Brazilian Journal
of Chemical
Engineering

ISSN 0104-6632
Printed in Brazil
www.abeq.org.br/bjche

Vol. 24, No. 03, pp. 325 - 336, July - September, 2007

CHARACTERIZATION OF CHEMICALLY MODIFIED CHITOSAN MICROSPHERES AS ADSORBENTS USING STANDARD PROTEINS (BOVINE SERUM ALBUMIN AND LYSOZYME)

M. A. Torres, M. M. Beppu* and C. C. Santana

School of Chemical Engineering, State University of Campinas, Phone: +(55)(19) 3521-3893,
Fax: +(55)(19) 3521-3922, CP 6066, CEP 13083-852, Brazil Campinas - SP, Brazil
E-mail: beppu@feq.unicamp.br

(Received: January 5, 2006 ; Accepted: June 27, 2007)

Abstract - Chitosan microspheres with a mean size of 140 ± 119 μm were produced by the spray and coagulation methods. The microspheres were chemically modified using the following routes: a) crosslinking with glutaraldehyde b) crosslinking with epychlorohydrin and c) acetylation. For investigation of their ability as adsorbents, the following standard proteins were chosen as adsorbates: bovine serum albumin - BSA (pI = 4.8 and MW = 66 kDa) and lysozyme (pI = 11 and MW = 14 kDa). The adsorption experiments were performed using a static method. The adsorption media and equilibrium concentration of adsorbates were varied in the ranges of pH 4-11 and 0.07-0.70 $mg.ml^{-1}$, respectively. The maximum adsorption capacities (q_m) and the constant of the Langmuir model (K_s) were shown to be dependent on charge interactions and on the kind of treatment performed on chitosan microspheres. The satisfactory fit of a kinetic model to the experimental data shows that the step that controls the adsorption kinetics is probably the initial adsorbate transport.
Keywords: Chitosan microspheres BSA lysozyme adsorption.

INTRODUCTION

The economical viability and the production on an industrial scale of bioproducts depend on the techniques employed in the steps of concentration and purification of these compounds (Arruda and Santana, 2003; Bohak and Sharon, 1979; Krigjaman and Jenkins, 1992). The most prominent bioproducts ones are proteins, which have applications in many areas such as food science, biochemistry, pharmaceuticals, medicine and others (Yoshida et al., 1994; Grzegorczyk and Carta, 1996; Hill and Nearath, 1975). Several chromatographic methods have been developed and are currently being applied to separation processes involving proteins; however, few publications demonstrate the fast and dynamic development of new adsorbents. The development and application of these biopolymeric adsorbents presupposes the control of characteristics such as internal porosity and specific chemical ligands on the surface. The versatility and uniform properties shown by these matrices determine their performance in the adsorption process. Furthermore, when the adsorbents are found on the market, mainly for use on a large scale, they are normally imported and expensive for many countries (Kamei et al., 2002).

Chitosan is an aminopolyssacharide, obtained from chitin deacetylation using alkaline medium, and has acquired a remarkable importance as an adsorbent in downstream processes involving bioproducts. Chitin and chitosan are the main components of crustacean shells and are thus widely

*To whom correspondence should be addressed

distributed in nature (Kurita, 1998). Due to its solubility in acidic solutions, hydrophilicity, chemical reactivity and moldability, chitosan is an excellent material for producing in different shapes (Tsigos et al., 2000).

An important advantage of chitosan is the possibility of chemically modifying its structure with agents that are able to improve its chemical and mechanical behavior. In this study, chitosan microspheres with a mean size of 140 ± 119 μm, obtained by the spray and coagulation methods, were chemically modified and their ability to adsorb standard proteins was investigated (Mi et al., 1999).

These microspheres were chemically modified in three ways:

a) Crosslinking of chitosan with the bifunctional glutaraldehyde agent (Muzzarelli, 1977). The crosslinking reaction occurs between primary amino groups and aldehyde groups, resulting in the formation of schiff bases. The complex reaction mechanism modifies the structure and functionality of chitosan, improving its chemical resistance;

b) Crosslinking with epychlorohydrin, which partially retains the cationic amino function and improves the mechanical properties (Wei et al., 1992);

c) Acetylation, which decreases the degree of chitosan deacetylation degree (Hirano et al.,1976). These macromolecular structures are depicted in Figure 1.

To characterize the adsorbents produced in this study, two standard proteins with different isoeletric points were chosen: bovine serum albumin - BSA - with pI = 4.8 and lysozyme with pI = 11.

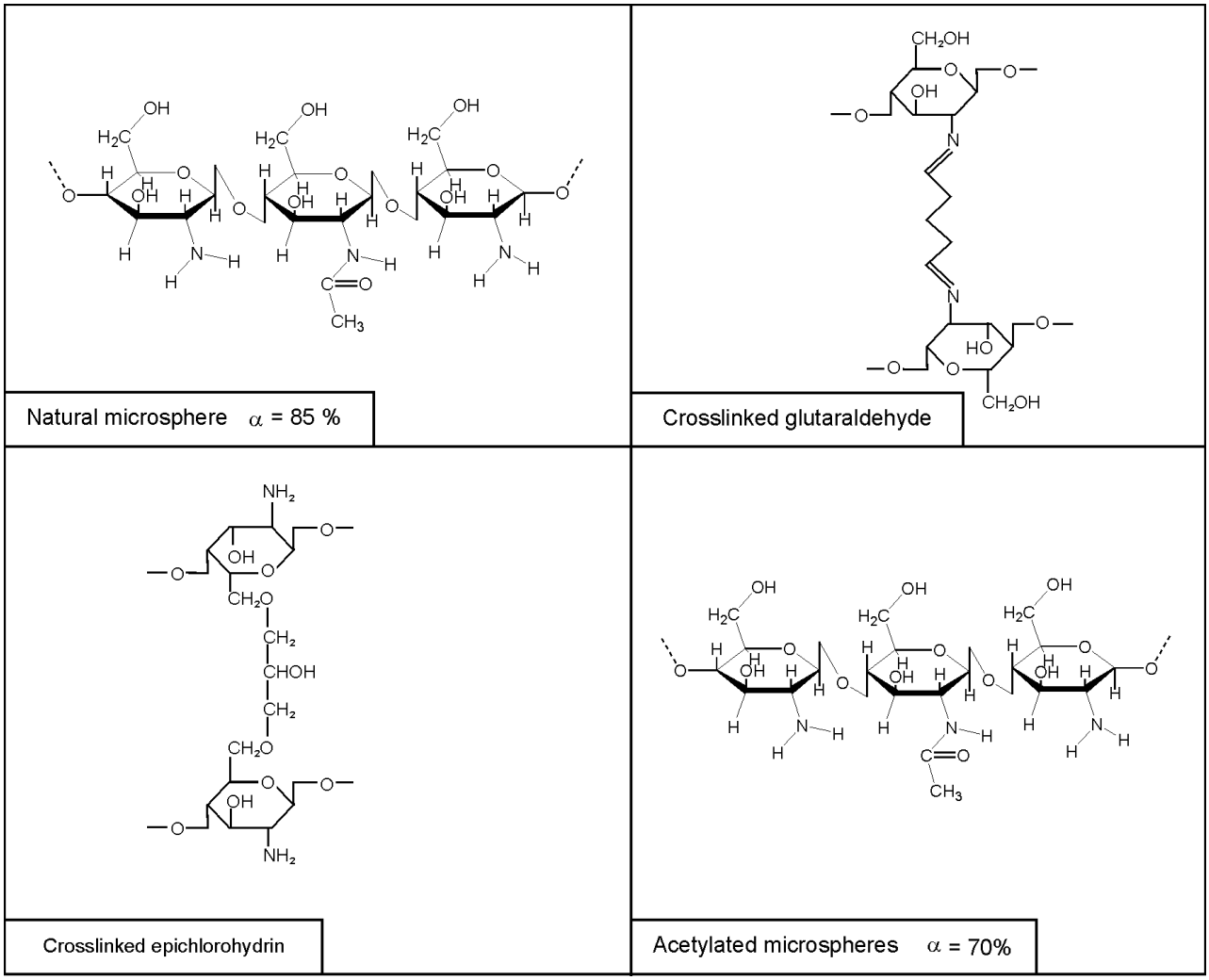

Figure 1: Structure of modified chitosan (α = degree of deacetylation).

MATERIALS AND METHODS

Materials

Chitosan of commercial degree, 85% deacetylated, was provided by Sigma-USA. Glutaraldehyde (25%) in aqueous solution was provided by Nuclear-Brazil. Epichlorohydrin was provided by Merck-Germany. Acetic anhydride and methanol were provided by Vetec and Synth (Brazil), respectively. BSA and lysozyme were provided by Sigma-USA. All other reagents were of analytical grade.

Preparation of Porous Chitosan Microspheres

Concentrated solutions were prepared by dissolving chitosan in a solution containing 3% (v/v) acetic acid in Milli-Q water. Porous chitosan microspheres were obtained using the spray and coagulation method. The solution fed into the nozzle with a peristaltic pump was sprayed using compressed nitrogen. Atomization occurred by the force of the compressed nitrogen, which breaks up the chitosan solution into small droplets. The sprayed particles were kept in contact with NaOH coagulating solution for 12 hours. The particles were then collected and washed with abundant Milli-Q water. The wet coagulated microspheres were separated using sieves (Arruda and Santana, 2003).

Crosslinking of Chitosan Microspheres

Natural chitosan microspheres (samples a) were modified using three different routes in order to increase their mechanical and chemical stability.

In the first method, microspheres were heterogeneously crosslinked in 0.75% (w/w) aqueous glutaraldehyde solution (5 g of wet chitosan microspheres in 50 ml of glutaraldehyde solution) at room temperature without agitation for 2 h. Then, they were rinsed using deionized water to remove the unreacted glutaraldehyde residues (samples b).

In the epichlorohydrin-crosslinking process, 5 g of wet natural chitosan microspheres were immersed in a 0.01 M epichlorohydrin solution, which was prepared in 0.067 M NaOH solution at 40°C under continuous agitation for 2 h. Microspheres were then rinsed with deionized water to remove the unreacted epichlorohydrin (samples c).

In the third method, microspheres were modified using a 0.6% (v/v) acetic anhydride solution in methanol at room temperature under continuous agitation for 3 minutes. Then, they were rinsed with methanol to remove the unreacted acetic anhydride (samples d).

All microspheres were stored in Milli-Q water at 7°C.

Porosity of Microspheres

The microspheres were tested in order to characterize their porosity. For chitosan and other biopolymers, porosity can be influenced by the following factors: route for the preparation of microspheres and type of chemical modification of microspheres. During the microsphere preparation step, the most important parameters are the concentration of chitosan solution and the concentration of NaOH solution (Beppu and Santana, 1999). In order to obtain the highest possible values of porosity and high concentration values, about a 2.5% mass concentration and a NaOH concentration of 1.0 M were chosen. Porosity can be estimated using a method adapted from Zeng and Ruckenstein (1996). The amount of water that occupies the pores was calculated weighing wet chitosan microspheres before and after extracting the water that filled their pores by using vacuum (at about 250 mmHg) for 30 minutes. Porosity (ε) can be estimated from Equation 1, where m_1 and m_2 are the weight of the microspheres before and after extraction of water from their pores, respectively; ρ_w is the density of the water at 20°C and ν is the volume of wet microspheres. This procedure was performed in triplicate.

$$\varepsilon = \frac{\left\{\frac{(m_1 - m_2)}{\rho_w}\right\}}{\nu}.100(\%) \quad (1)$$

pH_{zpc} Measurement

pH_{zpc} or zero point of charge, is often used as an important parameter to analyze the electrostatic surface change. The procedure used to calculate the pH_{zpc} of all samples was potentiometric titration based on the acid-base reaction (Davranche et al., 2003). To estimate the pH_{zpc}, *c.a.* 2 g of microspheres were mixed into 100 ml of 1 M $NaNO_3$ aqueous solution. During the potentiometric tritation, 0.1 M HNO_3 and NaOH aqueous solutions were used. A DM20 Digimed potentiometric instrument was used to measure the $[0H^-]$ and $[H^+]$ concentrations. The total charge in the samples was obtained through the difference between the base and acid added and the equilibrium. The total charge Q

(mol.g^{-1}) was calculated from the following equation:

$$Q = \frac{C_a - C_b + [OH^-] - [H^+]}{C_s} \quad (2)$$

where Ca (mol.l^{-1}) and Cb (mol.l^{-1}) are the acidic and basic concentrations and Cs (g.l^{-1}) is the sample concentration.

ADSORPTION EXPERIMENTS

BSA and Lysozyme Adsorptions

BSA and lysozyme were adsorbed using the static method: 4 ml of each solution with an initial concentration (C_0) ranging from 0.07 to 0.7 mg.ml^{-1} were placed in plastic flasks containing *c.a.* 0.25 g of chitosan microspheres. The pH of the solutions was adjusted to 6 and 7.5 (values close to 6.5, the pKa of chitosan amino groups). This pH corresponds to the pka value of the amino group in the chitosan structure. For acidic conditions, a 200 mM phosphate (NaH_2PO_4 and Na_2HPO_4) buffer solution was used. For basic conditions, a 400 mM Tris(hydroxymethyl)-aminomethane solution was used. Due to the fact that natural microspheres did not show chemical and mechanical resistance, they were not used in acidic adsorption experiments. The flasks were stirred in an orbital shaker at 150 rpm and room temperature for 12 hours. The protein concentrations in the samples were analyzed using a DU 640 Beckman Spectrophotometer at a wavelength of 280 nm. The maximum adsorption capacities (q_m) and constants of the Langmuir model (k_S) were then calculated.

Adsorption at Different pHs

To study the effect of solution pH on BSA and lysozyme adsorption only glutaraldehyde-crosslinked microspheres were used as they had shown the highest adsorption capacities in section 6.1. The pHs studied were 4, 5, 6, 7.5, 9 and 11.

Kinetics of Adsorption

Kinetic experiments were conducted at pH 6 and an initial protein concentration of 0.33 mg.ml^{-1}. The mixture was stirred using a shaker until the adsorption equilibrium was established. Samples were taken and analyzed periodically. The amounts of adsorbed proteins per unit weight of microspheres at time (t), q(t) (mg.g^{-1}), and equilibrium concentration C* (mg.ml^{-1}) were calculated from the mass balance. The kinetic rate constant was calculated using the Mckay and Poots (1980) model. Equation 3 shows that the amount of adsorption by diffusion-controlled dynamics as a function of time can be given as

$$q(t) = 2C_oS\sqrt{\frac{Dt}{\Pi}} = Kdt^{0.5} \quad (3)$$

where q(t) represents the amount of BSA and lysozyme adsorbed per unit weight of microspheres at time t ($s^{0.5}$), C_o is the initial concentration of proteins in the bulk solution, D is the diffusion coefficient and S is the specific surface area of the chitosan microspheres. Equation 3 indicates that under diffusion-controlled transport mechanisms, q(t) versus $t^{0.5}$ would have a linear relationship with $kd = 2CoS\sqrt{\frac{D}{\Pi}}$ expressing the intrinsic kinetic rate constant for diffusion-controlled adsorption.

BSA and Lysozyme Desorption Studies

The desorptions of both proteins from chitosan microspheres were investigated as follows: microspheres were first saturated with BSA or lysozyme using an initial concentration of 0.7 mg.ml^{1}. This was followed by elution at 4 ml of 1M NaCl at pH 6 for BSA and 7.5 for lysozyme, containing the respective buffers (200 mM phosphate and 400 mM "Tris"). The experiments were performed at room temperature under stirring for 12 hs. The concentrations of desorbed BSA and lysozyme were determined spectrophotometrically. The recovery of proteins from the microspheres was evaluated by comparing the amounts of BSA and lysozyme desorbed and adsorbed, as shown in Equation 4.

$$\text{Desorption(ratio)} = \text{protein}\left[\frac{\text{desorbed}}{\text{adsorbed}}\right].100\% \quad (4)$$

RESULTS AND DISCUSSION

Porosity values

After modifications, the microspheres had porosity values of between 48.5% and 88.5%, as presented in Table 1. The values were always lower than that found in natural microspheres. These values are coherent with the fact that treatment on natural chitosan microspheres changes not only the surfaces but also their internal structure. These values are related to the addition, in different degrees, of new segments the initial macromolecular chain of chitosan, changing chains and final hydrophobicity of the polymer.

Table 1: Porosity of chitosan microspheres.

Samples	Means porosity (%)	Standard derivation (%)
Natural	89.3	1.5
Glutaraldehyde	84	2.5
epichlorohydin	88.5	1.7
acetylated	48.5	3

pH_{zpc} Values

pH_{zpcs} of natural and modified chitosan microspheres were calculated as shown in Figure 2. All samples had pH_{zpc} values of about pH 6.5. The pk_a of the amino group in chitosan copolymer is known to be about 6.5 when $0.72 < \alpha < 0.87$ (Roberts, 1992). The deacetylation values of samples (a) and (d) are approximately 85% and 70%, respectively, as calculated by potentiometric tritation (Raymond et al., 1993). The total charge of samples depends on the pH values, indicating protonation and deprotonation of chitosan amino groups, as shown in Table 2. These values were obtained using the equation $pka=pH+\log[(1-\alpha)/\alpha]$. At pH values of lower than pH_{zpc}, the adsorption sites on the surface are protonated and the sample surface is positively charged, while at pH values of higher than pH_{zpc}, the sample surface is negatively charged. From the electrostatic interaction point of view, the positive charge of samples under acidic solution conditions would favor the adsorption of negatively charged species and, in basic solution, conditions may enhance the adsorption of positively charged species. In samples (b) and (c), a slightly reduced pH_{zpc} was observed. In addition, under acidic conditions, the modified samples are expected to have a considerable decrease in number of protonated amino groups, suggesting that some amino groups, mainly on samples (b) and (c), were consumed or blocked by the crosslinking reactions. However, for sample (d), where chitosan was partially acetylated ($\alpha = 70\%$), the results from the protonation of amino groups under acidic conditions did not demonstrate any important differences when compared to the natural sample.

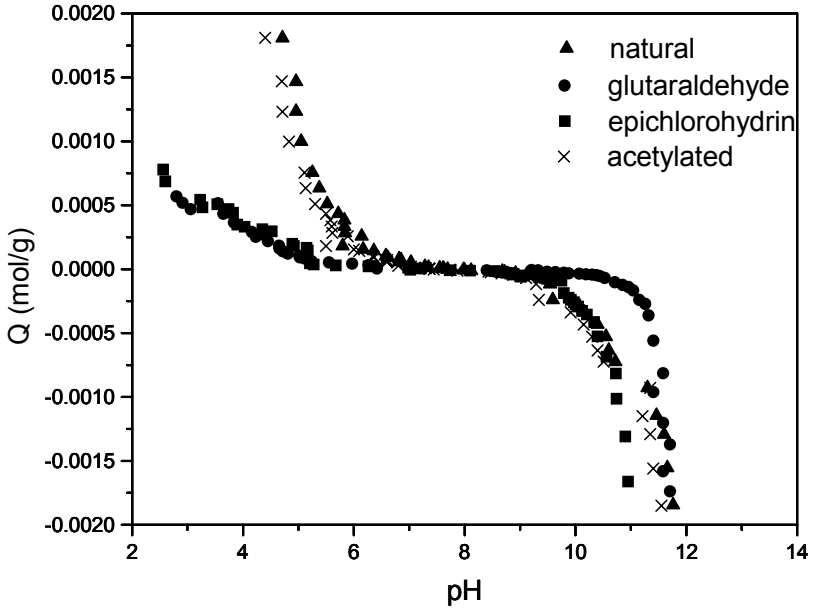

Figure 2: pH_{zpc} titration curves.

Table 2: Fraction of protonated amino groups at each pH.

	pKa = 6.5
pH	Amino groups (NH_3^+)
4	0.9968
6	0.7597
7.5	9.10×10^{-2}
9	3.2×10^{-3}
11	1×10^{-4}

Adsorption Isotherms

Adsorption isotherms describe how adsorbates, BSA and lysozyme interact with adsorbents (b), (c) and (d). Adsorption studies performed with proteins are usually more complex than those with a polyelectrolyte because proteins are complex macromolecules characterized by polar, hydrophobic and charged areas. The interactions between proteins and solid matrix can be electrostatic, hydrophobic or via hydrogen bond; however, the enthalpy involved in protein adsorption is usually positive, independently of the substrate (Fujimoto et al., 2002). Results show that with an increase in BSA and initial lysozyme concentration, the adsorption capacity of all adsorbents increased significantly. The experimental adsorption data are shown in Figs. 3, 4, 5 and 6. The adsorption capacities of BSA and lysozyme depend on molecular groups present on the adsorbent surface and on porosity. At lower initial BSA and lysozyme concentrations, the adsorption capacities increased linearly with the initial protein concentration, suggesting that there were enough adsorption sites on the microspheres and the amount of adsorption in these cases was dependent on the number of protein molecules transported from the bulk solution to the internal and external surfaces of the microspheres. However, at higher initial BSA and lysozyme concentrations, the adsorption capacity no longer increased proportionally with the equilibrium protein concentration, indicating that the number of adsorption sites on the surfaces of the microspheres actually limited the adsorption capacities.

Langmuir isotherms obtained from the batch experiments are shown in Table 3. The results, in general, indicate that the Langmuir model adequately describes the adsorption of BSA and lysozyme. The Langmuir isotherm model assumes that the adsorbed layer is one molecule thick and that the adsorbed layer has the same energies and enthalpies of adsorption. Therefore, the homogeneous surface conditions, assumed by the Langmuir isotherm model, may be approximately met. The differences in the comparative results are attributed to the specific interactions between adsorbents and proteins, ligands incorporated into the polymeric matrix and the charge on the adsorbents. Results showed higher values of q_m for glutaraldehyde-crosslinked microspheres (sample b) when compared with other samples. The related literature indicates that the adsorption capacity of the glutaraldehyde-crosslinked microspheres is favored due to the presence of imino groups (Monteiro and Airoldi, 1999). This probably occurs due to the electrostatic and hydrophobic effects between the adsorbents and protein surfaces. For samples (c) and (d), the adsorption capacities were lower than that of sample (b). The intermediate results of the adsorption of both proteins for the epichlorohydrin-treated chitosan microspheres can be explained by their partially blocked amino groups. The lower values of adsorption of both proteins for the acetylated chitosan microspheres may be attributed to lower porosity values, associated with a slight decrease in the quantity of protonated amino groups.

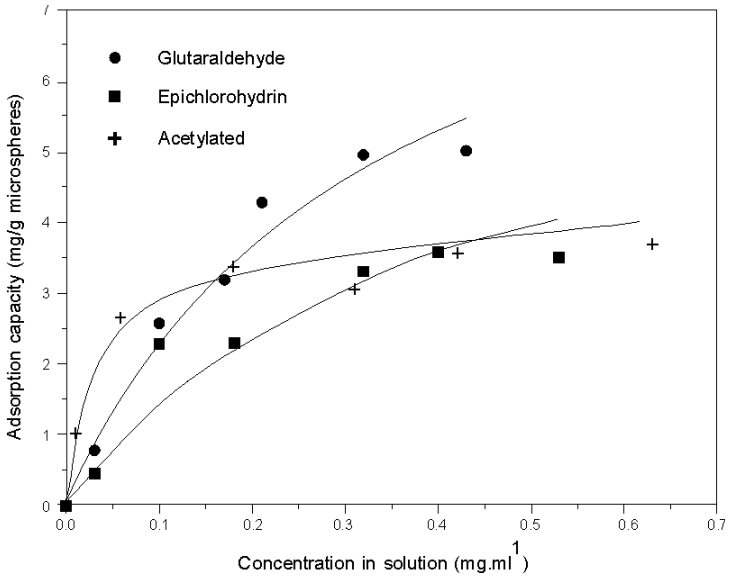

Figure 3: Adsorption isotherm of BSA using 200 mM phosphate buffer at pH 6 and 20°C.

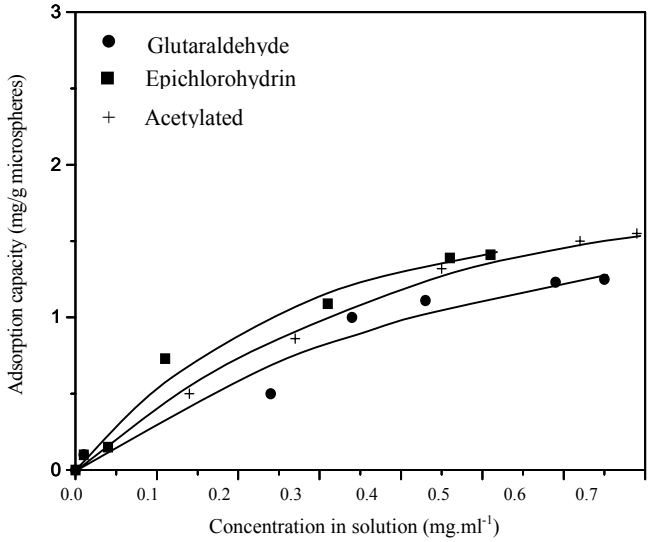

Figure 4: Adsorption isotherm of BSA using 400 mM Tris buffer at pH 7.5 and 20°C.

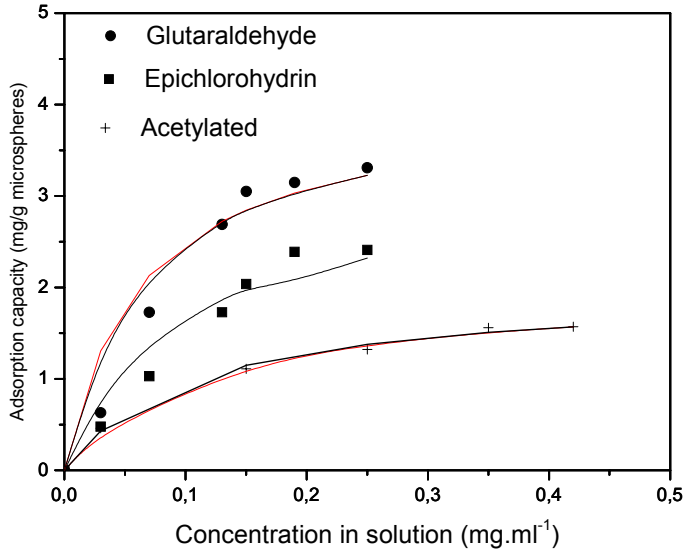

Figure 5: Adsorption isotherm of lysozyme using 200 mM phosphate buffer at pH 6 and 20°C.

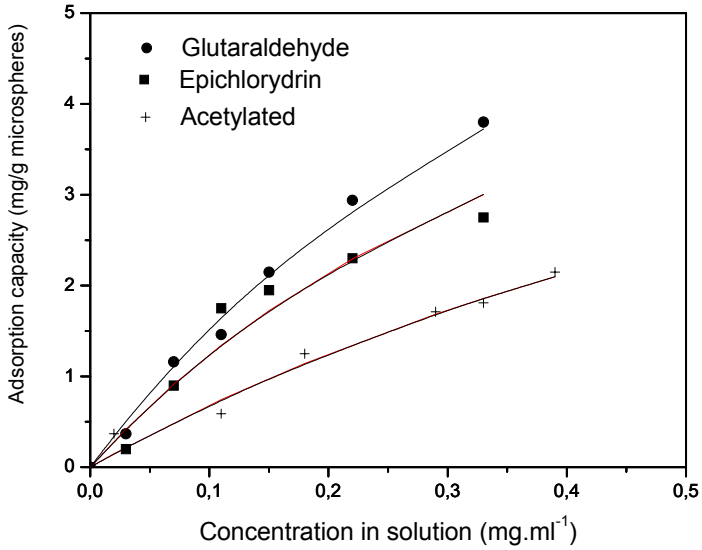

Figure 6: Adsorption isotherm of lysozyme using 400 mM Tris buffer at pH 7.5 and 20°C.

Table 3: Adsorption parameters obtained with the Langmuir model.

Proteins	pHs	Samples	q_m	K_s	R^2
BSA	6	Glutaraldehyde	9.24	0.29	0.97
	6	Epichlorohydrin	7.06	0.39	0.92
	6	Acetylated	6.93	0.36	0.65
Lysozyme	6	Glutaraldehyde	4.03	0.06	0.93
	6	Epichlorohydrin	2.84	0.09	0.98
	6	Acetylated	1.97	0.11	0.99
BSA	7.5	Glutaraldehyde	1.18	0.08	0.87
	7.5	Epichlorohydrin	1.59	0.12	0.95
	7.5	Acetylated	1.42	0.09	0.91
Lysozyme	7.5	Glutaraldehyde	11.95	0.79	0.99
	7.5	Epichlorohydrin	10.28	0.88	0.98
	7.5	Acetylated	7.37	0.97	0.97

Effect of pH on BSA and Lysozyme Adsorption

The presence of free amino groups in chitosan is responsible for its polycationic nature in acidic solutions. Hence, for pH < 6.5 (value of its pka), the amino groups of the chitosan are in the NH_3^+ protonated form, as shown in Table 4. Due to their amino group content, the crosslinked chitosan microspheres can serve as a weak anion exchanger. Consequently, it is not necessary to introduce ion-exchange groups, which often cause the chemical stability and thermal and mechanical resistance to deteriorate. The equilibrium $R\text{-}NH_3^+(aq.) \Leftrightarrow R\text{-}NH_2(aq)+H_3O^+$ occurs on the crosslinked chitosan microspheres. The imine R−C(=NH)−R' group is also in equilibrium on the glutaraldehyde-crosslinked chitosan microspheres. As shown in section 3.2, all samples were positively charged at pH < 6.5, with more NH_2 groups converted to NH_3^+. The electrostatic interactions between the microspheres and BSA (pI = 4.8) are repulsive at pH > 6.5, but become attractive at pH < 6.5. In addition, the electrostatic interactions between the microspheres and lysozyme (pI = 11) are repulsive at pH< 6.5, but become attractive at pH > 6.5 (Zeng and Ruckenstein, 1998). Figure 7 shows that the protein adsorption capacity of the microsphere surfaces strongly depends on bulk solution pH. The proteins and adsorbent charges are also strongly dependent on changes in solution pH. The BSA adsorption capacity of glutaraldehyde-crosslinked chitosan microspheres, in general, decreased with the increase in solution pH. Electrostatic interactions can explain the observed decrease in adsorption capacity at pH ranging from 6 to 7.5. However, the maximum adsorption capacity was observed at its isoeletric point of about 5.5 $mg.g^{-1}$. Under this condition (pH about 4.8), the substrate has a high charge density, as shown in Table 2, increasing the adsorption capacity. The lysozyme adsorption capacity of glutaraldehyde-crosslinked chitosan microspheres increased with the increase in solution pH, reaching a maximum adsorption capacity of 4.75 $mg.g^{-1}$ at around the isoeletric point of lysozyme. The electrostatic interactions may explain the behavior observed at pHs ranging from 7.5 to 11.

The maximum adsorption capacity for both proteins may be explained by the fact that these proteins do not have a net electrical charge at their isoeletric points, increasing the possibility of them making contact with any substrate, independently of charge (Denizli et al., 1999). During the crosslinking of chitosan microspheres with glutaraldehyde, their hydrophobic character was strengthened. Thus, at isoeletric points, the contribution of attractive and repulsive interactions is lower than the hydrophobic and hydrogen bond contributions. Both proteins interact with substrate (b), even at unfavorable solution pH. These results suggest other interactions between these proteins and the glutaraldehyde-crosslinked microspheres. For lysozyme, for instance, the adsorption capacities may be attributed to lyzozyme´s affinity for N-acetyl groups, which may be responsible for natural chitosan microsphere degradation (Colfen et al., 1998). Another type of well-known interaction is the protein-protein interaction between the amino acid chains that may occur in all pH ranges. In the case of BSA, the adsorption capacities are probably due to their van der Walls interactions.

Table 4: Distribution of charges on chitosan and proteins studied.

pHs	Chitosan (amine groups) charges	BSA	Lysozyme
pH > 11	negative	negative	negative
pH = 11	negative	negative	no charge
11 < pH < 6.5	negative	negative	positive
ph = 6.5	no charge	negative	positive
6.5 < pH < 4.8	positive	negative	positive
pH = 4.8	positive	no charge	positive
pH < 4.8	positive	positive	positive

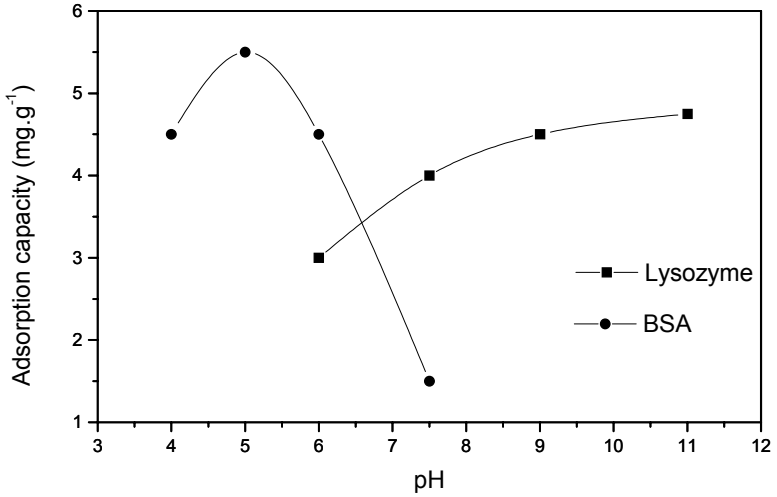

Figure 7: Effect of initial solution pH on protein adsorption capacity of glutaraldehyde- crosslinked chitosan microspheres.

Adsorption Kinetics

The kinetics results for experiments at pH 6 are shown in Figure 8. The adsorption of BSA was slower than that of lysozyme on chitosan. The adsorption equilibrium for BSA was reached at about 10 h and for lysozyme at about 7h. Stirring was kept constant at 150 rpm in all experiments. The adsorption of BSA and lysozyme consists of two steps: (a) the transport of proteins from the bulk solution to the surface of the microspheres (including external and/or intraparticular diffusions) and (b) the attachment of proteins to the active adsorption sites on the microspheres.

In the first step, the surfaces of microspheres were relatively free of protein and the protein that arrived at the microsphere surface could attach instantly to the surface sites. The adsorption rate may then be controlled by the number of BSA and lysozyme proteins that diffused from the bulk solution to the microsphere surface. Figures 9 and 10 contain a plot of q(t) versus $t^{0.5}$ for the experimental results in Figure 8. From the correlation analysis, the value of the kinetic constant rate for diffusion-controlled adsorption (kd) was found to be 0.1254 $mg.g^{-1}.s^{-0.5}$ and 0.1386 $mg.g^{-1}.s^{-0.5}$ for lysozyme and BSA, respectively. However, the experimental data do not obey rigorously a linear model. BSA curve presents systematic residues and lysozyme curve present deviation in the last step of adsorption. This fact suggests that other factors play an important role in the control of adsorptions rather than diffusion. Since most of the adsorption sites on the microsphere surfaces were occupied quickly by the previously adsorbed protein molecules, the proteins that were subsequently transported to the surface of the microspheres probably had difficulty in finding available adsorption sites for attachment to occur. The adsorption of BSA and lysozyme on the glutaraldehyde-crosslinked microspheres in the last step slowed down and would probably change from the initial diffusion-controlled process to a final attachment-controlled process. One possibility for decreasing this limitation would be to use faster stirring during the process. However, this was not possible due to limitations of the mechanical resistance of the microspheres.

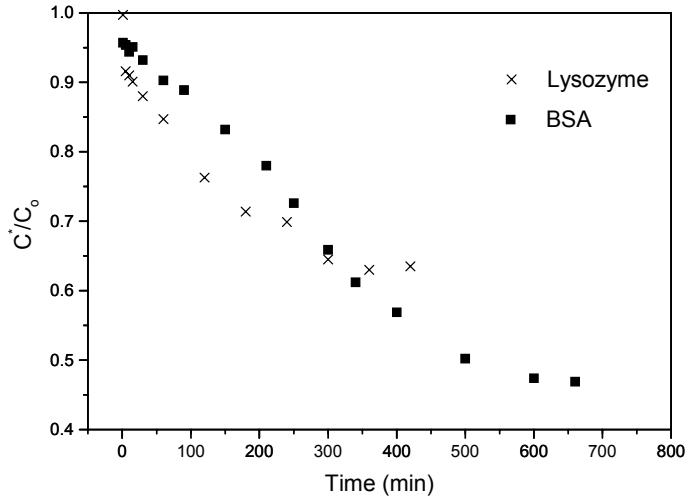

Figure 8: Adsorption kinetics of proteins on the glutaraldehyde-crosslinked chitosan microspheres at pH 6 and 20°C.

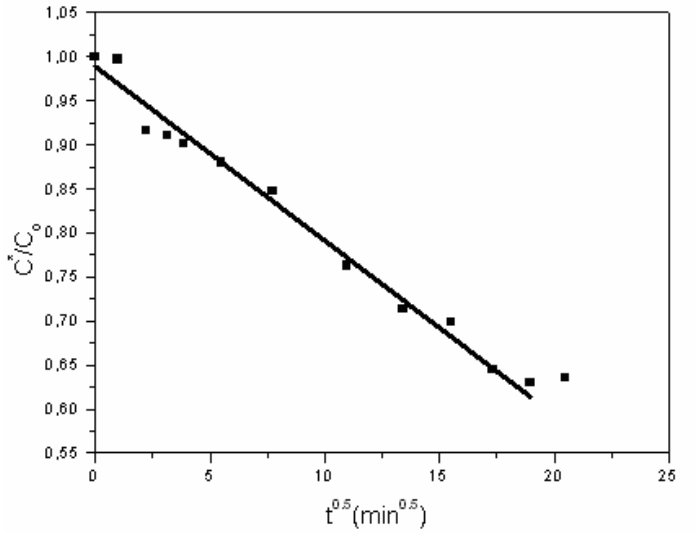

Figure 9: Diffusion-controlled kinetic model for lysozyme.

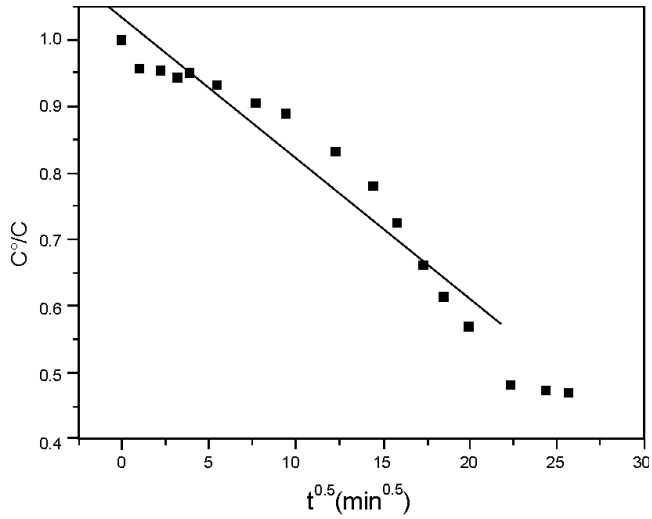

Figure 10: Diffusion-controlled kinetic model for BSA.

BSA and Lysozyme Desorption Studies

Adsorbed proteins were eluted by changing the ionic strength. In this study, microspheres were washed after adsorption with water and a buffer solution with NaCl 1 M was used as eluent. The results shown in Table 5 indicate a desorption ratio during the first cycle of around 23.38% to 73.23%. In order to test the regeneration capacity of the adsorbents, they were reused one more time. The desorption capacity decreased by about 50% compared to the first cycle. These results are related to the regeneration capacity of the adsorption sites, which sometimes attach amino acid residues and sometimes attach the ions from the eluent (Na^+ or Cl). Thus results suggest that the system at the pH studied is not dominated only by interactions of electrostatic nature.

Table 5: Desorption ratio of proteins from chitosan microspheres.

Protein	Sample	Desorption ration (%)	Satandard derivation (%)
BSA	b	33.13	3.14
	c	34.75	7.52
	d	23.38	5.7
Lysozyme	b	73.23	5.5
	c	43.64	16
	d	32.56	10.4

CONCLUSIONS

The proteins BSA and lysozyme may be adsorbed onto chitosan microspheres, but protein-adsorbent interactions must be considered. These interactions generally depend on adsorbate type, microsphere material and solution conditions, such as pH. The maximum adsorptions were observed, in our case, at the isoeletric point, which suggests that interactions are not predominantly of an electrostatic nature. The model of Mckay and Poots describes well the initial adsorption rate.

ACKNOWLEDGEMENTS

The authors thank FAPESP and CNPq for their financial support.

REFERENCES

Arruda, E.J. and Santana, C.C., Phenylboronate - chitosan resins for adsorption of beta-amylase from soybean extracts, Applied Biochemistry and Biotechnology, v. 105, n. 2, p. 829-842 (2003).

Bohak, Z. and Sharon, N. Biotechnological applications of proteins and enzymes. New York: Academic, Enzyme and Microbial Technology, v. 1, n. 4, p. 289-299, (1979).

Krigjaman, J. and Jenkins, O. Product recovery in bioprocess technology. Leicester Polytechnic, UK, 1992.

Yoshida, H., Nishihara H. and Kataoka, T. Adsorption of BSA on DEAE-Dextran; equilibria. Separation Science and Technology, v. 29, n. 17, p. 2227-2244 (1994).

Grzegorczyk, S. and Carta, G. Adsorption of amino acids on porous polymeric adsorbents – I. Equilibrium. Chemical Engineering Science, v. 51, n. 1, p. 807-817, (1996).

Hill, L.R. and Nearath, H. The proteins, New York: Academic, 1975.

Kamei, D.T., Wang, D.I.C. and Blankschtein, D. Fundamental investigation of protein partitioning in two-phase aqueous mixed (nonionic-ionic) micellar system, Langmuir, v. 18, n. 26, p. 3047-3057 (2002).

Kurita, K. Chemistry and application of chitin and chitosan. Polymer Degradation and Stability, v. 59, n. 1, p. 117-120 (1998).

Tsigos, I., Martinou, A.; Kafetzopoulos, D. and Bouriotis, V. Chitin deacetylases: New, versatile tools in biotechnology, TIBTECH, v. 18, n. 1, p. 5 (2000).

Mi, F., Wong, T.; Shyu, S.; Chang, S. Chitosan microspheres: Modification of polymeric chemicophysical properties of spray-dried microspheres to control the release of antibiotic drug. Journal of Applied polymer Science, v. 71, n. 1, p. 747-759 (1999).

Muzzarelli, R.A.A. Chitin Handbook, Plenum, Italy (1977).

Wei, Y.C., Hudson, S.M.; Mayer, J.M. and Kaplan, D.L. The cross-linking of chitosan fibers. Journal of Polymer Science, 30, p. 2187 (1992).

Hirano, S., Ohe, Y. and Ono, H. Selective N-acylation of chitosan. Carbohydrate Research, v.

47, n. 2, p. 315-320 (1976).

Arruda, E.J. and Santana, C.C., Phenylboronate - chitosan resins for adsorption of beta-amylase from soybean extracts. Applied Biochemistry and Biotechnology, v. 105 (2): p. 829-842 (2003).

Beppu M.M. and Santana C.C. Estudo da calcificação in vitro de quitosana. Doctoral Thesis, State University of Campinas (1999).

Zeng, X. and Ruckenstein, E. Control of pore size in macroporous chitosan and chitin membranes, Industrial Engineering Chemical Research, v. 35, n. 1, p. 4169-4175 (1996).

Davranche, M.; Lacour, A.C.; Bordas, F. and Bollinger, J.C. An easy determination of the surface chemical properties of simple and natural solids. Journal of Chemical Education, v. 80, n. 1, p. 76-78 (2003).

McKay, G. and Poots, R.E. Modelling interactions parameters. Journal Chemical Technology Biotechnology, v. 30, n. 2, p. 279 (1980).

Roberts, G.A.F. Chitin Chemistry, Macmillian, London, chapter 5, 1992.

Raymond, L., Morin, F.G. and Marchessault, R.H. Degree of deacetylation of chitosan using conductometric titration and solid-state NMR. Carbohydrate Research, v. 246, n. 1, p. 331-336 (1993).

Fujimoto, J., Reis, E.A.O. and Petri, D.F.S. Formação de multicamadas de polissacarídeos e proteína, Química Nova, v. 25, n. 5, p. 757-761 (2002).

Monteiro, O. A. C. and Airoldi, C. Some studies of crosslinking glutaraldehyde interaction in a homogeneous system. International Journal of Biological Macromolecules, v. 26, n. 2, p. 119-128 (1999).

Zeng, X. and Ruckenstein, E. Cross-linked macropouros chitosan anion-exchange membranes for protein separations, Journal of Membrane Science, v. 148, n. 2, p. 195-205 (1998).

Denizli, A., Kokturk, G.; Yavuz, H. and Piskin, E. Albumin adsorption from aqueous solutions and human plasma in a packed bed column with Cibacron Blue F36A - Zn (II) attached poly (EGDMA - HEMA) microbeads. Reactive Function Polymer, v. 40, n. 3, p. 195 -203 (1999).

Colfen, H., Harding, S.E.; Winzor, D.J. and Varum, K. A study of the interaction between chitosan and lysozyme, Abstracts presented at Biopolymer Mixtures, UK, 1998.