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Adsorption of Bovine Serum Albumin (BSA) on Clinoptilolite

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Abstract

The purpose of the present work was to investigate the adsorption of BSA on clinoptilolite in aqueous media. In this study, batch adsorption experiments were carried out and the effects of pH, protein concentration, ionic strength and modification of zeolite on the adsorption process were examined. The optimum pH for adsorption was found to be 4.0. It was found that various treatments applied to modify clinoptilolite has a substantial effect on the adsorption. In adsorption studies, residual BSA concentration reached equilibrium in a short duration of 60 min. Maximum adsorption capacity value for clinoptilolite in the original form is 388.3 mg BSA/g zeolite (23.2 mg BSA/m^2) and in the modified form ranges from 500-660 mg BSA/g zeolite (25-33 mg BSA/m^2) showed that this adsorbent, clinoptilolite, was suitable for the adsorption of BSA from aqueous media.

Key Words: Clinoptilolite, bovine serum albumin, adsorption

INTRODUCTION

Latest applications in biotechnology such as artificial implants, protein-purification strategies, biosensors, and drug delivery systems include the interactions of the proteins and non-biological surfaces [1,2]. Various chromatographic methods such as ion exchange, affinity, hydrophobic interaction, and so on have been used for the separation of various kinds of proteins, but these resins have many problems. Some of them are very weak chemically and physically (for example, strong acid and alkali, high and low temperature, and high pressure). Since the conventional adsorbents reach the limits of separation methods, it is impossible to further develop the methods for chromatography with these carriers [3,4]. Thus, new chemically and physically strong adsorbents are desired. Zeolites are known to be chemically and physically strong. Thus, these properties make zeolites attractive candidates for applications in separation technologies [5]. Zeolites are crystalline porous solids consisting of corner-sharing AlO_4 and SiO_4 tetrahedral with pores and channel system. These physicochemical functions in the catalysis reactions, separation, and characteristics are thought to be the basis for their ion-exchange and give the zeolites a very low bulk volume and thereby a material with very high adsorption capacity of low molecular weight molecules [6,7]. The zeolites adsorb also various

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macromolecules, such as proteins, can be adsorbed on the surface of the zeolite particles depending on the size of the pores. These properties of the zeolites make them potential carriers of various bio-molecules when their nano particle size and commercial availability are considered [8].

The most interesting feature of the zeolites is their well defined pore and cage system. The pore sizes and other properties of the pore system can be changed by thermal and chemical treatment. For example, Si/Al ratio of zeolites can be varied during synthesis or postsynthetically (e.g dealumination). The de-alumination process reduces the electrostatic charge in the structure of the zeolite and their adsorption properties become hydrophobic. Hydrophobic low molecular weight molecules show a high adsorption toward de-aluminated zeolites. Previous studies indicated that proteins tended to bind well at or around their isoelectric point (pI) to zeolites with a higher Si/Al ratio, suggesting that the hydrophobicity and the three-dimensional structure of zeolites have the strongest influence on adsorption. In one of these previous studies, it was found that zeolites adsorbed the proteins and that there were three physicochemical principles underlying the adsorption: (i) below the pI of each protein, it was mainly Coulomb's attraction, similar to ion-exchange chromatography, (ii) at the pI, it involved hydrophobic interactions (a kind of van der Waals attraction) together with the three-dimensional mesopore structure, and (iii) above the pI, it was the sum of Coulomb's repulsion and attraction, such as a hydrophobic interaction [9].

At high Si/Al ratios in the presence of a small amount of Al and with mesopores between the zeolite particles, maximal adsorption was seen at the pI, and it was thought to be dependent on the number of hydrophobic interaction points on the mesopores and their morphology [9,10].

Clinoptilolite is one of the zeolite species and is found in abundance in many deposits around the world. It belongs to the heulandite group, with a three-dimensional framework of silicon and aluminum tetrahedra, having the typical chemical formula $Na_6[(AlO_2)_6(SiO_2)_{30}].24H_2O$ [6].

Albumin is the most abundant protein in the circulatory system which is responsible for the blood pressure and pH [11-12]. BSA is a large protein containing 14% basic groups and 18% acidic groups, with a pI of 4.8. It is therefore negatively charged at pH 7.2 and positively charged at pH 4.7 [13]. There is not any study about the adsorption of BSA onto the natural clinoptilolite and its modified forms.

In this study, we studied the adsorption of BSA to the natural and modified clinoptilolite samples with different Si/Al ratios. The effects of pH, protein concentration, adsorption time and acidic and basic treatment on the adsorption of BSA were investigated.

EXPERIMENTAL

Zeolite sample

The zeolite sample used in this study was obtained from Bigadic region of Turkey. Zeolite was grinded to certain size and its particle size was determined. Surface areas of the samples were measured by Quantachrome NOVA 2200e series Surface Analyzer. The determination is based on the measurements of the adsorption isotherms of nitrogen at 77 K. Surface area of the samples were determined by using BET equation in the relative pressure range of between 0.05 to 0.3, seven adsorption points and BJH (Barrett-Joyner-Halenda) method was utilized for the measurement of pore size distributions. Before all of the measurements, moisture and gases such as nitrogen and oxygen

adsorbed on the surface or held in the open pores, were removed under reduced pressure at 100°C for 5 h.

Scanning electron micrographs were obtained using a Leo G34-Supra 35VP scanning electron microscope equipped with an EDX (Energy Dispersive X-Ray) analyser. The effects of adsorbent pore size and particle morphology on adsorption were also studied.

Adsorption studies

BSA was obtained from Sigma Chemical Co. (USA, catalog number A-7906, M.W. 69 KDa, pI 4.7). BSA solutions were prepared with deionised water with a conductivity value of 18.2 MΩ supplied from Barnstead Nano pure Diamond. The effect of pH on the BSA adsorption was investigated using BSA solutions ranging between 50 and 450 ppm over the pH range 3.0-7.0. The pH values of solutions were adjusted by appropriate using buffer solutions prepared by using phosphate (Na_2HPO_4 / NaH_2PO_4) and acetate (CH_3COOH / CH_3COONa) salts.

BSA adsorption was measured by the classical batch equilibration method. Adsorption isotherms were constructed by measuring the differences in the protein concentrations free in solution that resulted from the addition of a specific amount of zeolite and are averages of at least three experiments. Adsorption tests were conducted in polypropylene beakers. In each adsorption study, 30 mg zeolite (dry weight) was added to 50 ml of the BSA solution at 25°C and magnetically stirred continuously. After 1.5h, the aqueous phase was separated from the zeolite by centrifugation and the concentration of BSA in the solution was determined by diluting the solution with suitable proportions and than by adding Bradford solution. The protein concentration in the supernatant were analyzed with a UV-Vis spectrophotometer (UNICAM UV-Vis spectrometer) at 595.0 nm.

The effect of the initial BSA concentration on the adsorption capacity of the zeolite at the optimum pH was determined using solutions with concentrations ranging from 50 to 450 ppm. Again, 30 mg zeolite (dry weight) was added to 50 ml of the BSA solution at 25°C and magnetically stirred continuously. After 1.5h, the aqueous phase was separated from the zeolite by centrifugation and the concentration of BSA in that phase was determined by using UV-vis spectrometer.

The amount of adsorbed BSA (mg BSA/g zeolite) was calculated from the decrease in the concentration of BSA in the medium by considering the adsorption volume and amount of the zeolite in the adsorption study:

$$q_e = [(C_i - C_e) . V] / m \quad (1)$$

Here, q_e is the amount of BSA adsorbed to the unit mass of the zeolite (mg BSA/g zeolite) at equilibrium; C_i and C_e are the concentrations of the protein in the initial solution and in the aqueous phase after treatment for certain adsorption time, respectively (ppm BSA); m is the amount of zeolite used (in gram) and V is the volume of BSA solution (in liter).

To determine the adsorption rate of BSA from aqueous solution, same batch adsorption and analysis procedure given above was used and optimum adsorption time was determined.

To investigate the effects of the acidic and basic treatment, zeolite samples were treated with HNO_3, H_2SO_4, HCl, H_3PO_4 and NaOH solutions with concentrations ranging from 0.01-10 mol/l. To adsorb BSA from aqueous solution, these acid and base treated zeolite samples were used. Again, 30

mg treated zeolite samples (dry weight) was added to 50 ml of the BSA solution at 25°C and magnetically stirred continuously. After 1.5h, the aqueous phase was separated from the zeolite by centrifugation and the concentration of BSA in that phase was determined by using Bradford method and UV-vis spectrometer.

To determine the re-usability of the zeolite sample BSA adsorbed zeolite samples were treated with desorption agents, 0.01 M PEG, 1.0 M and 2.0M NaCl, 1.0 M and 2.5M NaSCN aqueous solutions and 0.1 M NaCl in ethanol / H_2O mixture (50 / 50, by volume). 30 mg (dry weight) portion of zeolite samples carrying 388 mg BSA /g zeolite were placed in desorption medium (50 ml) and stirred magnetically for 24h at 25°C. After 24h, the aqueous phase was separated from the zeolite and the concentration of BSA in that phase was determined by using Bradford method and UV-vis spectrometer.

RESULTS AND DISCUSSION

A) Characterization of the zeolite samples

The structural properties of the zeolite samples with different Si / Al ratio are listed in Table 1.

As can be seen, all the adsorbents have a mean pore diameter value around 39 angström except base treated zeolite samples which is smaller than all the principal dimensions of BSA 40*40*140 (in angström) [14].

B) Influence of variables on BSA adsorption

Effect of pH

The pH dependence of BSA adsorption onto zeolite is shown in Figure 1. Experiments were carried out using BSA solutions at different pH values adjusted by using buffer solutions. The stability of the zeolite samples were checked at the pH used and the structures were found to be stable. As it is seen in Fig. 1, the highest value of qe is obtained at pH 4.0 value near the protein isoelectric point. This result is in good agreement with the results obtained in previous studies reported adsorption isotherms with different adsorbents for different pH values [1,15].

Table 1. Propeties of the zeolite samples with different Si/Al ratio.

Type of solid (acid/base treated)	Si/Al ratio	S_{BET} (m^2/g)	Pore size (Å)
10.0 M HNO_3 treated	5.74	19.4	39.2
5.0 M HNO_3 treated	5.80	19.7	39.7
1.0 M HNO_3 treated	5.18	13.7	39.3
0.1 M HNO_3 treated	5.18	18.5	39.0
0.01 M HNO_3 treated	5.26	19.9	39.5
10.0 M H_2SO_4 treated	4.96	16.9	22.1
5.0 M H_2SO_4 treated	5.40	16.3	34.8
1.0 M H_2SO_4 treated	5.32	19.4	39.6
0.1 M H_2SO_4 treated	5.56	18.8	39.5
0.01 M H_2SO_4 treated	5.16	19.1	39.5
10.0 M H_3PO_4 treated	6.68	15.4	24.9
5.0 M H_3PO_4 treated	5.82	16.7	38.9
1.0 M H_3PO_4 treated	5.28	18.0	39.5
0.1 M H_3PO_4 treated	5.16	18.8	37.9
0.01 M H_3PO_4 treated	5.50	18.5	39.6
10.0 M HCl treated	5.94	20.4	39.6
5.0 M HCl treated	5.56	13.2	39.2
1.0 M HCl treated	5.08	20.6	39.7
0.1M HCl treated	5.13	20.2	38.1
0.01 M HCl treated	4.83	20.9	39.0
10.0 M NaOH treated	4.56	47.6	59.6
5.0 M NaOH treated	4.90	75.9	87.3
1.0 M NaOH treated	5.10	42.8	155.0
0.1 M NaOH treated	5.24	50.3	136.8
0.01 M NaOH treated	5.80	49.0	140.7
Original (no treatment applied)	4.46	16.8	39.7

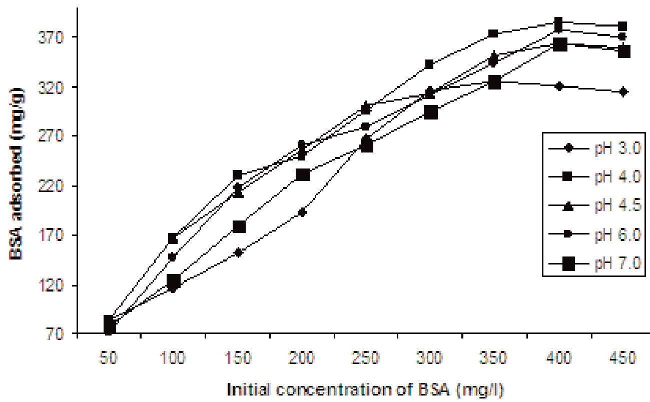

Figure 1. Variation of the adsorbed amount of BSA as a function of pH (BSA concentration = 50 to 450 ppm; temp. = 25°C); a) pH=3.0; b) pH=4.0; c) pH=4.5; d) pH=6.0; e) pH=7.0

pH has a significant effect on protein ↔ surface interaction [1,16,17]. The isoelectric point of BSA is 4.7, so when the pH = 4.0 protein is positively charged. In this case there is an electrostatic attraction between BSA and surface of the clinoptilolite. The maximum adsorption on the zeolites tended to occur when the pH was at or just below the pI of the proteins. With increasing pH (higher than pI) the electrostatic repulsion between the protein and surface increases as both are negatively charged. Additionally, repulsion between adsorbed BSA molecules also increases at higher surface coverage. These two effects combine to reduce the adsorption capacity when the pH is increased above pI [18]. Some proteins that adsorbed to the zeolites with high Si/Al ratios could bind to the zeolites at a pH above the pI value. BSA is known to be a soft protein with a low conformational stability meaning that BSA adsorbs onto negatively charged surfaces at pH values higher than the value of its isoelectric point [19]. Zeolites adsorbed biopolymers on their surface; this may be as a result of the following factors; 1) below pI, mainly the Coulombic attraction similar to ion-exchange chromatography; 2) at pI, probably hydrophobic interactions and the mesopore structure; 3) above pI, hydrophobic interactions and substitution of water at the Lewis acid sites of Al [9]. When the Si/Al ratio is high, but Al level low, and in the presence of mesopores between the zeolite particles the adsorption was maximal at pI; this suggests that the adsortion is markedly dependent on the number of hydrophobic interaction points on the mesopores and their morphology.

Effect of BSA concentration

Experiments conducted with different initial BSA concentrations showed that the amount of BSA adsorbed per unit mass of zeolite (i.e. the adsorption capacity) increases with the initial concentration of BSA (Fig.1). This increase continues up to 400 ppm BSA and beyond this value, there is not a significant change in adsorption capacity. This plateau represents saturation of the active sites available on the zeolite samples for interaction with BSA, the maximum adsorption capacity. It can be concluded that percentage adsorption for BSA decreases with increasing BSA concentration in aqueous solutions. The maximum adsorption capacity was calculated as 388 mg BSA/g zeolite which corresponds to 23.2 mg BSA/m^2 when the surface area of the zeolite sample was considered. Different adsorbents have been reported for the adsorption of BSA. Silica was used and adsorption capacity values of 2.2 mg/m^2 and 3.5 mg/m^2 were found for pH 4.7 and pH 5.0, respectively [1,15]. In another study, achieved adsorption capacities ranged from 3 to 32 mg BSA/m^2 for ZnSe and from 4 to 55 mg BSA/m^2 for polyurethane-coated ZnSe [20]. In a recent work, MCM-41 was used and BSA adsorption capacity range was found to be 124-255 mg/g MCM-41 [8]. It can be concluded that maximum adsorption capacities obtained in this study with natural and modifed zeolite samples are comparable to those obtained with other adsorbents.

Effect of adsorption time

Figure 2 illustrates the adsorption of BSA onto the zeolite as a function of time. The amounts of BSA adsorbed were calculated using equation (1). The adsorption conditions are given in the figure legends. The slopes of the lines joining the data points in the figure reflect the adsorption rates. As it is seen, high adsorption rates were observed at the begining and then plateau values were reached within 90 min. At pH = 4.0 , the protein has a strong affinity for the adsorbent and quickly attains its equilibrium capacity. In the previous studies in which different adsorbents; Al-MCM-41, SiO_2 and siliceous MCM-41 were used, 24h, 3h and 144h were given as optimum adsorption time [8,18,21]. Thus, the adsorption rate obtained with clinoptilolite seemed to be very satisfactory.

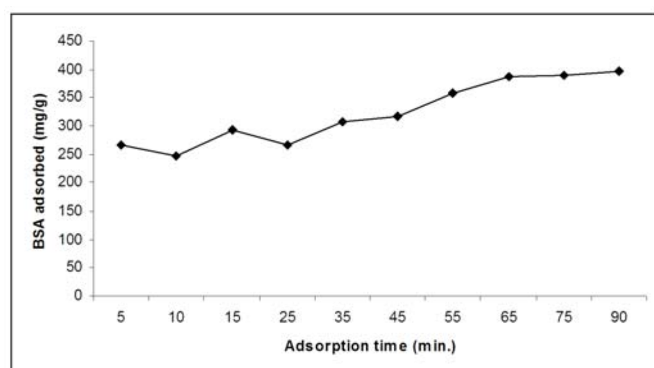

Figure 2. Variation of the adsorbed amount of BSA as a function of adsorption time (BSA concentration = 400 ppm, pH =4.0).

Desorption study

Although the proteins were most efficiently adsorbed on zeolites at their pIs, they were not desorbed by conventional eluents at their pIs. NaCl is widely used as desorption agent in the desorption of proteins. Na^+ and Cl^- ions could destabilize the hydrophobic interaction between the protein and the adsorbent [22]. Also, polyethylene glycol (PEG) could be used to desorb the proteins adsorbed on zeolites at their pIs without loss of activity. PEG is not usually used as a chromatographic eluent. PEG stabilizes protein structure, reduces protein aggregation, and enhances protein refolding. Therefore, it is suitable for the desorption of proteins adsorbed on zeolite [23,24].

In the desorption study in which 0.01M PEG, >1.0 M NaCl and 0.1 M NaCl in ethanol/water mixture (50/50, by volume) in buffer solutions were used as eluents, the zeolite samples loaded with the maximum amount of BSA were placed in the desorption medium and the amount of BSA desorbed with in 24h measured. It was observed that there is not any significant BSA release (0.25-2.50 mg BSA) from solid to the desorption medium. This result is in agreement with those obtained in previous studies used silica based adsorbents in which the adsorption of protein is said to be practically irreversible [21].

Effect of acidic and basic treatments

Acidic treatments of the zeolite samples were carried out with HNO_3, H_2SO_4, HCl and H_3PO_4 solutions with concentrations ranging from 0.01 to 10 mol/l. The adsorption of BSA onto modified zeolite samples with different Si/Al ratios and pore width about 39 angström was studied. Figure 3 shows the adsorption isotherms for BSA onto the zeolite samples with different Si/Al ratios at pH 4.0. H_3PO_4 - treated zeolite sample have a significantly higher adsorption capacity for BSA than the other adsorbents. The saturation adsorption capacity for BSA decreased with increasing concentration of acid used in acid treatment. During the acid treatment of the silica based materials the reaction depicted in (2) occurs.

$$\text{-Si-O-Si} + \text{HOX} \rightarrow \text{-Si-OH} + \text{-SiOX} \qquad (2)$$

In this reaction, siloxane bonds interacting with acid

are broken to yield the silanol group. This interaction increases the relative amount of the Si-OH groups used in the adsorption of BSA to the zeolite [25].

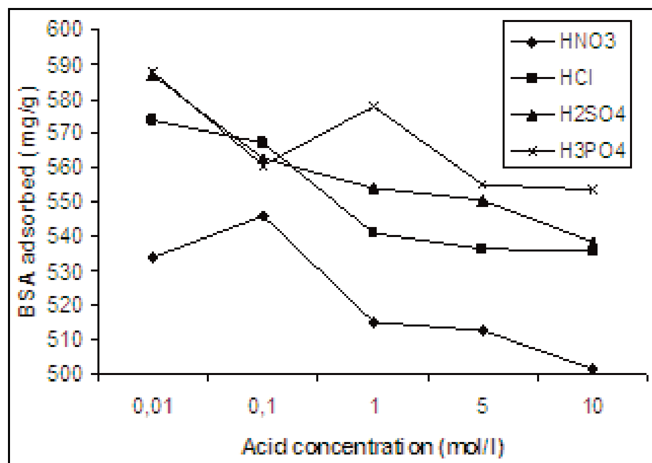

Figure 3. Variation of the adsorbed amount of BSA as a function of acid concentration (BSA concentration = 400 ppm, pH = 4.0)

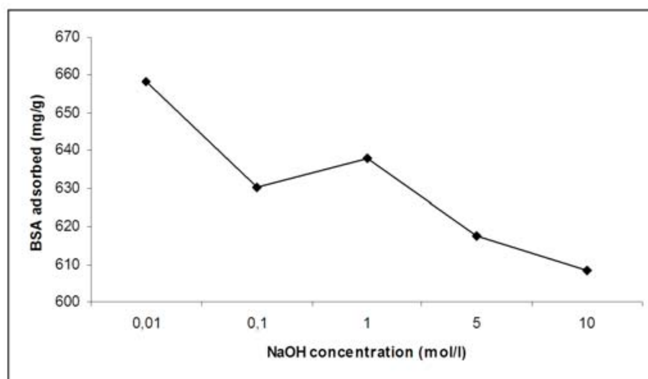

Figure 4. Effect of NaOH treatment on BSA adsorption (BSA concentration = 400 ppm, pH = 4.0).

It was shown that the adsorption capacity of clinoptilolite increased with decreasing of the Si/Al ratio, probably due to the increased affinity of BSA for the external surface of the adsorbents. The external area plays a predominant role in BSA adsorption on clinoptilolite [8]. As it is seen in Table 1, NaOH treated zeolite samples have the higher surface area and pore diameter values than the other zeolite samples.

But, when we use higher concentration of NaOH (greater than 0.01 M), adsorption capacity for BSA decreases. This is mainly due to the decreasing amount of hydroxyl groups on the surface of zeolites with increasing base concentration. In general, proteins with diameter larger than the pore diameter adsorb on the outer surface of the zeolite through hydrogen bonding interaction between the OH groups of zeolite and the carboxylic or amino groups in the protein structure [26].

Effect of ionic strength

By adding different amount of NaCl into the adsorption medium, effect of the ionic strength on the adsorption isotherm was quantitatively determined. Figure 5 shows that increasing salt concentration in adsorption medium has a detrimental effect on BSA adsorption but q_e values are greater than those obtained in the absence of NaCl. Previous studies showed that interactions between proteins and surfaces are both affected by the ionic strength. In the presence of salt charge-mediated repulsion is quenched by ions and excess adsorption occured [27-29]. Na^+ and Cl^- ions could destabilize the hydrophobic interaction between the protein and the adsorbent [22]. With increasing salt concentration this destabilization effect becomes stronger and the adsorption capacity decreases.

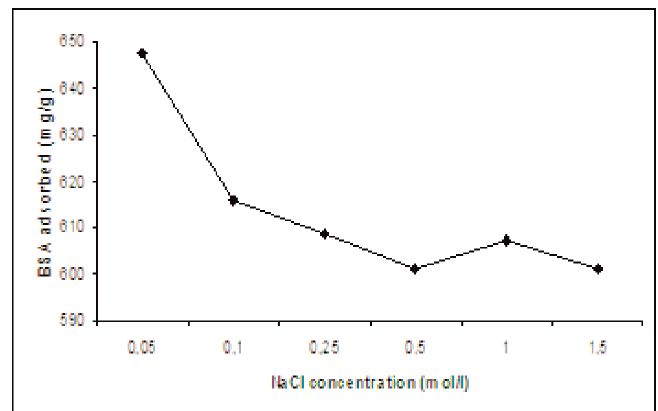

Figure 5. Variation of the adsorbed amount of BSA as a function of NaCl concentration (BSA concentration = 400 ppm; pH = 4.0).

CONCLUSION

In the present study, adsorption of BSA to zeolite samples was investigated. Adsorption isotherms were used to characterize the adsorption of BSA to zeolite in various buffer solutions. The method used in this study allows different angles of the protein/zeolite surface/solution system to be probed. It has been observed that zeolite adsorbents show a very high adsorption capacity for BSA. Maximum adsorption capacity values obtained in this study with natural zeolite and acid treated zeolite samples are comparable to the capacity of other adsorbents. Also, a significant increase was observed at the amount of adsorbed BSA as a result of the acid and base treatment of the clinoptilolite compared to the original zeolite.

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