Iron(III) Ion Removal from Solution Through Adsorption on Chitosan

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ABSTRACT: The potential of different forms of chitosan to adsorb iron(III) ion from a Jectofer [an iron(III)–sorbitol–citric acid complex] solution was investigated. The working solution was chosen as Jectofer, to mimic the composition of excess iron(III) as may be found in biological systems. The equilibrium studies showed that chitosan powder has the highest sorption capacity for the iron(III) ion when compared to chitosan flakes and microspheres. The amount of iron(III) adsorbed onto chitosan was found to increase with the contact time to reach equilibrium within 3 h at 37°C. A higher initial concentration of a Jectofer solution resulted in a higher amount of iron(III) adsorbed. Higher pH values, in the range 2–7, also resulted in an increase in iron(III) uptake from the solution. The data of adsorption from the Jectofer solution were found to correlate well with the Freundlich isotherm equation. *In vitro* adsorption experiments showed that chitosan is capable of adsorbing excess iron, measured in terms of the ferritin level, in human blood. © 2002 Wiley Periodicals, Inc. J Appl Polym Sci 84: 1185–1192, 2002; DOI 10.1002/app.10416

Key words: chitosan; chelation; iron(III); Jectofer; thalassemia; adsorption; biopolymers; metal-polymer complexes; polysaccharides

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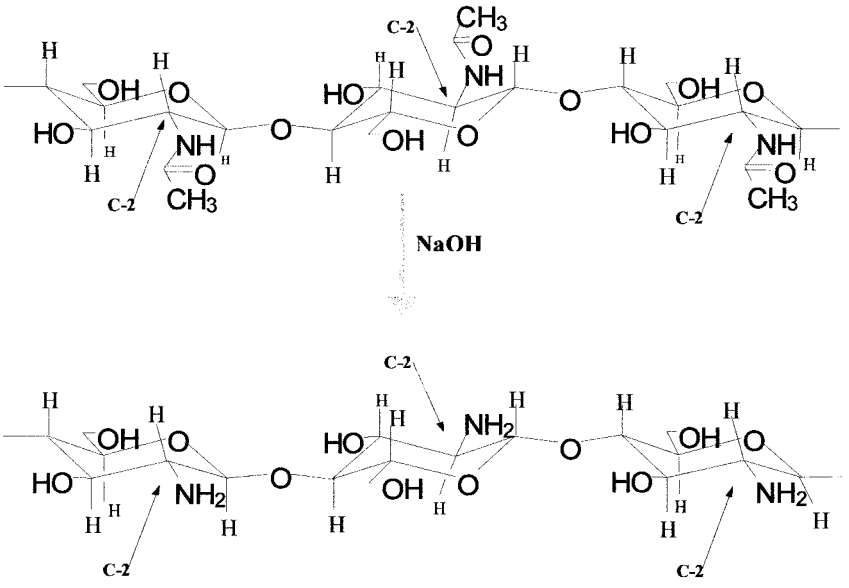
INTRODUCTION

Chitin is a natural biopolymer obtained from crab and shrimp shells or from similar natural sources. Chitosan (b) is the deacetylated form of chitin (a):



(a)

(b)

Chitin and chitosan have a very diverse range of applications including in the biomedical field and in cosmetic, food, and textile industries. Some biomedical applications are the use of chitin or chitosan as bone- and tooth-filling material, conjugates of drugs, and excipients in tablets and in microparticulate drug-delivery systems. Novel applications such as in transmucosal drug-transport vehicles, vaccines, and DNA delivery systems have been reported.[1]

Amino sugars and their polymeric derivatives, such as chitosan and chitin, are very efficient ligands, forming stable complexes with metal ions.[2–5] Chitosan is known to chelate metal ions in the following order: $Cr^{+3} < Co^{+2} < Pb^{+2} < Mn^{+2} \ll Cd^{+2} < Ag^{+} < Ni^{+2} < Fe^{+3} < Cu^{+2} < Hg^{+2}$. The cations coordinate to free electron pairs of nitrogen and oxygen atoms. Recently, it was proposed that the iron–chitosan complex formed by adsorption of iron onto chitosan is either penta- or hexa-coordinated Fe(III). It was concluded that, for each iron(III) ion, there are 2 mol of amino groups and 4 mol of oxygen atoms from two different chitosan chains. It is speculated that at least one water molecule takes part in the coordination complex.[6] The adsorption studies carried out on chitosan and its derivatives show that amino groups are important binding sites on the chitosan backbone. Generally, binding of metal ions onto chitosan obey Langmuir and Freundlich equations.[7–9] It has been reported that the pH of the medium, concentration of the ions, and size of the chitosan particles all play a role in the adsorption process.[4–12] In a previous work of the authors, the physicochemical parameters affecting the ability of chitosan flakes to adsorb iron(III) were studied.[13] It was observed that iron(III) ion adsorption onto chitosan increases with the amount and degree of deacetylation of chitosan, concentration of ferric ions in the solution, and pH of the medium. The amount of ferric ions that are adsorbed by the polymer increases with time until equilibrium is established between the adsorbed iron(III) ion and that in the solution. Preliminary *in vitro* results obtained in human blood serum indicate that chitosan is capable of adsorbing iron(III) in the body fluid medium and may be a suitable iron(III)-adsorbing agent in biological systems.[13]

The objective of this work was to evaluate different forms of chitosan for their iron(III)-binding capacity and its potential applicability as a new iron(III) chelating agent for the treatment of iron overload in biological fluids. The iron pool used in this research is a solution used for the treatment of iron(III) deficiency in humans. This solution

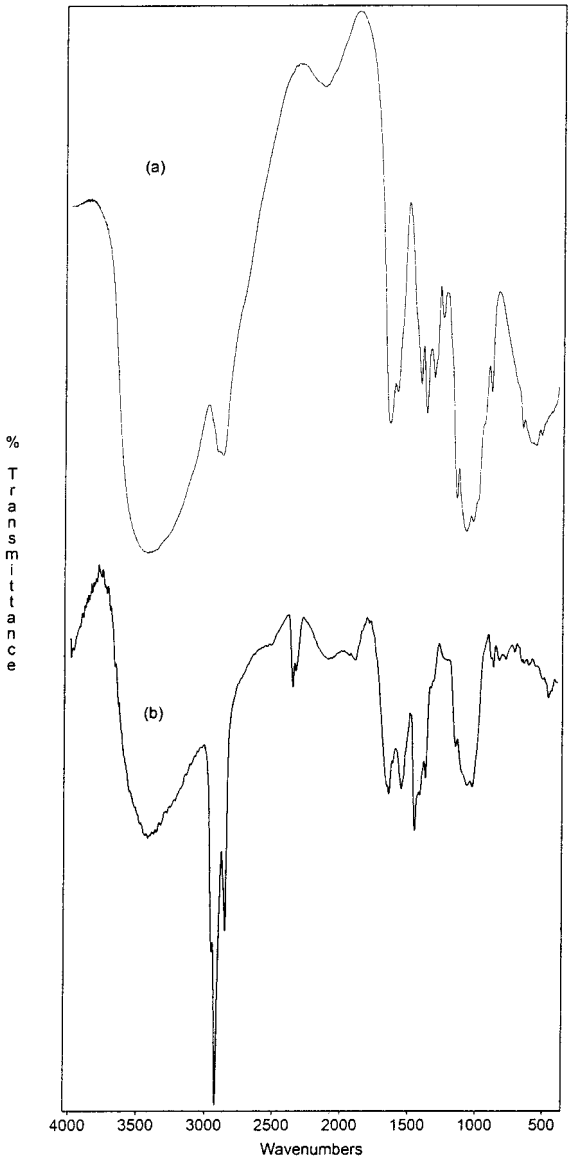


Figure 1 FTIR spectra of chitosan (a) powder and (b) microspheres.

was chosen because its composition is typical of excess iron(III) as found in human blood. The experiments were carried out at pH 2, 5, and 7 to simulate the acidity of the digestive system.

Development of a safe and effective oral-chelating agent is a major and urgent requirement for the treatment of patients with thalassemia (Cooley's anemia) who have to undergo frequent blood transfusions.[14] Since there is no natural means for the body to eliminate iron(III), the iron(III) contained in the transfused red blood cells builds up and eventually becomes toxic to tissues and organs of these patients. If excess iron(III) is not removed from thalassemia patients, they may not survive beyond the first decade of life. To accomplish this, an iron(III)-chelating drug, deferroxamine (Desferal), is used. Desferal therapy is extremely demanding, and many patients experience considerable discomfort from administration of the drug. The search for safer and more effective oral iron(III) chelators is in progress.[14] Chitosan could be a good candidate for this purpose since it is known for its high chelating, mucoadhesive[15] properties in addition to its high biocompatibility. Chitosan-coated dialdehyde cellulose has been found to be

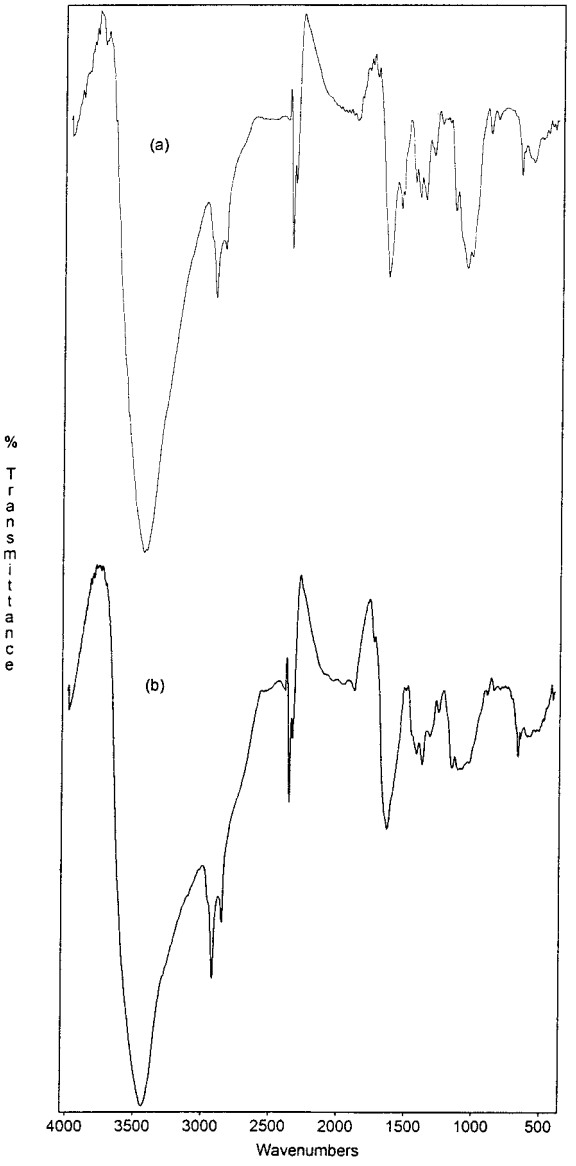


Figure 2 FTIR spectra of CHI-1 (a) before and (b) after contact with the Jectofer solution.

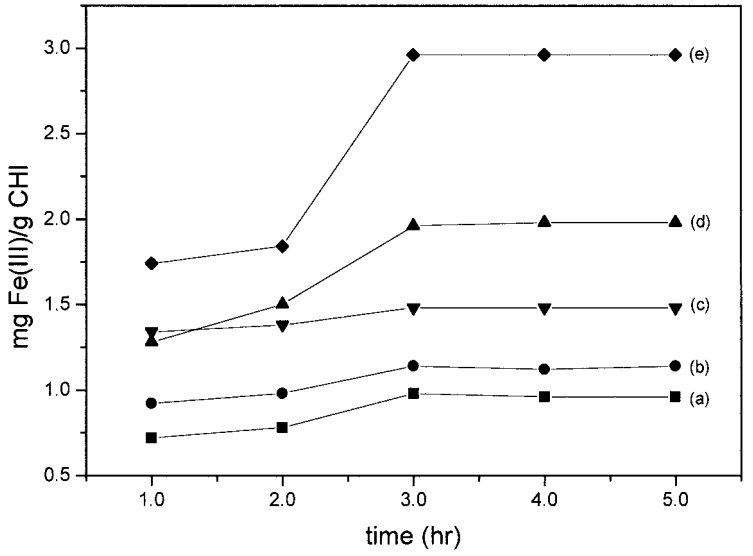


Figure 3 Time course of iron(III) uptake by chitosan (a) CHI-3, (b) CHI-2, (c) CHI-1, (d) CHI-M, and (e) CHI-P from 50 ppm Jectofer solution at 37°C, pH 2.

successful as an oral adsorbent for urea and ammonia.[16]

EXPERIMENTAL

Materials and Methods

Chitosan flakes (Fluka, Steinheim, Germany) of molar mass 1.5×10^5 (CHI-1), 4.5×10^5 (CHI-2), and 6.0×10^5 (CHI-3) g/mol and of degree of deacetylation of 85%; chitosan powder (Primex, Karmsund Fiskerihavn, Norway) of molar mass 3.0×10^5 g/mol (CHI-P) and degree of deacetylation of 90%; sodium hydroxide (Merck, Darmstadt, Germany); hydrochloric acid (BDH, Poole, England); hydrobromic acid (Analar); mineral oil (Fluka, Steinheim, Germany); glacial acetic acid (Analar); gluteraldehyde (Aldrich, Steinheim, Germany); potassium dihydrogen phosphate (Merck, Darmstadt, Germany); and sodium hy-

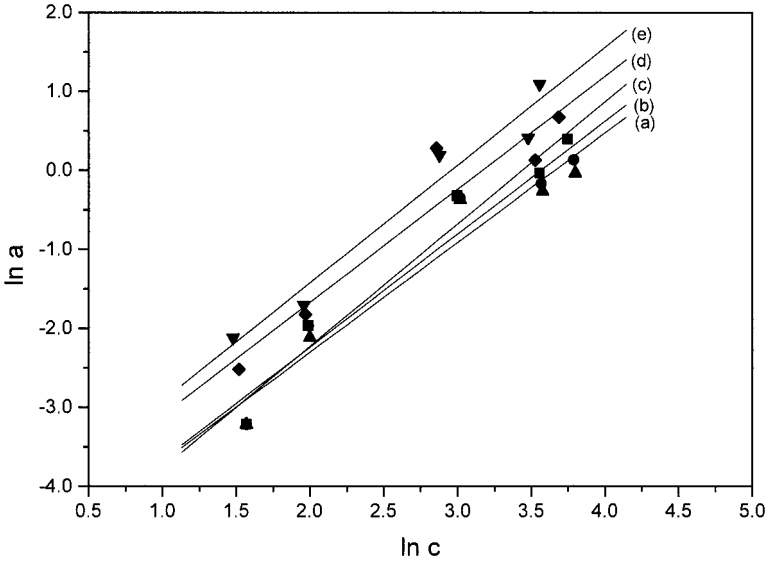


Figure 4 Freundlich isotherm of (a) CHI-3, (b) CHI-2, (c) CHI-1, (d) CHI-M, and (e) CHI-P from 50 ppm Jectofer solution at 37°C, pH 2.

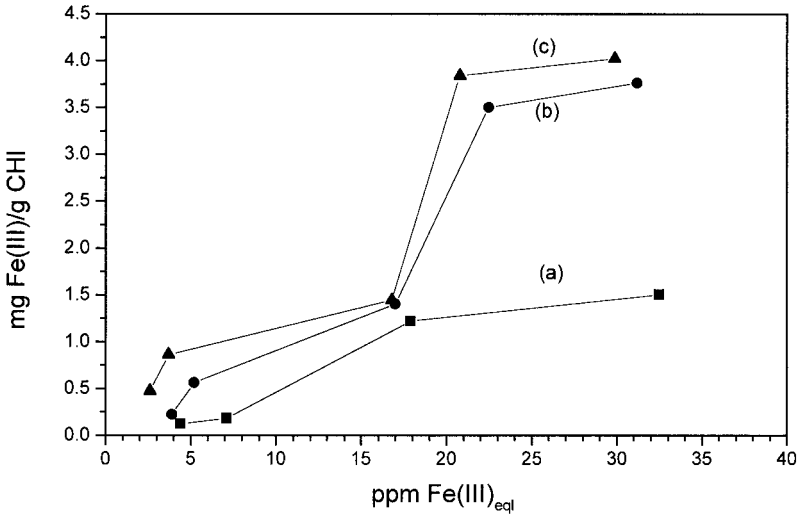


Figure 5 Adsorption equilibria of iron(III)/CHI-P system at 37°C in Jectofer solution for 3 h at (a) pH 2, (b) pH 5, and (c) pH 7.

drogen phosphate (Merck) were used without any further purification. Jectofer, which is an iron(III)–sorbitol–citric acid complex of an average molar mass of 5.0×10^3 g/mol, produced by Eczacıbaşı (Istanbul, Turkey), was used as the iron(III) pool. It contained 100 mg iron(III) in a 2.00 mL solution. The solution pH value was adjusted to 2, 5, and 7 by HCl, acetate, and phosphate buffer solutions.

Preparation of Chitosan Microspheres

Twenty-one milliliters of a 3% chitosan (CHI-P) solution in 5% acetic acid was dispersed in 150 mL of mineral oil and stirred with a glass paddle stirrer at 900 rpm for 30 min. Gluteraldehyde-saturated toluene, 2.00 mL, was added at 15-min intervals twice by stirring at room temperature. Then, 2.00 mL of 7% gluteraldehyde was added. The reaction was carried out for 5 h with stirring. The microspheres were filtered off, washed several times with ethanol, and then with plenty of ice-cold water and dried at 60°C.[17] The microspheres were fractionated by being sieved through standard test sieves. The size range of the microspheres (CHI-M) was 60–100 μm.

Determination of Adsorbed Iron(III) Ion

The effect of the size of the chitosan particles, the concentration of the iron(III) ion in solution, and the pH of the solution on the amount of ferric ion adsorbed were determined. Twenty milligrams of the chitosan sample, in flake, powder, or microsphere form, and a 4-mL sample of an aqueous iron(III) solution with a 5.0, 8.0, 24.0, 40.0, and 50.0 ppm concentration at pH 2, 5, and 7 were mixed in the reaction tubes and thermostatically maintained at 37 ± 0.5°C. After predetermined time intervals, the solutions were studied by atomic absorption spectrophotometry. Each experiment was repeated twice and the results for each sample agreed with each other within ±2% error. Precipitation of ferric salts was not observed under the given experimental conditions.

Determination of Adsorbed Iron(III) in Human Blood

To study the adsorption of iron(III) ions, some preliminary *in vitro* studies were carried with blood samples of thalassemia patients as taken for the routine assessment of these patients. Chitosan flakes or powder were brought into contact with 3 mL of a blood sample for 3 h at 37°C in a tube. The blood samples were analyzed for their iron(III) content by using an Irma-mat Ferritin test kit. Three hundred microliters of an iodine-125–antiferritin agent was added to 25 μL of a blood sample taken from the patient in a tube and incubated for 2 h at room temperature in a horizontal shaker. The liquid was aspirated and the tubes were washed with 2 mL of a 0.9 % NaCl solution. The radioactivity was measured in cpm using a Cap-Ria 16 multichannel gamma counter by Capintec Inc. The validity and precision of the results were checked by the control sample included in the kit.

Table I Freundlich Constants of Chitosan/Jectofer Systems

	CHI-3	CHI-2	CHI-1	CHI-M	CHI-P
n	2.2	2.3	2.0	2.0	1.8
$K \times 10^3$	1.22	1.22	1.25	1.83	8.31

FTIR Spectroscopy

FTIR spectra of the samples were taken in the solid state using KBr pellets with a Mattson Satellite 5000 FTIR spectrophotometer.

Atomic Absorption Spectrophotometry

A Phillips PU 9100 atomic absorption spectrophotometer was used.

RESULTS AND DISCUSSION

FTIR Spectroscopy

FTIR spectra of chitosan CHI-P and CHI-M are given in Figure 1(a,b), respectively. The evidence of a crosslinking reaction with gluteraldehyde to form an imine bond is clearly reflected in the spectrum of the microspheres by the absorption band at 1500 cm^{-1}, which is absent in the spectrum of chitosan powder.

Chitosan samples were analyzed by FTIR spectroscopy before and after contact with the Jectofer solution. Figure 2(a,b) shows the spectra of low molecular weight chitosan flakes, CHI-1, before and after treatment, respectively. The two spectra are identical except for the loss of the peak at 1556 cm^{-1} due to N—H bending vibrations of chitosan. This can be interpreted as the formation of an Fe(III)–chitosan complex through the involvement of N atoms on the chitosan backbone. The nonexistence of any new absorption band, which may characterize sorbitol or citrate groups, indicates the adsorption of iron(III) onto chitosan rather than the complex itself.

Adsorption of Iron(III) from the Jectofer Solution

The time course of iron(III) uptake of chitosan (CHI-3,2,1, CHI-M, and CHI-P) from the Jectofer solution is shown in Figure 3(a–e), respectively. It can be seen that the amount of iron(III) adsorbed increases in time for all samples studied, reaching equilibrium within 3 h. The amount of iron(III) adsorbed increases in the order CHI-3 < CHI-2 < CHI-1 < CHI-M < CHI-P. It is interesting to note that adsorbed iron(III) increases with a decreasing molar mass of the flakes. The improvement in the adsorption ability with a decreasing molar mass may be explained by better swelling of a lower molar mass polymer sample in the solution, hence, offering enhanced accessibility of the adsorption sites to the ion in solution. It is believed that adsorption of solute particles in solution on porous adsorbents such as chitosan involves three steps: transfer of the adsorbate to the external surface of the adsorbent, diffusion of the adsorbate into the pores of the adsorbent, and adsorption of the solute on the internal surface of the adsorbent.[7]

The validity of this model is well established in Figure 3. In all graphs from (a) to (e) in Figure 3, the first linear portion represents the external surface adsorption. The second linear portion, which has a steeper slope than that of the first one, is due to the intraporal diffusion in time, in addition to the external adsorption. The third linear portion with a zero slope is the final stage where the intraporal diffusion starts to slow down due to decreased ion concentration in the solution. A smaller particle size of the adsorbent increases the number of pores through which the solute can diffuse, increasing its capacity to adsorb. Therefore, the powder and microsphere forms could adsorb iron(III) much better than could the flake form. While 1 g of CHI-1 adsorbs

Table II Decrease in Ferritin Level in Blood Serums of Thaleassemics Treated with Chitosan

Sample	$(\text{Ferritin})_i$ (ng/mL)	$(\text{Ferritin})_f$ (ng/mL)	Decreased Ferritin (ng/mL mg chitosan)
Chitosan flake	658	620	1.9
Chitosan flake	3214	2264	47.5
Chitosan powder	882	620	13.1
Chitosan powder	598	452	7.3
Chitosan powder	2416	1714	35.1

Table III Blood Profiles Before and After Chitosan Contact

	Sample 1		Sample 2	
	Before	After	Before	After
Albumin	4.9 g/dL	4.8 g/dL	4.7 g/dL	4.6 g/dL
Total protein	8.8 g/dL	8.7 g/dL	8.0 g/dL	7.8 g/dL
Cholesterol	96 mg/dL	94 mg/dL	120 mg/dL	118 mg/dL
LDH	359 μ/L	339 μ/L	—	—
Uric acid	4.9 mg/dL	4.6 mg/dL	3.2 mg/dL	2.4 mg/dL
Glucose	92 mg/dL	94 mg/dL	76 mg/dL	87 mg/dL

1.5 mg iron(III), CHI-M and CHI-P adsorb about 2.0 and 3.0 mg of iron(III), respectively. Although CHI-P and CHI-M have a higher molar mass than that of CHI-1, they could still adsorb the iron(III) ion from a solution to a higher extent than could CHI-1. The particle size and degree of deacetylation of chitosan seem to be more effective parameters than is the molar mass in determining the maximum ion-uptake capacity of chitosan from a solution.

The adsorption equilibrium of the chitosan/Jectofer system studied did not obey the Langmuir equation, indicating that adsorption of iron(III) from the Jectofer solution onto chitosan does not follow a homogeneous, monolayer deposition mechanism. Figure 4 shows the Freundlich adsorption isotherms of the chitosan/Jectofer system. The Freundlich equation in linearized form is

$$\ln a = ln\ K + n\ ln\ c \quad (1)$$

where a is the amount adsorbed (mg/g adsorbent); K, the adsorption constant (L/g adsorbent); and c, the equilibrium (final) concentration of the adsorbate in solution (mg/L).

The value n is a measure of the affinity of the sorbent for the solute. Iron(III) adsorption data from the Jectofer solution was found to correlate well with the Freundlich isotherm equation. K and n values for each sample are given in Table I. The n values of the Freundlich equation obtained for chitosan flakes, powder, and microspheres are all comparable with each other since the chemical identities of the adsorbent and the solute are the same. The adsorption constant, which is a measure of specific adsorption of a particular adsorbent, decreases in the order powder > microspheres > flakes. Although the powder and the microsphere chitosan samples used in this study have comparable sizes, microspheres have a smaller adsorption capacity due to that they are crosslinked by imine linkages through the amine groups available on the chain. Due to the smaller number of free amine groups on the microsphere, adsorption of the solute on the internal surface of the adsorbent takes place to a lesser extent.

Figure 5 shows that a higher pH of the medium causes a higher amount of the adsorbed iron(III) ion. The increasing pH of the medium decreases the probability of having any soluble fraction of chitosan in the medium with protonated amino groups. As the fraction of free, unprotonated amino groups increases, the number of nitrogen atoms with free electrons increases in the medium. The sites available for complexation with iron(III) ions increase in the medium and this is reflected in an enhanced adsorption of iron(III) ion onto chitosan.

Adsorption of Iron(III) Ion onto Chitosan in Human Blood

The ferritin level is a direct measure of the amount of iron(III) stored in the body. Normal ferritin levels are 12–300 ng/mL for males and 10–150 ng/mL for females. Ferritin levels in blood serums of thalassemics before and after chitosan contact are given in Table II. The results show that chitosan is capable of adsorbing excess iron(III) stored in human blood. Depending on the sample, the adsorbing capacity changes between 6 and 30% per 0.02 g of chitosan. There is a trend in that the adsorption capacity of chitosan increases with increased iron(III) content in human blood as obtained in the chemistry laboratory experiments. Since a direct relation between the amount of the adsorbent and the amount of the solute adsorbed is well established, the results show that reasonable amounts of the order of hundreds of milligrams of chitosan could be

enough to restore the ferritin level back to its normal values in human blood. However, the biochemical changes that chitosan would undergo in a physiological solution and how these would affect its iron-uptake capacity are still to be established. Preliminary analyses carried out on the blood profiles of two thalassemics are summarized in Table III. The affinity of chitosan to proteins, fats, and acids is reflected by decreases in albumin, protein, cholesterol, LDH, and uric acid values. The level of glucose, on the contrary, increases, indicating enzymatic degradation of chitosan.

CONCLUSIONS

Chitosan seems to be a promising iron(III) chelator for the treatment of iron(III) overload. Physical and chemical properties of chitosan may be modified to make it a suitable medical iron(III) chelator. Further detailed studies are needed with respect to the specificity, selectivity, and biochemistry of the adsorption of ferric ions onto chitosan on oral administration or upon its direct injection in the form of microspheres of suitable size.

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