Preparation and Characterization of Porous Chitosan Tripolyphosphate Gel Bead

Egwuagu Ifeyinwa Nkechinyere

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Prof. Dr. Elvan Yilmaz
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 Yilmaz
Supervisor

Examining Commmittee

1. Prof. Dr. Elvan Yilmaz
2. Assoc. Prof. Dr. Mustafa Gazi
3. Asst. Prof. Dr. Mehmet Garip

ABSTRACT

In this study, Chitosan TPP beads and Chitosan TPP beads in the presence of PEG was prepared. PEG was added to achieve porosity after the removal of the polymer. The samples were characterized by FTIR and SEM analysis. The swelling behaviour was followed in pH 1.2, 7, and 11 and their Fe^{3+} ion adsorption rate from aqueous solution was determined at pH 1.2.

Beads prepared both in the presence and absence of PEG contained porosity. Chi-TPP porous beads prepared by PEG leaching method swelled more in aqueous media having a swelling ratio of about 3500% in acidic media, 350% in neutral media and 400% in basic media as compared with Chi-TPP beads with swelling ratio of 2000% in acidic media, 150% in neutral media and 200% in basic media. The beads served as effective adsorbents for Fe^{3+}. The Chi-TPP PEG beads showed a better adsorption capacity for Fe^{3+} and better swelling behavior as a result of increased hydrophilicity due to PEG treatment. The adsorption behavior obeyed Langmuir model and the kinetics followed pseudo second order.

Keywords: Chitosan, Polyethyleneglycol, Fe^{3+} adsorption.

ÖZ

Bu çalışmada kitosan TPP jel boncukların hazırlanması ve çözeltiden Fe^{3+} adsorplama davranışları incelenmiştir. Kitosan TPP jel boncuklar PEG porojen varlığında ve yokluğunda sulu çözeltiden koagülasyon yöntemi ile elde edilmiş ve birbirleriyle bazı fiziksel özellikleri bakımından karşılaştırılmıştır. Örnekler FTIR ve SEM analizi yöntemleri ile incelenmiş ve sulu asitli, nötr ve bazik ortamda şişme davranışları ile pH=1.2 tampon çözeltide Fe^{3+} adsorplama davranışları çalışılmıştır.

Jel boncuklar PEG den arındırıldıktan sonra gözenekli yapıda önemli bir değişiklik saptanamamıştır. Ancak bu işlemden sonra jel boncukların daha hidrofilik bir yapıya sahip oldukları şişme ve Fe^{3+} adsorplama kapasitelerinin arttığı gözlemlenmiştir. Jel boncukların Fe^{3+} adsorplama davranışlarının Langmuir modeline uyduğu ve ikinci dereceden kinetiğe sahip olduğu anlaşılmıştır.

Anahtar Kelimeler: polietilen glikol, kitosan, tripolifosfat, jel boncuk, Fe^{3+} adsorpsiyonu

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I thank God for giving me the precious gift of you all. God bless!

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Chapter 1

INTRODUCTION

Chiosan Tripolyphoshate beads may serve as useful supports for drug conjugation, enzyme immobilization, and protein recognition. The gel bead surfaces may further be functionalized via polymer grafting to provide moites with antibacterial or selective metal binding properties to name a few. Porous chitosan tripolyphosphate gel beads find applications in water treatment as dye removal, drug delivery, and in tissue engineering and preparation of biocomposites of chitosan.

Chitosan gel beads are formed when chitosan is dissolved in acidic solution alongside polyanions. (Shu & Zhu, 2000) Tripolyphosphate is one of the most widely used polyanions and it carries five negative charges in each molecule. These chitosan beads formed are very strong and long lasting and they find application in adsorption of metal from solution. Spectroscopic methods and electron microscopy have all been used to characterize these gel beads. (Cao, Yebang, Yugu, & Qiang, 2010). The surface area of the beads needed for interaction increases when porosity is introduced into the beads. The methods used in this thesis to form Chi-TPP and chitosan-TPP PEG beads have been reported in the literature before (Wu, Horng, Hsien, Long & Kuan; 2013, Chao, Anchong, Shu-Huei, & Guo; 2006, Santos, Neto, Fonseca, & Pereira; 2008, Feng, Shu, Xi, Cheng & Jing; 2012, Zeng & Fang, 2004, Cruz, Daniela, Joao, Jose, & Manuel, 2009). Porogen leaching method using polyethylene glycol (peg) was used to introduce porosity into Chitosan-TPP beads. Introducing

porosity to Chitosan TPP beads using PEG has not been reported anywhere in literature as far as I can tell. Hence preparation and characterization of porous Chitosan-TPP beads by porogen leaching method has been undertaken in this thesis as adsorbents for water treatment.

1.1 Chitosan; Occurrence, Properties and Application

Marine-based food products are easily digested and are made up of desirable source of vital minerals. Most of the materials comprising of carbohydrate found in nature occur in the form of polysaccharides. Polysaccharides are made up of not just glycosidic sugar moiety but also materials having polymeric structures connected through covalent linkages to proteins, amino acids, fats etc. (Giovanna, Malinconico, & Laurienzo, 2008). Polysaccharides, a class of very large complex molecule found in nature has been found to be remarkably useful and are generally gotten from agronomical or animal wastes. Cellulose, starch, pectin are examples of natural polymers gotten from plants while chitosan and chitin are found in lower animals (Harish & Tharanathan, 2007). Recently, sea foods have been recognized as nutraceuticals or functional foods.

Naturally chitin is found in the cell wall of fungi, green algae, yeast and protozoa as well as insect cuticles and especially in the exoskeleton of crustaceans. Chitosan is derived from chitin (Moury & Inamdar, 2008). Chitin is the 2nd most ubiquitous natural polymer found in nature with cellulose being the 1st in this ranking. While cellulose is found in plants chitin is synthesized in lower animals. Chitosan is mostly prepared by deacetylation of chitin in a coagulation bath of sodium hydroxide at a temperature range of 60℃ - 120℃, both natural polymers have chemical structures that are almost identical. While Chitin consists of a straight chain made up of acetyl

glucosamine groups, chitosan on the other hand is derived when acetyl groups on the molecule are eliminated and these results in solubility of chitosan in acids, a method commonly defined as deacetylation. (Rinaudo, 2006). This deacetylation process has to do with the elimination of acetyl groups from chitin, a linear chain and the resultant remnant being chitosan having an amino group ($-NH_2$) which is chemically very reactive. For this reason degree of deacetylation is considered very vital because it plays a huge role in the physiochemical properties of chitosan, hence it is a determinant factor as regards needed and necessary application of chitosan. It can therefore be said that degree of deacetylation (DD) and molecular weight are 2 important parameters determining the characteristics of chitosan (Tanveer**,** Peh & Ch'ng, 2002). Chitosan consists of two monosaccharide units; N-acetyl-glucosamine and D-glucosamine which are linked by β-(1→4) glycosidic bonds. It also contains 3 functional groups on the backbone; the amine group on the C2, the pry and secondary OH groups on the C3 and C6 positions. Figure 1 depicts the structure of chitosan. Chitosan is a polyfunctional polymer with amine and amide group whose fractions depend upon the degree of deacetylation of the polymer. When dissolved in dilute acid chitosan becomes positively charged as a result of the protonation of the amine groups as shown in figure 2.

Chitin is almost the only raw material used for the production of chitosan and chitosan derivatives. Chitosan has found many uses in large scale applications; in agriculture to stimulate the growth of plant (Ramesh & Tharanathan, 2003), in water treatment for removal of metal ions thereby helping to purify water, in biomedicine for wound healing, bone rebuilding, drug delivery etc (Jayakumar, Menon, Manzoor, Nair & Tamura, 2010), in food and beverage industries as food additives(Luo & Wang, 2013**)** , paper finishing industry, water engineering, photography and even in

cosmetic industries to maintain skin moisture and treatment of certain skin defects (Majeti & Ravi, 2000).

Other excellent features of chitosan are biodegradability, biocompatibility, mucoadhesivity, antibacterial activity (Yilmaz, 2004) and its potential capacity in film formation not forgetting to mention its metal ion chelating ability (Burke, Yilmaz, Hasirci & Yilmaz, 2001). The amino group in chitosan is an edge over cellulose since it gives room for broad range of modification reactions.

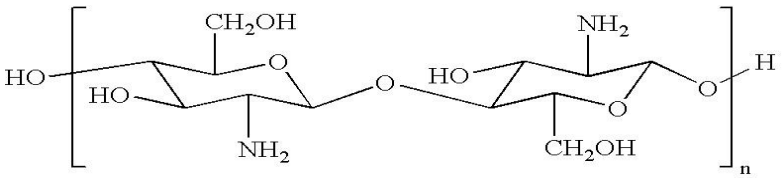


Figure 1: Structure of chitosan

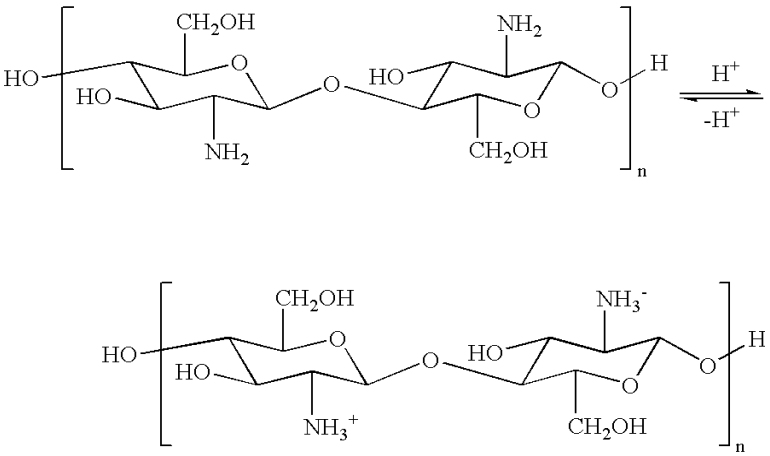


Figure 2: Protonation and deprotonation of chitosan

1.2 Chemically Crosslinked Chitosan

Membranes, fibers, powder, beads can be produced from chitosan but some of the drawbacks include their unstable nature especially in acid solutions, poor resistance to heat, low mechanical properties. Due to this drawback the need for modification

became vital and the best known process is crosslinking. Compounds with low molecular weight or high molecular both fall into these crosslinkers not forgetting ionic compounds. Crosslinkers can be defined as materials that contain relatively two functional groups which are active and they make way for the synthesis of bridges between polymers (Berger et al., 2003). Up until this time, the frequently used crosslinkers used for chitosan include glyoxal and glutaldehyde which are diadldehydes. Crosslinked chitosan has been studied extensively and bonds formed are imine bonds which occur due to the reaction of the aldehyde group and amino group of chitosan. When amine and aldehyde react a Schiff base (imine) is formed hence the reaction is described as a Schiff base formation and it is a chemical crosslinking reaction in which difunctional reagents are required(Kurita, 2006). The advantage of this chemical crosslinking reaction is that gels with higher resistance towards dissolution and disintegration and with better mechanical properties compared to physical gels are obtained. One of the disadvantages of dialdehyde is that they are seen to be toxic (Cenk & Akbuga, 1998, Lee, Mi, Shen & Shyu, 2000).

1.3 Physical Crosslinking of Chitosan with the Tripolyphosphate Ion (TPP)

Chitosan powders, films, membranes, beads are not stable in acidic media and this necessitates modification which is either physical or chemical, the commonest being crosslinking. Low or high molecular weight compounds which include ionic compounds are used as crosslinking agents. Ionic crosslinking is one of the simpler methods and it occurs under mild conditions. Low molecular weight sodium tripolyphosphate (TPP) was used as crosslinking agent. Modification results in the synthesis of ionic crosslinks amid the positively charged amino groups found in chitosan and sodium tripolyphosphate in its dissociated form. Chitosan forms

complexes with molecules that possess negative charges by electrostatic interaction. Chitosan having a pKa of around 6.5 can interact with negative counterions.eg tripolyphosphate and sodium sulphate. Tripolyphosphate is a polyanion (negatively charged compound) having five negative charges in each molecule (Li & Huang, 2012). When these two are brought together chitosan tripolyphosphate gel durable beads are formed. This is a reversible physical interaction. TPP is able to form either intermolecular or intramolecular linkages with chitosan and this is responsible for successful formation of chitosan TPP beads with lower crystallinity. Crosslinking density of beads is controlled by the pH value of sodium tripolyphosphate (Devika &Varsha, 2006). Crystallinity, hydrophilicity and crosslinking density bring about regulation of drug delivery hence its application in that field.

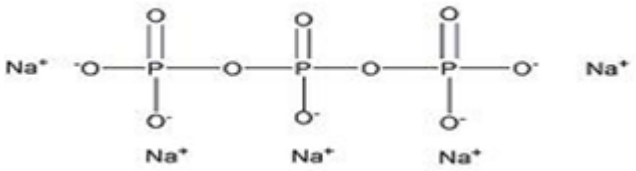


Figure 3: Structure of Sodium Tripolyphosphate

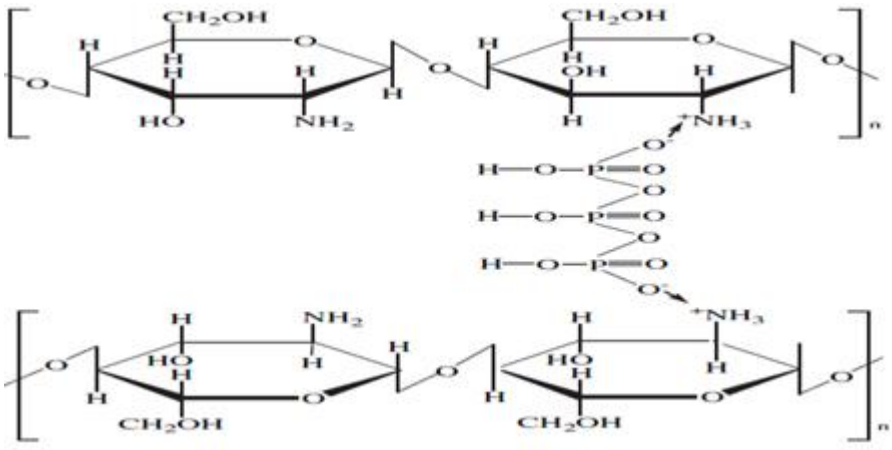


Figure 4: Chitosan Chain Crosslinking with the Tripolyphosphate Ion

1.4 Effect of pH on the Ionization of TPP

Na-TPP dissolves in water to produce OH ions and phosphoric ions. It has been studied that the crosslinking of chitosan depends on the available cationic sites and negatively charged sites; hence pH of TPP is expected to come into play. (Devika & Varsha, 2006).

Pentasodium tripolyphosphate dissolves in water at pH 9 producing several anions such as $P_3O_{10}^{5-}$, $HP_3O_{10}^{4-}$, $H_2P_3O_{10}^{3-,}$ $H_3P_3O_{10}^{2-}$ and $H_4P_3O_{10}^{-}$ in solution. The crosslinking of TPP ions with chitosan is affected by the OH- ions in solution at this pH. At acidic pH only phosphoric ions are present while at basic pH both phosphoric and hydroxyl ions are present. Hydroxyl and phosphoric ions compete for the NH^{+}_{3} site of chitosan and this leads to a decrease in crosslinking. Deprotonation dominated at basic pH while ionic interaction dominated at acidic pH of TPP (Druzynska & Czubenko, 2011).

1.5 Application of Chitosan-TPP Gels

Chitosan-TPP is of great importance for bone and tissue engineering whereby it complexes with metal ions that enhance bone grafting. (Pati, Adhikari & Dhara, 2011). Chitosan Tripolyphosphate (TPP) finds application in the pharmaceutical field where it encapsulates active molecules release; wound dressings etc. (Jiang, Wu, Xu, Wang, & Zeng, 2011). It is useful in the chemical industry for adsorption of metals e.g Fe^{3+}, Mn^{2+}, Cu^{2+}, and Pb^{2+} ions (via metal chelation). In the environmental industry they are useful for treatment of water because they aid removal of dyes.

1.6 Methods for Preparing Porous Chitosan

Diverse methods have been used in preparing porous scaffolds and they include porogen leaching methods, phase separating methods, three dimensional printing and

saturation and liberation of CO_2. The most common technique is the phase separation method which is based upon reducing solution temperature to initiate phase separation from solution. This method includes liquid-liquid demixing and solid-liquid demixing. The former gives rise to liquid phase which is either polymer poor or polymer rich. The growth of polymer poor phase leads to scaffold pores. The latter is achieved with very low temperature to the point it freezes the biodegradable polymer solution. As soon as the frozen part is eliminated, pores become visible in the remaining space (Ho et al., 2003). The porous structure is obtained during phase separation. As soon as the solvent which is frozen is eliminated, the open left over space initially filled by the solvent will emerge as pores in the already produced scaffolds. It is necessary to preserve the porous material during the solvent elimination stage; hence the need for freeze drying comes into play which is capable of removing solvent and preserving the porous nature. Loss of the porous material occurs when freeze drying is not applied. This leads to increase in temperature in the drying stage and can bring about remixing of the separated solution (phase separated) or disintegration of the frozen solution. Freeze drying is energy and time consuming even though it stops the breakdown of porous material and hence produces a procedure that is not effective and limited economically (chung & park, 2007).

Porogen leaching methods is also widely used to assemble scaffolds for tissue engineering and salt or polymer can be used as porogen. This method involves porogen powdering and screening of the particle to obtain a size that is desirable followed by casting a salt, polymer or organic solvent mixture into the mold. Afterwards water is used to leach away the porogen particles forming a porous material. Salt or polymer leaching method has to do with size regulation and control by controlling the amount of porgen added. (Azadeh & Murugan, 2012). Porous

chitosan gel beads were prepared using several different methods described by different authors and they are as follows;

Porous chitosan gel beads are obtained when inorganic silica is used as porogen. (Santos et al., 2008). This method basically deals with dissolving suitable porogen silica in this case in a polymeric matrix, evaporation allowed to take place and subsequent extraction of silica by completely immersing on any appropriate solvent for example aqueous NaOH. Chitosan solution was obtained when chitosan was dissolved in 2.0% CH_3COOH (aq) solution for about 24h with constant mixing. Solution obtained was filtered and allowed to cool for 2h to remove bubbles. Afterwards, small amount of solution was poured on a petri dish and dried for 3h in the oven. Silica dispersion was introduced into the petri dish and chitosan/silica membrane was obtained. To get a macroporous membrane, chitosan/silica membranes were treated with sodium hydroxide solution for about 4h at 60℃ and this resulted in dissolution of the silica particles. Washing and rinsing with distilled water at room temperature eliminated the excess alkali completely.

According to another method (Zeng & Fang, 2004) Porous chitosan gel beads were prepared using a polymer as a porogen and in this case polyethylene glycol was used. Into 2% acetic acid solution, chitosan and polymer porogen PEG were dissolved with stirring to obtain a solution. Solution obtained was poured on a casting apparatus and dried (50℃). The semi interpenetrating network membranes obtained after drying was neutralized with NaOH(aq) for about 30 min. Subsequent washing of membrane with water to eliminate NaOH remnant was allowed. The membrane was finally stored in water for about 10 h to remove PEG component and produce a structure that is highly porous. It was observed that the application of heat to the membrane did not

just eliminate PEG but it brought out the better mechanical quality of the membrane (Datta, 2007).

Using poly ε caprolactone as porogen in preparation of porous chitosan gel beads, a method described by Cruz (Cruz et al., 2009). This study dealt with the use of PCL (poly ε caprolactone) as porogen in preparation of chitosan beads and the work presented a procedure which is made up of blending PCL (melt) with chitosan (swollen) in dilute acetic acid solution. Chitosan is swelled in acetic acid solution (3%v/v) and blended with PCL at about 80℃. It was observed that swelling chitosan with the acidic solution before melt blending enhanced processability and stable structural samples were obtained as a result of using PCL which was the main constituent in the blends.

Porous chitosan tripolyphosphate beads are gotten when sodium tripolyphosphate and chitosan react and freeze drying is applied. Chitosan is first dissolved in acetic acid and then precipitation occurs when the solution is dropped into a tripolyphosphate solution (Wu et al., 2013). As soon as the chitosan solution came in contact with tripolyphosphate solution, durable gel beads were obtained right away. These beads were stored for 12 h before filtering and completely washing with water and appropriate solvent to rid acetic acid. Beads were freeze dried to bring forth porous chitosan tripolyphosphate beads after heating it up for 1h in an oven at about 35℃. These porous beads allow the diffusion of molecules and this finds application in adsorption of iron (iii), Cu (ii) or removal of certain dyes and ions from water. The degree of porosity is always determined using a porosimetry method and surface area of the chitosan available for adsorption is determined as well. It has been studied that non porous beads adsorb molecules at the surface especially but porous beads

containing pores this time around are able to absorb molecules to a large extent and are hence better preferred because their applications are varied when compared to non porous beads.

Chitosan with $\alpha\,\beta$-glycerol phosphate at a given temperature can form porous 3 dimensional gels (Dang et al., 2012). Dissolution of chitosan in 0.1M aqueous acid solution for 8 h at room temperature gives a clear solution. To know the extent of the strength of the solvent on chitosan $\alpha\,\beta$-glycerol phosphate hydrogels, this chitosan was dissolved in 0.8M lactic acid solution. It was allowed to cool for 30 min at 4℃. Preparation of $\alpha\,\beta$-glycerol phosphate solution 50 %(w/v) with distilled water is carried out and cooled same way as the chitosan solutions at4℃. Afterwards there is addition of $\alpha\,\beta$-glycerol phosphate solution into the chitosan solution and stirring is allowed until both solutions are homogenous for about 30 min. The resulting chitosan $\alpha\,\beta$-glycerol phosphate hydrogel solution was appropriately stored at 4℃.

Using NaCl salt as porogen alongside genipin as crosslinker resulted in the formation of porous chitosan beads (Chao et al., 2006). Preparation was achieved when 4 g of chitosan was dissolved in acetic acid, water (5% v/v) solution to get a 4% (w/w) solution. Into this solution, 25g NaCl particles and genipin aqueous solution (5mg/mL) was added and stirred. The solution was allowed to stand for 10 h after pouring onto a petridish. Evaporation of liquid at 50℃ was allowed, into a 10mL, 1M aqueous solution; dried membrane was immersed for complete 2 h to help neutralize the acid. Afterwards, into deionized water this same membrane was immersed to dissolve particles of NaCl and eliminate NaOH remnant. Chitosan porous membrane free from shrinkage due to vacuum drying was obtained.

1.7 Chitosan Tripolyphosphate as a Potential Fe^{3+} Ion Chelator

Porous and nonporous chitosan derivatives as chelators have been widely studied and these derivatives include chitosan and N-carboxymethyl chitosan (NCMC), Chitosan benzoyl thiourea derivative, chitosan containing phosphorus and sulfurs, chitosan crown ethers and chitosan ethylenediaminetetraacetic acid (EDTA)/ diethyl-triaminepentaacetic acid (DTPA) complexes, Glutaraldehyde cross-linked chitosan beads, Molybdate-chitosan gel beads.(Bhatnagar & Sillanpaa, 2009, Varma, Deshpande, & Kennedy, 2003).

Heavy metal ions found in waste water when not effectively handled can lead to exposure of harmful materials to both man and the environment at large. These materials are considered toxic to the biosystem and the are released out of industries and examples include mercury, lead, copper, nickel, zinc etc. Ongoing research as regards removal of these toxic wastes have intensified and chitosan has been particularly taken notice of and explored extensively (Ngaha, Teonga, & Hanafiaha, 2010).

1.8 Adsorption kinetics

Adsorption kinetic model is used in determining kinetics of adsorption and rate limiting steps. The first model is the pseudo 1^{st} order which tells us that uptake of solute with reference to time is directly proportional to the solid amount and saturation concentration difference (Oladipo, Gazi, & Samandari, 2014, Wu et al., 2012). It is expressed as;

$$\log qe - qt = \log qe - \frac{k_1 t}{2.302}$$

Where qe and qt represent amount of the solute adsorbed at equilibrium. (mg/g), t = time and K_1 is a constant (min^{-1}).

The pseudo 2nd order equation is shown below;

$$\frac{t}{qe} = \frac{1}{k_2 qe^2} + \frac{t}{qe}$$

intraparticle diffusion throws light on the thickness of the boundary layer and expressed thus;

$$qt = kpt^{1/2} + 1$$

Where $kp =$ intraparticle rate constant

1.9 Adsorption Isotherms

Adsorption is the binding of ions or molecules from a gas or liquid state to a surface. It gets to a point whereby equilibrium is reached between the adsorbate and adsorbent in solution and this is known as adsorption isotherm.

The interrelationship existing between adsorption capacity and concentration of ion or molecule can be interpreted by 2 models: the Langmuir isotherm and the Freundlich isotherm. The former describes a type of adsorption which is monolayer, and also dispersed uniformly at adsorption sites (Das, Sureshkumar, Radhakrishnan, Nuwar, & Pillai, 2011, Lee et al., 2000). It is expressed as;

$$\text{Langmuir model } = \frac{Ce}{qe} = \frac{1}{Q * K} + \frac{Ce}{Q}$$

Where Ce is the equilibrium metal ion concentration

qe = amount of adsorbed ions

Q = maximum metal ion adsorption capacity

K = Langmuir constant

Q and K can be obtained from the graph of Ce/qe against Ce

The latter assumes a multilayer adsorption for surfaces and is shown below;

Freundlich model;

$$\log qe = \log K_F + \frac{1}{n} * \log Ce$$

Where qe = equilibrium amount of adsorbed metal ion

Ce = equilibrium concentration of metal ions

K_F = maximum metal ion adsorption capacity

n = intensity of adsorption. K_F and n are both Freundlich constants and they can be obtained from graph of $log\ q_e$ against $log\ C_e$ which is expected to be linear. (Das et al., 2011).

The Freundlich isotherm defines adsorption on uneven surfaces. It is useful for both medium and high concentrations, but not fit for samples with low concentration. (Wu et al., 2013).

R_L is used to predict a favorable or unfavorable adsorption. R_L is represented as;

$$R_L = \frac{1}{1 + bC_O}$$

Where C_O is initial absorbance

If $R_L > 1$ it is unfavorable

If $R_L < 1$ it is favorable

If $R_L = 1$ it is linear

If $R_L = 0$ it is irreversible

The aim of this thesis is to prepare chitosan tripolyphosphate gel beads at different pH values in the presence and absence of a porogen, namely PEG. The effect of preparation conditions on the swelling and Iron (III) ion adsorption behavior is to be elucidated.

Chapter 2

EXPERIMENTAL

2.1 Materials

Table 1: List of chemicals and manufacturers

No	Chemicals	Manufacturers
1	Chitosan (medium molecular weight)	Aldrich-Germany
2	Acetic Acid	Riedel-deHäen-Germany
3	Sodium tripolyphosphate pentabasic	Sigma-Aldrich-Germany
4	5-sulfosalicylic acid dihydrate	Sigma-Aldrich-Germany
5	Potassium chloride	Sigma-Aldrich-Germany
6	Hydrochloric acid	Sigma-Aldrich-Germany
7	Sodium hydroxide	Sigma-Aldrich-Germany
8	Iron (III) chloride	Sigma-Aldrich-Germany
9	Polyethylene glycol	Sigma-Aldrich-Germany

2.2 Methods

2.2.1 Preparation of Chitosan Gel Beads

2.2.1.1 Preparation of Chitosan Tripolyphosphate Beads

A chitosan solution of concentration 2 %(w/v) was prepared in aqueous 1 %(v/v) acetic acid. A viscous solution was prepared and was added dropwise into 5 %(w/v) pentasodium tripolyphosphate coagulation bath and instantly durable gel beads were obtained. Sodium tripolyphosphate was prepared at the original pH of 8.6 and also at pH 3.0 by adding 1M hydrochloric acid. At both pH values chitosan solution was dropped and gel beads were obtained. The beads were left in the TPP solution for 2 h, removed and washed extensively with distilled water. They were placed in a glass plate and dried in the oven overnight at 50℃.

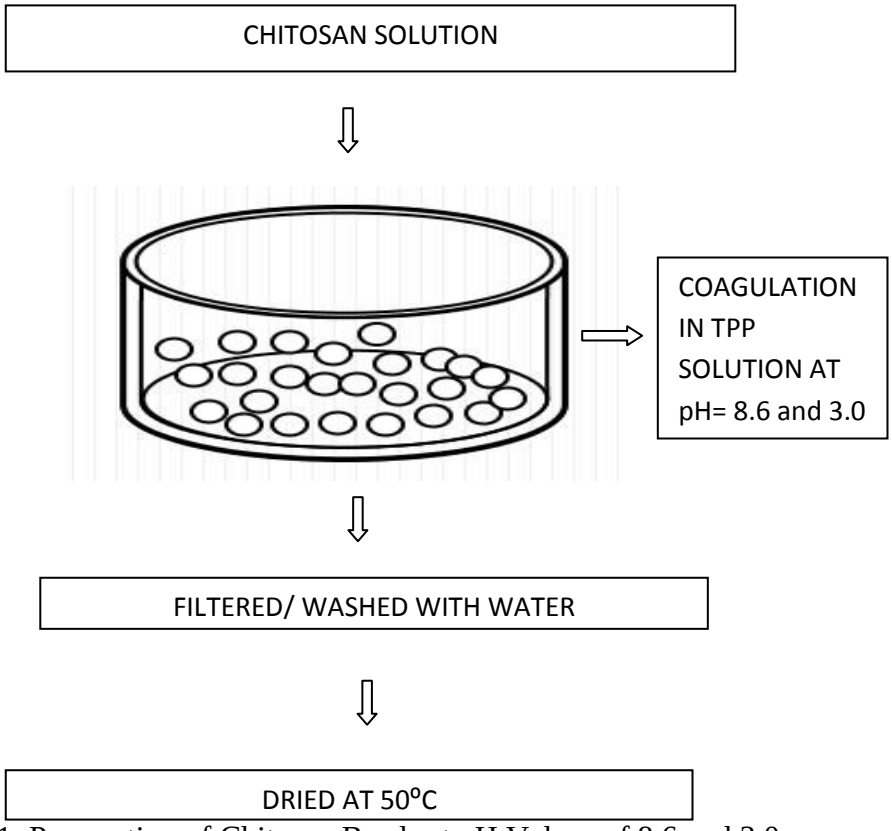


Figure 1: Preparation of Chitosan Beads at pH Values of 8.6 and 3.0.

2.1.1.2 Preparation of Porous Chitosan Tripolyphosphate Beads

To achieve porosity, 5g of PEG was added into 2 %(w/v) chitosan solution and stirred with a magnetic stirrer for about 3 hr. It was then dropped into the tripolyphosphate solution at the pH values of 8.6 and 3.0. The original pH of TPP is 8.6 but it was adjusted 3.0 with 1M hydrochloric acid. The beads were allowed to stand for 2 hr and afterwards washed extensively with distilled water. These beads were then dried overnight at 50℃.

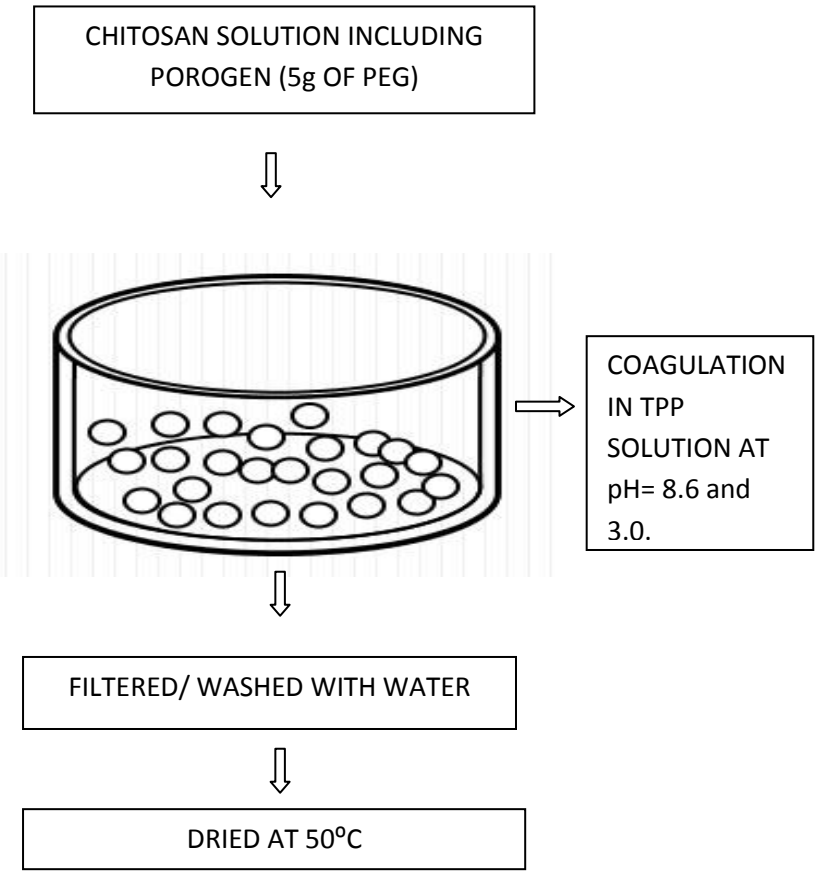


Figure 2: Preparation of Chitosan Beads with PEG at pH Values of 8.6 and 3.0

2.2.2 FTIR Analysis

FTIR spectra of prepared beads were determined with the aid of a Perkin Elmer Spectrum-65 FTIR machine.

2.2.3 Swelling Behavior of Beads

The swelling of beads were studied in aqueous buffers of pH values 1.2, 7.0 and 11.0 respectively. A given amount of the beads were placed in solution for a predetermined period of time, removed from solution, blotted with filter paper and weighed.

Table 2: Buffer Preparation

pH	constituents	Volume
1	125mL of 0.2M KCl and 335mL of 0.2M HCl	500ml
1.2	125mL of 0.2M KCl and 212.5mL of 0.2M HCl	500mL
7	122mL of 0.1M HCl and 378.0mL of 0.1M sodium hydrogen phosphate	500mL
11	1.05g sodium bicarbonate in 113.5mL of 0.10M NaOH	500mL

The swelling % was calculated as follows:

$$swelling\,(\%) = \frac{m_{swollen} - m_{dried}}{m_{dried}} * 100$$

2.2.4 Fe^{3+} Adsorption onto the Beads

25 mg of bead sample was added in a 25mL Fe^{3+} solution at pH of 1.2. The Fe^{3+} solution concentrations used for adsorption are; 0.25 mM, 0.5 mM, 1.0 mM, 2.5mM and 5.0 mM. 1mL of solution was taken at hourly intervals and by means of visible spectroscopy Fe^{3+} concentration was analyzed. The calculated difference between the

initial and final absorbance of the beads was taken as the amount of Fe^{3+} adsorbed from the solution.

2.2.5 Determination of Fe^{3+} in Solution

1.0mL Fe^{3+} solution, 1.0mL of sulfosalicylic acid dehydrate, (10% w/v) and 8 mL of buffer solution pH 1 was mixed together in a 10mL volumetric flask. Amount of Fe^{3+} was determined by an Ultra Voilet-1201 Visible spectrophotometer at 505nm. The initial and final absorbance values was used to determine the amount of Fe^{3+} adsorbed by the beads and calculated as mg Fe^{3+} adsorbed/gram of beads.

The adsorption capacity of prepared beads (Fe^{3+}mg/g of beads) was calculated as follows;

$$\frac{xmMolFe^{3+}}{Lsolution} * 0.025Lsolution * \frac{55.85gFe^{3+}}{1molFe^{3+}} * \frac{1000mgFe^{3+}}{1gFe^{3+}} * \frac{1}{0.025gchitosan}$$

The calibration curve was obtained at 10 different concentrations 10 mM, 7.5 mM, 5 mM, 3 mM, 2.5 mM, 1.5 mM, 1 mM, 0.8 mM, 0.4 mM, and finally 0.2 mM. Absorbance was plotted against concentration. Slope and intercept obtained from the graph was used to calculate initial and final concentration of absorbance.

Chapter 3

RESULTS AND DISCUSSION

Chitosan-TPP and chitosan-TPP PEG beads were prepared and characterized by FTIR spectroscopy and SEM microscopy. The swelling capacity of the beads was studied in buffers of pH 1.2, pH 7.0 and pH 11.0. The products were tested for their Fe^{3+} ion removal capacity.

3.1 Preparation of Chitosan-TPP and Chitosan-TPP PEG beads

The bead average diameter was found to be around 1mm measured with a standard ruler and illustrated as shown below in Figure 1. Chitosan-TPP and Chitosan-TPP PEG beads formation process has been illustrated in Figure (2), (3) and (4).

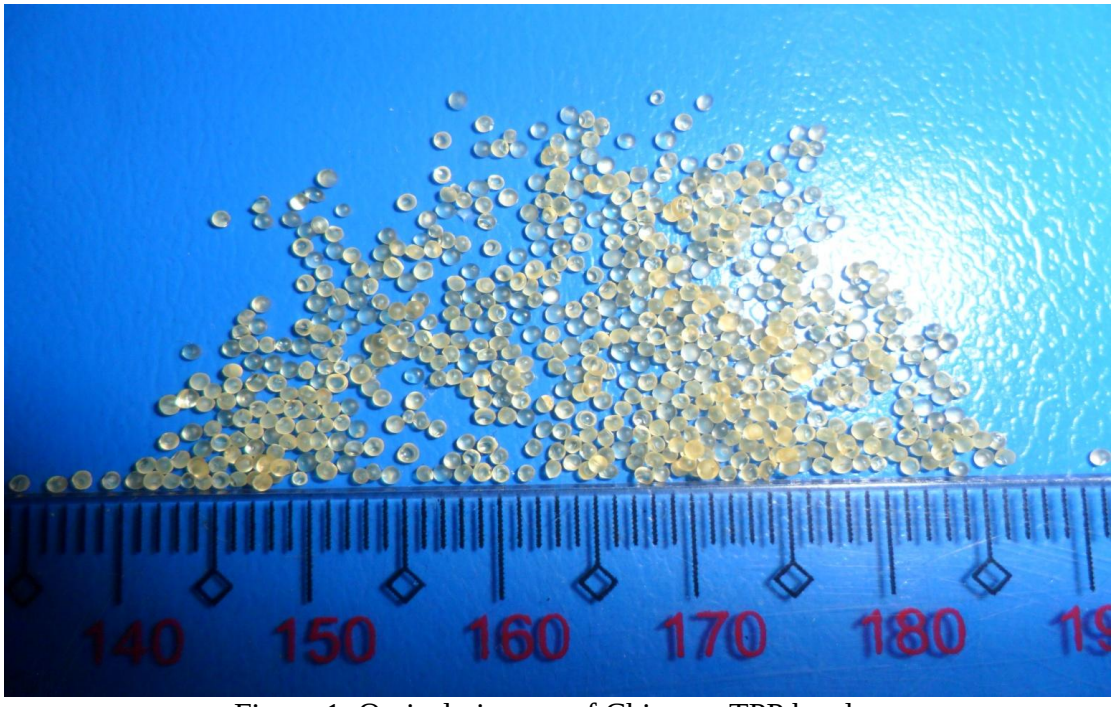


Figure 1: Optical pictures of Chitosan-TPP beads

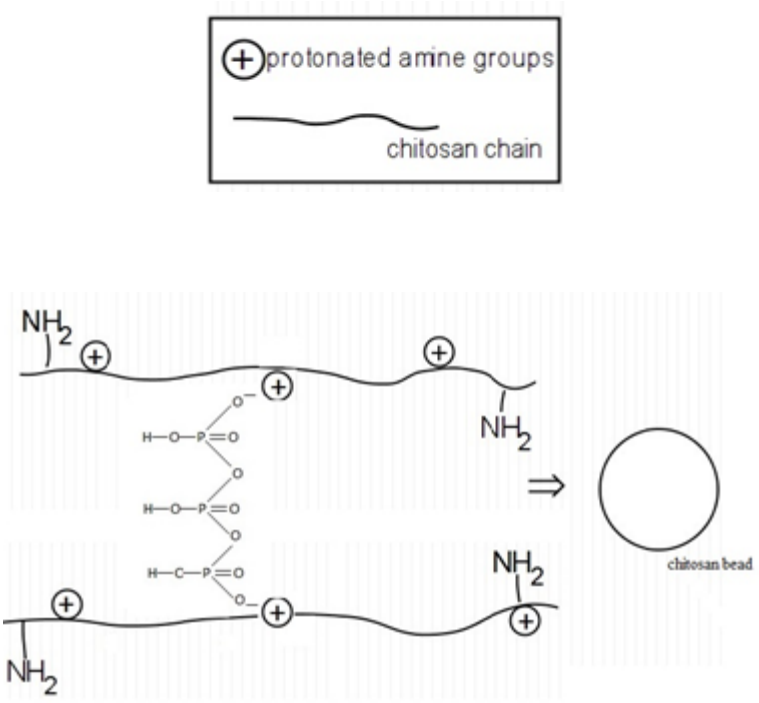


Figure 2: Chitosan-TPP Gel Bead Formation in the Absence of Porogen.

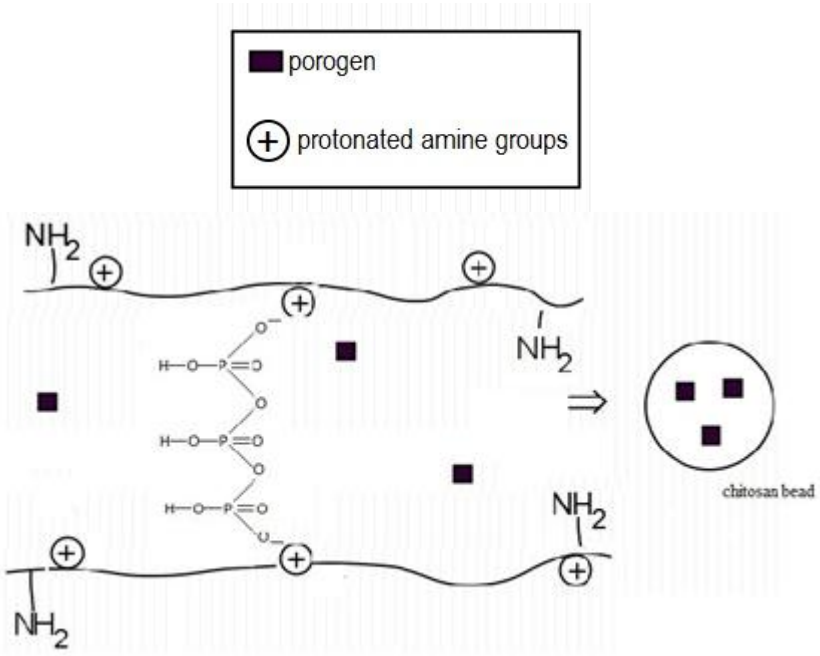


Figure 3: Chitosan-TPP Gel Bead Formation in the Presence of Porogen

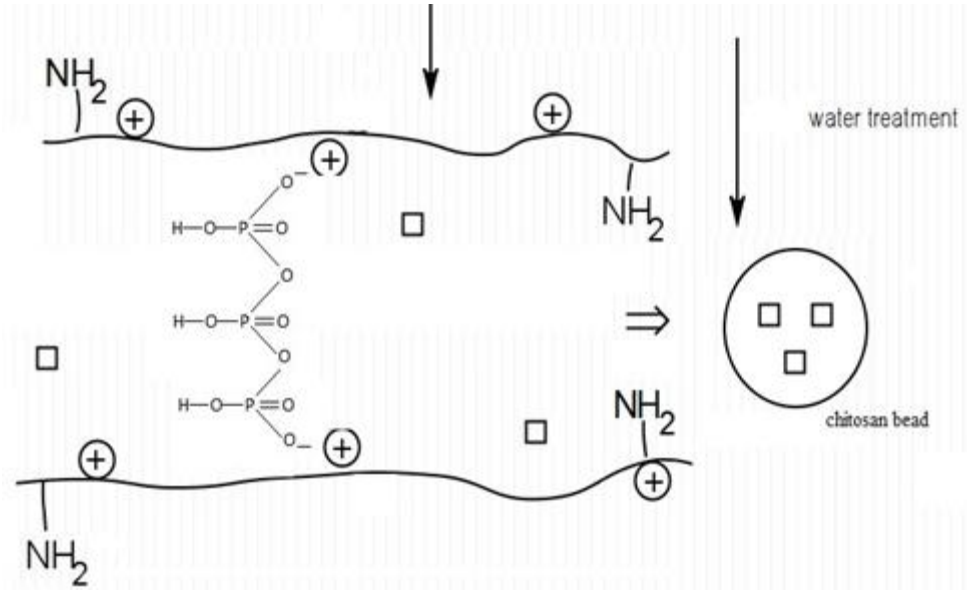


Figure 4: Chitosan-TPP Gel Bead after Removal of the Porogen

3.2 FT-IR Analysis

FT-IR spectroscopy of TPP (powder), chitosan (powder) and crosslinked chitosan-TPP at pH 3.0 and 8.6 were analyzed. The spectrum of Chitosan, TPP, Chi-TPP prepared at pH 3.0, Chi-TPP prepared at pH 8.6 and Chi-TPP after PEG removal is shown in figure 5 (a), (b), (c), (d) and (e) respectively.

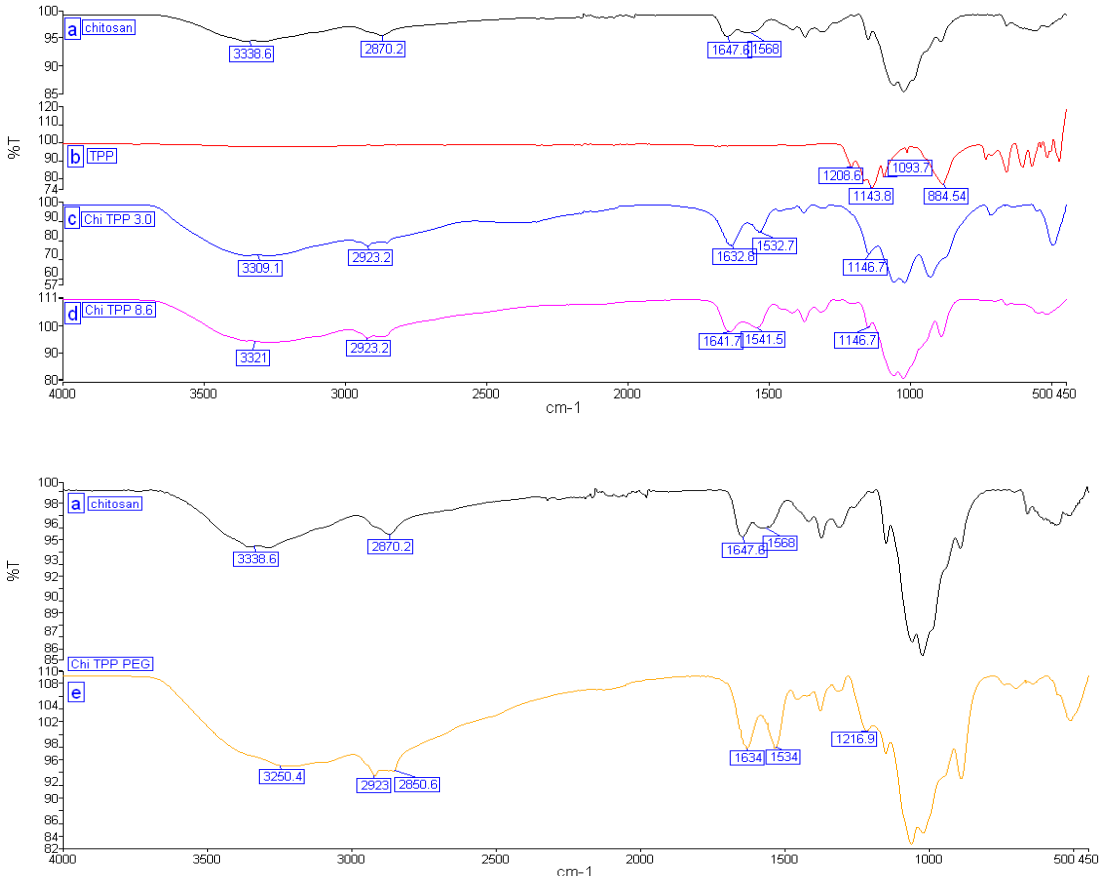


Figure 5: FTIR Spectrum of (a) chitosan (b) TPP (c) chi TPP 3.0 (d) chi TPP 8.6 (e) after PEG removal.

In chitosan sample FTIR spectrum showed peaks at $3338cm^{-1}$ indicating the presence of OH and NH stretching. Peak at $2876cm^{-1}$ is indicative of C-H group. Secondary amide group is shown at peak $1651cm^{-1}$ showing the stretching of CO (amide I). The band at $1567cm^{-1}$ is assigned to NH group (amide II). Peaks at $1034cm^{-1}$ indicate ether groups.

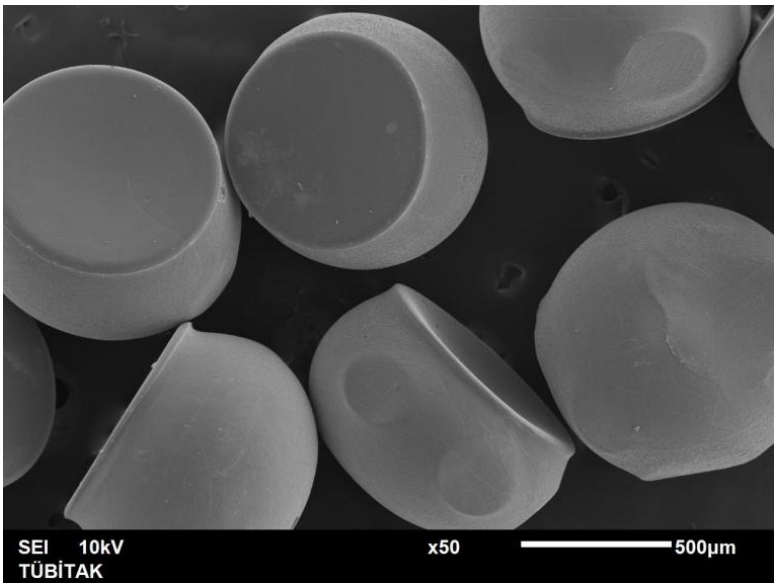
Peaks are seen at $1211cm-^{1}$ indicating the presence of P=O groups. Peak at $885cm^{-1}$ shows P-O-P stretching. Peak at $1148cm^{-1}$ and $1093cm^{-1}$ shows PO_2 and PO_3 groups respectively.

Chi-TPP 3.0 show peaks at $3308 cm^{-1}$ for OH and NH_2 stretching and C-H group is seen at $2924 cm^{-1}$. At peak $1636 cm^{-1}$ C=O group is observed. Peaks at $1536 cm^{-1}$ corresponds to NH group and finally the peak seen at $1149 cm^{-1}$ show PO_2 groups confirming crosslinking reaction. Chi-TPP 8.6 showed peak of OH and NH_2 overlap stretching at $3296\, cm^{-1}$ and C-H group at $2920\, cm^{-1}$. The amide carbonyl group is observed at $1644\, cm^{-1}$, NH group is seen at $1540\, cm^{-1}$ and PO_2 groups observed at $1149\, cm^{-1}$. Hence crosslinking is achieved via complexation of amine groups of chitosan and phosphate groups of tripolyphosphate. Chi-PEG after removal shows that PEG was incorporated into the beads and there are some remains in it indicated by the broadening of OH group at $3250\, cm^{-1}$ and two new peaks of C-H group at $2923\, cm^{-1}$ and $2850\, cm^{-1}$. The intensity of the peaks at $1634\, cm^{-1}$ and $1534\, cm^{-1}$ for amide I and amide II decreased indicating that some interaction with remains of PEG.

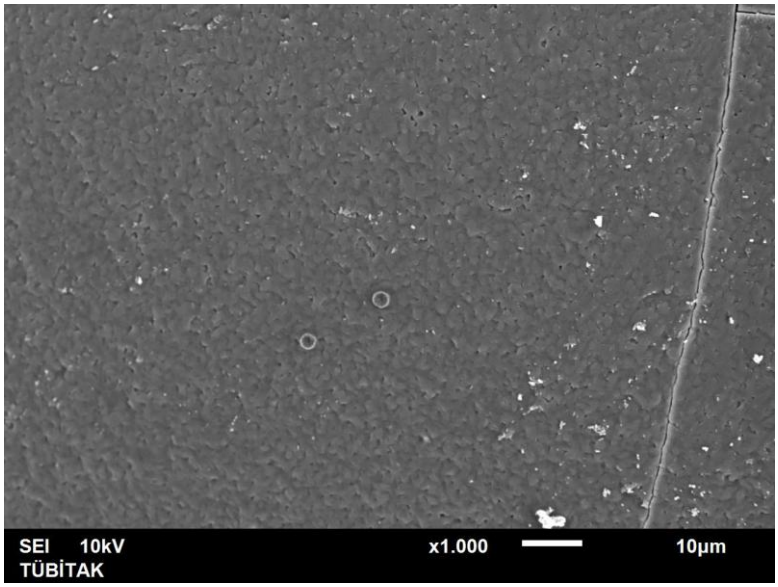
3.3 SEM Analysis

The morphology of the beads (Chi TPP pH 3.0 and Chi TPP PEG pH 3.0) was studied. SEM analysis shows that Chi-TPP has microporous surface and the pores have diameter of the order of 0.5- 1μm. Chi-TPP with PEG has a microporus surface similar to that of Chi-TPP. The presence of PEG results in less spherical shape and beads with tear drop are obtained as a result of high viscosity of the polymer also observed by Yilmaz & Bengisu (2003). There was also observed irregularities on the surface of the bead and this is due to cracking of the bead surface when subjected to drying. When bead prepared in the absence and in the presence of PEG are compared, not a clear difference in terms of porosity could be observed. Both samples bear porosity. The chains after PEG treatment are more closely packed. This fact can be taken as additional evidence for some PEG remaining in the structure.

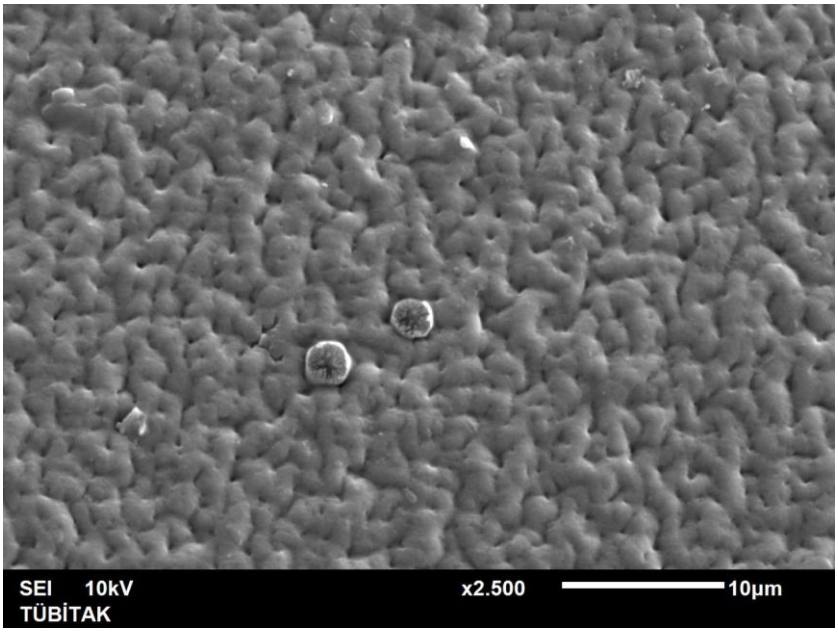
Figure 6: SEM micrograph of (a) Chi-TPP pH 3.0 ×50 (b) Chi-TPP pH 3.0 ×1000 (c) Chi-TPP pH 3.0 ×2500. (d) Chi-TPP PEG pH 3.0 ×50 (e) Chi-TPP PEG pH 3.0 ×1000 (f) Chi-TPP PEG pH 3.0 ×2500



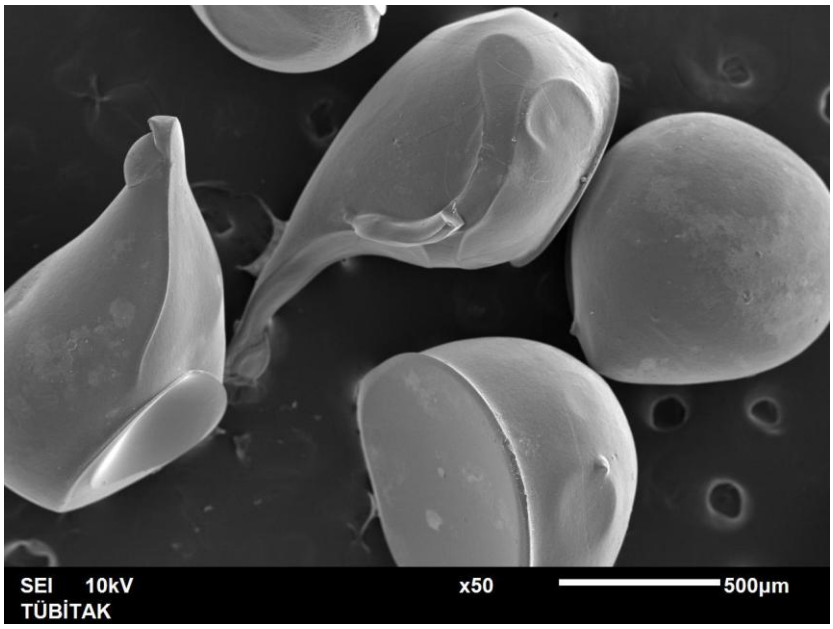
(a) SEM picture of chi-TPP at ×50 magnification



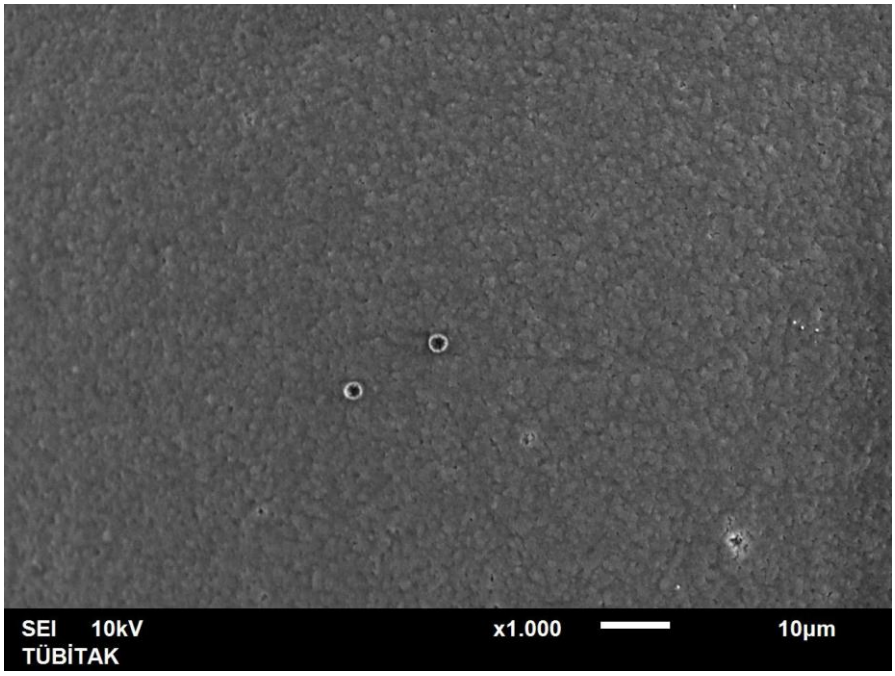
(b) SEM picture of chi-TPP at ×1000 magnification



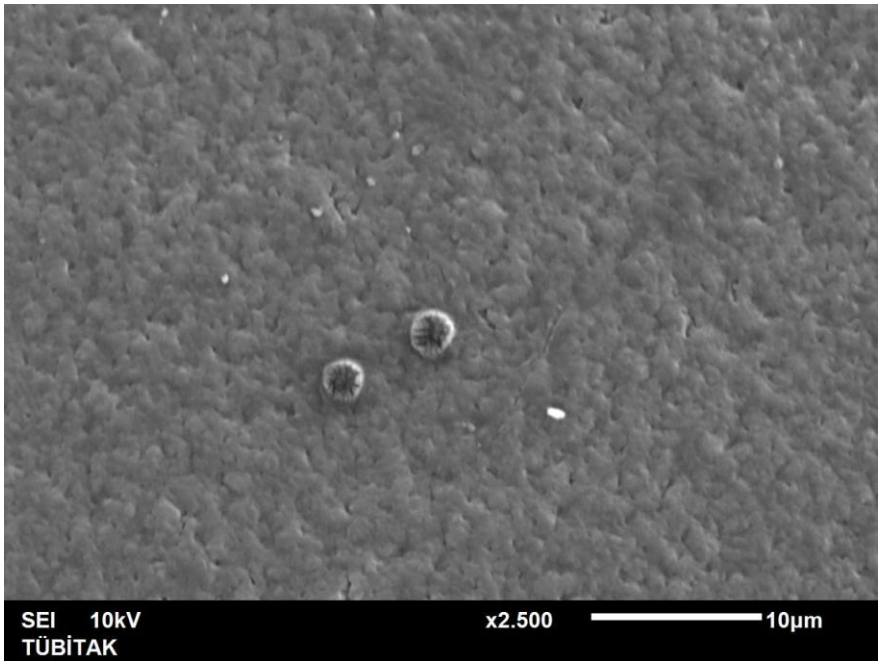
(c) SEM picture of chi-TPP at ×2500 magnification



(d) SEM picture of chi-TPP PEG at ×50 magnification



(e) SEM picture of chi-TPP PEG at ×1000 magnification



(f) SEM picture of chi-TPP PEG at ×2500 magnification

3.4 Swelling

To observe the swelling behavior of prepared chitosan-TPP and chitosan TPP PEG beads when exposed to different pH conditions, the beads were immersed in aqueous

medium of pH 1.2, pH 7.0 and pH 11.0 at 25℃. Depending on the degree of ionization of NH_2 and phosphate groups in the structure, the chitosan chain swells.

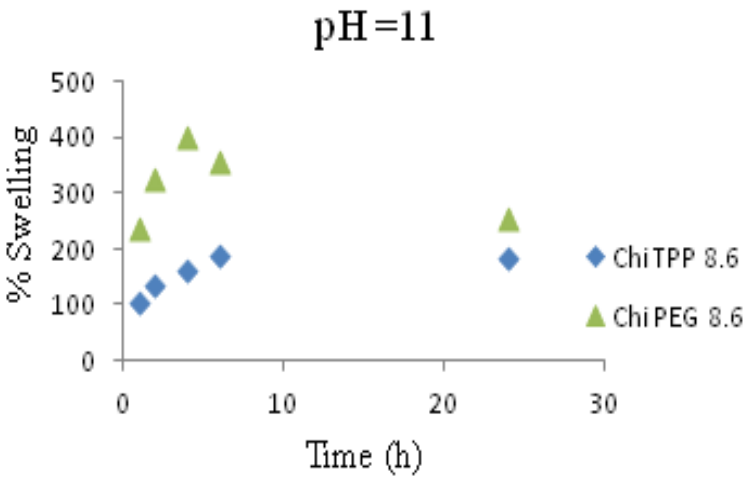


Figure 7: Swelling at pH 11 of Beads Prepared at pH 8.6

At pH 11 for Chi-TPP beads and Chi-TPP PEG beads prepared at pH 8.6, beads swelled and the highest swelling ratio of 410 % was obtained by chi PEG. This is because PEG is hydrophilic in nature hence its increased swelling ratio as evidenced in the FTIR spectrum where remains of PEG was left after washing. Porous beads showed better swelling when compared with Chi-TPP beads. Crystallinity may have been reduced during introduction and removal of PEG which could be another factor causing increased swelling in Chi-TPP PEG bead also observed by Yalinca, Yilmaz & Bullici. (2012). When the pH of TPP was adjusted from 8.6 to 3, swelling of beads prepared at acidic pH changed. The swelling ratio when compared with beads prepared at pH 8.6 was smaller and this is because of high crosslinking density that dominates at acidic pH. Therefore increase in crosslinking caused a decrease in swelling.

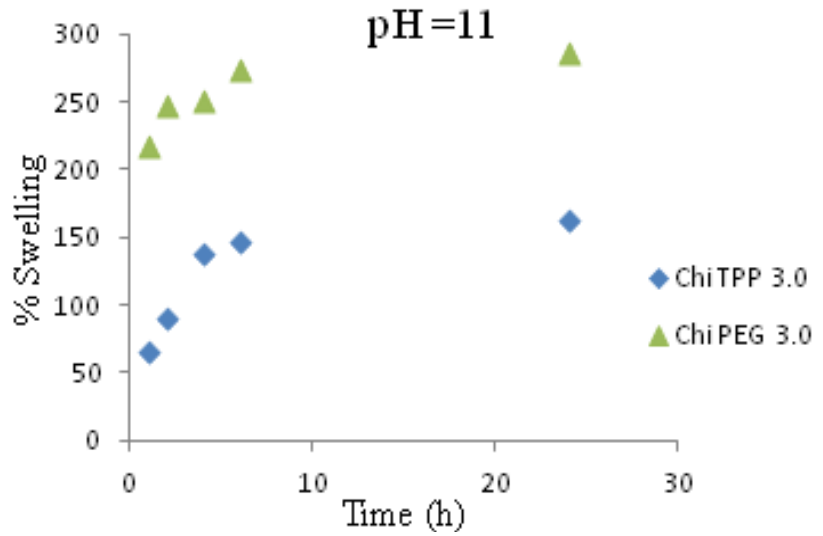


Figure 8: Swelling at pH 11 of Beads Prepared at pH 3.0

Neutral pH 7 with acid and alkaline conditions had the least swelling capacity because swelling is controlled by water molecules diffusing into gel beads rather than repulsive forces between ionic species and also as a result of hydrogen bonding. Beads prepared at pH 8.6 had the highest swelling ratio~392 % because it has lesser crosslinking than those prepared at pH 3.

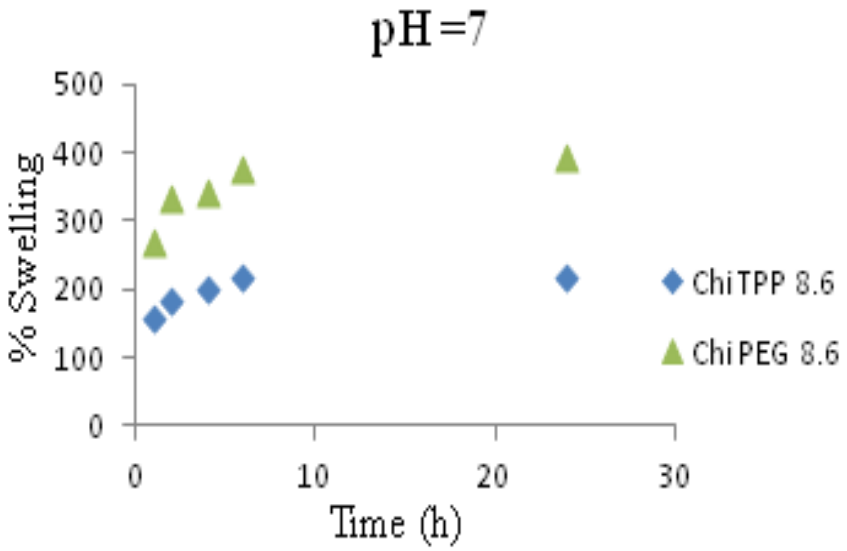


Figure 9: Swelling at pH 7 of Beads Prepared at pH 8.6

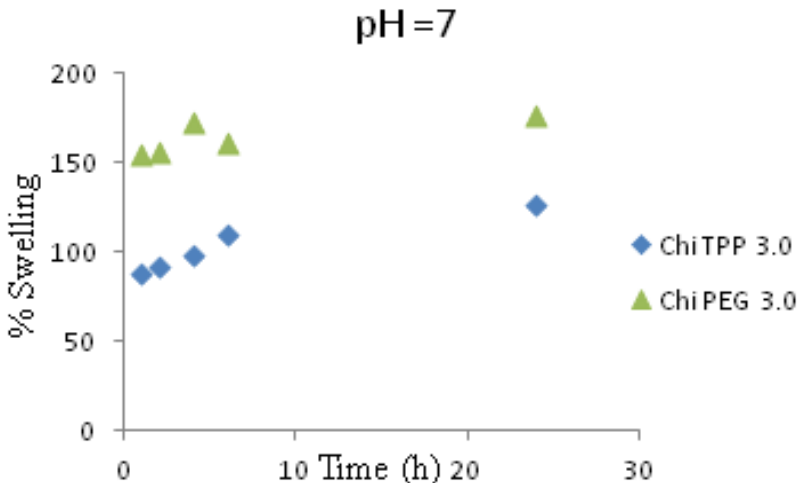


Figure 10: Swelling at pH 7 of Beads Prepared at pH 3.0

At pH 1.2 due to strong inter and intra electrostatic repulsion, swelling behavior of examined beads was not affected by pH of TPP (preparation pH). So there was considerable high degree of swelling ratio at both basic and acidic pH of TPP. Beads prepared using PEG as porogen showed a swelling ratio of~4455 %. Chi-TPP beads had a lower swelling ratio when compared with Chi-TPP PEG.

The prepared Chi-TPP and Chi-TPP PEG beads show polyampholyte characteristics since they are capable of swelling in both acidic and alkaline solutions. Repulsive interactions dominate at acidic and alkaline media. Swelling ratio decreased in time because it got to a point where the beads dissolved and this is because chitosan is soluble in acid.

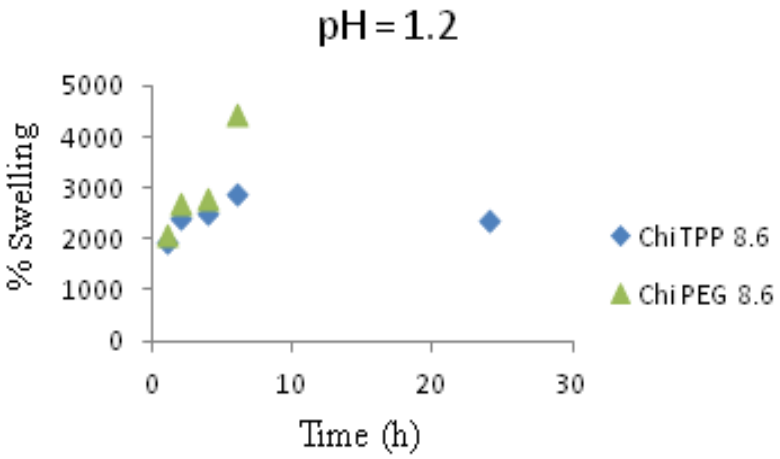


Figure 11: Swelling at pH 1.2 of Beads Prepared at pH 8.6

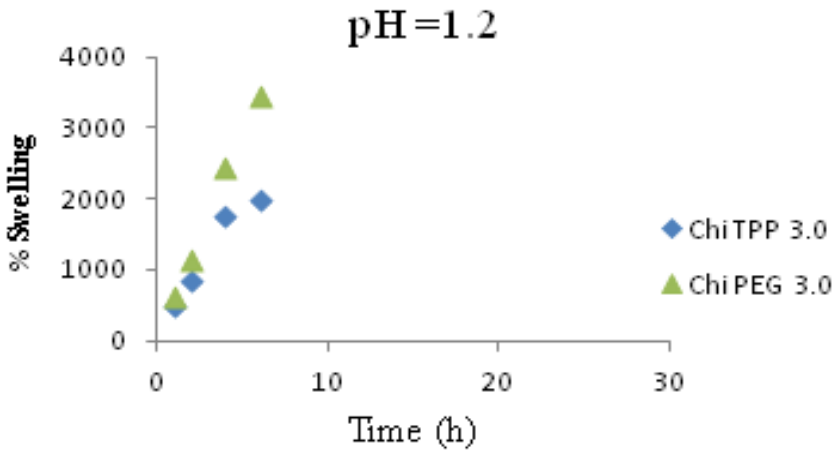


Figure 12: Swelling at pH 1.2 of Beads Prepared at pH 3.

3.5 Fe^{3+} Adsorption onto the Beads

Fe^{3+} adsorption unto the beads was studied in $FeCl_3$ solution at room temperature. First a calibration curve was prepared as illustrated in Figure 13.

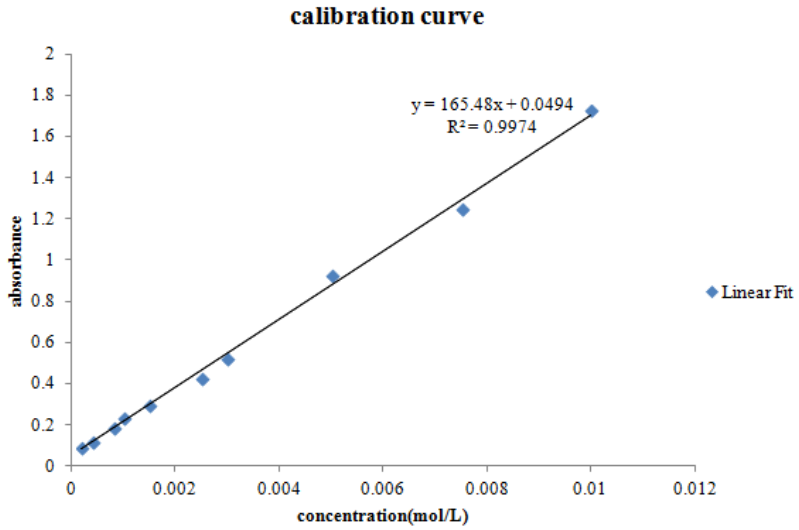


Figure 13: Calibration curve

The adsorption kinetics in 5mM Fe^{3+} solution is shown in figure 14.

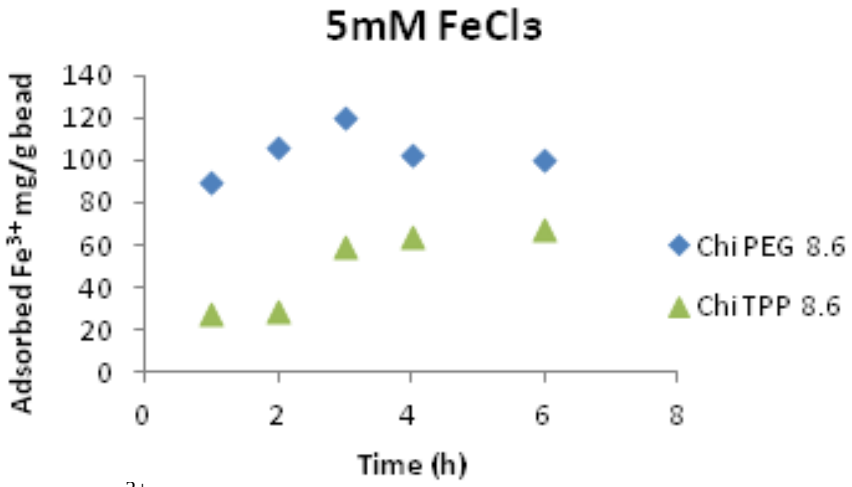


Figure 14: Fe^{3+} adsorption mg/g of bead in 5mM $FeCl_3$ with time at pH 8.6

At 5.0mM concentration of Fe^{3+} chloride ions, Chi-PEG showed the highest adsorption capacity showing good chelation with the metal Fe^{3+} ions. This can be attributed to the presence of PEG left in the beads which interact with Fe^{3+} ions, together with the porosity introduced after leaching the polymer.

The adsorption behavior in 2.5mM Fe^{3+} solution is shown below in figure 15.

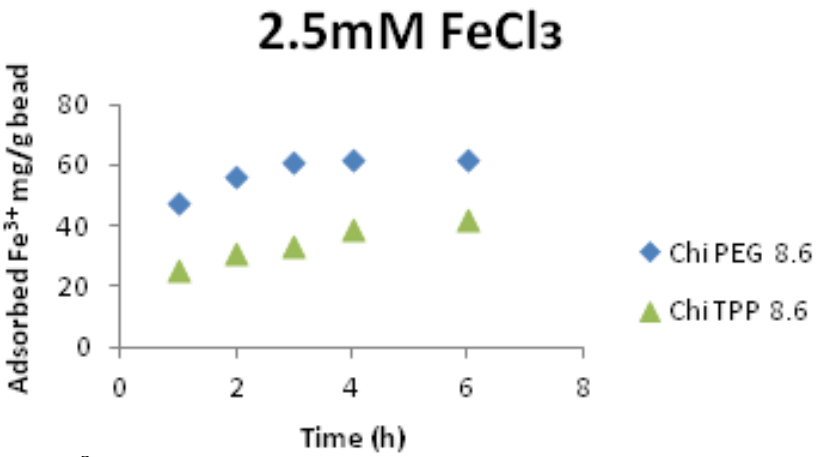


Figure 15: Fe^{3+} adsorption mg/g of bead in 2.5mM $FeCl_3$ with time at pH 8.6

At 2.5mM Chi- TPP PEG also showed highest adsorption capacity for Fe^{3+} ions. The adsorption capacity per gram of beads reduced as a result of reduction of the concentration of the $FeCl_3$ solution.

Illustration of the adsorption behavior in 1mM Fe^{3+} solution.

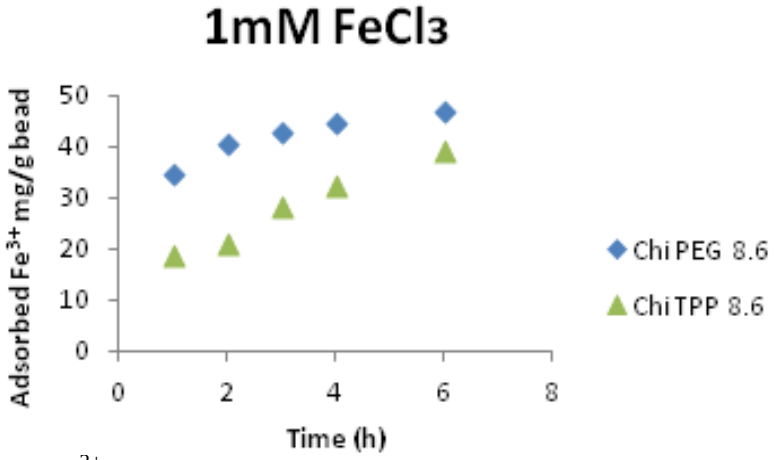


Figure 16: Fe^{3+} adsorption mg/g of bead in 1mM $FeCl_3$ with time at pH 8.6

The same order was observed at subsequent lower concentrations of Fe^{3+}chloride and it was observed that adsorption per gram of beads decreased as concentration

decreased, hence less Fe^{3+} ions available for the bead to absorb as shown in figure 16, 17 and 18.

Adsorption behavior in 0.5mM Fe^{3+} solution shown in figure 17

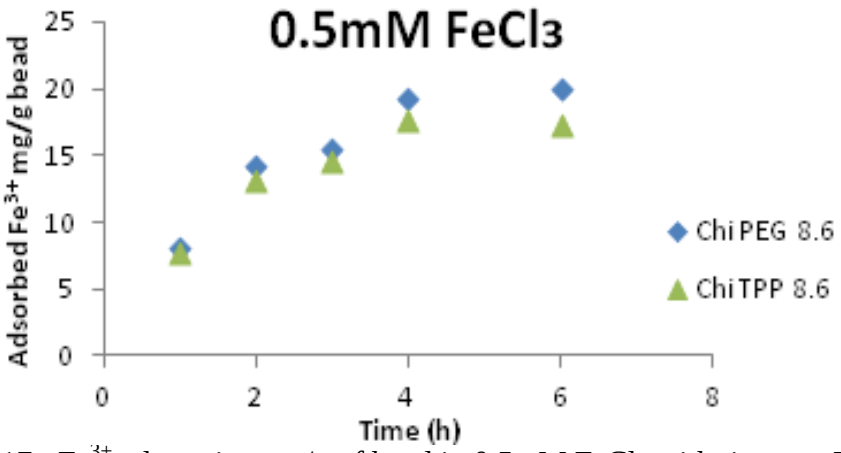


Figure 17: Fe^{3+} adsorption mg/g of bead in 0.5mM $FeCl_3$ with time at pH 8.6

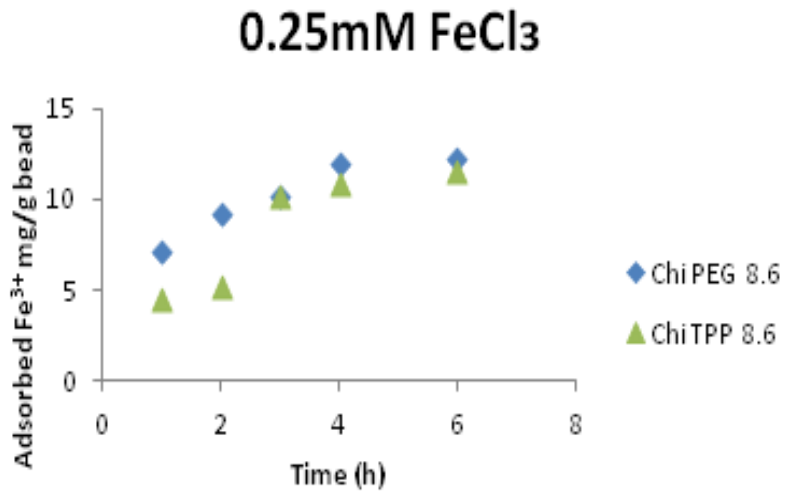


Figure 18: Fe^{3+} adsorption mg/g of bead in 0.25mM $FeCl_3$ with time at pH 8.6

It is also worth noting that adsorption capacity decreases as preparation pH decrease. In otherwords at acidic pH, because of higher ionic crosslinking that occurs at that pH, adsorption rate is lowered. Protonation occurs at acidic pH and the amino site (the free nitrogen electrons) which Fe^{3+} ions bind to are consumed and this leads to a

decrease in uptake of Fe^{3+} ions. On the other hand at basic pH, the reverse is the case as there is no competition with hydronium ions, hence the Fe^{3+} ions can bind to chitosan's unprotonated amino sites (the free nitrogen electrons) and a high adsorption capacity is observed. Fe^{3+} binds to 2 free electron pairs of nitrogen and 4 moles of oxygen atoms.

The adsorption kinetics in 5mM Fe^{3+} solution at pH 3 shown in Figure 18.

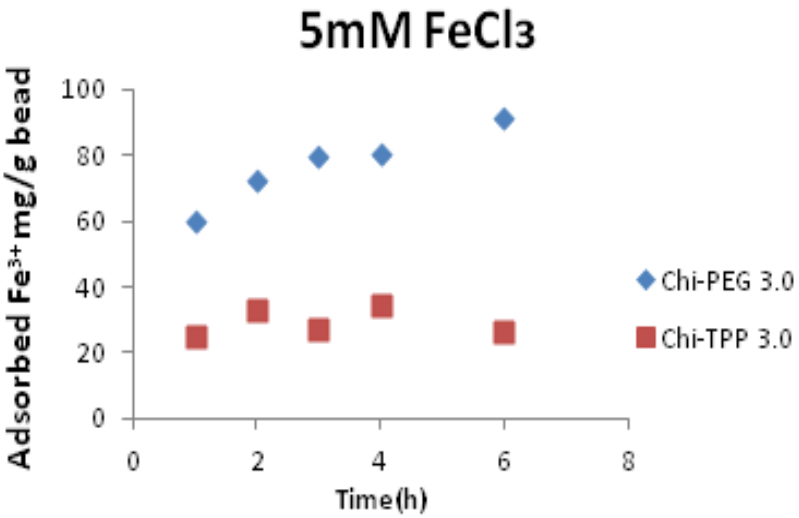


Figure 19: Fe^{3+} adsorption mg/g of bead in 5mM $FeCl_3$ with time at pH 3.0

At 5mM for beads prepared at acidic pH 3, adsorption capacity was about 80mg Fe^{3+} /g of bead and Chi-TPP beads adsorbed less when compared to Chi-TPP with PEG.

Adsorption behavior in 2.5mM Fe^{3+} solution is shown in Figure 20.

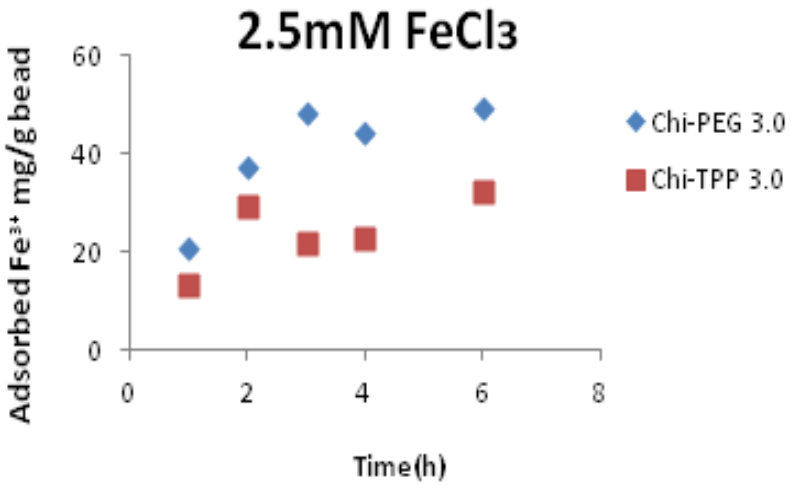


Figure 20: Fe^{3+} adsorption mg/g of bead in 2.5mM $FeCl_3$ with time at pH 3.0

At 2.5mM adsorption capacity rate reduced to about 50 mg Fe^{3+}/g of bead and Chi-TPP PEG still showed the highest absorption capacity. At 1mM the same phenomenon was observed as seen in figure 21.

Illustration of adsorption behavior in 1mM Fe^{3+} solution.

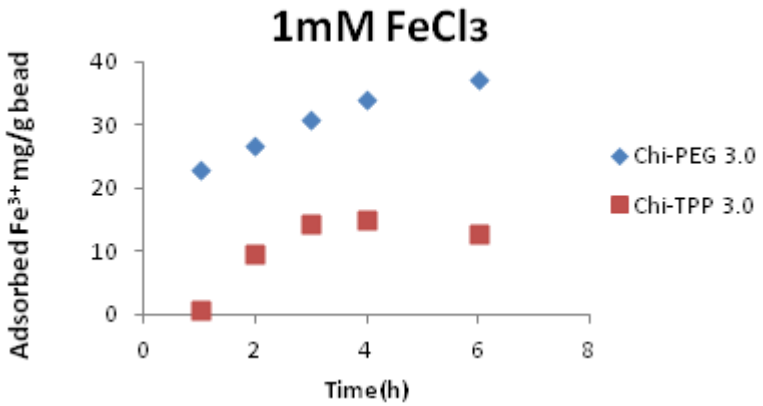


Figure 21: Fe^{3+} adsorption mg/g of bead in 1mM $FeCl_3$ with time at pH 3.0

Adsorption behavior in 0.5mM Fe^{3+} solution

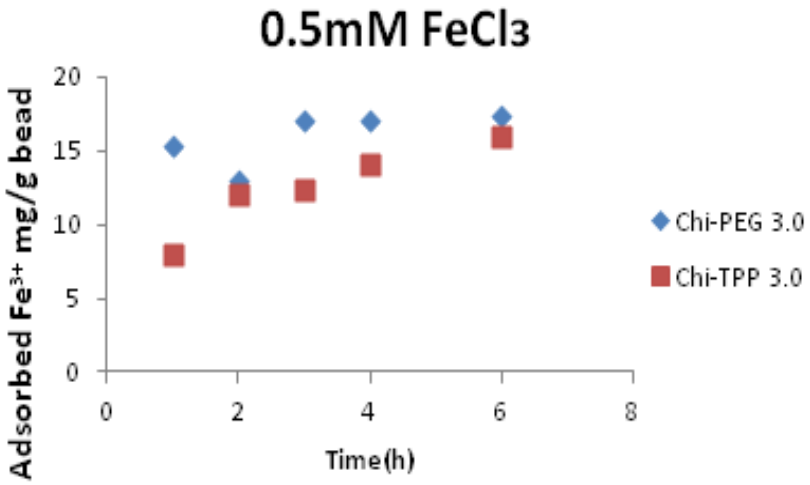


Figure 22: Fe^{3+} adsorption mg/g of bead in 0.5mM $FeCl_3$ with time at pH 3.0

Adsorption behaviour in 0.25mM of Fe^{3+} solution

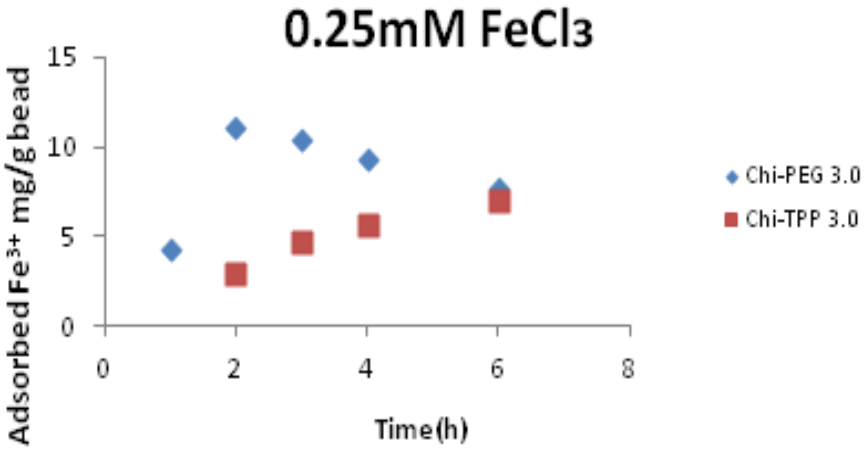


Figure 23: Fe^{3+} adsorption mg/g of bead in 0.25mM $FeCl_3$ with time at pH 3.0

At 0.5mM and 0.25mM, adsorbed capacity of beads reduced as concentration reduced and this is because the amount of Fe^{3+} ions available for adsorption reduced as seen in Figure 22 and 23. At concentration of 0.25mM, the results obtained were not so reliable as a result of working near the limit of detection limit of the instrument with a highly dilute concentration.

3.5.1 The Effect of pH of TPP on Iron Adsorption

At higher pH adsorption rate increases and this is attributed to lower crosslinking that occurs at that pH during the formation of the beads. As pH of coagulation medium decreases, adsorption rate decreases because higher crossslinking dominates at that pH medium and these results in adsorption rate decrease.

3.5.2 The Effect of Porogen on Iron Adsorption

The results show that Chi TPP PEG has a much higher adsorption capacity when compared with Chi-TPP. The polymer PEG might have reduced crystalinity on chitosan. This factor together with increased hydrophilicty introduced by remains of PEG increased Fe^{3+} adsorption. When results are compared to iron imprinted Chi-TPP beads prepared by Yalinca et al (2012). The beads prepared by PEG treatment in this study proved to have higher equilibrium swelling values and higher Fe^{3+} adsorption capacities. Iron imprinted beads were reported to have equilibrium swelling percent of the order of 1000-1700 while the PEG treated in this study swelled up to 4000%. The iron adsorption capacity of iron imprinted beads in 5mM $FeCl_3$ was in between 30-50 mg/g, PEG treated beads on the other hand had 120 mg/g under similar conditions.

3.5.3 Adsorption Isotherms

Langmuir and Freudlich equations were tested to find out the adsorption model that fits the Chi-TPP/ Fe^{3+} system.

Table 1: Table of Isotherm constants for Fe3+ adsorption onto chitosan beads

pH	chi tpp 3.0	chi peg 3.0	chi tpp 8.6	chi peg 8.6
Qe, exp (mg/g)	32	91	67	101
Langmuir model				
Qo (mg/g)	28	112	79	131
K_L (L/mg)	17.06	53.7	50	64.2
R_L	0.402	0.176	0.186	0.152
R^2	0.9513	0.9243	0.93	0.9057
Freudlich model				
$K_{F\ (}$mg/g)	4.58	3.603	4.687	4.77
1/n	0.3943	0.5859	0.6794	0.6069
R^2	0.8596	0.9112	0.8679	0.8678

From the table above, Langmuir isotherm model was suitable to describe the adsorption that took place. The qe calculated was closer to the qe experimental when compared with Freudlich. The correlation coefficient obtained from Langmuir is closer to 1 when compared to that of Freudlich that is much less than 1. The Langmuir plot for Chi-PEG 3.0, Chi-TPP 3.0, Chi-PEG 8.6, and Chi-TPP 8.6 is shown in figure 23, 24, 25 and 26

respectively.

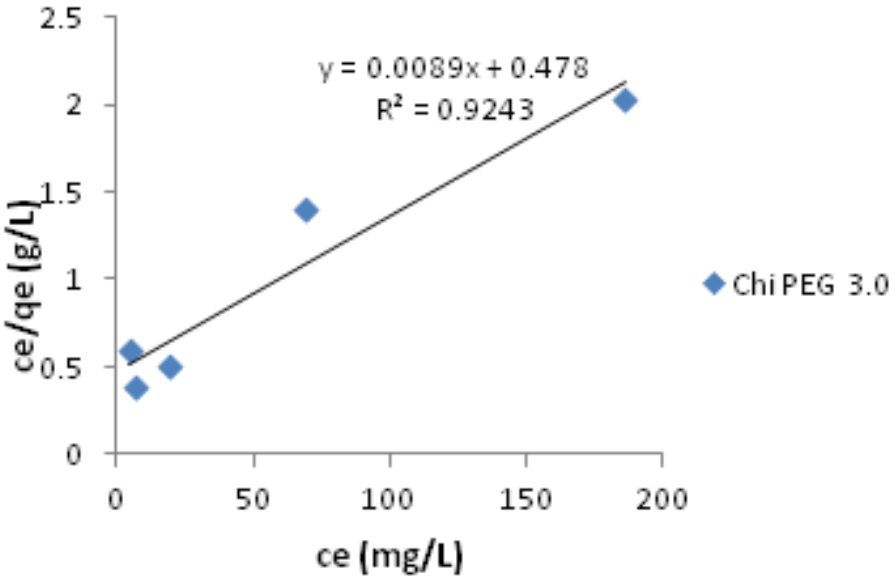


Figure 24: Langmuir plot of chi peg 3.0

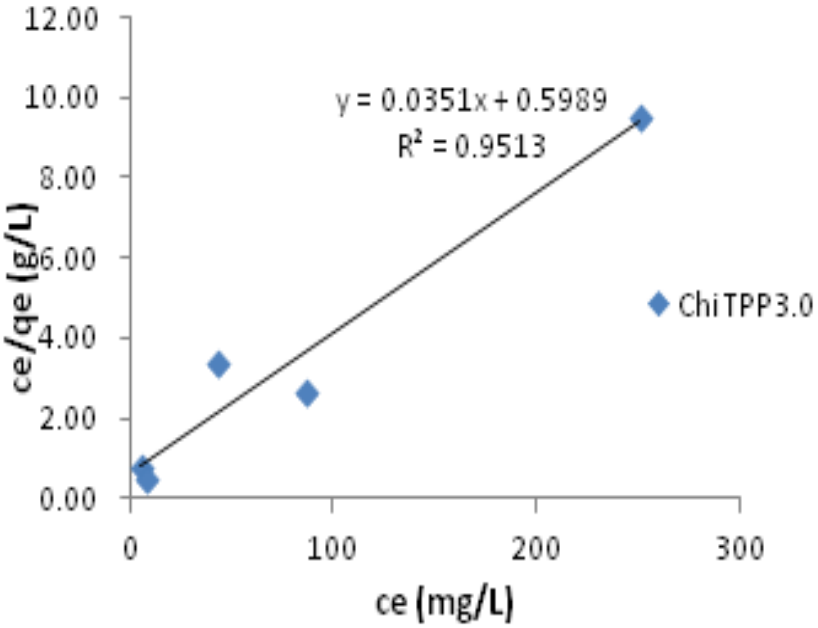


Figure 25: Langmuir plot of chi tpp 3.0

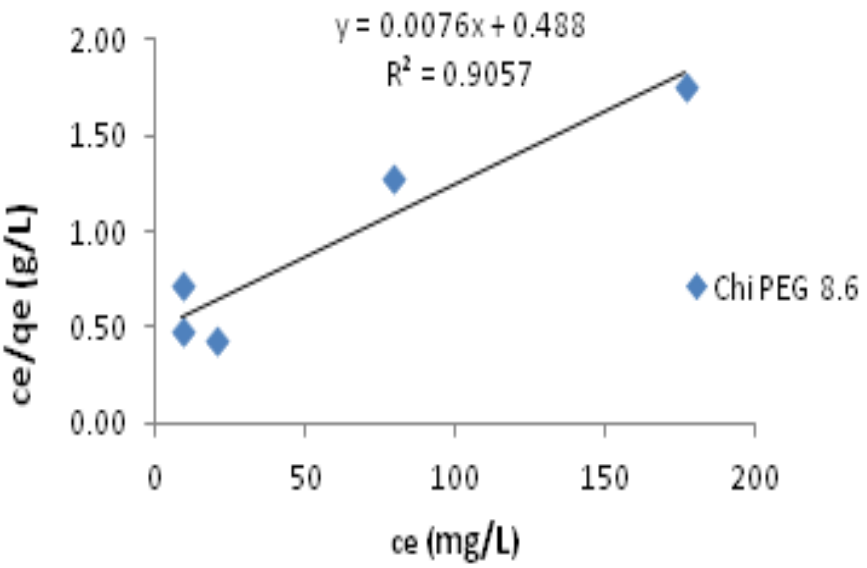


Figure 26: Langmuir plot of chi peg 8.6

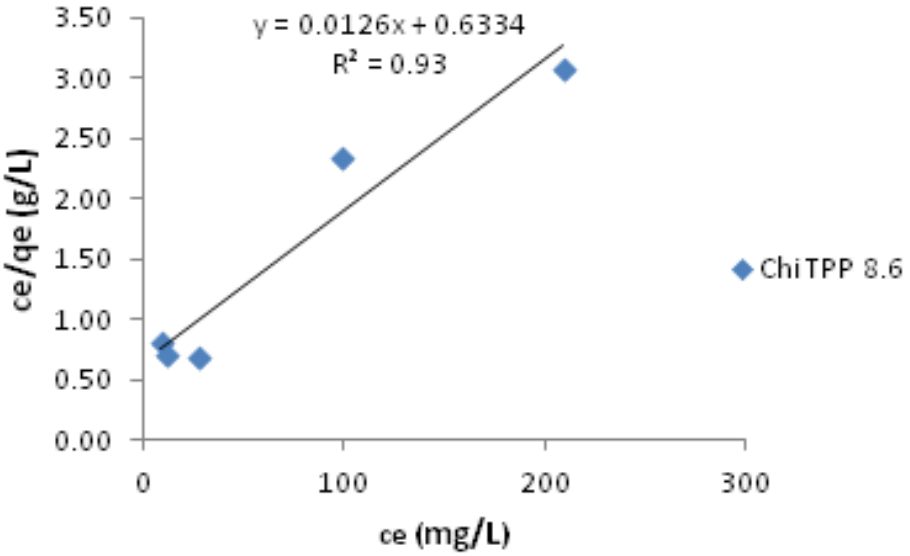


Figure 27: Langmuir plot of chi tpp 8.6

The Freudlich plots are shown in figure 28, 29, 30 and 31 respectively.

As can be followed from the figures this model does not fit the chi-TPP/Fe^{3+} System in this work.

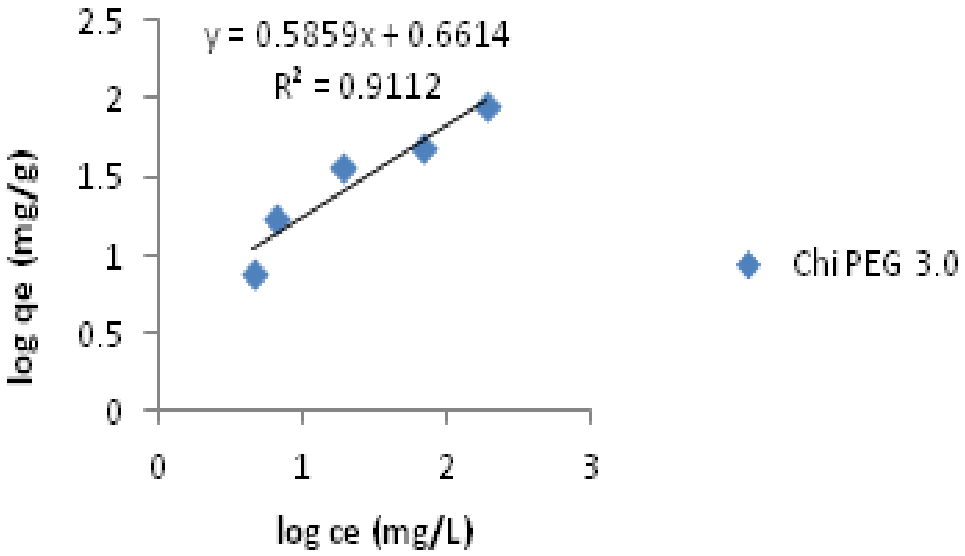


Figure 28: Freudlich plot of chi peg 3.0

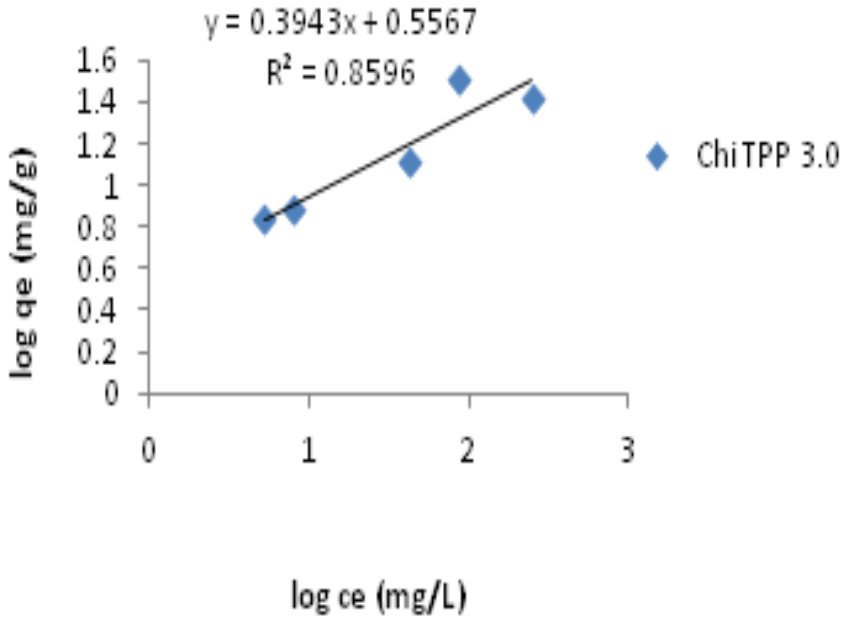


Figure 29: Freudlich plot of chi tpp 3.0

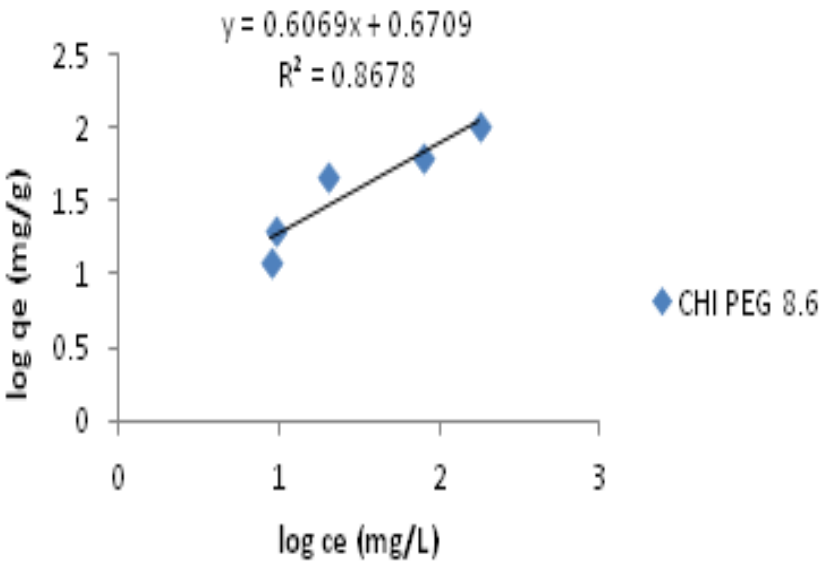


Figure 30: Freudlich plot of chi peg 8.6

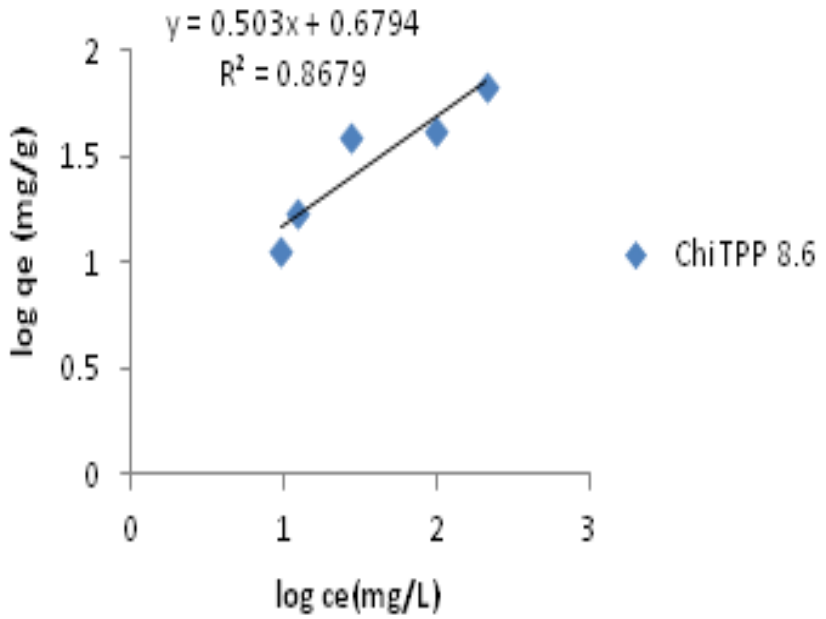


Figure 31: Freudlich plot of chi tpp 8.6

Adsorption kinetics of the beads was carried to find out the best model for chi-TPP/Fe^{3+} system and the kinetic correlation coefficients for Fe^{3+} adsorption onto chitosan beads is given in table 2.

Table 2: Table of Kinetic correlation coefficients for Fe^{3+} adsorption onto chitosan beads.

	pH 3.0	pH 8.6
Pseudo 1st order R^2	0.5049	0.9466
Pseudo 2nd order R^2	0.9068	0.9697
Intraparticle diffusion R^2	0.5232	0.9866

From table 2 above the correlation coefficient for pseudo 2nd order was closer to 1 when compared to pseudo 1st order and intraparticle diffusion.

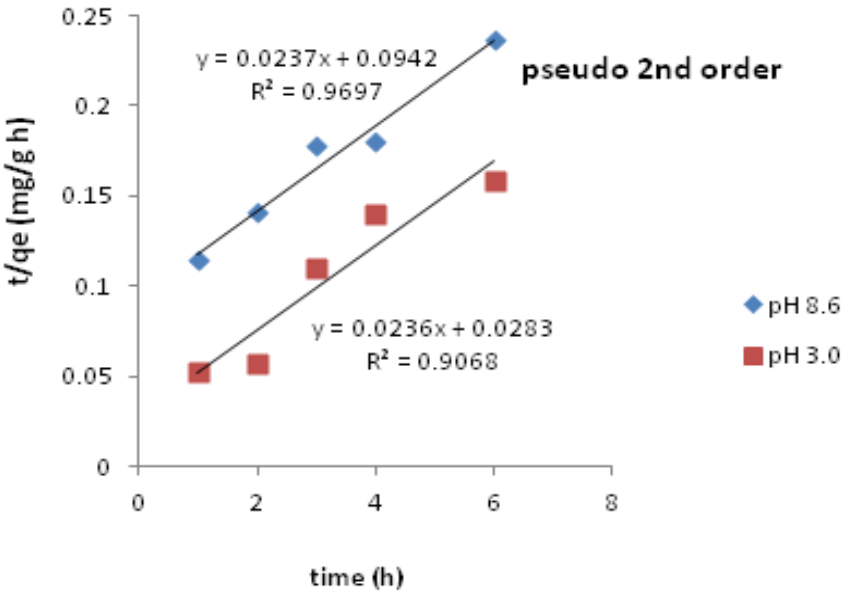


Figure 32: pseudo 2nd order plot of pH 3.0 and 8.6

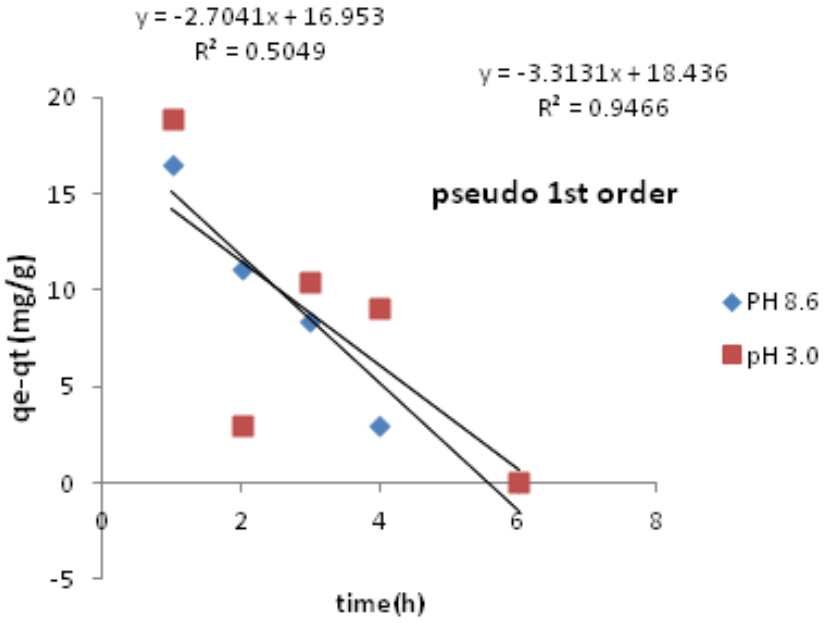


Figure 33: pseudo 1st order plot of pH 3.0 and 8.6

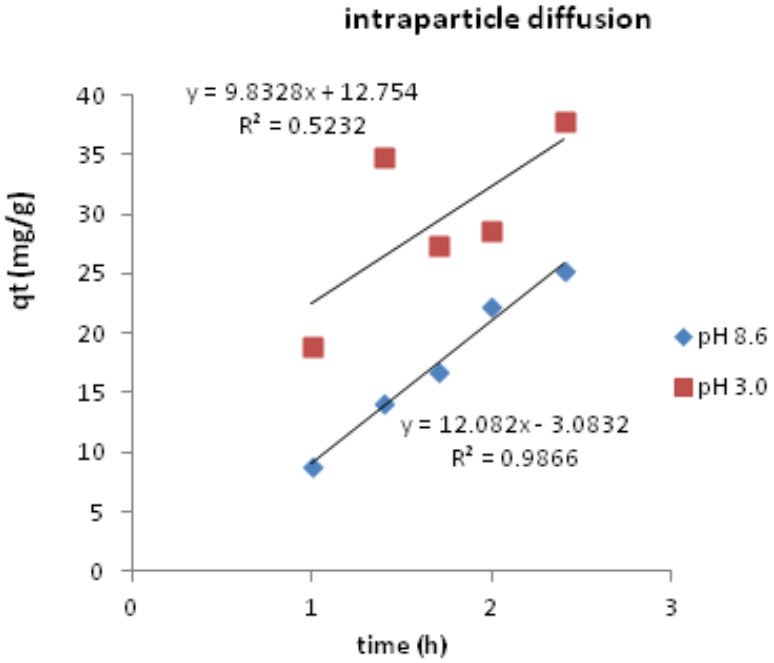


Figure 34: Intraparticle diffusion plot of pH 3.0 and 8.6

Langmuir model fits better than Freudlich model. The Langmuir isotherm established that the type of adsorption that occured with the material used here is monolayer and there was no side interactions. Pseudo 2nd order model gave the best description for

the adsorption kinetics stating that the type of adsorption that occurred with Chi-TPP /Fe^{3+} system is chemical.

Chapter 4

CONCLUSION

Chitosan TPP is a good adsorbent for Fe^{3+} ions. The beads adsorb considerable amount of the range 40mg -130mg of Fe^{3+} ions within the first 3h. The amount of Fe^{3+} adsorbed was found to vary with the concentration of $FeCl_3$, contact time, the pH of coagulation medium (TPP) and the presence of PEG on the bead. The uptake amount of Fe^{3+} ions on prepared beads increased with contact time, concentration of $FeCl_3$, pH of coagulation medium. Uptake of Fe^{3+} ion is significantly greater in Chi TPP PEG beads when compared to Chi TPP beads. Also beads prepared at coagulation media of 8.6 worked much better with swelling and iron adsorption. Intraparticle diffusion model showed that it is not the only rate controlling step but other models took part in adsorption. The Langmuir model showed the best adsorption isotherm indicating that chi TPP and Chi TPP PEG adsorption was monolayer. Pseudo 2^{nd} order kinetics went ahead to prove this adsorption type describing it as chemisorption. It can therefore be seen that chitosan finds application in removal of iron (iii) ions which is toxic to the environment. It detects and removes it even at the least concentration as observed in this work. Adsorption rate increased per gram of beads with chi- TPP PEG therefore polymer leaching method of achieving porosity is suitable for adsorption studies. However under the conditions studied not a sufficient improvement could be obtained in terms of porosity according to SEM pictures.

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