Korean J. Chem. Eng., **24**(4), 641-647 (2007)

Binary protein adsorption to DEAE sepharose FF

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(*Received 18 October 2006 • accepted 8 January 2007*)

Abstract–Equilibrium between ion exchangers and proteins is one of the most important factors in ion exchange chromatography. A model system was used to simulate the adsorption of human serum albumin (HSA) and ovalbumin (OVA) as a binary protein system to the DEAE Sepharose FF as an anion exchanger. Two models, one based on a competition between adsorbing molecules and the other a non competitive model have been compared to experimental results. Competitive adsorption was seen in experiments in which breakthrough curves and the profiles of adsorbed proteins in packed beds were determined. However, although the results for packed bed experiments were more closely predicted by the competitive model, some discrepancies were found, suggesting that when considering multicomponent protein adsorption to ion exchangers it may also be necessary to take account of factors such as the molecular size of adsorbing proteins and any potential inter protein interaction, which may hinder the development of a general model of multicomponent protein adsorption to ion exchangers.

Key words: Ion Exchange Chromatography, Protein Adsorption, Langmuir Isotherm, HSA, OVA

INTRODUCTION

The ability to characterize adsorption equilibria accurately is important in a number of chemical processes, ranging from the study of adsorption at fluid interfaces to the design of heterogeneous chemical reactors. Ion exchange chromatography has been extensively employed for the recovery and the purification of proteins because proteins are desorbed under non-denaturing conditions - generally at high salt concentrations in the buffer [1-3]. The design of a purification process, especially in large scale production of pharmaceutical proteins, requires the use of a model which is able to predict the required parameters. A theoretical discussion of an approach to modeling multicomponent protein has been published by Velayudhan [4], although they did not present any associated experimental data. Experimental results from a non protein system have been published [5]. This paper presented two methods for determining competitive adsorption isotherms from the results of frontal analysis chromatography and included a theoretical discussion of the derivation and the use of the parameters of the Langmuir isotherm. Results of studies of the adsorption of a mixture of albumin and β-lactamase to encapsulated ion exchange have been published [6]. They report that competition between the two adsorbing proteins occurred.

To start studying multicomponent adsorption, a model system consisting of two adsorbing proteins, human serum albumin (HSA) and ovalbumin (OVA), and the anion exchanger DEAE Sepharose FF has been examined. The characteristics of the adsorption of each pure protein to the DEAE Sepharose FF have been studied and the results were reported in a previous paper [7]. Those studies showed that the Langmuir adsorption isotherm could be used to describe the equilibrium adsorption characteristic of both proteins.

In this paper, the results of studies of two component adsorption of HSA and OVA to DEAE Sepharose FF are presented and two different models, one based on competitive adsorption to ion exchanger and the other based on non competitive adsorption, are compared.

THEORY

Proteins adsorb to ion exchangers as a result of ionic interaction between charge groups on the surface of the protein and oppositely charged groups on the ion exchanger. A protein molecule carries many charged groups and multiple ionic interactions will occur with the adsorbent [8]. As three dimensional distributions of ionic groups on the surface of the adsorbent are random, the actual protein site is not a unique entity. So, the adsorption site on a protein ion exchanger cannot strictly be treated in the same manner as that postulated for affinity adsorption where molecules of the immobilized affinity ligand constitute adsorption sites with identical properties. A Langmuir type isotherm might not be excepted to describe the adsorption of single proteins to ion exchangers as fundamental thermodynamic conditions such as the identical nature of the adsorption sites and an absence of lateral interaction between adsorbed solute molecules are not obeyed. However, the Langmuir equation is still the most commonly used expression in the study of chromatographic process [9], and experimental results from systems in which a single protein is adsorbed to ion exchanger yield equilibrium isotherms which can be described by a Langmuir equation of the form shown below [10-12]:

$$q^* = \frac{c^* q_m}{c^* + K_d} \quad (1)$$

Where q represents the concentration of protein adsorbed to the ion

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Table 1. Values of constants for adsorption equilibrium of pure HSA and OVA to DEAE Sepharose FF [7]

	K_d (mg/ml)	K_d (mol)	q_m (mg/gr)	q_m (mg/ml)
HSA	0.604	9×10^{-6}	108.2	114.7
OVA	0.423	9.5×10^{-6}	62.2	65.9

exchanger, q_m represents the maximum concentration of protein that can be adsorbed to the ion exchanger, c the concentration of protein in the bulk solution and K_d is the dissociation constant for the complex of protein with ion exchange. The superscript * indicates equilibrium values.

Our previous experiments [7] involving the single component adsorption of HSA and OVA to DEAE Sepharose FF show that the equilibrium adsorption isotherms are of the above form and the equilibrium adsorption parameters determined in those studies are presented in Table 1. The observation of a Langmuir type shape can probably be explained by protein adsorption to the ion exchanger continuing until there is no longer room on the surface of the adsorbent of further molecules of adsorbate to bind. Hence further adsorption ceases once monolayer coverage has occurred. This eventual saturation of the adsorbent surface leads to adsorption isotherms of a similar shape to the Langmuir isotherm even though the underlying thermodynamic assumptions are not strictly obeyed, and the Langmuir isotherm therefore is widely used as a simple empirical model of the equilibrium adsorption characteristics of various protein adsorbent systems.

In addition to uncertainties arising from the undefined nature of the adsorption sites on an ion exchanger for the adsorption of a single pure protein, the situation is further complicated when the adsorption of two or more proteins is being considered. As a result of the different sizes and distribution of charges on the surfaces of different proteins, the number of ionic groups that will participate in the adsorption interaction and the amount of adsorbent surface which interacts with the different proteins will vary. Whilst recognizing the complexities of multicomponent adsorption of proteins to the ion exchangers, the Langmuir isotherm is the simplest model available to construct two component isotherms when single component isotherms are available, so in this study we have adopted two extreme views to analyze two components protein adsorption, namely a non competitive model and a competitive model. Both of these models are based on the Langmuir adsorption isotherm, an approach supported by our earlier single component studies of the adsorption of HSA and OVA to DEAE Sepharose FF [7]. It is shown that the competitive model more accurately describes the experimental observations.

1. Non Competitive Adsorption Model

One extreme view of the adsorption of two proteins to an ion exchanger is to assume that the adsorption of one type of protein to the ion exchanger in no way affects the adsorption of the other spices and there is therefore no competition between the proteins for the adsorption sites. If there is no competition between the proteins for adsorption, the adsorption characteristics of each protein will be the same as if the other protein were not present, so:

$$q_1^* = \frac{c_1^* q_{m1}}{c_1^* + K_{d1}} \quad \text{and} \quad q_2^* = \frac{c_1^* q_{m2}}{c_2^* + K_{d2}} \tag{2}$$

Where the subscripts 1 and 2 indicate adsorbate species 1 and 2.

2. Competitive Adsorption Model

In overloaded chromatography, the feed components compete for access to the stationary phase, and as a result we need the competitive multicomponent isotherm to account for the band profiles in chromatography. The other extreme approach to the analysis of two component adsorption is to assume that there is competition between proteins for adsorption to the ion exchanger. Although the exchanger shows different maximum capacities for the two proteins (q_{m1} and q_{m2}), a competitive model can be developed which involves a fractional occupancy of the adsorption capacity for each type of protein and uses Langmuir parameters derived from a single component experiment.

Let α represent the fractional occupancy of the adsorbent at equilibrium with a particular protein, such that:

$$\alpha_1 = \frac{q_1^*}{q_{m1}} \quad \text{and} \quad \alpha_2 = \frac{q_2^*}{q_{m2}} \tag{3}$$

The fractional of unoccupied sites is therefore given by $(1-\alpha_1-\alpha_2)$. At equilibrium:

$$K_{d1} = \frac{c_1^*(1-\alpha_1-\alpha_2)}{\alpha_1} \quad \text{and} \quad K_{d2} = \frac{c_2^*(1-\alpha_1-\alpha_2)}{\alpha_2} \tag{4}$$

Giving

$$c_1^* = \alpha_1(K_{d1} + c_1^*) + c_1^*\alpha_2 \quad \text{and} \quad c_2^* = \alpha_2(K_{d2} + c_2^*) + c_2^*\alpha_1 \tag{5}$$

And

$$(1-\alpha_1-\alpha_2) = \frac{K_{d1}\alpha_1}{c_1^*} = \frac{K_{d2}\alpha_2}{c_2^*} \tag{6}$$

From Eq. (6):

$$c_1^*\alpha_2 = \frac{K_{d1}}{K_{d2}} c_2^* \alpha_1 \tag{7}$$

Substituting into Eq. (5) for $c_1^*\alpha_2$ from Eq. (7) and for α_1 from Eq. (3) gives:

$$q_1^* = \frac{q_{m1} c_1^*}{K_{d1} + c_1^* + \frac{K_{d1}}{K_{d2}} c_2^*} \quad \text{and} \quad q_2^* = \frac{q_{m2} c_2^*}{K_{d2} + c_2^* + \frac{K_{d2}}{K_{d1}} c_1^*} \tag{8}$$

The equilibrium position of a batch system can be determined by solving Eq. (8) simultaneously with the mass balance equations [13]:

$$Vc_{01} = Vc_1^* + vq_1^* \quad \text{and} \quad Vc_{02} = Vc_2^* + vq_2^* \tag{9}$$

Where V is the volume of the liquid phase and v is the volume of ion exchanger in the system. c_{01} and c_{02} are the initial concentrations of the two proteins. A computation program was written to solve Eqs. (8)-(9) for values of c_i^* and q_i^* for a particular set of initial condition V, v and c_{0i} by an iterative method using the values of K_{di} and q_{mi} determined in single component adsorption isotherm measurements.

EXPERIMENT

1. Materials

HSA and OVA were obtained from the Shanghai Research Institute of Biochemistry and Bio Life Science & Technology Co., LTD.

catalogue number G0070, respectively. HSA has a relative molecular weight of 67,000 Daltons and an isoelectric point (pI) of 4.9, while OVA have a relative molecular weight of 44,000 Daltons and pI of 4.7.

All solutions were buffered with 0.01 M phosphate buffer, pH 6. The buffer solution as well as the sample has been filtered with a micropore filter (at least 0.45 μm and preferably 0.22 μm) before use to prevent the ion exchange columns from fouling.

2. Determination of Protein Concentration in the Liquid Phase

In experiments in which only one protein was present in solution, it was possible to determine protein concentration by measuring the optical density at 280 nm. In experiments in which both HSA and OVA were present in solution together, quantization of the concentrations of the individual protein was achieved by analytical separation of the protein by molecular exclusion chromatography by using a Fast Protein Liquid Chromatography system (FPLC) (Pharmacia LKB). 100 μl protein samples were loaded onto a Superose® 12 HR 10/30 FPLC column with 0.01 M phosphate buffer, pH 7. The column was eluted with 0.1 M sodium chloride in 0.01 M Phosphate buffer, pH 7, at flow rate 0.5 ml/min. Integration of the peaks on the resultant chromatogram was performed by the LCC-500 chromatography controller unit of FPLC system. The concentration of each protein was then determined from the areas of their peaks by reference to calibration data.

3. Batch Equilibrium Adsorption Studies

A number of experiments were performed in which samples of DEAE Sepharose FF were equilibrated with different mixtures of HSA and OVA. The experiments were performed in flasks prepared according to the protocol for determining the adsorption isotherm described previously [7], with the difference that each flask contained not one protein but a mixture of the two proteins. The amount of HSA and OVA used in each flask was always equal on a mass basis. The flasks were incubated overnight in a shaking water batch at 25 °C to allow equilibrium to be established. At equilibrium the amount of each protein present in the liquid phase was determined by FPLC, allowing the amounts of each protein that were adsorbed to the ion exchanger to be calculated by mass balance.

4. Packed Bed Experiments

All of the column experiments were performed with 2 g of DEAE in buffer solution packed in a chromatography column with an internal diameter of 1.5 cm and bed height about 1.2 cm, mounted vertically. Optical density at 280 nm of the outlet stream was recorded and fractions were collected at the column exit as required for FPLC analysis.

5. Consecutive Application of Single Protein Solutions to Packed Bed

Packed bed experiments, in which a feed solution containing only one of the proteins was applied, were performed. When the protein concentration of the outlet stream (c), as determined from optical density measurements equaled, or was approaching, that of the inlet stream (c_0), the incoming feed stream was switched to a solution containing only the other protein. Fractions were collected at the column exit for analysis by FPLC.

RESULTS

The results of the batch equilibrium adsorption experiments are plotted in Fig. 1. From each adsorption experiment a pair of equilibrium adsorption results was obtained, one for each of the proteins present. Each result represents the concentration of protein in solution that was in equilibrium with an adsorbed amount of the same protein. In Fig. 1, each HSA point in the order of increasing soluble protein concentration pairs with an OVA point also in the order of increasing soluble protein concentration.

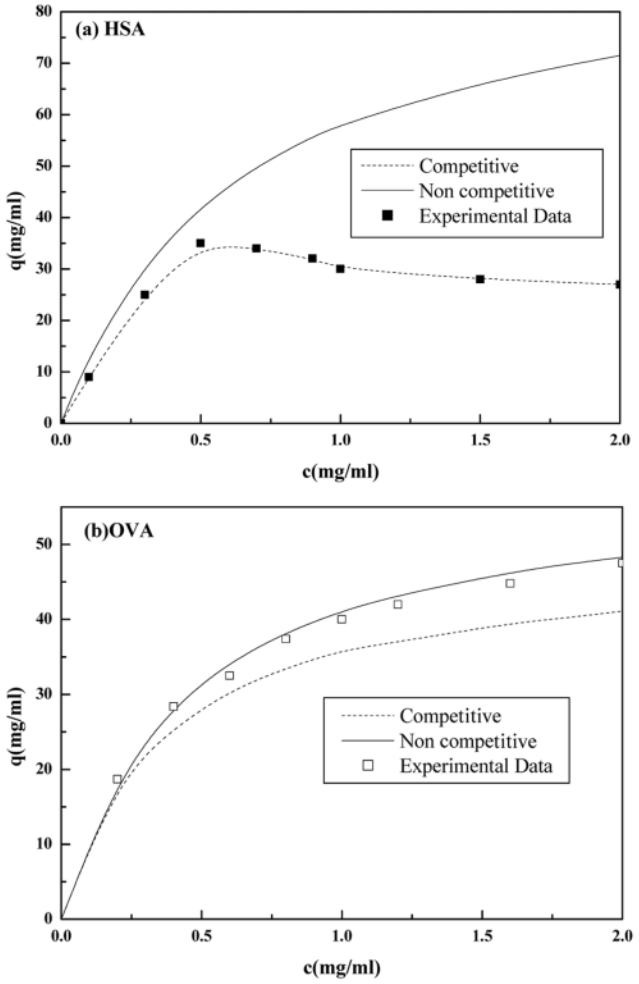

Fig. 1. Batch adsorption of a mixture of HSA and OVA to DEAE Sepharose FF in 0.01 M phosphate buffer, pH 6 at 298 K. The boxes present experimental result which are plotted with the results calculated by the competitive (dash line) and the non competitive model (solid line). (a) HSA (b) OVA.

The experimental data are compared to the results predicted by the two models of two component adsorption. The non competitive model gave a fairly accurate prediction of the OVA adsorption results but greatly over predicted the amount of HSA that would be adsorbed to the ion exchanger. Conversely, the competitive model gave a good prediction of the amount of HSA adsorbed but underpredicted the amount of OVA adsorbed.

1. Frontal Analysis

The development of the breakthrough profiles for HSA and OVA when a solution containing a mixture of each protein at a concentration of 2 mg/ml was passing through a bed of DEAE Sepharose

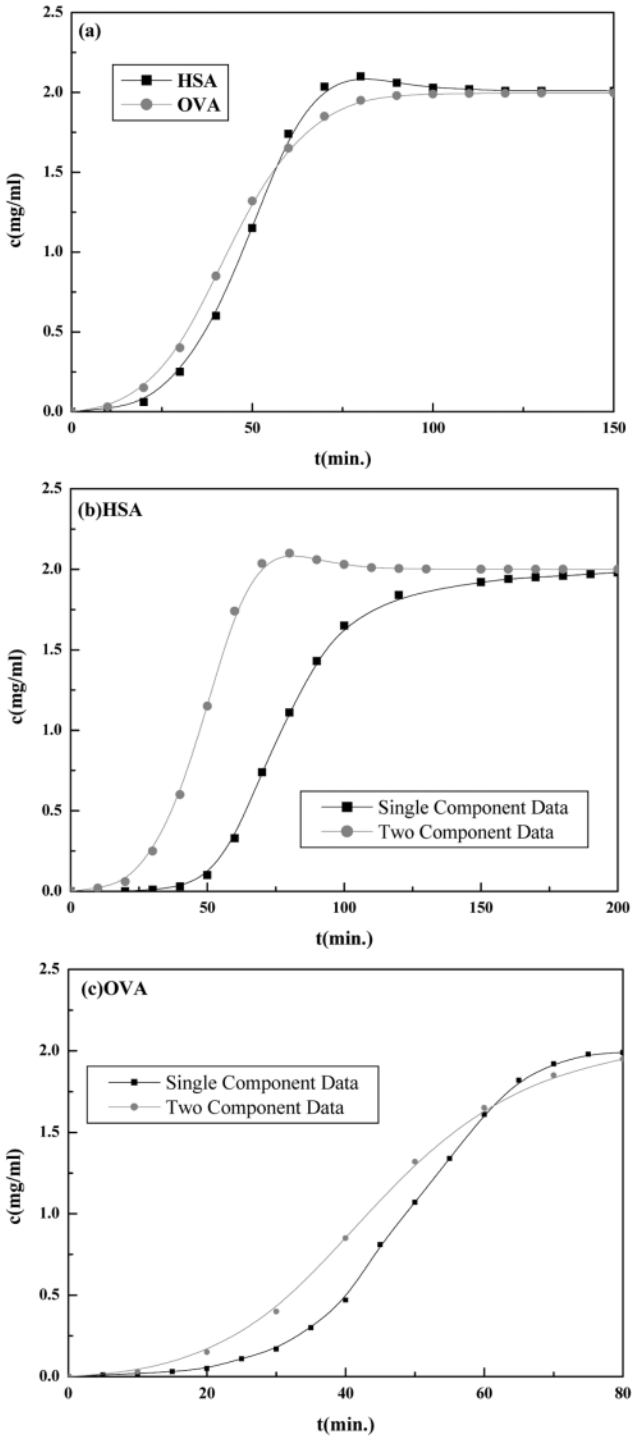

Fig. 2. Breakthrough profiles for the adsorption of OVA and HSA to DEAE Sepharose FF in packed beds. (a) The breakthrough profiles of OVA (●) and HSA (■) for a solution containing both proteins. (b) Breakthrough curves for HSA from experiments in which pure HSA was applied to the bed (■) and when the mixture of HSA and OVA applied (●). (c) Breakthrough curves for OVA from experiments in which pure OVA was applied to the bed (■) and when the mixture of HSA and OVA applied (●).

FF is shown in Fig. 2a. This figure shows that the concentration of HSA is raised above that of the inlet concentration before it falls back towards it. This profile indicates that OVA is able to displace, and thereby elute, a certain amount of adsorbed HSA. The amounts of each protein that had bound to beds were determined by integrating the area above the breakthrough curves for each component. It is possible to use the two models of adsorption to calculate the amounts of protein that would be expected to the ion exchanger, since at equilibrium the values of c_{01} and c_{02} may be substituted for c_1^* and c_2^* in Eqs. (2), (8) and (9). The amounts of each protein bound to the packed bed from experiments and the calculated values from the two models of adsorption are shown in Table 2. It can be seen that the experimentally determined figure of 49.4 mg HSA adsorbed per ml DEAE Sepharose FF was not accurately predicted by either model but is closer to the value predicted by the competitive approach. In the case of OVA the experimentally determined figure of 32.4 mg/ml is almost completely consistent with the competitive model.

Table 2. Amounts of HSA and OVA bound to a packed bed of DEAE Sepharose FF at equilibrium

	Experimental (mg/ml)	Non competitive model (mg/ml)	Competitive model (mg/ml)
HSA	49.4	71.5	37.8
OVA	32.4	46.3	31.1

In order to compare more easily the two component breakthrough profiles with those obtained from the single component experiments for each protein, the profiles of two component and single component experiments have been plotted in the same figures, those for HSA in Fig. 2b and those for OVA in Fig. 2c. The single component breakthrough curves are those determined previously in beds of the same size, under identical conditions and presented in our earlier paper [7]. Fig. 2b clearly shows that the breakthrough profile of HSA in the presence of OVA is shifted considerably towards the origin compared to the position of the breakthrough curve when pure HSA is applied to the column. This is a result of the fact that significantly more HSA was able to bind to the packed bed in the absence of OVA than in the two component experiment. The amount of HSA that was calculated to have adsorbed in the single component was 114.7 mg of HSA per ml DEAE Sepharose FF compared to the 49.4 mg HSA per ml DEAE Sepharose FF that was bound in the two component experiment. The slope of the two component HSA breakthrough profile is seen to be much sharper than that observed in the single component experiment.

The breakthrough profile of OVA obtained in the two component experiments is shifted towards the origin by a much smaller amount that was the case for HSA (Fig. 2c). The position of the two component curve indicates that less OVA bound to DEAE Sepharose FF in the presence of HSA than was the case when OVA alone was present, 32.4 mg of OVA per ml DEAE Sepharose FF having been bound in the two component experiment compared to an adsorbed OVA concentration of 65.9 mg/ml in the single component experiment. Also, in contrast to the result for HSA, the gradient of the two component OVA breakthrough curves is shallower than that obtained in the respective single component experiment.

2. Consecutive Application of Single Protein Solutions to Packed Beds of DEAE Sepharose FF

Two experiments were performed in which a packed bed of DEAE

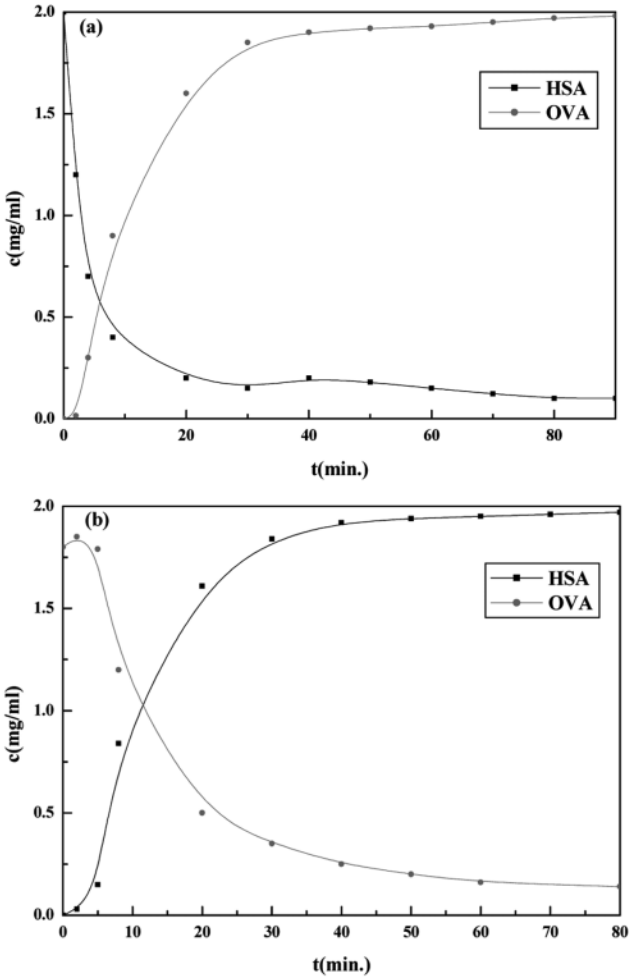

Fig. 3. Adsorption of proteins to packed beds already loaded with another protein. (a) The profile of HSA (■) and OVA (●) in the exit stream of a bed initially loaded with HSA and subsequently loaded with OVA. (b) The profile of HSA (■) and OVA (●) in the exit stream of a bed initially loaded with OVA and subsequently loaded with HSA.

Sepharose FF was loaded with one protein and then the inlet stream switched to a pure feed of the other protein. The results of these experiments are shown in Fig. 3. The protein profiles are plotted from the point at which the second feed solution first entered the bed. The amounts of protein bound or eluted after the feed was switch were determined from the concentration profiles shown in Fig. 3. In the case of loading a bed with HSA and switch the feed to OVA, (Fig. 3a), the concentration of HSA in the exit stream rapidly fell to a low level which during the subsequent FPLC analysis resulted in a peak height that was below the detection limit when the integrator was set at the sensitivity required for OVA quantitation. From Fig. 3a, it was calculated that approximately 48 mg of OVA bound to a bed which initially contained over 210 mg of adsorbed HSA and of which only 41 mg of HSA was eluted from the column. The result of the complementary experiment in which a bed was loaded with OVA and then the feed was switched to HSA is shown in Fig. 3b. In this case the amount of HSA that was adsorbed to ion exchanger, 63 mg, was less that the amount of OVA eluted, 89 mg.

DISCUSSION

The equilibrium adsorption characteristics of the two proteins, HSA and OVA, adsorbing to DEAE Sepharose FF were determined from the single component adsorption isotherm described previously [7]. The Langmuir isotherm parameters determined are presented in Table 1. The dissociation constant, K_d, is a measure of the strength interaction between the protein and the ion exchanger. In the case of HSA and OVA adsorbing as single components to DEAE Sepharose FF, OVA has a smaller dissociation constant than HSA on mass basis. This is probably a reflection of the greater negative charge density found on the OVA molecule at pH 6. From consideration of the Langmuir isotherm parameters and the molar concentrations of OVA and HSA used in these studies, it was expected that if competitive adsorption occurred, OVA would act as the more strongly binding component.

Neither of the models of multicomponent adsorption which were considered correctly predicted the amounts of each protein that bound in either stirred vessel or packed bed experiments. Since the nature of the adsorption mechanisms of different proteins to an ion exchanger is considered to be based on interactions with the same charged groups on the ion exchanger surface, it was expected that experimental results would be in close agreement with the competitive model. The major reasons for discrepancies between the observed results and those predicted by the competitive Langmuir adsorption model are discussed below.

In the batch adsorption studies, only the adsorption of HSA was similar to the results of the competitive model, with this model greatly under predicting the amounts of OVA that were bound. In contrast, the non competitive model gave a prediction which agreed closely with the amount of OVA that were bound in these batch adsorption experiments, whilst over predicting the amount of the amount of bound HSA.

The two extreme models of multicomponent adsorption were also used to calculate the amounts of each protein that would be expected to bind to a packed bed of ion exchanger at equilibrium. In this case the results of the competitive model gave a good correlation with the amounts of OVA that were bound, but the amount of HSA bound was underestimated. These results, however, were closer to the competitive model than the predictions of non competitive model, which over predicted the amounts of both proteins which should bind, with the discrepancy between predicted and observed results being greatest in the case of HSA.

Despite the lack of exact agreement between the experimental results and those calculated from the competitive model, the results of the packed bed experiments provided substantial evidence, in the form of breakthrough profile of OVA, that was noticeably less sharp in experiments in which a mixture of the two proteins was applied to a pack bed than was the case in single component exeriments. This suggests that the adsorption of OVA was hindered by the presence of HSA. In contrast the breakthrough profile of HSA from two component experiments was sharper than that observed when pure HSA was applied to a bed of DEAE Sepharose FF. The two-component HSA profile was seen to rise above a c_0 (2 mg/ml), which is that the concentration of HSA in the exit stream was greater than that in the inlet stream. This type of profile is caused by a proportion of the more weakly binding component, in the case HSA,

being eluted from the adsorption by the more strongly binding component, in this case OVA.

In order to investigate the characteristics of the adsorption of protein to an ion exchanger which is already loaded with another protein, experiments in which packed beds of DEAE Sepharose FF were first loaded with one protein and then the feed solution switched to a solution of the other protein were performed. From consideration of a competitive model of adsorption, supported by the evidence of the breakthrough profiles, it was expected that OVA would replace a proportion of the adsorbed HSA. Conversely, it was expected that HSA, the less strongly binding component, would be less effective at replacing adsorbed OVA. Similar experiments of protein adsorption/displacement in a reversed-phase liquid chromatography system [14] showed just such behavior. However, in this study the results of applying OVA to a bed near to saturation with HSA were found not to correspond to this expectation. The amount of HSA which was eluted from the bed was approximately 20 mg/ml DEAE Sepharose FF with the final adsorbed concentration of HSA being just under 105 mg/ml DEAE Sepharose FF, whilst an additional 25 mg OVA was bound per ml DEAE Sepharose FF. These results are in contrast to those reported [14] that demonstrated complete elution of more weakly binding proteins by more strongly adsorbing proteins. In the complementary experiment, in which HSA was applied to a bed saturated with OVA, as expected the more weakly binding HSA was found to be less effective at eluting and replacing adsorbed OVA.

The results of the experiments described have clearly shown that the adsorption of HSA and OVA to the DEAE Sepharose FF, to some extent, was competitive in nature. However, the discrepancies between the experimental results and those predicted by either model of multicomponent adsorption indicate that the adsorption process is more complex than those models described. One of the primary assumptions of the competitive model is that all adsorption sites are equally accessible to all adsorbate molecules. However, it is likely that due to the smaller size of the OVA in comparison to HSA, OVA is able to penetrate regions of the ion exchanger particle which may be restricted for HSA to enter. Such differential access of proteins of different molecular sizes to adsorbent particles is which molecular exclusion chromatography is based and Sepharose marked as a gel filtration material. Any OVA adsorption which occurred at sites which are inaccessible to HSA would be non competitive and would result in the amount of adsorbed OVA being under predicted by the competitive model. The presence of large quantities of HSA adsorbed within the particles might be expected to hinder the access of OVA to these sites.

A further adsorption mechanism could be due to protein-protein interactions within the ion exchanger particles. Electrostatic interactions between proteins in free solution have been reported [15-16]. Under these circumstances the amount of OVA predicted to adsorb by the competitive model would indeed be an underestimate of the experimental results.

CONCLUSION

The studies presented here have demonstrated some of the experimental techniques and a possible theoretical approach that can be used to investigate multicomponent protein adsorption. In this study, two component adsorption isotherms were not determine such isotherms by performing frontal analysis experiments using solutions containing mixtures of varying compositions [5].

Two extreme models of multicomponent protein adsorption have been considered and neither of these accurately predicted the adsorption characteristics of HSA and OVA in either batch adsorption or packed bed experiments. Clear evidence that a competitive model is the better approach to modeling multicomponent protein adsorption to ion exchangers was provided by the breakthrough curves. Competitive adsorption was demonstrated in those experiments by the observation that OVA could elute adsorbed HSA from the ion exchanger. However, the discrepancies between the observed and predicted results suggest that in studies of the multicomponent adsorption of proteins of different sizes, it may be necessary to include in the model contributions from non competitive adsorption in order to allow for the adsorption of small proteins in regions of particles inaccessible to large molecules. In the particular case of HSA and OVA, the development of such a multicomponent adsorption model is yet further complicated by the possibility that OVA may become bound to adsorbed HSA molecules as a result of electrostatic interactions.

In conclusion, the results presented here suggest that the theoretical modeling of multicomponent protein adsorption is a complicated task and that an accurate model may require contributions from the theories of molecular exclusion chromatography and protein-protein interaction in addition to adsorption chromatography.

NOMENCLATURE

c : concentration of protein in the bulk solution [mg/ml]
K_d : dissociation constant for the complex of protein with ion exchange [mg/ml]
q : concentration of protein adsorbed to the ion exchanger [mg/ml] (adsorbent)
q_m : maximum concentration of protein that can be adsorbed to the ion exchange [mg/ml] (adsorbent)
V : volume of liquid phase [ml]

Greek Letters

α : fractional occupancy of the adsorbent at equilibrium with a particular protein
ν : volume of ion exchanger

Subscripts

i : component index

Superscripts

0 : initial value
* : equilibrium value

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