Investigation of Adsorption–Desorption Dynamism of Bovine Serum Albumin on Crosslinked *N,N′*-Diethylaminoethyl Dextran Microbeads: Solution Phase

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ABSTRACT: New hydrogel microspheres based on crosslinked dextran containing *N,N′*-diethylaminoethyl (DEAE) groups with different chemical structures have been used in adsorption–desorption studies. Bovine serum albumin (BSA) is frequently used in biophysical and biochemical studies. BSA has a well-known primary structure that has been associated with binding of many different categories of small molecules. Both adsorption kinetics and equilibrium isotherms for the adsorption of BSA on crosslinked DEAE dextran have been determined experimentally. These were only slightly dependent on the initial concentration of BSA but were considerably affected by the pH of the medium. The results fitted the Freundlich–Langmuir isotherm model for pH 6.9. The adsorption capacity factor and the adsorption equilibrium constant were obtained and mathematical modeling of adsorption, adsorption rate constants, and maximum adsorption were determined. Swelling kinetics of crosslinked DEAE dextran and optimum ionic strength, pH, and mass of hydrogel were also investigated. Desorption studies were finally determined under optimum medium conditions. © 2005 Wiley Periodicals, Inc. J Appl Polym Sci 99: 2288–2299, 2006

Key words: DEAE dextran microbeads; adsorption; proteins; UV–vis spectroscopy; polysaccharides

INTRODUCTION

Industry has a significant interest in the design, optimization, and control of large-scale affinity adsorption systems that are to be employed in the purification of biologically active macromolecules for use as pharmaceuticals or in other applications where the purification of the product is a very important consideration. Certain fundamental mechanisms underlying the affinity adsorption separations have been identified and constitutive expressions that may be used to quantify these mechanisms and their effects have been suggested and constructed.[1,2] Many conventional separation processes (i.e., gel filtration, ion-exchange, precipitation, ultrafiltration, electrophoresis) and their combinations (multistep purification procedures) may not be applicable to purification of proteins from complex biochemical mixtures.[3] However, biospesific adsorption (affinity chromatography) seems to have the greatest potential to succeed in the very difficult task of purifying to homogeneity many biochemical substances from dilute mixtures containing a large number of proteins exhibiting small differences in solubility, charges, and molecular size. This separation technique utilizes small differences in biological activities of molecules to achieve the purification of proteins. Often, very high levels of purity as well as high product yields may be obtained in a single purification step. The chromatographic separation of proteins is important not only for analysis but also in such large-scale applications, as in the food and the drug industries.[4] Bioaffinity chromatography is a highly specific separation technique used for the isolation and purification of biomolecules. However, some limitations exist such as biological instability and leakage desorption conditions, and cost may limit its applications. A number of ion exchangers for protein separation have been developed to use for analysis, and a large number of chromatograms have been presented to show that they are useful for protein separation. When these ion exchangers are applied to large-scale chromatographic separation, it is necessary to investigate in the detail the equilibria, kinetics, and dynamics of the system.[5] Yamamoto et al. examined the adsorption of bovine serum albumin (BSA) on crosslinked *N,N′*-diethylaminoethyl (DEAE) dextran (a weakly basic dextran-type ion exchanger) decreases with decreasing pH.[6] Tsou and Graham showed the effect of the

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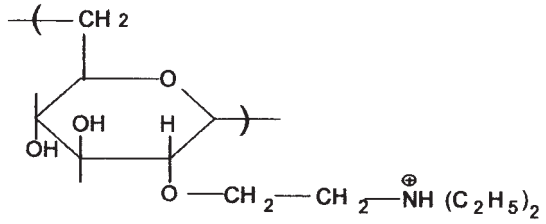
concentration of NaCl (c_e) in the BSA solution on the adsorption isotherm of BSA on DEAE at pH 6.9.[7] They showed that the isotherm for $c_e = 0$ is much more favorable than that for $c_e = 1\%$. However, these reports are fragmentary and systematic experimental investigations for adsorption isotherms of protein have not been reported. Yoshida et al. presented experimental equilibrium isotherms of adsorption of BSA on crosslinked DEAE dextran ion exchanger using Na^+ and Cl^- ions.[5] BSA is the most abundant protein in blood plasma. It has many important physiological functions that contribute significantly to colloid osmotic pressure and aid in the transport, distribution, and metabolism of many endogenous and exogenous substances.[8–13]

In this work, adsorption kinetics, equilibrium isotherms for adsorption of BSA on crosslinked DEAE dextran, have been determined experimentally. The adsorption isotherm model was found as Freundlich–Langmuir isotherm model. Adsorption capacity factor and adsorption equilibrium constant were obtained and mathematical modeling of adsorption, adsorption rate constant, and maximum adsorption was performed. Adsorption mechanism of a BSA on crosslinked DEAE dextran was estimated according to the experimental results of the adsorption. It was proved both experimentally and theoretically that protein is adsorbed by electrostatic attraction, ion-exchange, hydrophobic interaction, and/or hydrogen bonding. Besides the adsorption mechanism, swelling kinetics of crosslinked DEAE dextran and optimum ionic strength, optimum pH, and optimum mass of hydrogel were investigated. Desorption studies and the desorption ratio of the system were determined for optimum medium conditions.

EXPERIMENTAL

Chemicals

Commercial DEAE dextran microbeads were used (Sephadex A-50, Pharmacia Fine Chemicals).



(BSA was purchased from Sigma (lyophilized, fraction V). Adsorption studies were performed in a BSA reservoir by constant stirring rate at120 rev/min. The pH of the adsorption medium was changed in the range pH 4.5–7.2 using different buffer systems. (0.1*M* CH3COONa-CH_3COOH for pH 4–6 and 0.1*M* K_2HPO_4-$KHPO_3$ for pH 7–7.5). The ionic strength of the medium was adjusted by NaCl. The initial concentration of BSA solution in aqueous phase was varied between 0.1 and 0. 01 g/100 mL.

Adsorption studies

Adsorption experiments were carried out at a constant temperature of 25°C. Microbeads were contacted with the BSA solution and gently mixed. The amount of BSA adsorbed on the particle was measured after a certain time and until it was confirmed that there was no further adsorption. The adsorption capacity was determined by measuring the initial and final concentrations of BSA within the reservoir at $\lambda = 279$ nm using a Schimadzu-100 double beam UV–vis spectrophotometer. Using the formula the adsorbed phase concentration of BSA was calculated with

$$q = V(c_0 - c_e)/W, \quad (1)$$

where c_0 and c_e are the initial and equilibrium concentrations of BSA in liquid phase (mg/mL), respectively, q denotes the microbeads-phase concentration of BSA (mg BSA/g of crosslinked DEAE dextran microbeads), and W is the mass of the microbeads (g) and V is the volume of the solution (mL).

Desorption studies

Desorption of the adsorbed BSA from the crosslinked DEAE dextran microbeads was studied in a reservoir. The microbeads carrying different amounts of BSA were placed with in the desorption medium containing 1.0*M* NaCl at medium pH and an amount of BSA was released in 5 h. Desorption ratios for BSA were calculated by use of the expression:

$$\text{Desorption ratio (\%)} = (\text{amount of BSA released})/(\text{amount of BSA on the microbeads}) \times 100. \quad (2)$$

Swelling studies

Swelling percentage value volumetric measurements were made to measure the microgels. For this purpose a constant weight of crosslinked DEAE dextran microgels was left into the volumetric vessel and the increment of the volume was followed as the function of time. Microgels were immersed in distilled water at room temperature. The percentage of swelling (%) was determined by the equation

$$S(\%) = V_{\text{swollen}}(\text{mL})/V_{\text{dry}}(\text{mL}) \times 100, \quad (3)$$

where V_{dry} is the initial volume of the microgels at $t = 0$ and V_{swollen} is the volume of the swollen microgels at time t.

TABLE I
Properties of Crosslinked DEAE Dextran Microbeads (Sephadex A-50)[5]

Crosslinked DEAE dextran (Sephadex A-50)
Description: Weakly basic anion exchanger
Functional group: Diethylaminoethyl (DEAE)
Diameter in water[a] (mm)
Free: 0.2364
Saturated by BSA: 0.2182
Diameter of dry particle[a] (mm): 0.0851
Water content (% wt): 94.1
Effective pH range: 2–9
pK of X-DEAE dextran group: 9.5
Density
True (kg/m^3): 1690
Apparent (kg/m^3): 604
Porosity: 0.963
Total capacity: 3.5 ± 0.5 mg/g
Available capacity
Albumin (MW = 67,000): 7566 mg/g
Hemoglobin (MW = 69,000): 5000 mg/g
Ferritin (MW = 440,000): 74 mg/g

[a] Average value of 50 particles.

Scanning electron microscopy (SEM) studies

To observe the surface topography of the crosslinked DEAE dextran microspheres, we took scanning electron micrographs of the gold-coated samples with a scanning electron microscope (Raster Electronen Microscopy, Leitz-AMR-1000, Germany).

RESULTS AND DISCUSSION

Properties of crosslinked DEAE dextran microbeads

An ideal microbead for protein separation must have the following properties: high hydrophilicity and low nonspecific adsorption, fairly large pore size and narrow pore distribution, chemical and mechanical resistance, as well as enough reactive functional groups. Crosslinked DEAE dextran microgels have an appropriate distribution, good mechanical stability, and adsorption capacity.[4] Table Isummarizes the physical properties of crosslinked DEAE dextran used in this study as obtained commercially.

The SEM micrographs of the dry, swollen, and adsorbed forms of microbeads can be seen in Figure 1. This figure shows the surface structure of microbeads in dry, swollen, and adsorbed BSA forms. Crosslinked DEAE dextran microbeads have a spherical shape and a smooth surface. These surface characteristics of the microspheres favor better adsorption of the BSA as a result of an increase in surface area. Moreover, crosslinked DEAE dextran had initially a uniform and spherical form with smooth surface characteristics. After swelling and adsorption, the smooth surface of the spheres was rapidly covered with BSA molecules, followed by the gradual binding of the latter to BSA as observed in the SEM micrographs (Fig. 1). There is a small difference volume swelling between the SEM micrographs and the percentage swelling value. This can be explained as experimental error, such as drying of the microbeads.

A fundamental relationship exists between the level of swelling of a crosslinked polymer in a solvent and the chemical nature of both the polymer and the sol-

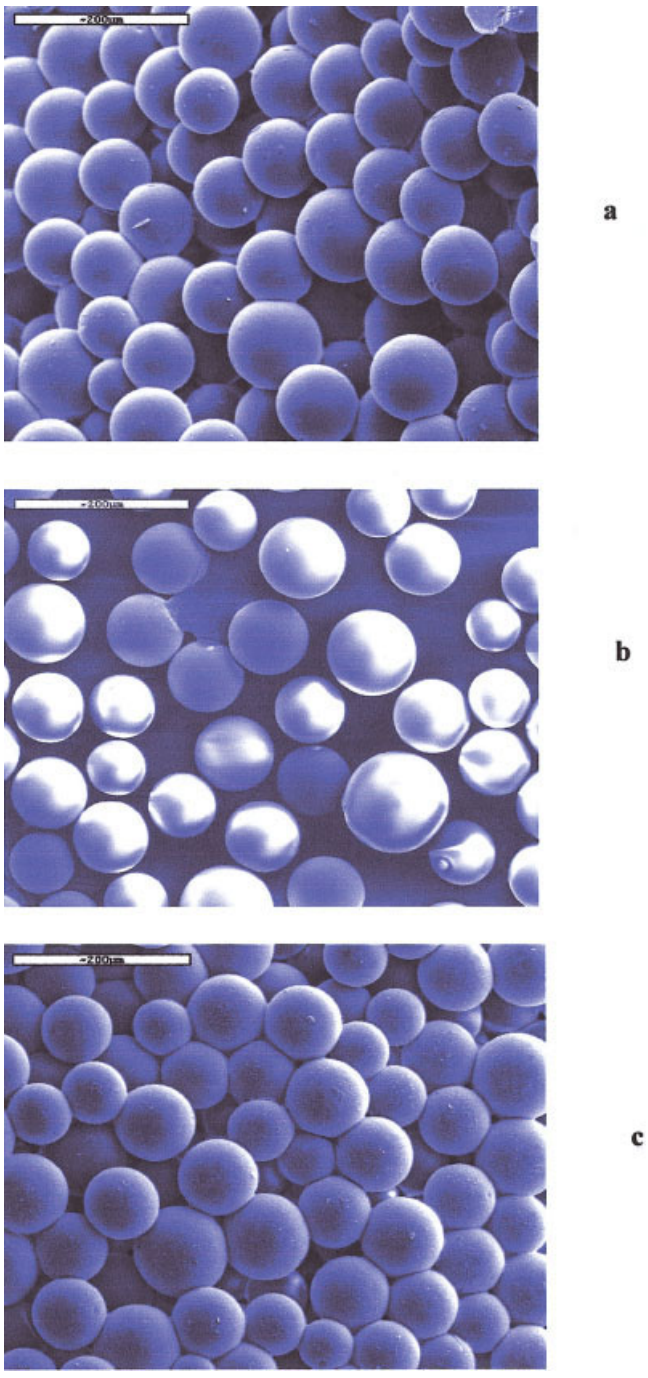


Figure 1 Representative SEM micrographs of crosslinked DEAE dextran in the form of (a) dry, (b) swollen, and (c) adsorbed BSA.

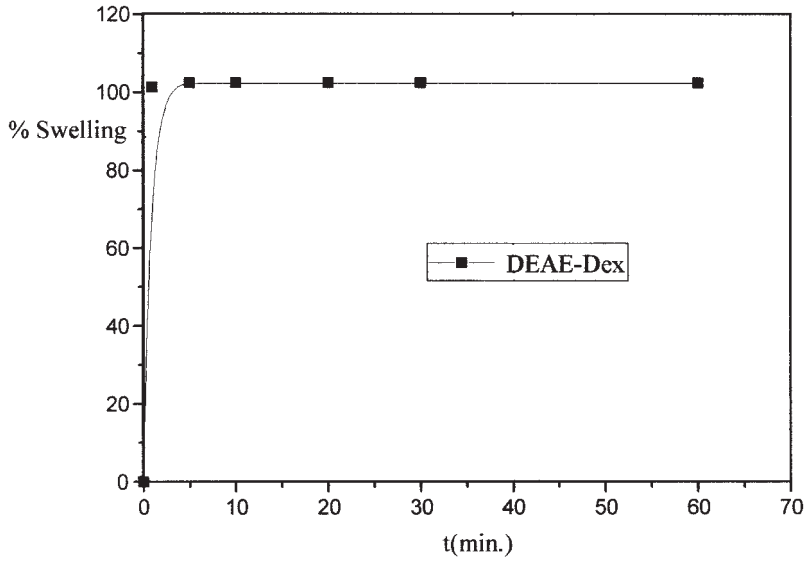


Figure 2 Swelling (%) –time curve of the crosslinked DEAE dextran microsphere in water.

vent. Swelling of the three-dimensional network structure in a suitable solvent is the most important parameter (other especially important parameters are mass and volume swelling).[14,15]

Charged groups attached to the polymeric network structure play an essential role in swelling properties. Several attempts to establish the water content of microspheres have been described in the literature, e.g., by determination of the difference in total volume of wet and dry microspheres or the difference in total weight either with or without blotting. In another study, the wet microspheres were filtered and the change in filter mass was determined. Another approach for determination of the solvent content of microspheres is based on their size upon swelling.[16,17] The increase in size can be determined using static and dynamic light scattering techniques as well as with microscopic methods. The percentage swelling of the dextran microbeads can be derived by the difference between volume in the dry and swollen states.

The presence of the hydrogen bonding groups in the polymer matrix causes the network to be less swollen, compared to a less polar structure. Figure 2 shows the swelling kinetics of the crosslinked DEAE dextran in water. Equilibrium swelling of crosslinked DEAE dextran value for microspheres was approximately 105%. The equilibrium degree of swelling of ionic hydrogels is controlled by three major factors: mixing of the polymer with a swelling medium (miscibility), the elastic-refractive force exerted on the network, and the ionic pressure generated from the mobile counterions onto the charged ion in network. Because of the low equilibrium swelling value of the crosslinked DEAE dextran microbeads, this matrix can be easily used in the column separation applications.[18]

Adsorption studies

Effect of mass of microspheres on adsorption

To determine the optimum mass of the microspheres, several masses of crosslinked DEAE dextran microgels were used for the BSA adsorption studies. By increasing the mass of the microbeads, the adsorption of BSA increased up to a certain value; however, when very large amount of microspheres were employed the adsorption could not be measured. The optimum mass of crosslinked DEAE dextran microspheres was estimated to be 0.005 g (Fig. 3).

Adsorption kinetics

Figure 4 implies the curves of adsorption kinetics obtained in medium at pH 6.9. High adsorption rates were observed at the beginning of the adsorption, and saturation values were then gradually achieved in about 5 h. This may be because of the decrease in the BSA concentration in the reservoir with time due to adsorption. As expected; when BSA concentration in the mobile phase (i.e., the aqueous phase) decreases (which also corresponds to an increase in the stationary phase, i.e., the sorbent microbeads) the driving force (i.e., the concentration difference between the two respective phases) decreases, which brings about a decrement in the adsorption rate.

Based on the adsorption kinetics of BSA/dextran system, it may be concluded that the protein adsorption can be observed on anion- and cation-exchange resins and semiplateau values vary with the experimental conditions; the semiplateau or kink cannot be observed in the isotherm and is not directly related to the surface coverage of protein molecules on resin.

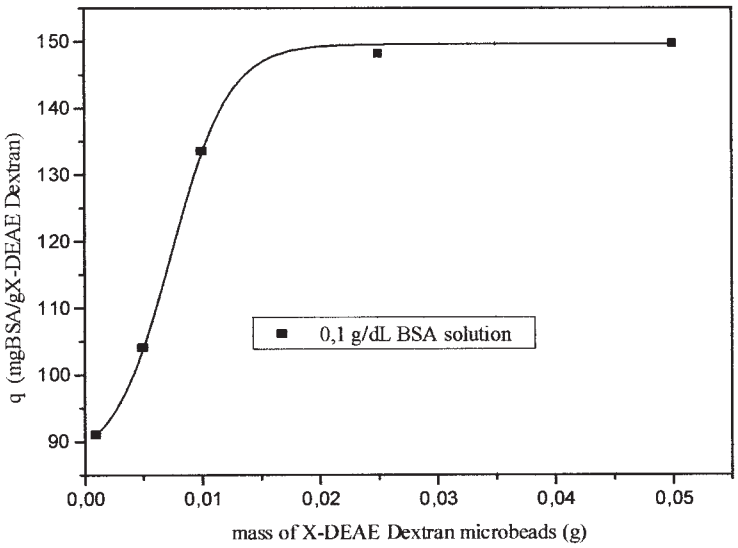


Figure 3 Effect of mass of crosslinked DEAE dextran on the BSA adsorption at constant BSA concentration 0.1 g/100 mL, temperature 25°C; and total protein concentration 10 mL.

This effect probably reflects a conformational alteration of the BSA molecules adsorbed.

Effect of initial BSA concentration on adsorption

A number of data on the adsorption isotherm thus far observed indicate that the higher the bulk concentration, the greater the amount of protein adsorbed, showing a saturation curve. Figure 5 shows the adsorption of BSA on the crosslinked DEAE dextran microbeads. Note that one of the main requirements in bioaffinity chromatography is the specificity of the sorbent. The nonspecific interaction with the carrier matrix (crosslinked DEAE dextran) should be a minimum for high specificity. As shown Figure 5, increasing BSA concentration in solution results in an increase in the BSA amount adsorbed by the microbeads, reaching a plateau at protein concentration of 0.2 mg/mL (0.005 g/25 mL).

The BSA adsorption capacity of the microbeads varies between 44.2 and 13.6 mg/g, implying that the increase in the BSA adsorption capacity is due to the increase in the BSA concentration in the initial state. Adsorption capacity values of crosslinked DEAE dextran microbeads are given in Table II. This can be explained in terms of specific interactions (electrostatic, hydrophobic,

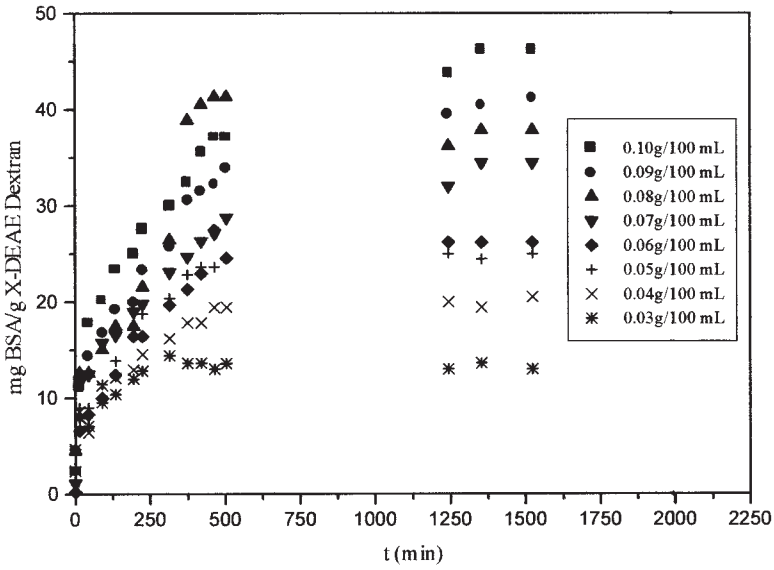


Figure 4 Crosslinked DEAE dextran microbeads–BSA adsorption kinetics curves at different BSA concentration (at 25°C pH 6.9).

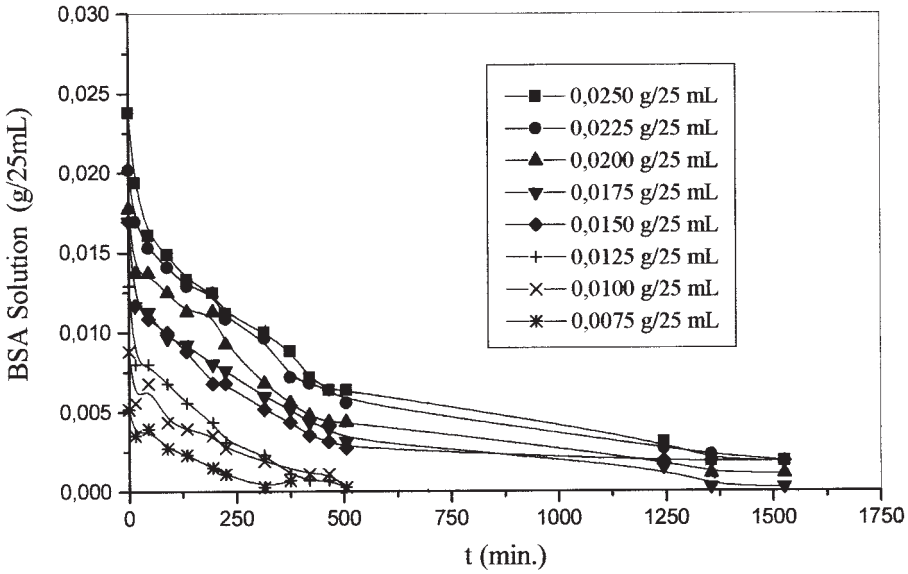


Figure 5 Dependence of the adsorption on initial concentration of the bovine serum albumin (BSA) solution on the adsorption.

H-bonding, and salt bridge) between microbeads and the BSA molecules. The cationic groups of DEAE dextran microbeads interact with BSA molecules from which it can be concluded that the BSA molecules were adsorbed on the microbeads via functional groups.

Different sorbents with varying adsorption capacities were reported in the literature for albumin adsorption. Nigel et al.[20] used Sepharose CL-6B-200 as the carrier matrix and they reported BSA adsorption capacities around 1–3 mg BSA/g gel. Denizli et al.[21] obtained 35 mg BSA/g adsorption capacity using Cibacron Blue F_3GA attached to PVA particles. Boyer and Hsu[22] used Sepharose beads carrying different amounts of Cibacron Blue F_3GA and reported adsorption values up to 55.9 mg BSA/g polymer. McCreath et al.[23] found a 9.7 mg HSA/g equilibrium adsorption capacity with the PVA-coated particulate perfluoropolymer containing anion exchange and cation exchange groups. Chase[24] was able to reach 14 mg BSA/g with Cibacron Blue F_3GA attached to Sepharose CL-6B. Compared with these results it is clear that crosslinked DEAE dextran microbeads exhibit a high albumin adsorption capacity in the present system.

TABLE II
Adsorption Capacities of the DEAE Dextran Microbeads for Protein Adsorption at Constant BSA Concentrations 0.1–0.03 g/100 mL, pH 6.9, at 25 °C; Total Protein Volume is 10 mL

BSA concentration (g/100 mL)	q_{eq} (mg BSA/g DEAE dextran microspheres)
0.10	46.2
0.09	41.2
0.08	37.8
0.07	34.4
0.06	26.2
0.05	24.4
0.04	19.4
0.03	13.6

Effect of pH

Proteins are best known as amphoteric molecules capable of existing in solution as both cations (pH<pI) and anions (pH>pI); their negative charge increases with an increase in pH and its positive charge increases with a decrease in pH. Adsorption capacities of the proteins increase with a decrease in pH. Proteins can be defined as copolymers of some 22 different amino acids of varying hydrophobicity; as a consequence, proteins are amphiphilic molecules. For ion-exchange resin containing weak acid or weak base groups, the degree of the dissociation of groups is dependent on the pH of the solution; the number of dissociated DEAE group decreases with the increases of pH. The adsorption process involves the transfer of a protein molecule from solution to resin surface, and the environmental change upon adsorption results in change in the structure of the proteins.

Table III represents the effect of pH on the equilibrium adsorption of protein. In all cases, the highest adsorption was observed at pH 4.72 and 7.2. Significantly, lower adsorption capacities were obtained in more acidic or more alkaline pH regions. In all investigated cases, the maximum adsorption of BSA was observed at pH 4.8, which is the isoelectric point of BSA for DEAE dextran. The maximum adsorption was

TABLE III
Effect of pH on Bovine Serum Albumin (BSA) Adsorption from Microspheres at Constant BSA Concentration 0.1 g/100 mL, at 25 °C; Total Protein Volume is 10 mL

Medium pH	Adsorption capacity of the microbeads q_{eq} (mg BSA/g X-DEAE dextran gel)
4.50	0.80
4.72	1.60
4.92	0.80
5.98	0.80
6.80	0.80
6.90[a]	46.2
7.20	1.60

[a] pH 6.9 is the nonbuffered protein solution.

observed at pH 6.9, which is nonbuffered protein solution. At pH 6.9 the value was used hereafter as the medium pH of the protein solution. The effect of pH on the adsorption can be explained as the electrostatic repulsion effects of the charges groups. The decrease in the BSA adsorption capacity can be attributed to electrostatic repulsion effects between the identically charged groups at higher and lower pH levels. At the isoelectric points, proteins have no net charge and, therefore, the maximum adsorption from solution is usually observed at this point. When the negative charges of the BSA molecule increase, the high pH value for the number of fixed groups needed to neutralize the negative charges of one BSA molecule increases. Consequently, the adsorption capacity changes according to upper and lower pH limits. Because the dissociation of anionic groups of the BSA molecule is completed at a pH value within the alkaline region, the adsorption capacities increase the maximum values as of BSA/crosslinked DEAE dextran and its dependence on pH [(pH 4.72 (approximately isoelectric point of BSA) and pH 7.2 (anionic character of BSA)] are shown Table IV. According to the data in Table IV an increase in initial concentration of BSA can cause also increment the adsorption capacity of the microbeads.

Effect of the ionic strength

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

(i) The salt counterions compete against the protein ions for binding sites.

(ii) The salt coions shield the protein ions and uncharged binding sites from each other.

(iii) The change of ionic strength changes the folding and configuration of the protein molecules, resulting in a variation of the hydrophobic interactions between the protein and the resin matrix.

(iv) 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 the binding sites.

There is clearly a need to develop a model to incorporate the effects of ionic strength on protein adsorption, but it is very difficult to develop a theoretical model for the prediction of such a complex effect.[25] The effects of the ionic strength (IS) (adjusted by adding NaCl) on BSA adsorption are presented in Figure 6, which shows that the adsorption capacity decreases with increasing ionic strength of the adsorption medium. It should be noted that in the experimental data set, the salt concentration refers to 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 cationic diethyl amino ethyl groups of the dextran resins and 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 the ionic strength occurs as the NaCl concentration increases.

The effects of the IS (adjusted by adding NaCl) on BSA adsorption are presented in Figure 6, which shows that the adsorption capacity decreases with increasing ionic strength of the adsorption medium.

In addition, given 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 adsorption capacity of BSA decreases by an amount 13% at 0.1 g/100 mL BSA (46.2% in the absence of salt reducing to 33.4% at the minimum salt concentration) the adsorption capacity drops rapidly as the salt concentration is increased. The decrease in the adsorption capacity with the increasing ionic strength can be attributed to the decreased attraction between cationic amine group of dextran and protein molecules.

When the salt concentration increases in the adsorption medium, this can cause cation–cation interactions

TABLE IV
Adsorption Capacity of BSA/Crosslinked DEAE Dextran Microbeads Dependent on the pH Values

Initial BSA concentration (g BSA/100 mL)	q_{eq} (mg BSA/g X-DEAE dextran gel) at pH 4.72	q_{eq} (mg BSA/g X-DEAE dextran gel) at pH 7.20
0.10	2.60	3.20
0.09	2.50	2.40
0.08	1.60	1.60
0.07	1.50	1.60
0.06	0.80	0.80
0.05	0.76	1.60
0.04	2.44	2.40
0.03	0.80	1.60

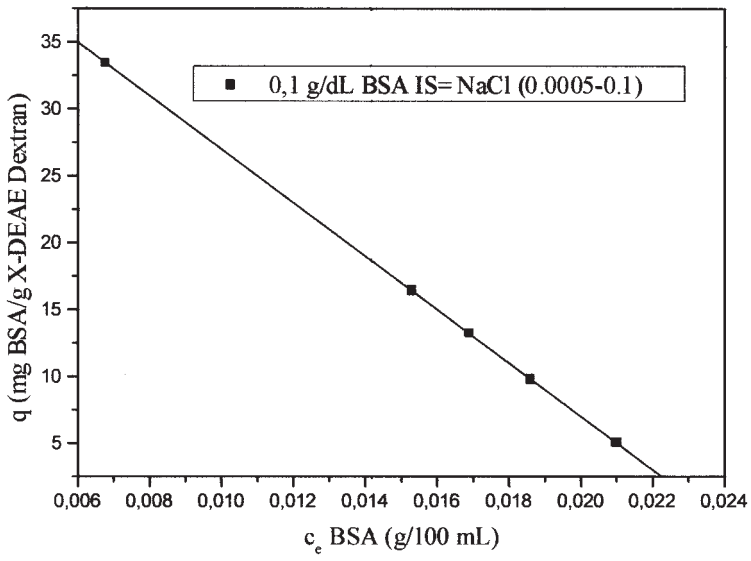


Figure 6 Effect of ionic strength on BSA adsorption on the crosslinked DEAE dextran microspheres at constant BSA concentration 0.1 g/100 mL, temperature 25°C, pH 6.9, and total protein concentration 10 mL.

with the sodium salt, which leads to low protein adsorption. The distortion of existing salt bridges in the presence of sodium salt also contributes to the low protein adsorption at high ionic strength. Tsou et al.[26] showed the effect of the concentration of NaCl in the BSA solution on the isotherm for the adsorption of BSA on DEAE dextran at pH 6.9.

Adsorption equilibrium isotherms

The most common presentation of the adsorption data is the adsorption isotherm, where, at constant temperature, the amount of sorbate (q_{eq}) is plotted against the steady-state concentration c_{eq} of the sorbate in the bulk solution after adsorption. Fully measured adsorption isotherms provide a convenient method for determining whether an adsorption process can be treated as reversible. Investigations on simple model systems, consisting of a well-characterized protein, a well-characterized sorbent, and an aqueous solvent containing only simple ions, have provided the most reliable and meaningful data on the thermodynamics of protein adsorption. In most cases, the system is unbuffered so that pH changes and proton transfer reactions can be monitored as a function of adsorbed amounts. Results from such studies are the basis for most of the general principles that are thought to govern the protein adsorption process; unfortunately, even for these simple systems, a complete picture has not yet emerged. Nevertheless, resolution of the interactions governing adsorption in these model systems will provide the only reliable foundation for understanding protein adsorption in more complex, technologically important processes. For protein adsorption at solid/liquid interfaces, some model system studies and many different isotherm models, such as the linear Langmuir, the bi-Langmuir, the Fowler, the Redlich–Peterson, the Javanovic, the *S*-shaped isotherm, the Freundlich, and the Freundlich–Langmuir isotherms, have been proposed for the adsorption of solutes in a liquid solution onto solid surfaces.[27] Most of these models are essentially empirical although theoretical derivations have been accomplished in some cases. Among all, this model is probably the most popular due to its simplicity and its good agreement with the experimental data. Modeling of the adsorption processes on crosslinked DEAE dextran microbeads was realized by applying different adsorption isotherms.

For determining equilibrium isotherms of BSA/crosslinked DEAE dextran microbeads Freundlich–Langmuir isotherm models were used.

$$(X\text{-DEAE Dextran}) + Cl^- + BSA \underset{k_2}{\overset{k_1}{\Leftrightarrow}} \text{complex} + Cl^- \quad (4)$$

After applying the Langmuir and Freundlich adsorption isotherm models, these two models were not valid for the interpretation of adsorption kinetics of the system. Thus, a third model was employed for the investigation of adsorption equilibrium trend.

The third adsorption isotherm was fitted to the Langmuir-Freundlich isotherm using a nonlinear regression method, the same as in the case of previous studies.[28] Although the Freundlich–Langmuir equation (Fig. 7) can be used to model adsorption cooperativity, the combination of the Langmuir and the Freundlich expressions is more often applied on a strictly empirical basis, since the extra parameter (n)

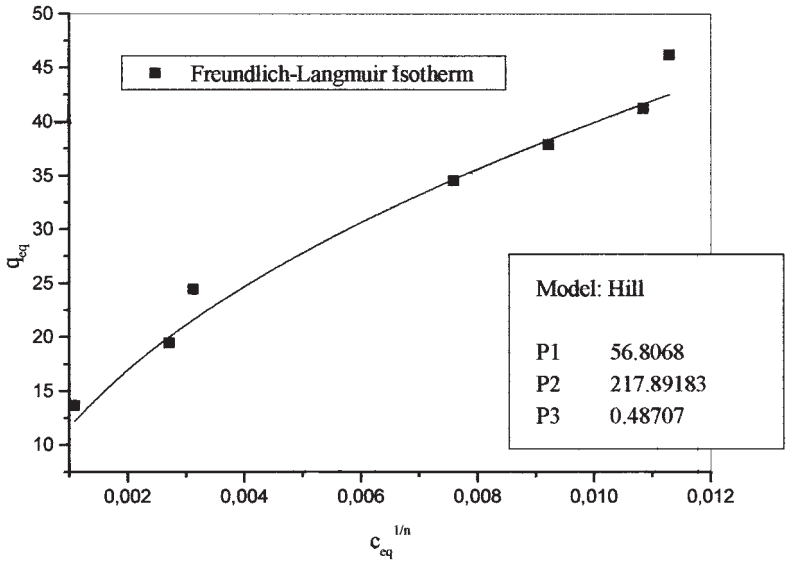


Figure 7 Freundlich–Langmuir isotherm model for adsorption of BSA on crosslinked DEAE dextran microspheres.

ensures an improved correlation with the equilibrium data compared to the simple Langmuir isotherm,

$$q_{eq} = q_{max}(K_d c_{eq}^{1/n})/(1 + K_d c_{eq}^{1/n}), \quad (5)$$

where q_{max} is the plateau adsorbed amount (maximum amount of adsorption) and K_D is the adsorption constant. In the Langmuir isotherm, K_d is the ratio of the adsorption to the desorption rate constant. In the Langmuir–Freundlich isotherm this definition does not apply but its meaning is almost the same; a high value of K_d represents faster rates of adsorption over desorption. Figure 7 represents the Langmuir–Freundlich isotherm. From the isotherm of the BSA/crosslinked DEAE dextran microspheres K_d and q_{max} values were as high as 7.9 mg/mL and 56.81 mg/g, respectively ($R^2 = 0.987$).

Mathematical modeling of the adsorption

To examine the controlling mechanism of the adsorption process of such a mass transfer and chemical reaction, kinetic models are used to test the experimental data. The large number and different chemical groups on the BSA (e.g., –COOH, -NH_2, = NH, -SH, -OH) imply that there are many types of protein–DEAE dextran microgels interactions. Kinetic models including the pseudo first-order and pseudo second-order equations can be used in this case assuming the measured concentrations. The first-order rate equation of the Lagergren is one of the most widely used for the sorption of a solute from a liquid solution. For determining kinetic modeling of adsorption the following equation was used,[29]

$$t/q_t = (1/k_2 q_{eq}^2) + (1/q_{eq})t, \quad (6)$$

where k_2 (g mg min^{-1}) is the rate constant of pseudo second-order adsorption and q_{eq} denotes the amounts of adsorption at equilibrium (mg g^{-1}) and t is time (min). The kinetic model based on concentration is represented in Figure 8, which displays a second-order kinetic. According to this model two different behaviors can be proposed. Due to the first assumption, it is considered that the empty sites of the microspheres are first filled with BSA molecules, after which they move closer toward each other due to the electrostatic interactions among the DEAE dextran, protein, and water molecules (anionic–cationic, hydrophobic–hydrophilic), subsequently followed by further binding of other BSA molecules in several layers onto the microspheres previously covered with BSA. The latter explanation can be interpreted by the concentration of BSA molecules. As the BSA concentration increases, the adsorption capacity of the microspheres also increases where the adsorption rate is very high at the beginning of kinetics (due to the empty sites of microspheres) and at high BSA concentrations (Table V). However, with proceeding time, the adsorption rate slows down since the adsorption of the latter approaching BSA molecules becomes more difficult for the microspheres and consequently the adsorption rate constant should increase to provide further adsorption of approaching BSA molecules. The theoretical q_{eq} values, which can be seen from Table V, estimated from the kinetic modeling gave significantly close values to that of the experimental values, and correlation coefficients were also found to be higher (greater than 0.995). All these results suggest that the crosslinked DEAE dextran mi-

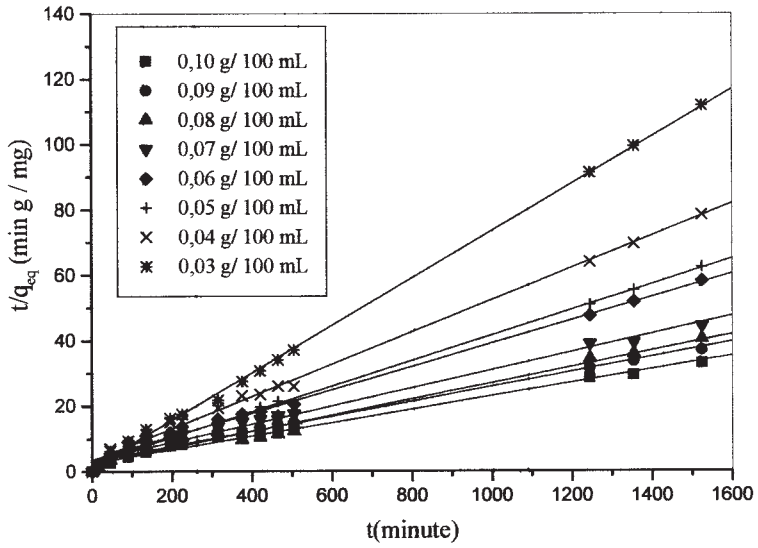


Figure 8 Mathematical modeling of adsorption kinetics of BSA on crosslinked DEAE dextran microspheres using adsorption capacity at 25°C, pH 6.9, and total protein volume 10 mL.

crospheres of BSA system are described with the pseudo second-order kinetic model, based on the assumption that the rate-limiting step may be adsorption involving the electrostatic interactions among the DEAE dextran, protein, and water molecules as (i) hydrophobic forces, (ii) electrostatic interactions, and (iii) hydrogen bonding through sharing or exchange of electrons between sorbent and sorbate, providing the best correlation of the data.

Desorption studies

The adsorption of proteins on solid surfaces is often described as irreversible since, after attachment is established, which has usually reached a final value within an hour, subsequent replacement of the protein solution by pure solvent, as a rule, does not lead to any significant desorption on a time scale of hours or even days.[30] However, it does not mean that protein molecules remain attached to the surface whatever the conditions of the solution in which the surface is immersed. With respect to reversibility of adsorption/desorption of proteins, distinction should be made among reversibility toward dilution of the solution, changes in pH and ionic strength, addition of other types of surface-active substances, and exchange against dissolved proteins. The usual procedure for desorption is to remove the adsorbed protein rapidly by increasing the salt concentration or changing the pH. However, under conditions of reversible adsorption, as evidenced by a Langmuir isotherm, desorption can also be achieved (although more slowly) merely by removing protein from the feed solution, i.e., switching back to a solution of buffer and NaCl.[31,32] However, in the Freundlich–Langmuir isotherm system, high protein recovery can be obtained by increasing the pH and/or the ionic strength of the eluent. Desorption experiments were carried out in a reservoir. Albumin adsorbed crosslinked DEAE dextran microspheres were placed in the reservoir and NaCl

TABLE V
Adsorption Kinetic Constants and Adsorption Capacity of the System of Crosslinked DEAE Dextran Microspheres in Different Concentration of BSA Solutions

BSA concentration (g/100 mL)	Experimental q_{eq} (mg BSA/g microspheres)	Theoretical q_{eq} (mg BSA/g microspheres)	k_2 (g mg^{-1} min^{-1}) $\times 10^4$
0.10	46.2	48.9	1.59
0.09	41.2	43.9	1.61
0.08	37.8	40.5	2.58
0.07	34.4	36.3	2.20
0.06	26.2	28.2	3.46
0.05	24.4	25.7	5.56
0.04	19.4	20.3	7.29
0.03	13.6	13.9	39.5

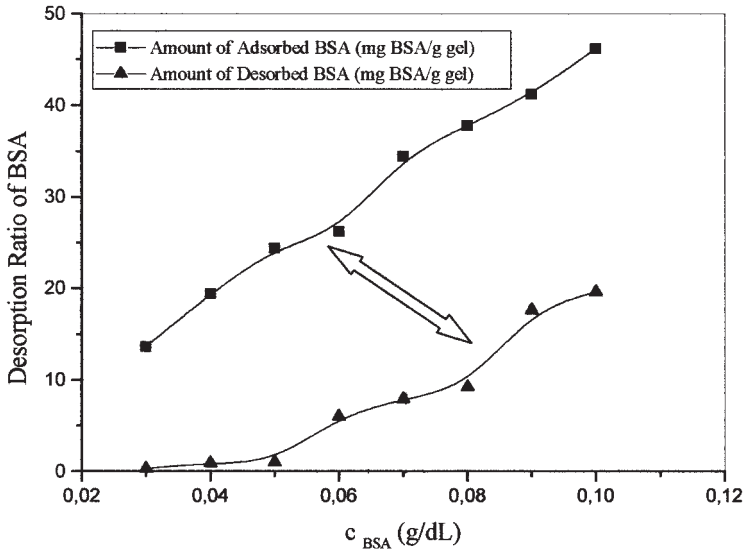


Figure 9 Desorption ratio of the percentage of BSA from crosslinked-DEAE dextran microspheres.

solution (0.2*M* NaCl) was added. The amount of albumin released in 1 h was determined. The desorption ratio for BSA was calculated using the equation

Desorption ratio (%) = (amount of BSA desorbed/amount of BSA adsorbed microbeads) × 100.

It can be concluded that the adsorption may be categorized within a chemisorption process since the maximum desorption value reaches only 42.7% where the recovered amount access seems to be very low (Table IV). One may simply imply that only those BSA molecules attached to the surface in the upper may have been desorbed and not those in the lower layers or in the empty sites of microspheres. The trend validated by the Freundlich–Langmuir isotherm may also be another solid proof for classification of our adsorption behavior as a chemical–physical adsorption process. Figure 9 shows the difference between the adsorbed amount of BSA and the desorbed amount of BSA from the crosslinked DEAE dextran. As can be seen from Table VI; approximately 20 mg BSA adsorption capacity/g crosslinked DEAE dextran microbeads cannot be desorbed from the microbeads. One may simply imply that only those BSA molecules attached to the surface in the upper layers have been desorbed and not those in the lower layers or in the empty sites of microspheres.

CONCLUSIONS

Unlike small molecules, protein possesses specific secondary and higher levels of structure. These higher levels of structure are maintained by relatively weak noncovalent interactions. Distribution of these weak interactions can be caused by factors such as temperature (heat or cold), pH, salt, shear, surface interactions, and freeze-drying. Results in the desorption parts indicate that bovine serum albumin attachment to the crosslinked DEAE dextran microbeads occurs rapidly at medium pH. Desorption occurs with NaCl but the adsorption process is not fully reversible. These results support the interpretation that bovine serum albumin has a moderate affinity for crosslinked DEAE dextran.

TABLE VI
Desorption Percentage (%) of BSA from Crosslinked DEAE Dextran Microspheres

BSA solution (g/100 mL)	Desorption ratio (BSA %)	Amount of adsorbed BSA (mg BSA/g gel)	Amount of desorbed BSA (mg BSA/g gel)	BSA amount on gel (mg BSA/g gel)
0.10	42.4	46.2	19.6	26.6
0.09	42.7	41.2	17.6	23.6
0.08	24.2	37.8	9.20	28.6
0.07	23.0	34.4	7.90	26.5
0.06	22.7	26.2	6.00	20.3
0.05	4.10	24.4	1.00	23.0
0.04	4.70	19.4	0.90	18.5
0.03	2.10	13.6	0.30	13.3

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