levenberg-Marquardt methods. The same nonlinear least-squares curve fitting technique was utilized to identify q_m and K_d in Eq. (3), by fitting the equation to the measured equilibrium data.

RESULTS AND DISCUSSION

In this study, we have attempted to evaluate the possibility of identifying the Langmuir isotherm parameters, q_m and K_d, for the adsorption of BSA to the cation exchanger Fractogel EMD, by fitting a rate model predicated on the kinetic form of the Langmuir isotherm to a single kinetic profile, which was acquired from a batch apparatus.

Batch Adsorption Kinetics

Fig. 1 shows the batch concentration decay profile for BSA which is expressed in normalized solution concentrations (c/c_i). As can be seen in this figure, the dynamic profile manifests a slow approach towards equilibrium.

Table 1. Experimental conditions for batch kinetic experiment

c_i (μmol/L)	V (L)	v (L)
22.5	5×10^{-2}	2.5×10^{-4}

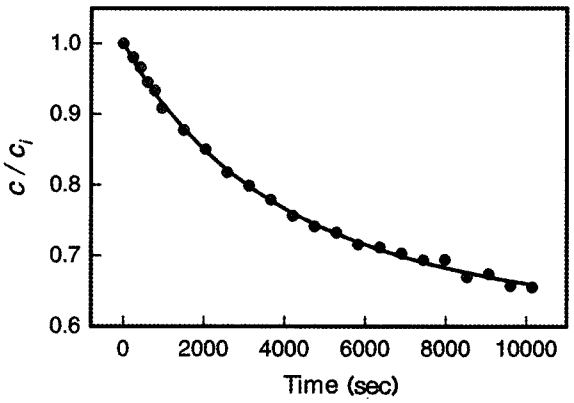

Fig. 1. Kinetic profile for BSA in solution showing experimental points and fit of Eq. (6) (solid line) with the parameters given in Tables 1 and 2.

The solid line in Fig. 1 represents the fit obtained using the rate model (Eq. (6)). It is evident that, using this approach, an excellent fit over the entire time course of the protein uptake could be obtained.

The best-fit values of the three adjustable parameters in Eq. (6) are shown in Table 2. As can be seen in this table, the saturation capacity of the cation exchanger (q_m) for BSA is substantial. Because the pH of the experiment was fixed at a value of 5, the protein with an isoelectric point of approximately 5 would have a zero net charge. The fact that appreciable BSA uptake occurs at a pH of 5 emphasizes that the ion exchange mechanism relies on specific interactions on the surface of the protein, rather than relying on the net properties of the protein [18].

The forward rate constant, k_1, is an apparent rate constant, which includes contributions from intrinsic adsorption kinetics, film diffusion, and intraparticle diffusion, because the rate model (as presented above) is a simplified approach, which lumps all kinetic and mass transport processes into a single rate constant.

Unfortunately, a unique K_d value could not be determined, because the minimization algorithm always returned the initial guess of K_d, thereby indicating that the model was not at all sensitive to K_d. By way of contrast, the q_m and k_1 parameters always converged to the values listed in Table 2, without regard to the starting guesses. Consequently, the systematic investigation of the identifiability of the three adjustable parameters in Eq. (6), which have been shown to provide an excellent fit to the kinetic data of Fig. 1, is of paramount importance.

Parameter Sensitivity Analysis

The preceding discussion illustrates the difficulty inherent to the simultaneous acquisition of unique esti-

Table 2. Best-fit values of rate model parameters (Eq. (6))

q_m (μmol/L)	K_d (μmol/L)	k_1 (L/μmol·s)
1767	-	1.17×10^{-5}

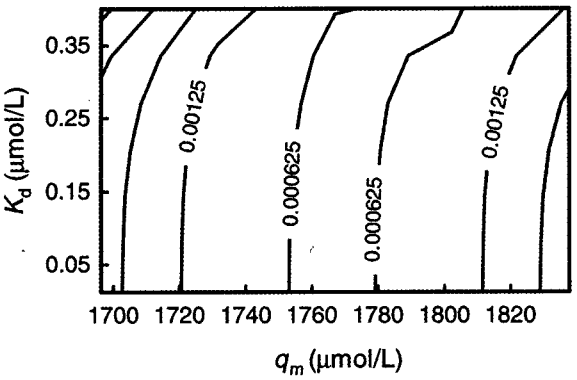

Fig. 2. Contours of K_d versus q_m with constant $k_1 = 1.17 \times 10^{-5}$ L/μmol·s.

mates of q_m, K_d, and k_1 by fitting Eq. (6) to a single batch kinetic profile. In this section, we consider the ability of the nonlinear minimization algorithm to generate unique estimates of the individual parameters of the rate model, via the performance of a parameter sensitivity analysis. Several approaches to parameter sensitivity analysis have been developed in the past [19,20]. One such approach is predicated on the analysis of the residual distribution, plotted as contours [21]. The approach involves fixing all other parameters of a multi-parameter model and altering pairs of two parameters to generate model responses. The corresponding residual distribution is then calculated via comparisons with the experimental data. In this study, the residual distribution is expressed in terms of the sum of squares of error (*SSE*) values:

$$SSE = \sum \left[\left(\frac{c}{c_i} \right)_{exp} - \left(\frac{c}{c_i} \right)_{model} \right]^2 \quad (7)$$

Figs. 2 and 3 display the *SSE* values, plotted as contours for various combinations of K_d and q_m and of K_d and k_1, respectively. The ranges of the parameters used in the generation of the contours are the best-fit values of q_m and k_1 (see Table 2) ± 4%, as well as a reference value of K_d (0.1 μmol/L) ± 400%. According to these results, a common feature is clearly apparent. A comparison of Figs. 2 and 3 clearly shows that the contours in both figures are aligned with the K_d axis. This implies that the *SSE* values do not change substantially when K_d varies between 0.025 and 0.4 μmol/L (*i.e.*, by a factor of 16) at a given value for q_m or k_1. Under the given conditions, the rate model is clearly insensitive to the K_d value, within the selected range of the parametric space. This is consistent with the parameter estimation exercise, which always returned the initial guess of K_d as the best estimate. The

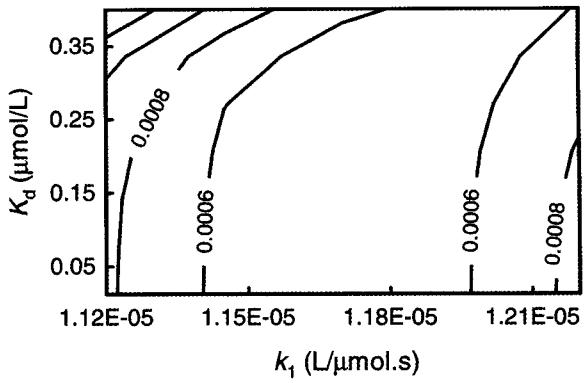

Fig. 3. Contours of K_d versus k_1 with constant q_m = 1767 μmol/L.

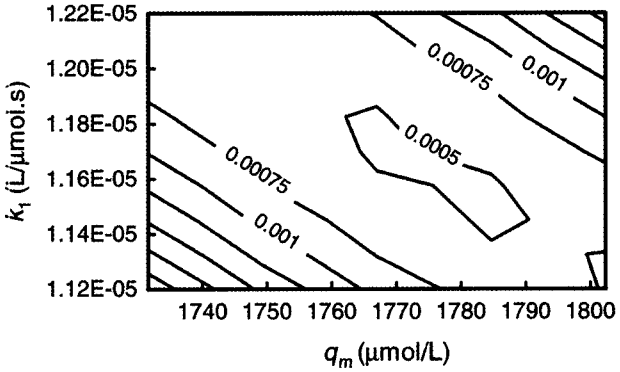

Fig. 4. Contours of k_1 versus q_m with constant K_d = 0.1 μmol/L

sensitivity analysis presented above shows that, without considering parameter identifiability, simple multi-parameter curve fitting methods may provide erroneous parameter values for nonlinear models.

Fig. 4 depicts the contours of k_1 versus q_m, which are diagonal, thereby indicating that the two parameters are correlated to some degree. Nevertheless, the innermost contour does close, enclosing a minimum *SSE* value. It may be concluded that both parameters can be estimated with significant reliability. The accuracy of the q_m estimate can be assessed via comparison with the estimate obtained from the batch equilibrium data, as is discussed below.

Batch Adsorption Equilibrium

Fig. 5 shows the equilibrium isotherm for BSA on Fractogel EMD, as determined by conventional batch equilibration. From Fig. 5, it is clear that the isotherm is nearly rectangular, indicating a highly favorable degree of protein adsorption. The solid line in Fig. 5 represents a description of adsorption equilibrium behavior in terms of the Langmuir isotherm (Eq. (3)). The two parameters in the Langmuir model, q_m and K_d, were obtained from a nonlinear fit of the data, and are shown in the second row of Table 3. As can be observed in Fig. 5, the agreement between the calculated and the experimental data is ex-

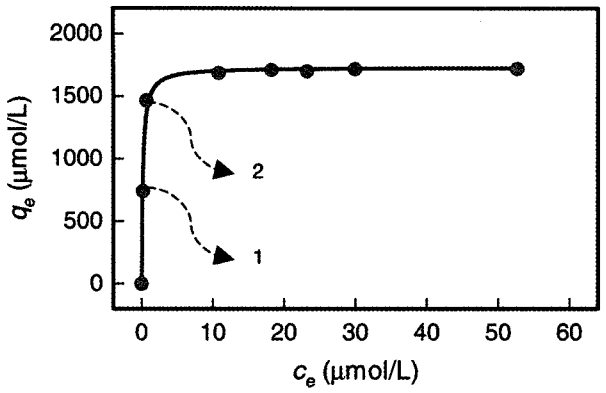

Fig. 5. Equilibrium isotherm for BSA on Fractogel EMD resin showing experimental points and fit of Langmuir isotherm expression (solid line) with the parameters given in the second row of Table 3.

cellent. A comparison of Tables 2 and 3 shows that the resulting q_m value is 2% smaller than the previous estimate derived from the kinetic data. This high degree of agreement serves to verify the accuracy of the q_m estimate acquired from the kinetic data and provides convincing evidence of the validity of the proposed approach, subject to the requirement that the resin in the batch kinetic system is fully saturated.

As discussed above, the K_d value was not able to be retrieved from the kinetic profile, as the result of model insensitivity towards this parameter. By way of contrast, K_d can be readily identified from the equilibrium data obtained via batch equilibration. However, as the result of the nearly rectangular shape of the isotherm, it is postulated that the estimation of K_d by nonlinear regression could be quite sensitive to the initial slope of the isotherm. To verify this hypothesis, the effect of omission of a single data point within the ascending part of the isotherm from the regression analysis was evaluated.

Table 3 indicates that the omission of one data point (see Fig. 5) from the regression had no significant effect on the Langmuir model's ability to fit the measured data, as indicated by the similar R^2 correlation coefficients, and on the q_m values. However, the K_d values evidence a considerable degree of scatter. The K_d value obtained in the case in which data point 1 was omitted in the regression was 47% smaller than the K_d value obtained in the case in which all data points were used. Similarly, the omission of data point 2 from the regression resulted in a K_d value 16% larger than that obtained when all data points were considered. It is apparent that the K_d value is altered according to the number of such data points used in the regression analysis.

Accurate data describing the ascending portion of a highly rectangular isotherm are not easy, in practice, to obtain, as this would require the utilization of very low protein concentrations in the batch equilibrium experiments. As a result, it may prove impossible to retrieve unique K_d estimates in practice, although K_d is *a priori* identifiable from the batch equilibrium data. The issue of the practical retrievability of K_d has been, for the most part, neglected in the field of isotherm determinations via batch equilibration.

Table 3. Best-fit values of equilibrium model parameters (Eq. (3))

	q_m (μmol/L)	K_d (μmol/L)	R^2
Complete data set	1732	0.19	0.994
Omission of data point 1[a]	1716	0.12	0.999
Omission of data point 2[a]	1724	0.22	0.999

[a] See Fig. 5.

CONCLUSION

In this study, we have attempted to describe the process by which the Langmuir isotherm parameters could be estimated from a single kinetic profile obtained from a batch apparatus. The saturation capacity (q_m) of the cation exchanger Fractogel EMD for bovine serum albumin identified by this approach was quantitatively consistent with the q_m value derived from the equilibrium data generated via conventional batch equilibration. Unique q_m estimates can, therefore, be retrieved from batch kinetic data with a high degree of confidence, subject to the requirement that the resin is fully saturated. However, sensitivity analysis indicated that it was impossible to identify a unique value for the dissociation constant, K_d, under the experimental conditions selected for study. The insensitivity of the rate model to K_d could be attributed to either the nature of the model itself, or to the experimental design (initial conditions of the batch apparatus). The influence of initial conditions (c_i, V, and v) on the identifiability of K_d was not considered in this study, but will certainly become the subject of further study.

Acknowledgements We dedicate this work to the memory of Grace P. S. Tsan, in recognition of her contribution to this research. We also acknowledge the constructive comments of the editors.

NOMENCLATURE

a	parameter defined in Eq. 6(a), μmol/L
A	adsorption site on adsorbent surface
b	parameter defined in Eq. 6(b), μmol/L
c	protein concentration in the solution phase at time t, μmol/L
c_e	protein concentration of solution phase at equilibrium, μmol/L
c_i	initial protein concentration of solution phase, μmol/L
k_1	forward rate constant, L/μmol·s
k_2	backward rate constant, 1/s
K_d	Langmuir dissociation constant, μmol/L

P protein molecule in the solution phase
PA protein-adsorbent complex
q protein concentration in the adsorbent phase at time t, μmol/L
q_e protein concentration in the adsorbent phase at equilibrium, μmol/L
q_m saturation capacity of the adsorbent, μmol/L
SSE sum of squares of error
t time, s
v settled volume of adsorbent, L
V volume of protein solution, L

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[Received August 16, 2005; accepted November 17, 2005]