Chitin/Chitosan and Alginates Based Two Component Systems for Fe^{3+} Adsorption

Chidozie Kingsley Nwokeji

Submitted to the
Institute of Graduate Studies and Research
in Partial Fulfilment of the Requirements for the Degree of

Master of Science
in
Chemistry

Eastern Mediterranean University
July 2013
Gazimağusa, North Cyprus

Approval of the Institute of Graduate Studies and Research

Prof.Dr. Elvan Yılmaz
Director

I certify that this thesis satisfies the requirements as a thesis for the degree of Master of Science in Chemistry.

Prof. Dr. Mustafa Halilsoy
Chair, Department of Chemistry

We certify that we have read this thesis and that in our opinion it is fully adequate in scope and quality as a thesis for the degree of Master of Science in Chemistry.

Prof. Dr. Elvan Yılmaz
Supervisor

Examining Committee

1. Prof. Dr. Elvan Yılmaz _____

2. Assist. Prof. Dr. Mehmet Garip _____

3. Assoc. Prof. Dr. Mustafa Gazi _____

ABSTRACT

In this study chitin and chitosan were blended with sodium alginate. The blends were characterized by FTIR and SEM analysis. The swelling ratios of obtained products in different pH (1.0, 7.0, 11) were determined and their adsorption properties in pH=1.3 were tested for Fe^{3+}. The desorption capacities of the products in acidic buffer (pH=1.2) and alkaline media (0.06 M ammonia) were also compared.

Chitin/Alginate and Chitosan/Alginate beads which swelled in aqueous media up to 200-300% were obtained. The beads proved to be effective Fe^{3+} adsorbents. Chitin/Alginate and Chitosan/Alginate beads showed an adsorption capacity of the order of 80mg/g bead towards Fe^{3+} in 100ppm Fe^{3+} solution within 3hours contact time. Ammonia (0.06M) was found to work better than acid buffer as a desorption agent for Fe^{3+}. It was possible to obtain 100% desorption using ammonia solution.

Keywords: Chitosan, alginic acid, Fe^{3+} adsorption

ÖZ

Bu çalışmada, kitin ve kitosan aljinat ile karıştırılmış olup fiziksel özellikleri FTIR ve SEM analizleri ile karakterize edilmiştir. Elde edilen ürünlerin farklı pH lardaki tampon çözeltilerde (pH=1.0, 7.0, 11.0) şişme oranı belirlendi ve adsorpsiyon özellikleri, pH=1.2 Fe^{3+} için test edildi. Asidik tampon çözeltide (pH=1.2) ve alkali çözeltide (0.06 M amonyak) desorpsiyon kapasiteleri karşılaştırıldı.

Sulu ortamda 200-300 % mertebesinde şişme kapasitesine sahip kitin/aljinat küreler elde edilmiştir. Ürünlerin 100 ppm Fe^{3+} çözeltisinde 3 saatte 80 mg Fe^{3+}/g küre adsorplama kapasitesine ulaşabildikleri gözlemlenmiştir Seyreltilmiş amonyak çözeltisi ile 100% desorpsiyon sağlanabildiği saptanmıştır

Anahtar Kelimeler: Kitosan, aljinik asit, Fe^{3+} adsorpsiyonu

ACKNOWLEDGMENTS

My sincere gratitude to Prof.Dr.Elvan Yilmaz for whom without, this work would not come to completion.

I extend my deepest gratitude to my parents, siblings and friends and also to Dr.Zulal Yalinca and all my polymer chemistry colleagues. Thank you all.

TABLE OFCONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SCHEMES

LIST OF ABBREVIATIONS

DMAC..... ....Dimethylacetamide

NMP.....N-methylpyrrolidinone

NMR.....Nuclear Magnetic Resonance

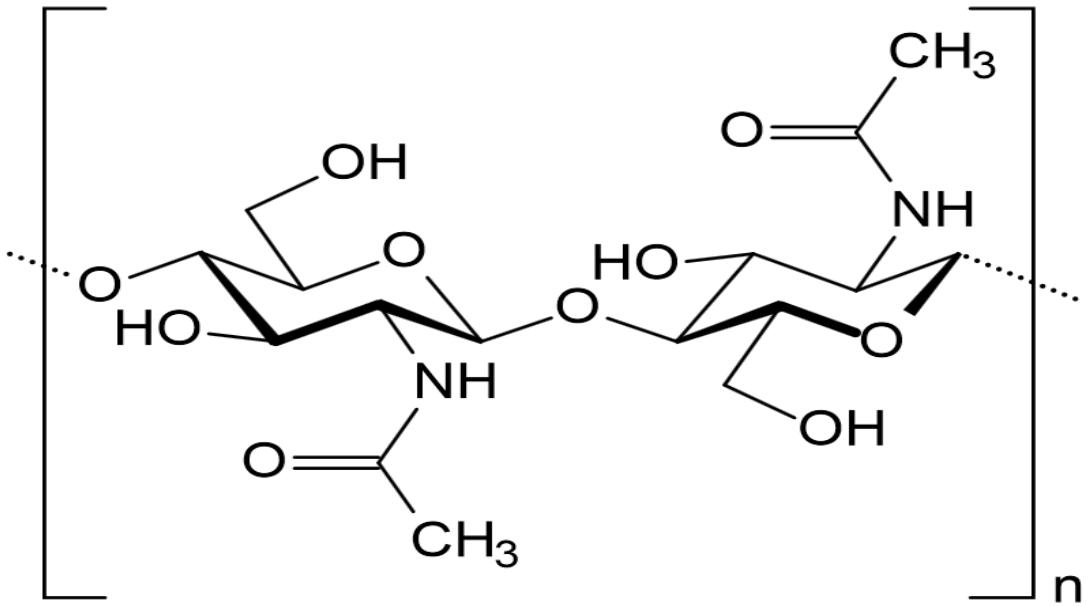
SEM……Scanning Electron Microscopy

Chapter 1

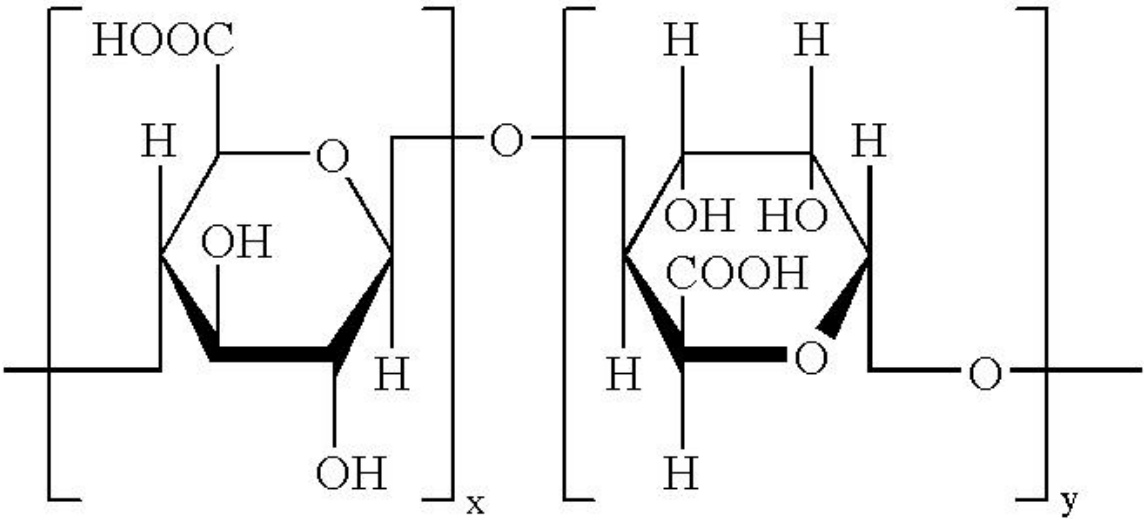
LITERATURE REVIEW

1.1 Introduction

Chitin and alginic acid are two natural polymers with basic and acidic functionalities respectively. While chitin bears the acetamido group at C-2, alginic acid contains carboxylic acid group at C-6. The chemical structures of the polymers, chitin and alginic acid are shown in scheme 1 and 2 respectively. They are both intractable in nature, chitin being soluble only in a limited number of special solvents such as dimethylacetamide/lithium chloride (DMAC/LiCl) or N-methyl-pyrrolidinone/lithium chloride (NMP/LiCl) and alginic acid not being soluble at all. Alginic acid can only be brought into the dissolved state by alkaline treatment to form sodium alginate. Although these polymers may form blends with other polymers, it is not easy to find a second component to accompany them to form miscible blends in solution due to the limitations with regards to their solubilities. The primary requirement for solution blending is to have both polymers dissolved in a common solvent.



Scheme 1: Structure of Chitin



Scheme 2: Structure of Alginic Acid

Both chitin and sodium alginate are capable of sol-gel transition in solution upon contact with a coagulating medium to form physical gels. Preparation conditions and several properties for various applications of calcium alginate, the alginate in the gel form have extensively been studied in the literature [1] [2]. Reports on the properties and applications of chitin gels, on the other hand, are rather scarce.

The aim of this thesis work is to form a new two-component gel system by blending chitin and alginic acid/alginates via coating chitin gel with an alginate layer. Chitin gels are formed by coagulating a solution of chitin in ethanol. The preparation conditions of chitin gels had been established in our laboratory previously [3]. Chitin gels have a small swelling capacity in aqeous solution which limit their applicability as adsorbents for water treatment or for biomedical applications. By being coated with an alginate layer chitin gels are expected to be imparted with a more hydrophilic character, and acid fuctionality which will open up new routes for useful end uses for these biobased gels. To illustrate one application for the new gels, Fe^{3+} adsorption from solution has been investigated.

1.2 Chitin

Chitin, poly (β-(1-4)--N-acetyl-D-glucosamine), which is found abundantly in nature is the world's second most abundant naturally occurring polymer. It was first identified in 1884. Chitin is a linear biopolymer that occurs as an important constituent in the exoskeleton of many organisms. Chitin is ordered crystalline micro fibrils that form the structural components in the cell walls of yeast and fungi or in the exoskeleton of arthropods. Chitin is also produced by a large number of living organisms in the animal and plant kingdoms giving rigidity, strength and reinforcement where required [4].

Chitin is the second most abundant polymer after cellulose, it structurally resembles cellulose with an acetamido group replacing the C-2 hydroxyl group of cellulose. It is non-toxic, biodegradable, and biocompatible, it also has biofunctionality, hydrophicility, and is ecofriendly. These properties have attracted more scientific research into application and modification of this polymer [5]. These modifications

come in the form of grafting and crosslinking, blending or copolymerization using ultraviolet, microwave, heat etc.

The major commercial sources of chitin, despite its wide spread availability, has been shrimp, crab shells, lobsters, krill and squid. Industrially chitin is extracted from waste marine foods. Chitin extraction usually involves two steps, deproteinization and demineralization, these steps being conducted by two methods, biologically or chemically. Chemical extraction of chitin requires the use of acids and bases, while biological extraction involves microorganisms. Chemical extraction of chitin is done by acid treatment to dissolve calcium carbonate, then alkaline extraction is used to solubilise the proteins. Decolorization is then used to remove other impurities and pigments to get the colorless product. The product is then graded in terms of color and purity. These treatment and extraction of chitin vary from the source of raw material used [4].

Chitin usually occurs as two allomorphs, the α and β forms, which can be differentiated using X-ray diffraction, solid state NMR spectroscopy and infrared spectroscopy. The α form of chitin is by far the most abundant of the allomorphs. It is found mostly in yeast and fungal cell walls, crab and shrimp shells, etc. While the other less abundant β form is found in squid pens, seaweeds, protozoa etc [6]. The intra crystalline swelling of β chitin in concentrated hydrochloric acid or nitric acid results in irreversibly α chitin.

Chitin is potentially useful for applications in pharmacy, medicine, textile, cosmetics and agriculture and also as a biosorbent material for the uptake of metal ions from polluted water as well as for analytical applications [7].

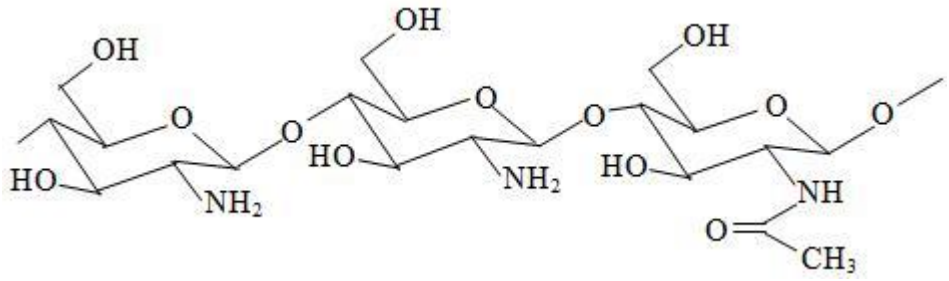
Chitin can be blended with other synthetic or natural polymers and can be cross-linked using crosslinking agents also used for cellulose (i.e glutaraldehyde, epichlorhydrin, bisacrylamide etc.). Chitin and its modified forms can be grafted in using ceric salt, potassium persulphate or any other redox initiators.

1.3 Chitin Gels

Generally, polysaccharides form physical and thermoreversible gels with different solvents. Some examples of gelling polysaccharides are amylopectin and amylose from starch, derivatives of cellulose, and marine polysaccharides such as chitin. Of all these gelling polysaccharides, chitin which has poor solubility in most known solvents is one of the least studied. The complex formed between DMAc/LiCl or NMP/LiCl is a suitable solvent for chitin which would allow for the preparation of chitin solutions, chitin gels and fibers. This complex between NMP/LiCl or DMAc/LiCl dissolves chitin through ion–dipole interactions. Decreasing the power of the solvent, by adding a non solvent such as water or ethanol causes gelation of the biopolymer because the ion-dipole interaction between [NMP–Li]+ or [DMAc–Li]+ and chitin are destroyed [8].

1.4 Chitosan

Chitosan is the most important derivative of chitin; it is obtained by N-deacetylation of solid chitin under hot alkaline conditions or by enzymatic hydrolysis using chitin deacetylase. Aqueous or alcoholic NaOH solution is used with care, the reaction mixture is protected from oxygen, with a nitrogen purge or by addition of sodium borohydride in order to avoid undesirable reactions such as depolymerization and formation of other reaction species [4].



Scheme 3: Chemical Structure of Chitosan.

Chitosan is usually less crystalline than chitin and this invariably makes it more accessible to reagents. One major difference between chitin and chitosan usually lies in their solubilities. Chitin is soluble in very few organic solvents while chitosan is soluble in aqueous acid solutions. The solubility of chitin usually depends on the degree of deacetylation, pH, the nature of acid used for protonation and also on the distribution of the acetyl groups along the main chain. Dilute solutions of acetic acid and formic acid are some common solvents for chitosan [9].

Since chitosan is easier to process than chitin even though its stability is lower, it has found uses in many small to large scale applications. Chitosan has found uses in agriculture as a defensive mechanism in plants, seed coating, stimulation of plant growth etc. In water treatment it is used in the removal of metal ions, flocculants to purify drinking water. In cosmetics it is used to maintain skin moisture, oral care and in treatment of acne. It is also used in biomedical applications such as dental implants, bone rebuilding, wound healing, and as a preservative in food and beverage industries [10].

Due to its very high molecular weight which results in its high viscosity, chitosan use in several biological applications has been hindered. However chitosan can be

depolymerised to produce chitosan of lower molecular weight, oligosaccharide and monomers. Since these oligomers have excellent solubility their applications are numerous in fields of cosmetics, pharmaceuticals and biomedicals [4].

1.5 Alginic Acid

Alginic acid is a naturally occurring biopolymer obtained mostly from the different species of brown seaweed. It is a linear copolymer consisting mainly the residue of β-1 , 4-linked D-mannuronicacid and a-1 , 4-linked L-guluronic acid which are covalently linked together in different blocks or sequences The monomers could appear in homopolymeric blocks of consecutive G-blocks, consecutive M-blocks or alternating M and G-residues [11]. It usually has a yellowish color although it could be whitish or yellowish-brown depending on the source.

The solubility and water-holding capacity of alginates is pH dependent. They precipitate at around pH of 7 and below. Alginates generally show high water absorption capacity and are used as low viscosity emulsifiers and shear-thinning thickeners [12].

Alginates are generally hydrophilic, nontoxic and biocompatible, although during commercial manufacturing of alginates a purification step where acid treatment precipitates the alginate is required. Its ability to retain water and it's gelling, stabilizing and viscosifying properties are a major advantage utilized in its industrial application. Alginates are widely used in pharmaceutical, cosmetic, food, textile/paper-printing industry, and used clinically as a drug delivery vehicle [12].

1.6 Alginic Gels

Polymeric hydrogels are a class of materials that largely swell in water and still maintain its three dimensional network structure in the swollen state. Solvent permeability, swelling, mechanical, hydrophobic and hydrophilic properties of these hydrogels can be adjusted by the method of synthesis and polymer/monomer combinations. Quite a number of biopolymers can form hydrogels. These include alginates collagen and chitosan.

Alginates are regarded as nonimmunogenic, biodegradable and nontoxic polymers, which has made them an attractive candidate for use in biomedical applications.

Alginates form hydrogels in the presence of divalent cations, like calcium (Ca^{2+}), which also acts as crosslinkers between the alginate chains and functional groups. Slowl addition of hydrolyzing D-glucono-lactone (GDL), acidifies alginates to form alginic acid gel. It has been proposed that these gels are stabilized by intermolecular hydrogen bonds [13].

Alginate contains hydroxyl groups and carboxylic acid groups, these groups are used as functionalities to form covalent cross-links. Ester linkages are formed between hydroxyl groups and carboxylic acid groups of alginate by using water-soluble carbodiimide as a crosslinking agent. But, these ester bonds are prone to hydrolysis, making it unstable. Crosslinkers of different structures and sizes, like lysine and adipic dihydrazide, have also been used in production of alginate hydrogels. Experimental results have shown that the mechanical properties of hydrogels are controlled mainly by its cross-linking density, and the type of crosslinking molecules involved [14].

1.7 Biopolymer Beads Coated with another Biopolymer

The coating of biopolymers has received alot attention in recent year. This coating is done to improve the properties of one of the biopolymers or to produce a product with desireable properties from both biopolymers.

Preparation, characterization and application of novel drug delivery devices based on biopolymer/inorganic composites have attracted much attention recently, owing to their unique properties such as biodegradability, easily achievable controlled release characteristics and high encapsulation efficiency. Several studies on biopolymer coating and blends have been conducted. For example composite blend microbeads of sodium alginate (NaAlg) with sodium carboxymethyl cellulose (NaCMC) containing magnesium aluminum silicate (MAS) particles and enteric coated with chitosan have been prepared to achieve controlled release (CR) of amoxicillin in stomach environment [15].

Jiang jiang Duan prepared a new chitosan bead by simply dropping acidic chitosan solution into 0.5wt % alkaline cellulose solution. The bead reveals porous microstructure of regenerate cellulose in the surface. Owing to the cellulose coating, the bead displays improved mechanical properties and acid resistance. Adsorption of Fe^{3+} onto the new chitosan beads was also investigated, and the results show that the cellulose coating has little influence on the adsorption of the chitosan [16].

1.8 Adsorption

Adsorption is a process of transferring from fluid phase to a solid phase. Adsorbents are materials that are capable of adsorbing atomic or molecular species while atomic or molecular species which are adsorbed onto surfaces are known as adsorbates.

Adsorption may be physical (physisorption) or chemical (chemisorption), sometimes they have been found to occur simultaneously. Intermolecular forces are the only cause of bonding in physical adsorption, which is related to polarity and molecular mass, while chemisorption involves bond formation (covalent/ionic) between the adsorbate and the adsorbent.

1.8.1 Adsorption Isotherms

When an adsorbate and adsorbent are kept in contact for enough time, equilibrium is established between the adsorbate and adsorbent in solution. This equilibrium relationship is known as adsorption isotherm. The adsorption isotherm is a curve which relates the equilibrium concentration of a solute on the surface of an adsorbent $\mathbf{q_e}$ to the concentration of the solute in the liquid, **Ce**, of which it is in contact with. Adsorption isotherm is also an equation that relates the amount of solute adsorbed onto a solid and the equilibrium concentration of the solute in solution at a given temperature [17].

The relationship between $\mathbf{q_e/Ce}$ is dependent on the type of adsorption chemical, physical or multilayer adsorption.

1.8.2 Isotherm Models

Different models have been used for predicting equilibrium distribution. Of these, two of them are the most commonly observed. They are Langmuir and Freundlich.

Original form

1. Langmuir model:

$$q = \frac{q_m.K_L.C}{1+K_L.C} \qquad (1)$$

Linearized form

$$\frac{C}{q} = \frac{1}{K_L.q_m} + \frac{1}{q_m}.C \qquad (2)$$

2. Freundlich model:

$$q = K_F.C^{\frac{1}{n}} \qquad (3)$$

Linearized form

$$\log q = \log K_F + \frac{1}{n}.\log C \qquad (4)$$

Where q_m = the maximum adsorption capacity.

*K*F and *n* are the Freundlich constants related to the adsorption capacity and adsorption intensity of the adsorbent, respectively.

The main difference between these two isotherm models is in the variation of heat of adsorption within surface coverage. The Langmuir model assumes uniformly while the Freundlich model assumes logarithmic decrease. In determining which model to use to describe the adsorbant-adsorbate system, data from the isotherms are analyzed using different methods that are based on linearization of these models [18].

The Langmuir Equation: This is usually the most important model for monolayer adsorption. It is based on the assumption that adsorption only occurs at a fixed number of localized sites, that each of these sites can hold only one adsorbate molecule (monolayer) and there is no interaction between adsorbed molecules, even on adjacent sites. Maximum adsorption will correspond to a saturated monolayer of solute molecules on the adsorbent surface. It is assumed that the energy of adsorption is constant, and no transmigration of the adsorbate in the plane of surface.

Langmuir equation which is valid for monolayer sorption onto surface is given by;

$$\frac{C_e}{q_e} = \frac{C_e}{\mathrm{Q}} + \frac{1}{\mathrm{Qb}} \quad (5)$$

A linear plot $\frac{Ce}{q_e}$ against C_e gives Q and b. Where Q is the maximum adsorption, Ce is the equilibrium concentration of the adsorbate, qe is the amount of adsorbate adsorbed per unit weight of adsorbent at equilibrium concentration and b is the Langmuir constant related to the affinity of binding sites.

R_L is used to predict if an adsorption is favourable or unfavourable (Table 1). R_L is given by;

$$R_L = \frac{1}{1+bCo} \quad (6)$$

Where C_0 is the initial adsorbate concentrations [19].

Table 1: Effect of R_L on the isotherm shape.

R_L value	Type of isotherm
$R_L > 1$	unfavourable
$R_L = 1$	Linear
$0 < RL < 1$	favourable
$R_L = 0$	Irreversible

The Freundlich Equation: Freundlich equation model assumes exponential decay in energy distribution function. The widely applied empirical Freundlich equation is based on adsorption onto heterogeneous surfaces is given by;

$$\log_{qe} = \log_{Kf} + \frac{1}{n} \log_{Ce} \quad (7)$$

A linear plot of log q_e against log C_e gives K_f and n (18).

Chapter 2

EXPERIMENTAL

2.1 Materials

Chitin (Sigma, Germany) and Chitosan (Fluka, molar mass$1.5x10^5$ (MM_w) with degree of deacetylation (85%) was used. Acetic acid (Aldrich), sodium hydroxide (Aldrich), Ferric chloride (Aldrich), 5-sulfosalicyclic acid dehydrate (Riedel-de Haen), food grade ethanol (Sema, Northern Cyprus), LiCl (Sigma, Germany), N,N-dimethylacetamide (DMAc) (Aldrich, Germany), alginic acid (Sigma, Germany), N-methyl-pyrrolidinone (NMP) (Aldrich, Germany), were used as received.

2.2 Methods

2.2.1 Purification of Chitin

Chitin was treated with 1 M NaOH for 3 hours at 80ºC, neutralized with water and was digested in solution of 1M HCl for 12 hours, after which it was neutralized. This process was repeated twice. The chitin powder which was recovered was soluble in DMAc/5% LiCl (w/w) and in NMP/5%LiCl solutions.

2.2.2 Preparation of the Solvent System

The solvents NMP or DMAc were dried for 48 h over molecular sieves of 400 Å activated at 280ºC for at least 4 hours. LiCl salt was dried at 130ºC. A 5% (w/w) solution was prepared. Dissolution of the salt was completely made possible by stirring the solution overnight.

2.2.3 Preparation of Chitin and Chitosan Solution

Chitin solution of two different concentrations 2.5% and 1.0% (w/v) was prepared by dissolving an appropriate amount of chitin in the DMAc/5%LiCl solution. A clear and transparent solution of chitin was obtained after stirring the solution for at least 48 hours at room temperature.

Chitosan solution 2.5%w/v was prepared by dissolving chitosan flakes in 1% (v/v) acetic acid solution and kept under stirring at room temperature for 24hours.

2.2.4 Preparation of Alkaline Solution of Alginic Acid

1.5 g alginic acid was dispersed in 6 mL double distilled water. 0.5 g NaOH was dissolved in 4 mL double distilled water. The NaOH solution was then added to the alginic acid solution under powerful magnetic stirring for 15 minutes at 60°C. A dark brown coloured homogeneous solution was obtained.

2.2.5 Preparation of Chitin Beads

The chitin beads used were prepared by dissolving the chitin in NMP/LiCl or DMAc/LiCl. The solution was left under constant stirring for 48hours. Then the chitin solution of different concentrations (1-2.5%) were sucked into a pipette and dropped slowly into the nonsolvent ethanol. Beads formed instantaneously. The beads were then left in the nonsolvent for 24 hours, to allow solvent exchange. The nonsolvent was then filtered off. The beads formed were immersed and rewashed in the same nonsolvent and then dried at room temperature [3].

2.2.6 Preparation of Chitin Blend Alginate Beads

The 0.5 g chitin beads were then dropped into the alginate solution which was formed by adding 30 mL water to the 30 mL of sodium alginate. It was then kept at 60^{o}C for 3 and 6 hours under constant stirring. The coated beads were then picked out and left to dry for 48 hours.

2.2.7 Preparation of Chitosan Beads

Chitosan beads were formed by coagulation of chitosan solution in alkali medium,of 4%NaOH solution. The beads formed were left in the coagulation bath for 2 hours. They were then filtered and washed extensively using distilled water. The chitosan beads were dried overnight at about 60^{o}C.

2.2.8 Preparation of Chitosan Blend Alginate

50mg of the chitosan bead was added into the alginate solution which was formed by adding 30 mL water to the 30 mL of sodium alginate. It was then kept at 60^{o}C for 3 and 6 hours under constant stirring hours to obtain chitosan/alginate blend beads. The coated beads were then picked out and left to dry for 48 hours. This sample is denoted as sample 5.

2.2.9 Calculation of Extent of Blending

The extent of blending was calculated with respect to increase in mass of chitin beads. The equation below was used:

$$\% \text{ Extent of Blending} = \frac{Mblend - Mchitin}{Mchitin} \times 100\% \qquad (8)$$

Where M is= mass in grams.

Table 2: List of Samples Prepared and Sample Identification.

Sample ID	Sample
Sample 1	2.5% Chitin /alginate beads 6hours
Sample 2	2.5% Chitin / alginate beads 3hours
Sample 3	1% Chitin / alginate beads 6hours
Sample 4	1% Chitin / alginate beads 3hours
Sample 5	2.5% Chitosan / alginate beads
Sample 6	Pure chitin
Sample 7	Alginic acid
Sample 8	1% Chitosan solution
Sample 9	3% Chitosan solution

2.3 Swelling Tests

The swelling behaviour of beads was studied in aqueous buffer solutions with pH values of 1, 7, and 11, respectively at 37 ^{o}C. The %swelling was calculated as follows:

$$\text{Swelling } (\%) = \frac{m_s - m_d}{m_d} \times 100 \qquad (9)$$

m_s and m_d abbreviated for mass of swollen and initial mass, respectively.

2.4 Adsorption of Fe^{3+} By Chitin and Chitosan Blend Alginates

5mg of beads were placed in 50 mL iron solutions at pH=1.2 of different concentrations 25mM, 100mM and 1000mM respectively. The beads were stirred at 60 rpm at room temperature for 24 hours in the Fe^{3+} solution. 1 mL aliquots were taken at 2 hours intervals and analyzed for Fe^{3+} concentration by visible spectrophotometry. The iron absorbed was calculated from the difference between

concentrations of the initial and final readings from the spectrophotometer. These adsorption experiments were all done in duplicate for accuracy.

2.5 Determination of Fe^{3+} in Solution

1 mL of Fe^{3+} solution was mixed with 1mL of 5-sulfosalicylic acid dehydrate, (10%w/v), and completed to 10 mL with pH=1 buffer solution. The amount of residual iron was determined by visible spectrophotometry at 505 nm, and then the amount of Fe^{3+} ion absorbed by the chitin and chitosan alginate blends was calculated. The Fe^{3+} concentration was analyzed using Shimadzu UV-1 201V visible spectrophotometer.

Chapter 3

RESULTS AND DISCUSSION

3.1 Formation of Chitin Coated Alginate and Chitosan Blended Alginate Beads

A chitin solution with 1-2.5% (w/v) and chitosan solution with 2% (w/v) concentration is viscous enough to lead to spherical gel beads and relatively dilute to produce gel beads easily. Optical pictures of chitin beads and chitin/alginate beads are shown in Figure 1(a) and (b) respectively.

Table 3: % Modification of Chitin Blended Alginate

Sample ID	Chitin, w/v	Sodium alginate w/v	Treatment Time	Extent of Blending
Sample 4	1	50	3	156
Sample 3	1	50	6	200
Sample 2	2.5	50	3	69
Sample 1	2.5	50	6	85

It can be observed from Table 3 that chitin solution with 1% (w/v) concentration resulted in significantly higher extent of blending than chitin solution with 2.5% concentration. The higher extent of blending observed for 1% chitin is attributed to the fact that beads formed by chitin solution of concentration 1% chitin are soft and flexible. As a result of this, the alginate solution can penetrate into the beads with much ease but 2.5% chitin is thicker, harder and less flexible, hindering diffusion of alginate solution into the bead.

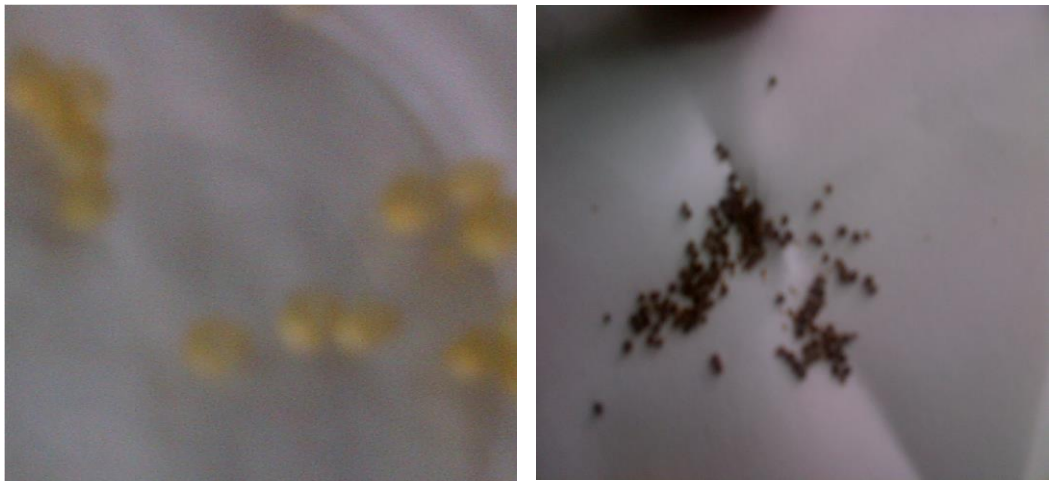


Figure 1: Optical Pictures of Chitin Beads and Chitin Alginate Beads.

Table 4: % Modification of Chitosan Blended Alginate

Sample ID	**Chitosan, w/v**	**Sodium alginate w/v**	**Treatment Time**	**Extent of Blending**
Sample 8	1	Too soft		
Sample 5	2	50	6	35
Sample 9	3	Too viscous		

3.2 FTIR Analysis

All the samples and products were characterised by FTIR and evidence of blending was observed for these spectra.

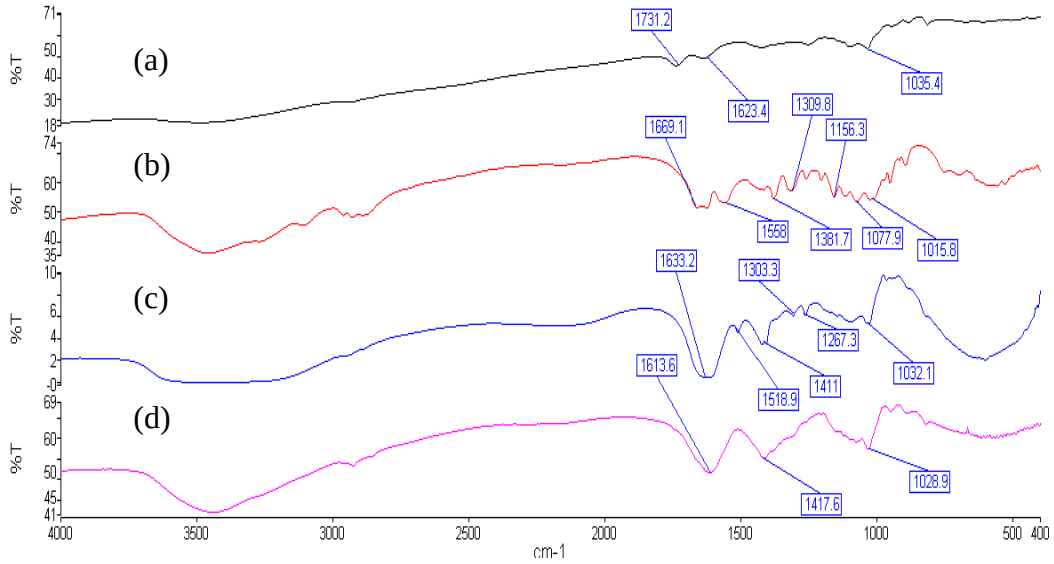


Figure 2: FTIR Spectra of (a) Alginic acid, (b) Chitin, (c) 1% Chitin/Alginate and (d) 2.5% Chitin/Alginate.

FTIR spectrum of alginic acid, chitin, 1% chitin/alginate and2.5% chitin/alginate are shown in Figure 2 (a), (b), (c), and (d) respectively. It is observed from the spectra that the C-O ether stretch for alginic acid is at 1035.4 cm^{-1} while that of chitin is at 1015.8 cm^{-1} to 1077.9 cm^{-1}. These peaks are then observed at 1032cm^{-1} for 1%chitin blend alginate and 1029 for 2.5%chitin blend alginate. The weak carboxylate peak of alginic acid observed at 1623.4 cm^{-1} and the N-H bending observed for pure chitin at 1558cm^{-1} shifted to 1518.9 cm^{-1} for 1% chitin blend alginate and 1417.6 cm^{-1} for 2.5% chitin blend alginate. It is interesting to note that a new amide peak appears at 1633cm^{-1} and 1613 cm^{-1} in the spectrum of 1%chitin blend and 2.5% chitin blend alginate respectively. These peaks may be due to an amide formation reaction between deacetylated chitin and alginate or hydrogen bonding interactions.

3.3 SEM Analysis

Scanning Electron Microscopy (SEM) results of chitin bead outer surface (Fig 3-4), chitin bead inner surface(Fig 5-6), chitin / alginate bead outer surface (Fig 7-8), chitin / alginate bead inner surface(Fig 9-10), and chitin / alginate bead iron adsorped (Fig 11-12) at 250 and 2500 respectively are shown in Figure 3- 12

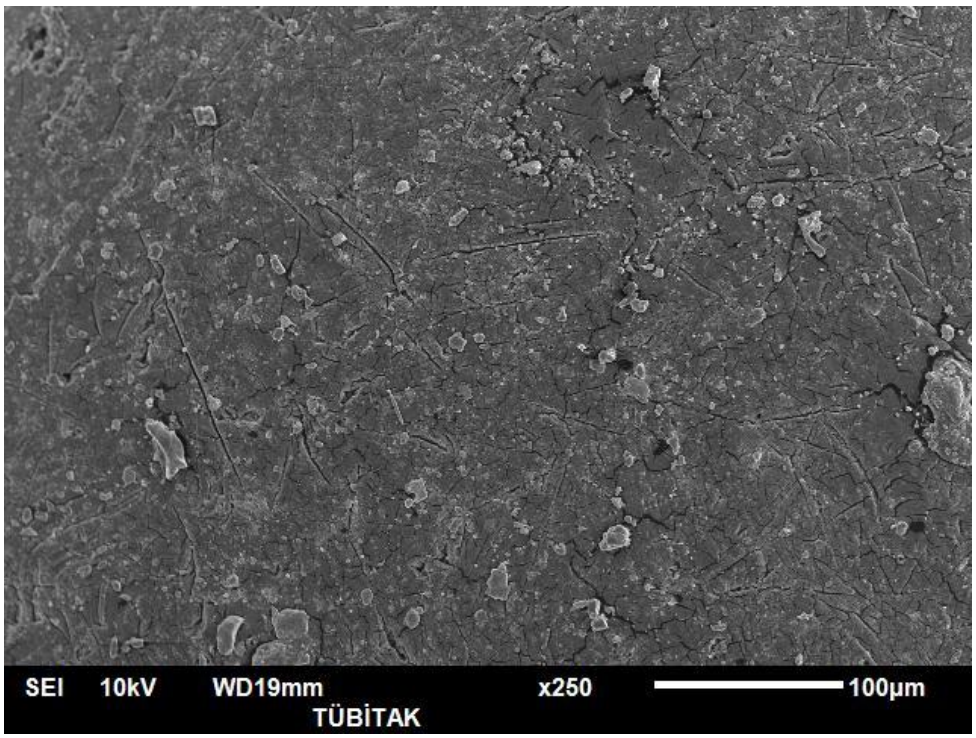


Figure 3: SEM picture chitin bead outer surface at ×250 magnification

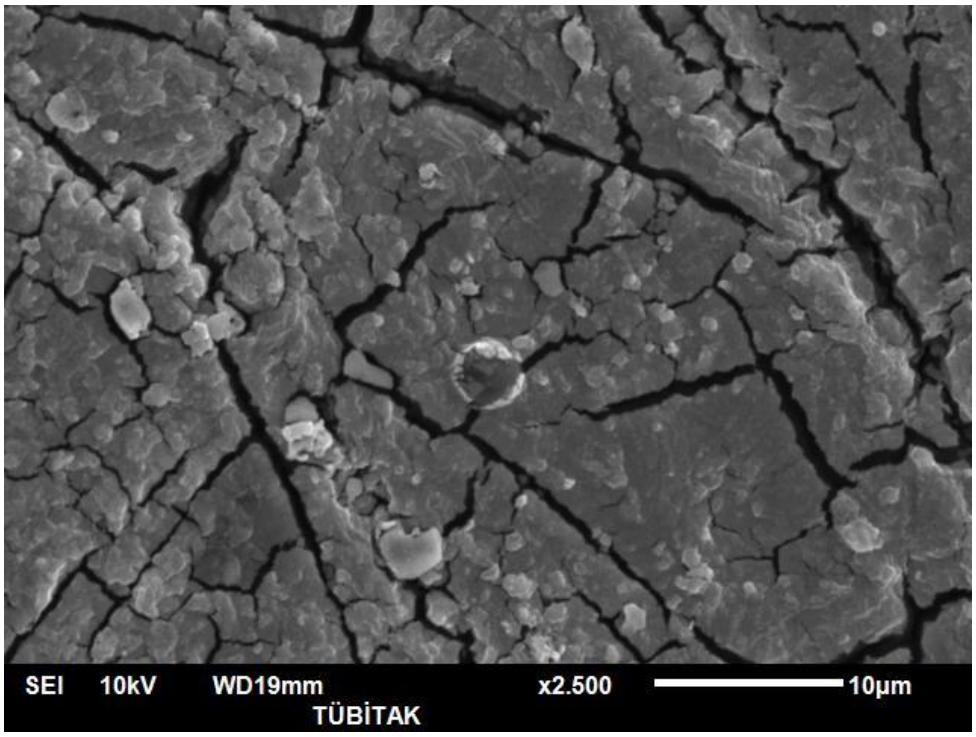


Figure 4: SEM picture of chitin bead outer surface at ×2500 magnification

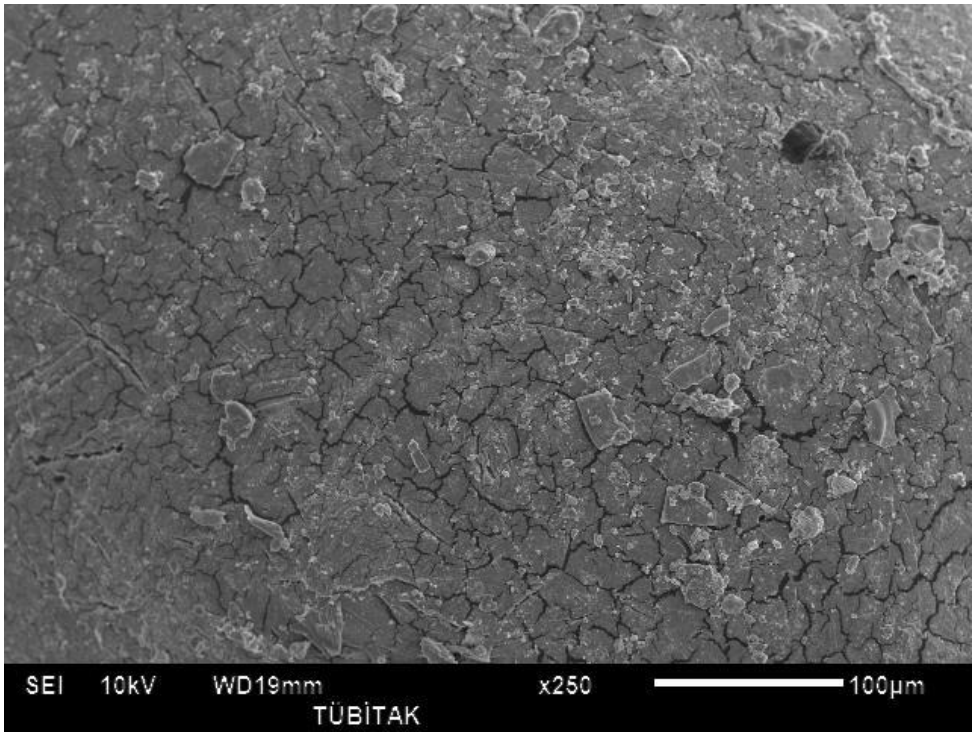


Figure 5: SEM picture of chitin bead inner surface at ×250 magnification

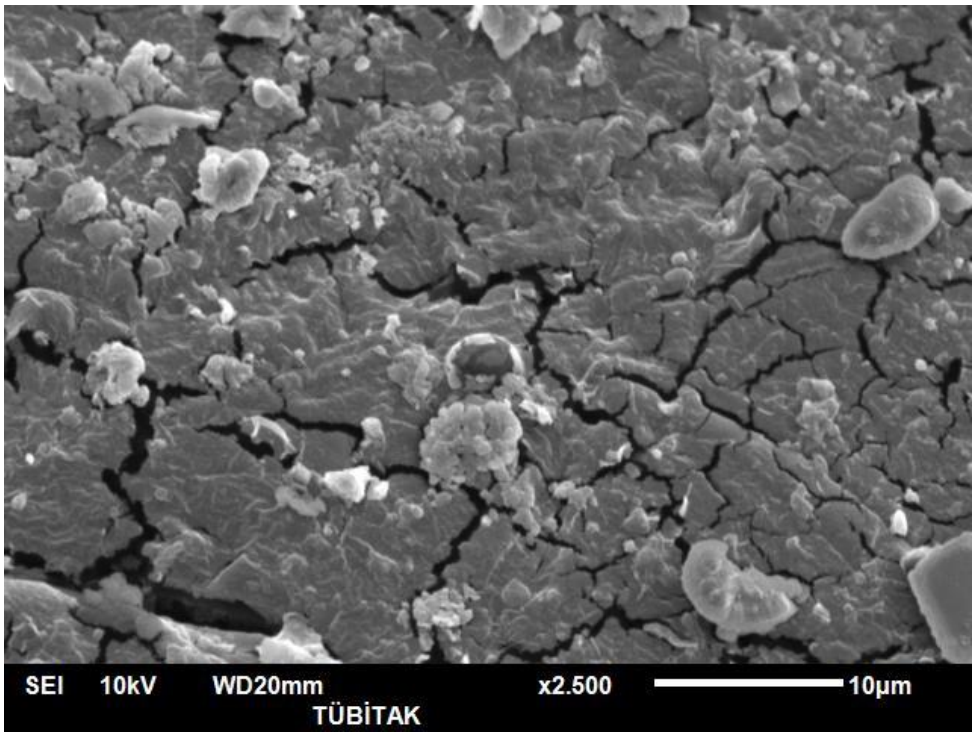


Figure 6: SEM picture of chitin bead inner surface at ×2500 magnification

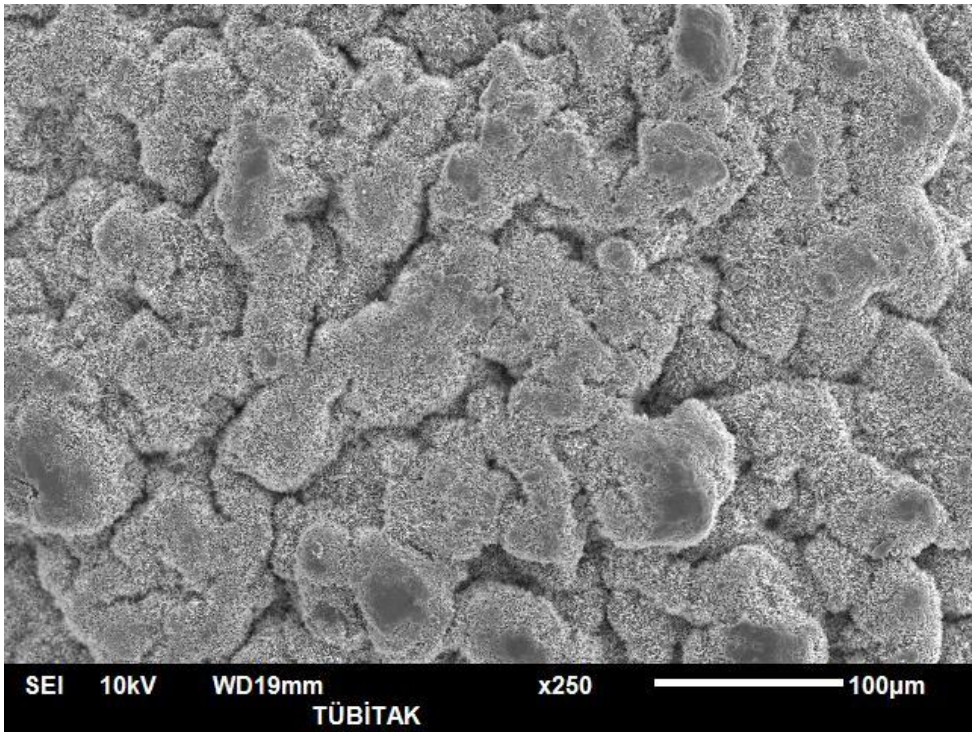


Figure 7: SEM picture of chitin / alginate bead outer surface at ×250 magnification

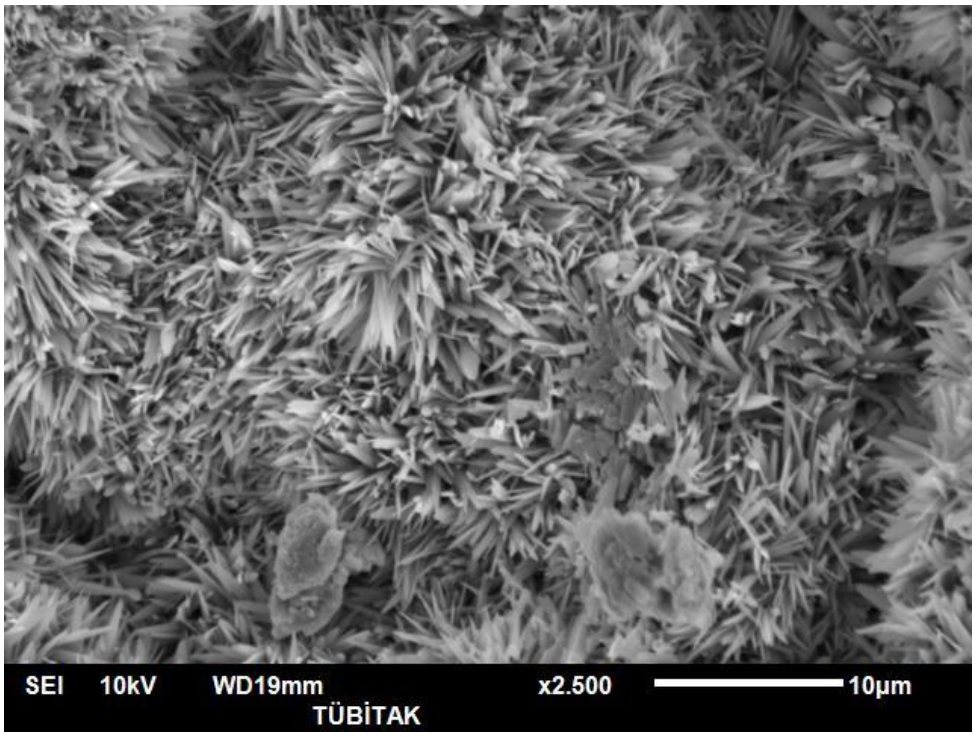


Figure 8: SEM picture of chitin / alginate bead outer surface at ×2500 magnification

Figure 7 and 8 which shows the outer surface of chitin/alginate bead. From the SEM pictures grain like particles are observed at 250µm, which are shrub like at 2500µm. When these are compared to Figurs 3 and 4 which look plain and cracky it shows large morphological difference. This difference shows that the alginate blended into the chitin beads.

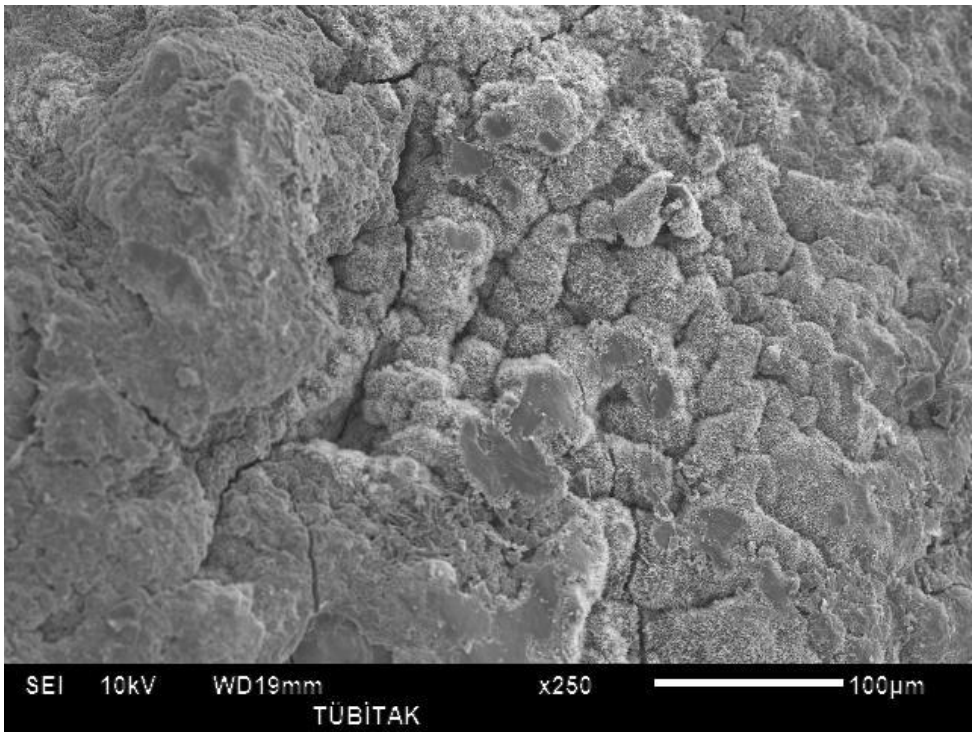


Figure 9: SEM picture of chitin / alginate bead inner surface at ×250 magnification

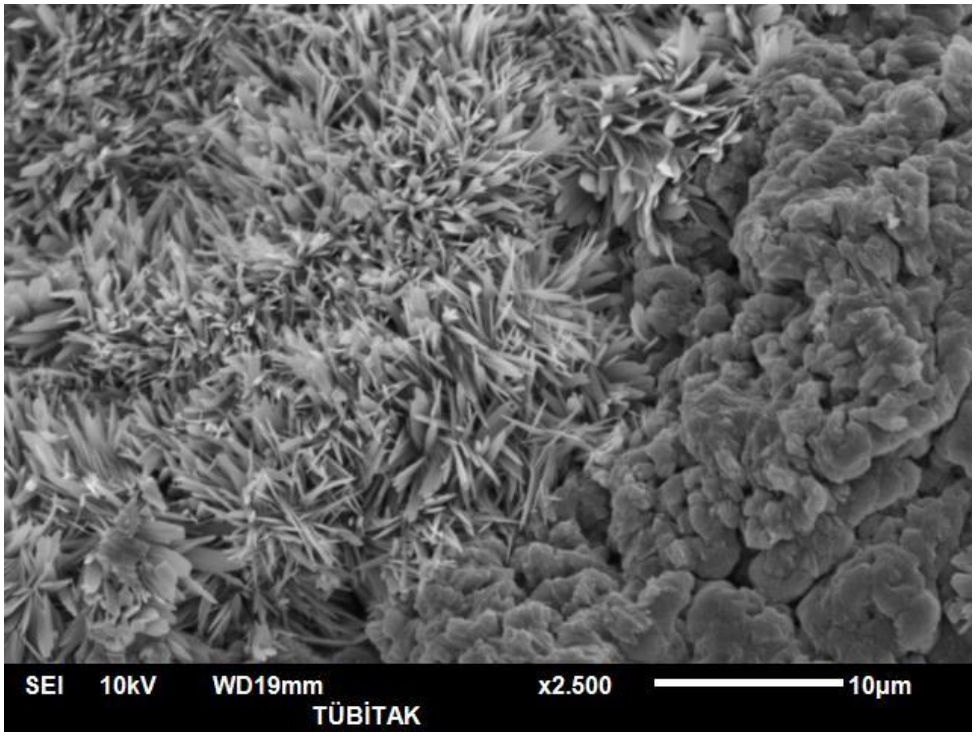


Figure 10: SEM picture of chitin / alginate bead inner surface at ×2500 magnification

Comparing figure 9 and 10 with fig 5 and 6, it is noted that a furry clothe like layer is observed for chitin/alginate inner surface bead. This morphological difference shows that the alginate penetrated into the chitin beads.

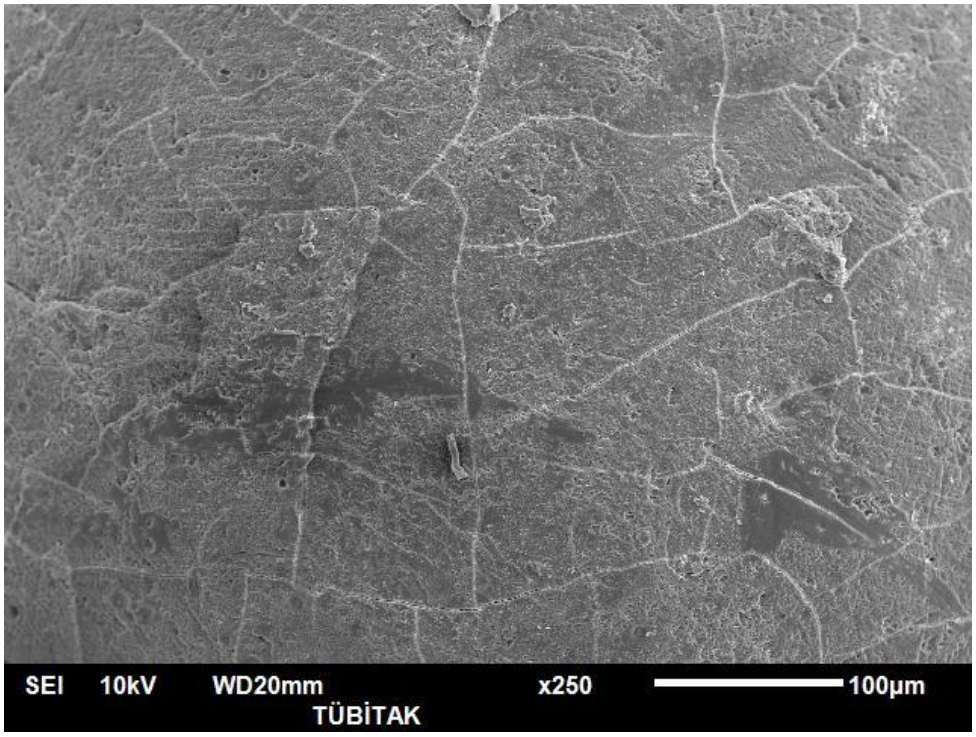


Figure 11: SEM picture of chitin / alginate bead iron adsorped at ×250 magnification

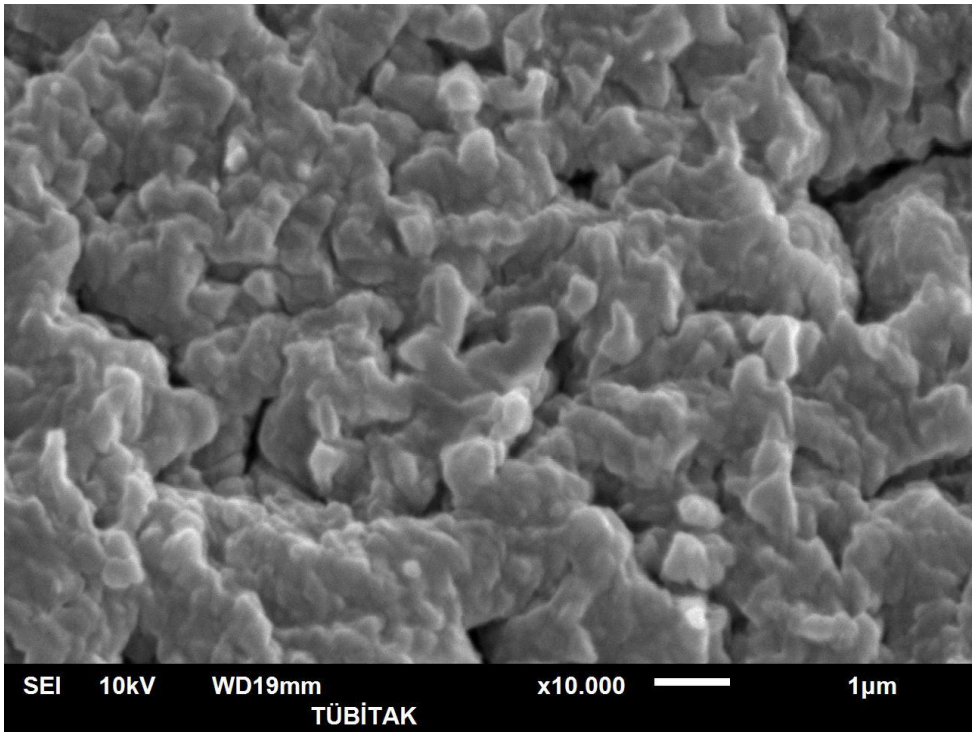


Figure 12: SEM picture of chitin / alginate bead iron adsorped at

×10000magnification

Figure 11 and 12 shows chitin /alginate bead iron adsorped at 250 and 10.000µm. There is complete change in morphology of the chitin /alginate bead which looked hairy in Fig 9 and 10, the beads after iron adsorption has a mould particle growth on the surface compared to chitin /alginate bead which looks hairy.

3.4 Swelling Characteristics

The swelling kinetics of the samples prepared were followed in aqueous solutions of pH=1, pH=7 and pH=11 values. The results are shown for pH=1, pH=7 and pH=11 in Figure 5, 6 and 7 respectively. Tables 4, 5 and 6 gives the result of the swelling percentage of the samples at pH=1, pH=7 and pH= 11 respectively.

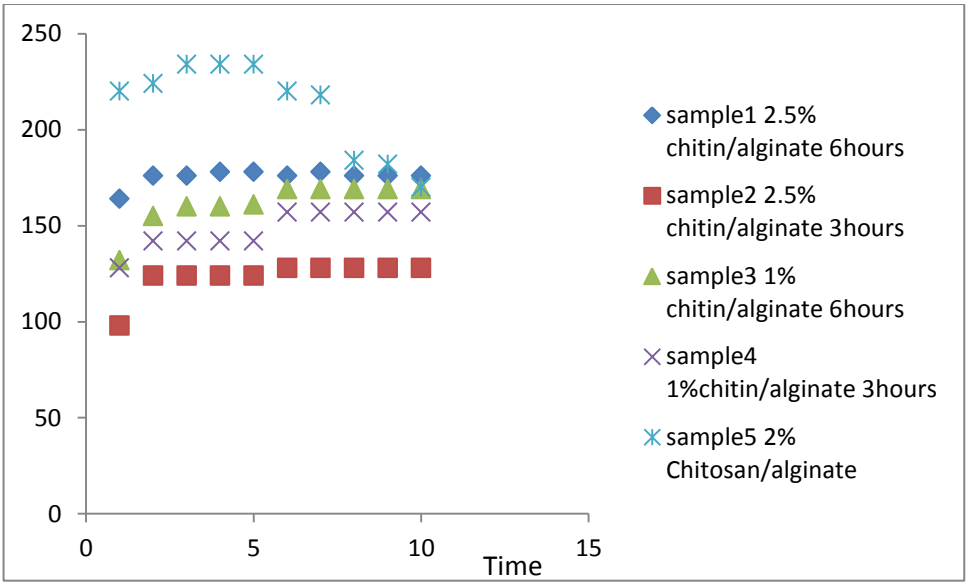


Figure 13: Graph of Swelling Effect Effect of Samples at pH=1

Table 4: % Swelling ratio of Chitin (2.5% and 1%) Blend Alginate and Chitosan Blend Alginate at pH=1

Sample ID	**Time(minutes)**						
	60	120	180	240	300	360	480
Sample 1	31	145	154	153	153	153	153
Sample 2	70	115	128	128	128	128	128
Sample 3	90	141	176	176	174	176	180

Sample 4	120	208	213	213	216	216	216
Sample 5	164	204	204	204	204	204	200

Table 5 and figure 13 gives the swelling of all samples at pH=1.0. It is observed that all samples swell with increase in time, because of the penetration of the aqueous acidic medium into the polymer network. Sample 5 which is composed of chitosan and alginate swells to about 200% in acidic medium because it contains the highest fraction of free $-NH_2$ and free carboxylate functionalities. Sample 4 swells almost as much as sample 5 due to the deacetylation of chitin to chitosan for 6hours treatment time under alkaline conditions. Sample 3 swells less than sample 5 and sample 4 due to less amount of deacetylation than sample 4, this is due to smaller treatment time of 3hours. Sample 2 and 1 has less $-NH_2$ groups because of the higher concentration of chitin solution (2.5%). Hence, less swelling in acidic conditions.

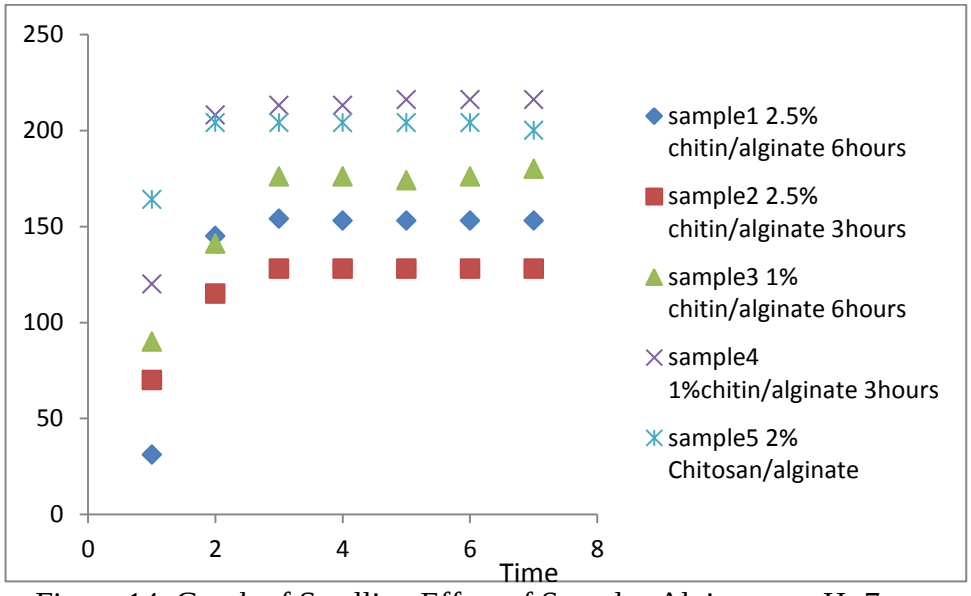


Figure 14: Graph of Swelling Effect of Samples Alginate at pH=7

Table 5: Swelling Effect of Samples at pH=7

Sample ID	**Time(minutes)**						
	60	120	180	240	300	360	480
Sample 1	164	176	176	178	178	176	178
Sample 2	98	124	124	124	124	128	128
Sample 3	132	155	160	160	161	169	169
Sample 4	128	142	142	142	142	157	157
Sample 5	220	224	234	234	234	220	218

Table 6 and Figure 14 show the swelling behaviour of the samples in neutral pH buffer. Under neutral conditions, all samples swell less than or almost equally as they do under acidic conditions. They all swell less than in basic conditions. They swelling capacity at this pH is determined by osmotic pressure developed by water molecules diffusing into the gel beads, rather than repulsive forces between ionic species.

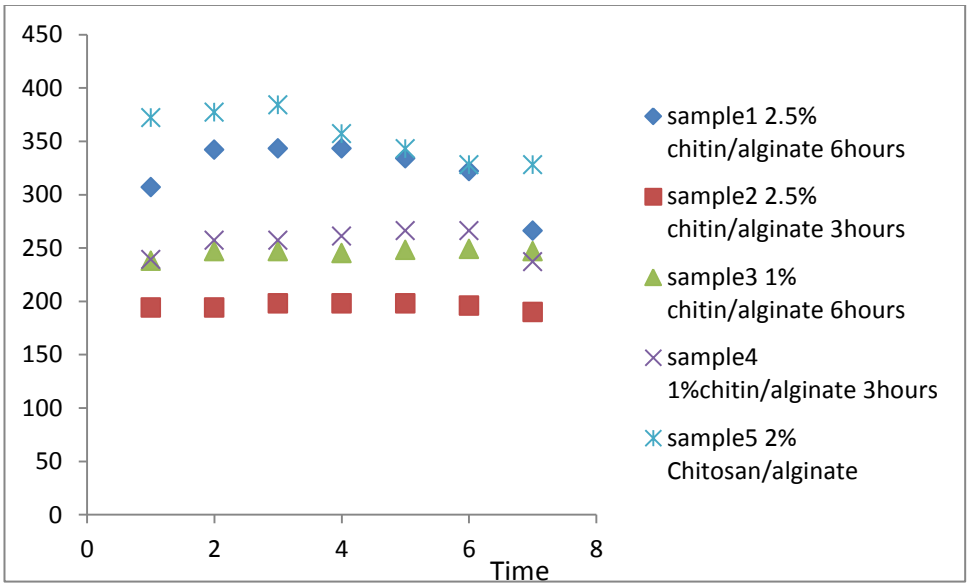


Figure 15: Graph of Swelling Effect Effect of Samples at pH 11

Table 6: Swelling Effect of Chitin (2.5% and 1%) Blend Alginate and Chitosan Blend Alginate at pH=11

Sample ID	**Time(minutes)**						
	60	120	180	240	300	360	480
Sample 1	307	342	343	343	334	322	266
Sample 2	194	194	198	198	198	196	190
Sample 3	238	247	247	245	248	249.2	247
Sample 4	239	257	257	261	266	266	237
Sample 5	372	377	384	357	343	328	328

It is observed that chitin blend alginate and chtosan blend alginate swelled more in basic media than in the acidic and neutral medium with the least swelling was observed at neutral pH. The highest swelling observed in sample5 shows that it contains the highest amount of –COOH-. The higher swelling observed in the basic medium for all samples when compared to other media,can be attributed to the functional groups –OH, –COOH and $–COO^-$ present in the adsorbent since the blends has an outer layer with the anionic polysaccharide alginate.

3.5 Fe^{3+} Adsorption/Desorption Behavior

The iron adsorption capacity of the prepared chitin/chitosan coated alginate was studied using Fe^{3+} solutions of 2.5 mM, 25 mM, 50 mM and 100 mM. The results for 2.5mM, 25mM, 50 mM and 100mM are given in Figure 12, 13 and 14 with Tables 8, 9 and 10 presenting the data respectively. It is important to study Fe^{3+} removal from aqueous solution to provide people with clean water free from toxic components. In addition to the unpleasant taste of drinking water even with low concentration of Fe^{3+} (1.8mg/L), problems regarding iron toxicity may also develop [20]

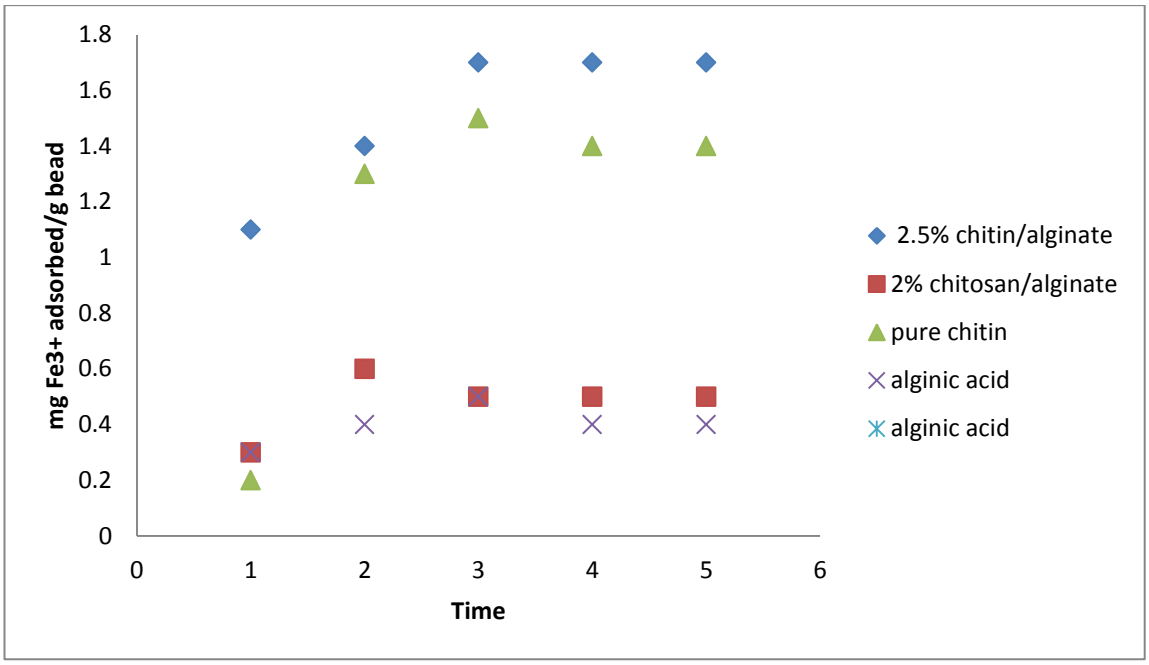


Figure 16: Fe^{3+} adsorption by bead (mg Fe^{3+}/g bead) in 2.5mM $FeCl_3$ with respect to time.

Table 7: Fe^{3+} adsorption (mg Fe^{3+}/g bead) in mM $FeCl_3$ with respect to time

Sample ID	**Time(minutes)**				
	30	120	240	360	1440
Sample 1	1.03	1.4	1.7	1.8	1.7
Sample 5	0.3	0.56	0.56	0.54	0.54
Sample 7	0.2	1.3	1.5	1.4	1.4
Sample 6	0.28	0.44	0.48	0.45	0.45

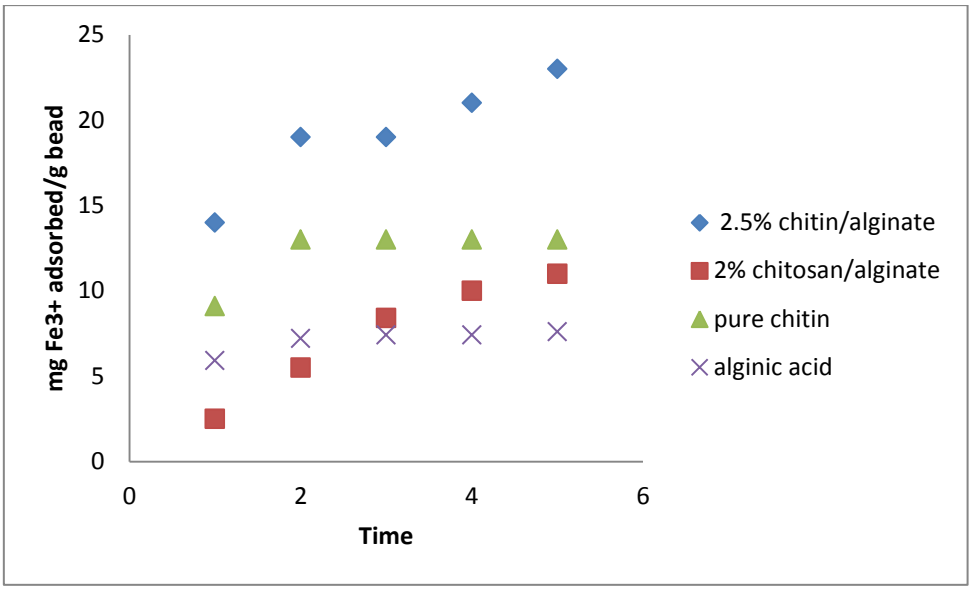


Figure 17: Fe^{3+} adsorption by bead (mg Fe^{3+}/g bead) in 25mM $FeCl_3$ with respect to time.

Table 8: Fe^{3+} adsorption (mg Fe^{3+}/g bead) in 25 mM $FeCl_3$ with respect to time

Sample ID	**Time(minutes)**				
	30	**120**	**240**	**360**	**1440**
Sample 1	14.2	19.6	19.6	21.1	23.5
Sample 5	0.15	5.49	8.38	10.05	11.6
Sample 7	9.00	13.6	13.2	13.2	13.0
Sample 6	5.90	7.20	7.40	7.40	7.60

Figure 17 and Table 9 shows that comparing with other time intervals, equilibrium Fe^{3+} adsorption is reached within 2 hours.

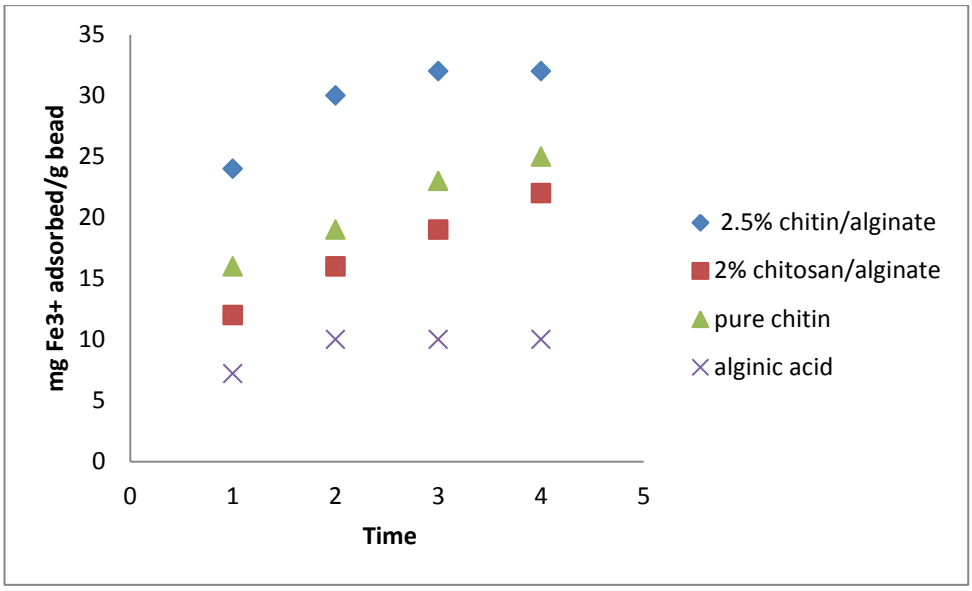


Figure 18: Fe^{3+} adsorption by bead (mg Fe^{3+}/g bead) in 50mM $FeCl_3$ with respect to time.

Table 9: Fe^{3+} adsorption (mg Fe^{3+}/g bead) in 50 mM $FeCl_3$ with respect to time

Sample ID	**Time(minutes)**			
	60	**180**	**360**	**1440**
Sample 1	24	30	32	32
Sample 5	12	16	19	22
Sample 7	16	19	23	25
Sample 6	7.2	10	10	10

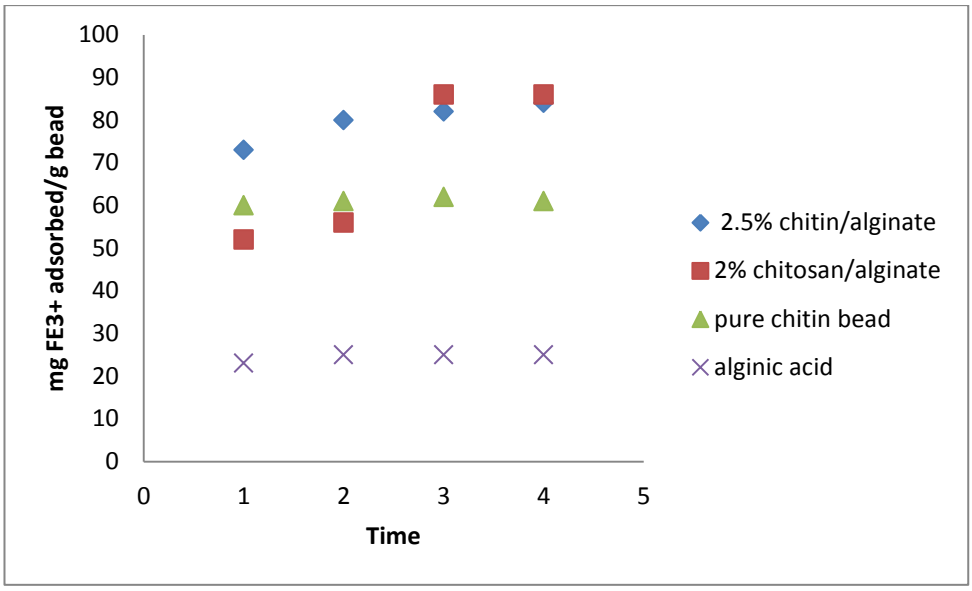


Figure 19: Fe^{3+} adsorption by bead (mg Fe^{3+}/g bead) in 100mM $FeCl_3$ with respect to time.

Table 10: Fe^{3+} adsorption (mg Fe^{3+}/g bead) in 100 mM $FeCl_3$ with respect to time

Sample ID	Time(minutes)			
	60	180	360	1440
Sample 1	73	80	82	84
Sample 5	52	56	86	86
Sample 7	60	61	62	61
Sample 6	23	25	25	25

From Figure 8, 9 and 10 it is observed that chitin bead blended with alginate increased in iron adsorption than pure chitin for all four concentrations. This is also observed for chitosan where the beads blended with alginate has higher iron adsorption capacity than pure chitosan. The experiments which where done for 2.5mM, 25mM, 100mM, and 1000mM $FeCl_3$ for all four samples showed that the

beads adsorbed more iron at 100mM concentration with chitin bead blend alginate having the best and highest adsorption than other samples.

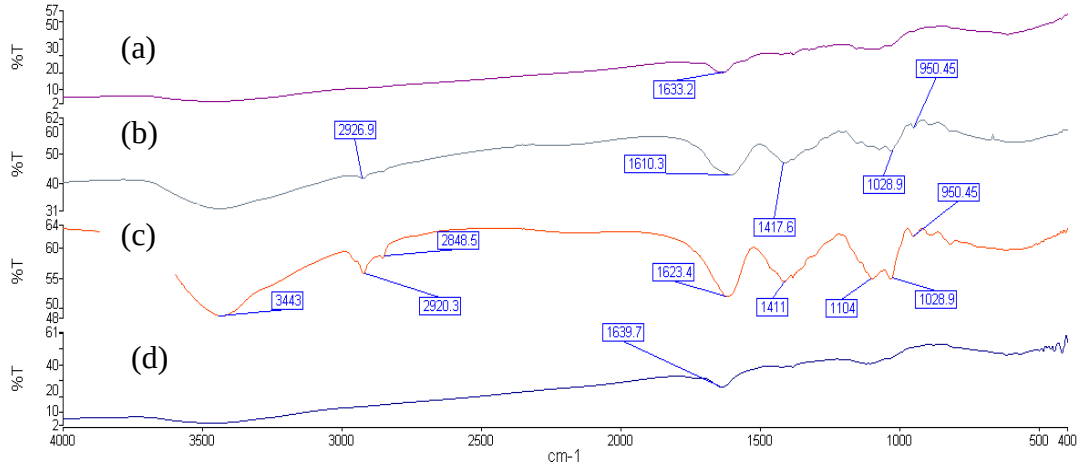


Figure 20: FTIR Spectra (a) Iron Adsorbed 2.5% Chitin/Alginate Beads with Iron, (b) 2.5% Chitin/Alginate Beads, (c) Chitosan/Beads (d) Iron Adsorbed onto Chitosan Alginate Beads.

The FTIR spectrum above shows that 2926.9cm^{-1}, 1417.6cm^{-1}, 1028.9cm^{-1} and 950.45cm^{-1} peaks in chitin were hidden when it adsorbed iron. The spectra shows that for chitosan adsorbed iron the peaks at 3443cm^{-1} ,2920.3cm^{-1} , 2828.5cm^{-1} , 1411cm^{-1} , 1104cm^{-1} and 1028. ˉ9cm $^{-1}$ found in chitosan blend alginate of the third spectra were masked.

3.6 Desorption

Desorption studies for the iron adsorbed by the beads were carried out in pH-1.2 and 0.06M ammonia the results are shown in Figure 21 and Figure 22 below.

3.6.1 Desorption in pH 1.2

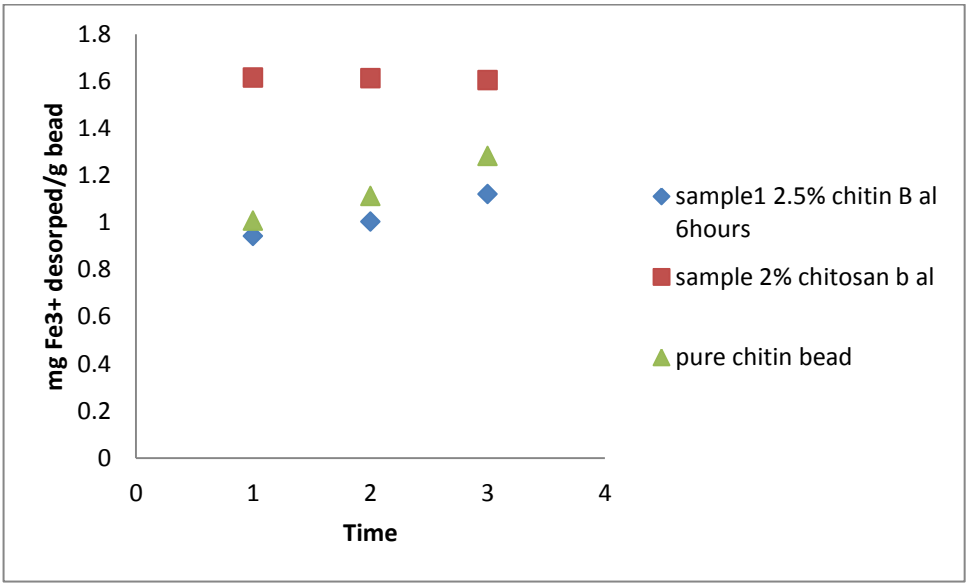


Figure 21: 100mM Fe^{3+} desorption (mg Fe^{3+}/g bead) in pH=1.2 buffer solution after 3, 6 and 24 hours.

Table 11: Fe^{3+} desorption (mg Fe^{3+}/g bead) in pH=1.2 buffer solution after 3, 6 and 24 hours.

Sample ID	**Time(minutes)**		
	180	**360**	**1440**
Sample 1	0.896	1.003	1.12
Sample 5	1.615	1.612	1.604
Sample 6	1.008	1.112	1.282

3.6.2 Desorption in 0.06M Ammonia

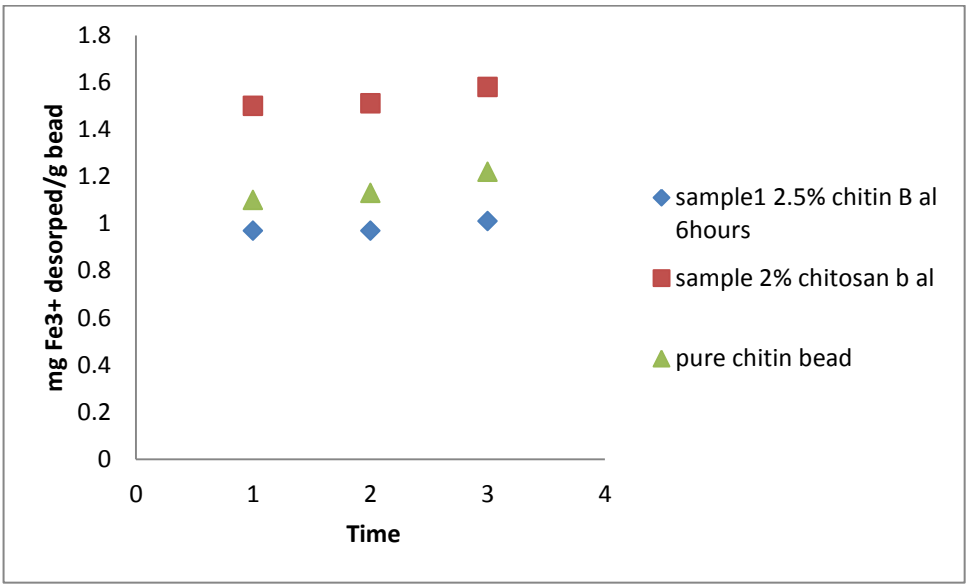


Figure 22: 100mM Fe^{3+} desorption (mg Fe^{3+}/g bead) in 0.06M Ammonia after 3, 6 and 24 hours.

Table 12: Fe^{3+} desorption (mg Fe^{3+}/g bead) in 0.06 Ammonia after 3, 6 and 24 hours

Sample ID	**Time(minutes)**		
	180	**360**	**1440**
Sample 1	0.97	0.97	1.01
Sample 5	1.5	1.5	1.6
Sample 6	1.1	1.1	1.2

From the desorption in Figure 19 and 20 its shows that all three samples released iron in pH and 0.06M ammonia but the iron tends to be released more in ammonia. The results showed that from both desorption studies not all iron was released with pure chitin releasing more iron than the blends. This lesser release of iron by the blends is as a result of the beads (chitin and chitosan) bein entangled by the alginate.

The main advantage of chitosan blend alginate is that chitosan bead alone cannot be used as an absorbent for Fe^{3+} under acidic conditions, as it dissolvesin acidic conditions.

3.7 Isotherms

From the adsorption studies chitin blend alginate showed higher Fe^{3+} adsorption than pure chitin bead. The alginate blends of both chitin and chitosan showed higher adsorption compared to their pure forms. Pure alginate gave very negligible absorption for iron but it still increased the iron adsorption capacity of pure chitin and chitosan. The greatest adsorption was observed between 3 to 6hours for all samples, between these hours adsorption increased by about 30 percent.

3.7.1 Isotherm Graphs of Samples

The isotherm of the iron adsorbed samples was observed and plotted using the Freundlich model. It was plotted using log C_e against log q_e for all samples. The samples obeyed Freundlich model better than other models.

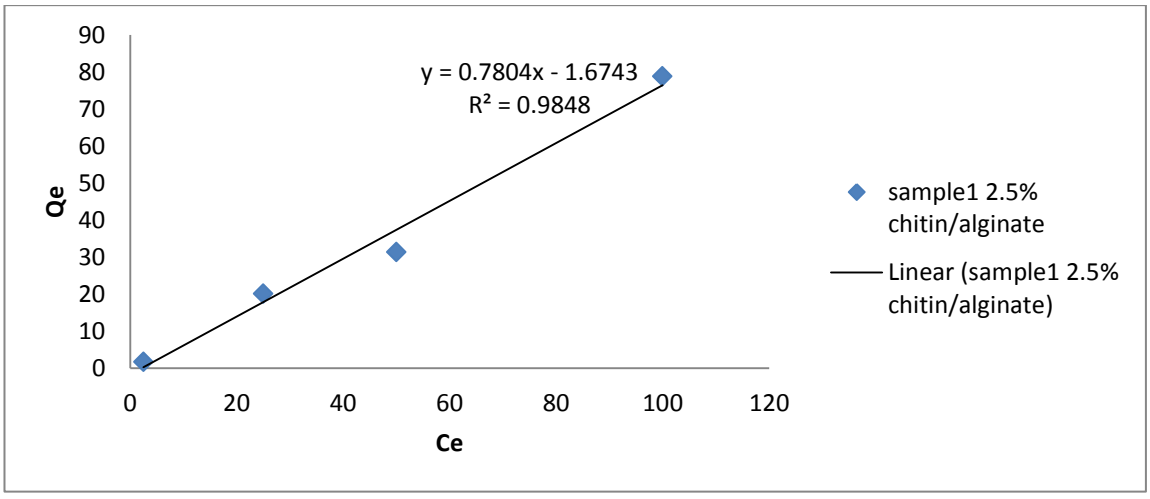


Figure 23: Freundlich plot of 2.5% chitin/alginate bead

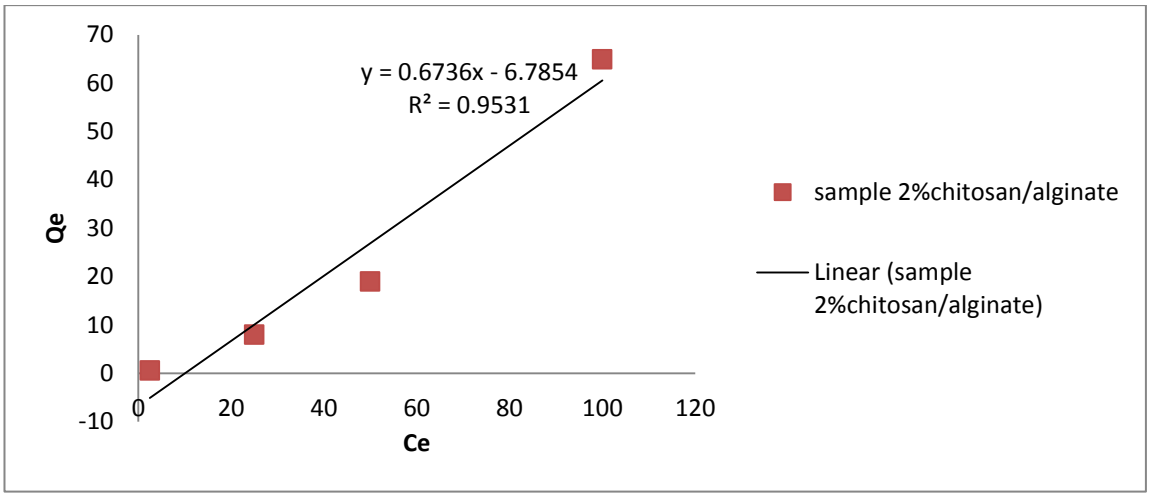


Figure 24: Freundlich plot of 2.5% chitosan/alginate bead

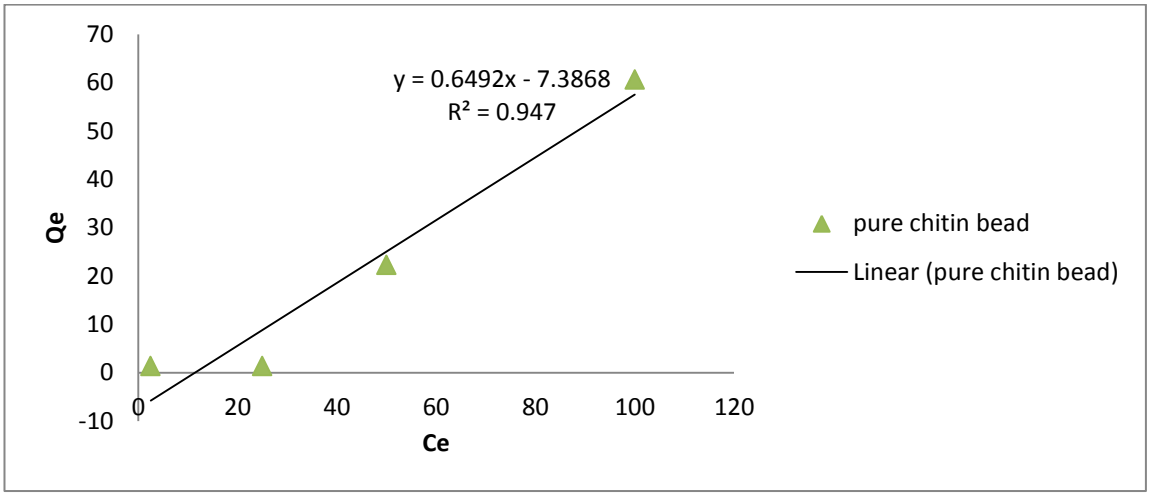


Figure 25: Freundlich plot of 2.5% chitin bead

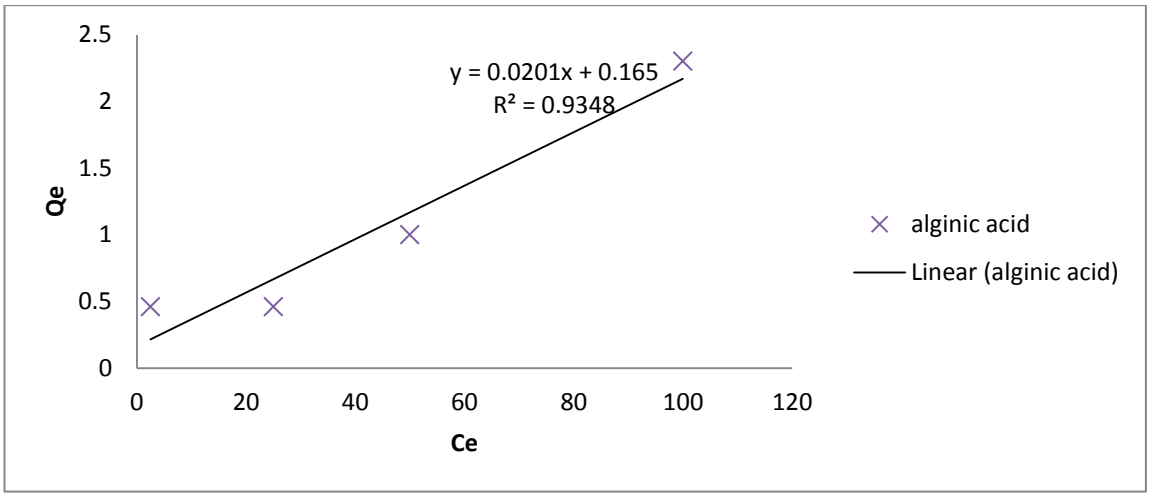


Figure 26: Freundlich plot of alginic acid

From the graphs above Figure 16-19 it shows that Freundlich gave a linearised explanation for iron adsorption into all the samples.

Chapter 4

CONCLUSIONS

Alginate modified chitin and chitosan beads vary greatly in their ability to adsorb Fe^{3+} ions from solution. These biosorbents have considerable capacity to uptake iron (III) ions. The iron (III) ions adsorbed is readily desorbed from alginate beads using both acidic media (pH=1.2) and alkaline diluted ammonia (0.06M).

Chitin/Chitosan blend alginate biopolymers generally improved the adsorption capacity of chitin and chitosan probably due to increase in the number of active binding sites after modification, better ion-exchange properties and formation of new functional groups that favours Fe^{3+} ions uptake.

The system has shown that by coating chitosan with alginate, chitosan beads which are soluble in acidic media can adsorb Fe^{3+} under acidic conditions. Also, chitin when blended with alginate swelled to a great extent in acidic media compared to pure chitin which rarely swells in acidic media.

Chitin, chitosan and alginate beads have uptake and desorption performances that encourage further studies on the industrial application of this biosorbent.We hope this study may provide a cost effective, simple and environmentally friendly adsorbent for Fe^{3+} removal from solution.

REFERENCES

[1] . D. I. Kurt and S. Olav , "Alginate based new materials," *International Journal of Biological Macromolecules,* vol. 21, pp. 47–55, 1997.

[2] I. Kurt and . C. Taylor, "Physical and biological properties of alginates and their biomedical implications.," *Food Hydrocolloids,* vol. 25, pp. 251–256, 2011.

[3] M. B. Elvan Yilmaz, "Preparation and characterization of physical gels and beads from chitin solutions," *Carbohydrate Polymers,* vol. 54, pp. 479–488, 2003.

[4] M. Rinaudo, "Chitin and chitosan: Properties and applications," *Progress in Polymer Science,* vol. 31, pp. 603–632, 2006.

[5] K. Rudall, "Chitin and its association with other molecules," *Journal of Polymer Science,* vol. 28, pp. 83-102, 1969.

[6] K. Rudall and W. Kenchington , "The chitin system," *Biological Reviews,* vol. 40, pp. 597-636, 1973.

[7] B. Olivera , D. Hillyard , M. Marsh and D. Yoshikami , "Lesson from venomous cone snails," *Combinatorial Peptide Libraries in Drug*

Design, vol. 13, pp. 422–6, 1995.

[8] M. &. C. A. Terbojevich, "Molecular weight determination of chitin and chitosan," *Chitin handbook*, pp. 87-101, 1997.

[9] M. Rinaudo , G. Pavlov and J. Desbrieres, "Influence of acetic acid concentration on the solubilization of chitosan.," *Polymer*, vol. 40, pp. 7029–32, 1999.

[10] M. Thanou and H. Junginger , "Pharmaceutical applications of chitosan and derivatives," *Polysaccharides*, vol. 2, pp. 661–77, 2005.

[11] T. H. Hanne and J. Karlsen, "Alginate in Drug Delivery Systems," *Drug Development and Industrial Pharmacy*, vol. 28, pp. 621–630, 2002.

[12] K. Prasad and J. Kadokawa, " Alginates: Biology & Applications," *Springer*, vol. 13, pp. 175–210., 2009.

[13] W. Gombotz and S. Wee, "Protein release from alginate matrices.," *Drug Delivery Rev*, vol. 31, pp. 267–85, 1998.

[14] M. Leonard , D. Boisseson , P. Hubert and F. Dalencon , "Hydrophobically modified alginate hydrogels as protein carriers with specific controlled release properties.," *J Control Release*, vol.

98, pp. 395–405., 2004.

[15] C. Sudha , Angadi, S. Lata , Manjeshwar and Tejraj M, "Novel composite blend microbeads of sodium alginate coated with chitosan for controlled release of amoxicillin," *International Journal of Biological Macromolecules,* vol. 51, pp. 45-55, 2012.

[16] D. Jiangjiang , W. Yue , W. Yudi and X. Xiaopeng , "Preparation and Characterization of Cellulose-Coated Chitosan Beads with Improved Strength and Acid Resistivity," vol. 1, pp. 1-2, 2012.

[17] P. Atkins , Physical chemistry, processes at solid surfaces, third edition, 1986, pp. 771-781.

[18] A. Dabrowski, "Adsorption-From theory to practice," *Colliod and Interface Science,* vol. 93, pp. 135-224, 2001.

[19] W. Ngah, "removal of copper (II) ions from Aqueous Solution onto Chitosan and Cross-linked Chitosan Beads," *Reactive and functional polymers,* vol. 50, pp. 181-191, 2002.

[20] G. Karthiyekan, "Adsorption studies of iron(iii) on chitin," *Journal of Chemical Sciences,* vol. 117, pp. 663-672, 2005.