Covalent Chitosan Gels for Efficient Iron (III) Ion Adsorption

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ABSTRACT

Covalent Chitosan Gels for Efficient Iron (III) Ion Adsorption

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MS in Chemistry

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Synthetic and natural polymers are suitable metal ion adsorbents for various purposes like wastewater or drinking water treatment, biomedical applications, and industrial applications like production of some household chemicals. Synthetic polymers have advantages like being durable under severe conditions but they are not suitable especially for biomedical applications since they usually lack biocompatibility. Furthermore almost all synthetic polymers are petrochemical derivatives.

Chitin is the second most abundant natural polymer, which can be obtained from the shells of sea animals. These shells contain 30% of chitin; the rest being different proteins and minerals. Chitosan that can be obtained by N-deacetylation of chitin, is a copolymer of β-linked 2-acetamido-2-deoxy-α-D-glucose and 2-amino-2-deoxy-α-D-glucose residues. Chitosan is a natural aminopolysaccharide and it has complex formation and ion adsorption properties. It is also a biocompatible, biodegradable and mucoadhesive natural polymer and therefore has a great potential for biomedical applications.

In this study, N-phthaloylated chitosan was phosphorylated by a chemical reaction using sodium triphosphate ($Na_5P_3O_{10}$) as the phosphorylating agent and urea as a catalyst. The stability of the product in aqueous medium was improved by applying chemical crosslinking using ethylene glycol diglycidyl ether (EGDE) as the crosslinking agent. The product was then dephthaloylated to obtain an amine rich phosphorylated chitosan. All products obtained were characterized by FTIR spectrometry. Phosphorylated and EGDE – crosslinked chitosan was tested as an Fe^{3+} adsorbent in aqueous solution. The Fe^{3+} adsorption was followed by visible spectrometry at 505 nm. The EGDE – crosslinked phosphorylated chitosan product proved to be a successful Fe^{3+} adsorbent and was calculated to have an equilibrium adsorption capacity of 140 mg Fe^{3+}/g adsorbent.

ÖZET

Demir (III) İyonunun Etkin Adsorpsiyonu İçin Kovalent Kitosan Jelleri

ERARSLAN, Hande

Yüksek Lisans, Kimya Bölümü

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Temmuz 2009, 55 sayfa

Sentetik ve doğal polimerler metal iyon adsorbanı olarak değişik amaçlarla; kirli su ve içme suyunu temizlemede, biyomedikal uygulamalarda ve bazı sık kullanılan kimyasalların üretiminde olduğu gibi endüstriyel uygulamalarda kullanılmak için uygundurlar. Sentetik polimerler zor şartlara dayanıklı olduklarından dolayı avantajlıdırlar fakat biyouyumluluk gibi özellikleri olmadığından özellikle biyomedikal uygulamalarda kullanılmaları uygun değildir. Sentetik polimerler ayrıca petrokimyasal türevleridir.

Kitin; deniz hayvanlarının kabuklarından elde edilen doğada bulunma yüzdesi olarak ikinci sırada gelen doğal bir polimerdir. Söz konusu kabukların 30%' u kitinden, geriye kalan kısmı ise protein ve minerallerden oluşmaktadır. Kitosanβ -(1,4)-2-asetamido-2-deoksi-α-D-glükoz ve -(1,4)-2-amino-2-deoksi-α-D-glükoz birimlerinden oluşan doğal bir aminopolisakkarit olup N-deasetilasyon yoluyla kitinden elde edilmektedir. Kitosan kompleks yapıcı ve iyon adsorplama özelliğine sahiptir. Biyouyumluluğa sahip, biyobozunur, mukozayapışkan ve toksik madde içermeyen bir polimer olduğundan biyomedikal uygulamalarda kullanılmak üzere büyük bir potansiyeli vardır.

Bu çalışmada; N-fitaloilkitosan, sodyum tripolifosfat ve üre varlığında kimyasal şartlarda fosforilasyona uğratılmıştır. Sulu ortamda ürünün kararlılığı, çapraz bağlayıcı olarak etilen glikol diglisidil eter (EGDE) kullanılarak sağlanmıştır. Ürün son olarak defitaloilasyona uğratılıp amin grupları açısından zengin fosforlanmış kitosan elde edilmiştir. Bütün ürünler FTIR spektrometre ile karakterize edilmiştir. Fosforlanmış ve EGDE ile çapraz bağlanmış kitosan sulu ortamda Fe^{3+} adsorbanı olarak test edilmiştir. Fe^{3+} adsorpsiyonu visible spektrometre ile 505 nm de takip edilmiştir. EGDE ile çapraz bağlanmış ve fosforlanmış ürünün başarılı bir Fe^{3+} adsorbanı olduğu ispatlanmış ve denge adsorpsiyon kapasitesi 140 mg Fe^{3+}/g adsorban olarak hesaplanmıştır.

ACKNOWLEDGMENTS

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CHAPTER 1

INTRODUCTION

Metal ion adsorbents are needed for various reasons like waste water or drinking water treatment, biomedical applications, and industrial applications like production of some household chemicals. Research has been focused on the development of new modified synthetic and natural polymers as metal ion adsorbents.

Synthetic polymers have advantages like being durable under severe conditions but they are not suitable especially for biomedical applications since they usually lack biocompatibility. Furthermore almost all synthetic polymers are petrochemical derivatives. Therefore scientists are in continuous search of new materials from renewable natural resources to replace petroleum products for the 21st century.

Chitin is the second most abundant natural polymer, which can be obtained from the shells of sea animals on the industrial scale. These shells contain 20-50% of chitin; the rest being proteins and minerals. Chitin can be extracted by applying very cheap and simple deproteination and demineralization procedures. Chitin can be used for several purposes like separation of proteins from diary waste waters and metal removal from solution. Its insolubility in water hinders its applications in a broader scale.

On the other hand, chitosan, a derivative of chitin, is soluble in dilute acidic solutions and has found diverse applications ranging from contact lens production to weight control pills. It can easily be produced from chitin by complete or near to complete deacetylation of chitin.

Chitosan is known to have very high affinity towards proteins, fats, and metal ions. Adsorption of various metal ions by either pure chitosan or its derivatives in various forms such as powder, bead or membrane has been reported in the literature. However, the selectivity and adsorption capacity problems still remain intact and need the attention of researchers.

Although chitosan is an effective metal ion adsorbent compared to its synthetic counterparts, its efficiency and effectiveness may further be improved via chemical modification. Chitosan owes its unique cationic character among natural polymers to the presence of the amine ($-NH_2$) group in its chemical structure. The cationic character allows chitosan to form complexes with negatively charged ions, thus forming physical gels in aqueous solution. These gels can be used for drug loading purposes in controlled drug release formulations as well as ion exchange resins. Mucoadhesive, protein adsorbing and antibacterial properties are also attributed to the cationic character. Free amine groups, on the other hand, provide chitosan with chemical functionality available for chemical modification and chemical crosslinking. So, during chemical modification at least some fraction of free amine groups is lost. Hence, it is critical in chitosan modification to save free amine groups for further useful applications. This is achieved by applying N-protection methods.

Previous study of our group showed that chitosan flakes and powder were useful as Fe^{3+} adsorbents with an equilibrium adsorption capacity of 1.12 mg Fe^{3+}/g adsorbent [1, 2].

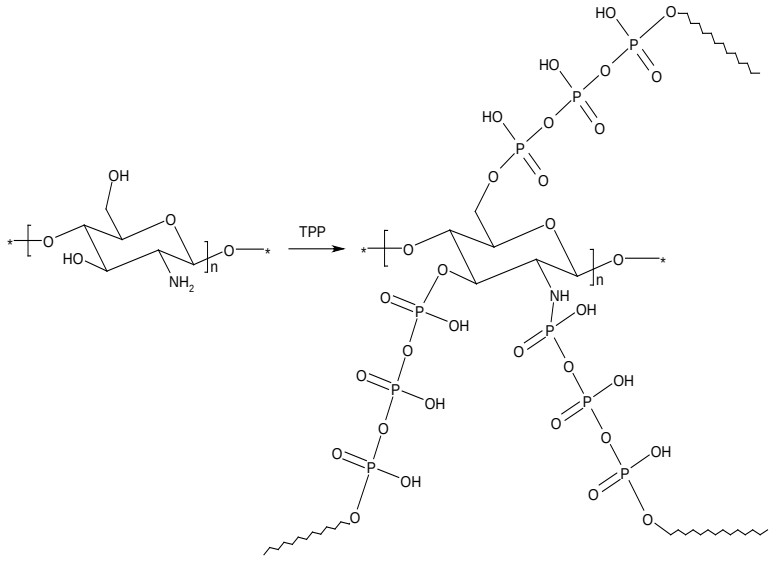
The Fe^{3+} adsorption ability of chitosan was attributed to a complex formation between Fe^{3+} ion and the amine and hydroxyl groups of chitosan. It was observed that Fe^{3+} adsorption capacity of chitosan decreased with decreasing degree of deacetylation. Hence, amine groups play a crucial role in metal ion adsorption.

Further studies with chitosan tripolyphosphate physical gels of chitosan showed that although some fraction of the amine groups were used for ionic crosslinking with the phosphate, Fe^{3+} adsorption capacity was improved significantly due to the presence of the phosphate group on the adsorbent. [3,4]

This thesis aims at preparing covalently phosphorylated chitosan without sacrificing the amine groups for the modification reaction. Thus, the modified chitosan will preserve its amine-group-related biological and physiochemical properties, whilst having enhanced Fe^{3+} ion adsorption capacity due to the presence of both amine and phosphate groups on the structure.

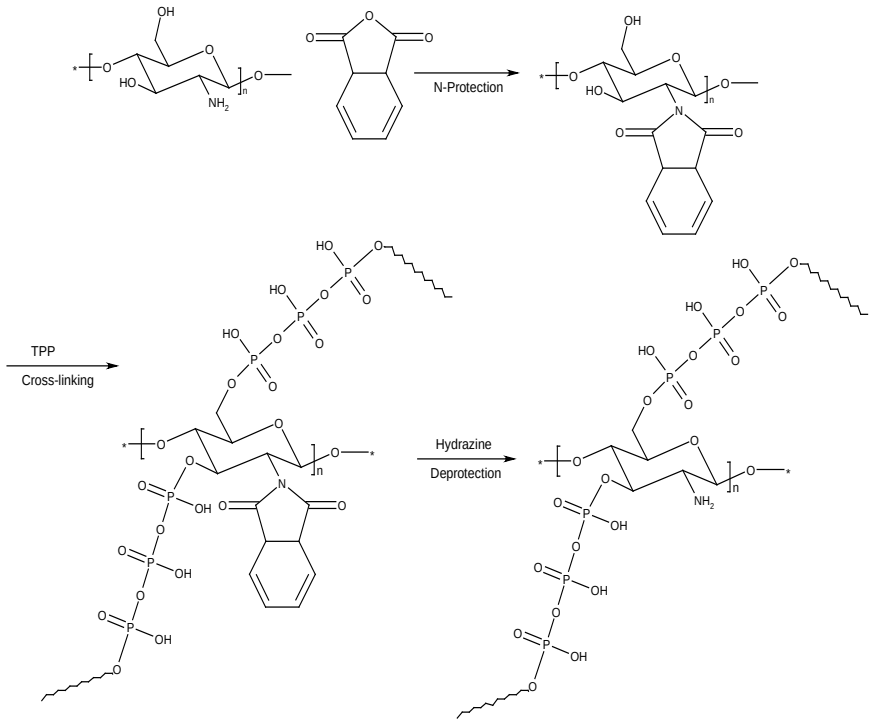
The subject of this thesis is the synthesis and characterization of biocompatible chitosan systems which will act as efficient metal adsorbents. The strategy developed to improve the metal ion adsorption capacity of chitosan was to synthesize tripolyphosphate esters of chitosan by a phosphorylation reaction. The multifunctional tripolyphosphate group was chosen to serve a dual purpose; to act as a cross-linker to form chitosan gels and to enhance the metal ion binding capacity of chitosan due to the presence of the phosphate group. Furthermore, insertion of

biologically essential phosphate groups on the chitosan structure will enhance the biocompatibility.



Scheme 1.1: Cross-linking with TPP

The reaction scheme given in Scheme 1.1 shows phosphorylation of chitosan via the –OH and $–NH_2$ groups. Phosphorylation from all three susceptible sites on carbon-2, carbon-4 and carbon-6 will probably not occur simultaneously due to steric and electrostatic hindrances. Phosphorylation via $-NH_2$ groups is not desired since this will decrease the fraction of free $-NH_2$ groups which in turn will adversely affect both the adsorption capacity and other related biological properties**.** To prevent this, the amine groups were protected before phosphorylation with TPP. This was achieved by treating chitosan with phthalic anhydride. After phosphorylation, the amine groups were deprotected by treatment with hydrazine. The protection/deprotection mechanism is shown in Scheme 1.2. Whenever needed, ethylene glycol diglycidyl ether was used as an additional crosslinker.



Scheme 1.2: Protection / Deprotection of amine groups

The synthesis strategy in this study can be summarized as follows:

N-Protection (Phthaloylchitosan)

Chitosan

↓ 130 ^{o}C in DMF under argon atmosphere

N-phthaloylchitosan

Phosphorylation

↓ DMF, TPP solution sodium triphosphate ($Na_5P_3O_{10}$) in urea-EGDE medium at 70 ^{o}C

Crosslinking phosphorylated N-phthaloylchitosan

Deprotection

↓ DMF, hydrazine monohydrate at 100 ^{o}C

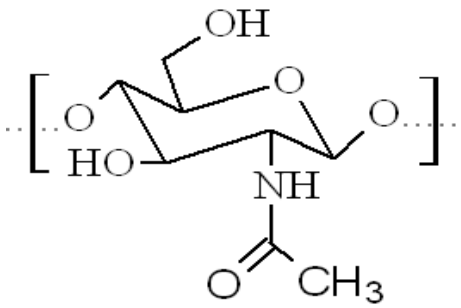
Dephthaloylated chitosan (crosslinked and phosphorylated)

↓

Adsorption Experiments

1.1 Chitin, Chitosan and their Applications

Chitin is a high molecular weight linear polymer of 2-acetamido-2-deoxy-D-glucopyranose units linked together by 1, 4-glycosidic bonds.



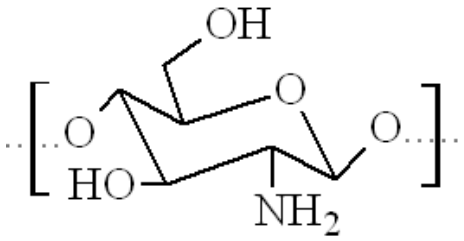
Scheme 1.3: Structure of Chitin

Chitin is the second most abundant natural polymer, which can be obtained from the shells of crustaceans such as crabs and shrimps. These shells contain %30 of chitin the rest being proteins and minerals [5]. Chitin is the supporting material of invertebrates such as crustaceans, insects and cell wall component of fungi. Chitin can be extracted by applying very cheap and simple deproteination and demineralization procedures.

Chitin can be used for several purposes like separation of proteins from diary waste waters and metal removal from solution. Furthermore, it is a good chelating agent.

Chitin is a highly insoluble material resembling cellulose in its solubility and chemical reactivity. Its insolubility in water hinders its applications in a broader scale. It is soluble only in special solvents like DMAc containing 5-10% LiCl and in weak acids.

Chitosan; is a copolymer of β-linked 2-acetamido-2-deoxy-α-D-glucose and 2-amino-2-deoxy-α-D-glucose residues.



Scheme 1.4: Structure of Chitosan

Chitosan can be obtained by N-deacetylation of chitin. It has complex formation and ion adsorption properties. Chitosan is a very versatile material in terms of its chemical, physical and biological properties. It is a non-toxic, mucoadhesive, biodegradable and biocompatible polymer [5].

Biological properties and activities of chitosan depend largely on its physiochemical properties such as the degree of deacetylation, crystallinity, molecular weight, and high charge density in solution, as well as chemical reactivity and ease of fabrication into different forms. Metal adsorption capacity of chitosan depends largely on the degree of deacetylation. Free amine groups have an affinity for metal cations such as copper, nickel, lead and iron [5].

Chitosan has poor solubility in both water and organic solvents due to its rigid crystalline structure which limit its effective utilization. To overcome this drawback, much attention has been paid on modification and utilization of chitosan, due to its good biodegradability, biocompatibility, and bioactivity. The presence of amino groups on chitosan makes the material soluble in dilute organic acids such as acetic acid, formic acid, lactic acid and in dilute inorganic acids like hydrochloric acid [5].

Chitin and chitosan have found a wide range of applications in medicine and in industry. Some of these applications are in photography [6], cosmetics [7], drug delivery systems [8], gene delivery [9, 10], vaccine delivery [11, 12], tissue engineering [13], biological iron chelator [5], ophthalmology [14], waste water treatment [15, 16] and in paper finishing [17].

1.2 N-Protection of Chitosan

Before the phosphorylation amine groups were protected, because amine groups are very efficient to adsorb metal ions and many unique biological and physical properties of chitosan are due to the presence of amine groups. If amine groups are not protected, phosphate groups will react with amine groups. Related studies in the literature are summarized below.

In their study, Nishumura et. al. was prepared N-phthaloylchitosan by heating the mixture of purified chitosan and phthalic anhydride in *N*,*N*-dimethyl-formamide (DMF) at 130°C with magnetic stirring under a nitrogen atmosphere. After 7 h, the resulting clear solution was precipitated into 1000 ml of ethanol, after cooling to room temperature and collected by filtration. The resulting product was dried in vacuum at room temperature to give N-phthaloylchitosan (yield: 96%). Results are recorded like this: IR (KBr): ν 3450 (O–H), 2930 (C–H, pyranose), 1773 (C=O, imide), 1716 (C=O, imide), 1394 (C=C, phth), 1150–1010 (C–O, pyranose), 721 cm^{-1} [18].

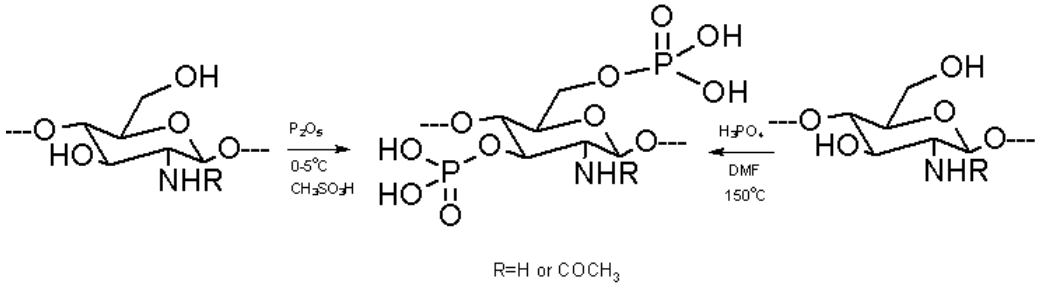
In another study by Kurita et. al. and Nisumura et. al, chitosan was heated with excess phthalic anhydride in dried DMF to give phthaloyl-protected chitosan (PHCS). A yellow powdery material was obtained and the degree of substitution

(DS) of phthaloyl group was calculated to be about 1.05 from the C/N value of elemental analysis and ^{1}H–NMR integral. Results are recorded like this: FTIR (KBr, CM^{-1}): ν 3460 (O–H), 1777 and 1712 (C=O, anhydride) and 721 (aromatic ring). ^{1}H-NMR (δ, ppm): 3.0-5.0 (pyranose) and 7.4-7.9 (aromatic ring) [19, 20].

1.3 Phosphorylation of Chitosan

Recently there has been a growing interest about the chemical modification of chitin and chitosan to improve their solubility and widen their applications. Among them phosphorylated chitin and chitosan have attracted considerable interest because of their various advantages: ability to form metal complexes, anti-inflammatory property, blood compatibility and formation of anionic polyelectrolyte hydrogels.

Surface phosphorylation of chitosan membranes was performed using the H_3PO_4 / Et_3PO_4 / P_2O_5 / butanol reaction system [21]. In another study, phosphorylated chitin (P-chitin) and chitosan (P-chitosan) were prepared by heating chitin or chitosan with orthophosphoric acid and urea in DMF [22].

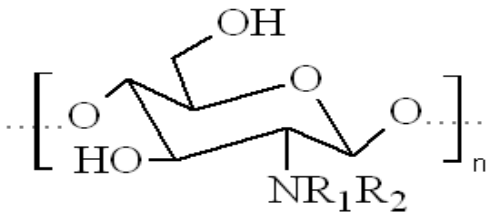


Scheme 1.5: Synthesis of phosphorylated chitin and chitosan [22]

The P-chitin and P-chitosan were also synthesized by mixing chitin or chitosan with sodium pyrophosphate [23]. or by grafting mono (2-methacryloyl oxyethyl) acid

phosphate onto chitosan [24]. It was observed that the grafting reaction improved the antimicrobial activities of chitosan. It was also observed that the antimicrobial activity of chitosan and graft copolymer against Candida albicans, Trichophyton rubrum, and Trichophyton violaceum depends largely on the amount and type of grafted chains, as well as on the changes of pH.

A water soluble *N*-methylene phosphonic chitosan (NMPC) was synthesized using chitosan, phosphorous acid and formaldehyde.(Scheme 1.11) [25].



R_1=H, R_2=$CH_2PO_3H_2$

R_1=R_2=CH_2-PO_3H_2

Scheme 1.6: Chemical structure of N-methylene phosphonic chitosan

Nishi and co-workers prepared P-chitin and P-chitosan by the cross-linking reaction with adipoyl chloride in methanesulphonic acid with phosphorous pentoxide for adsorbing metal ions. Obtained products were completely insoluble in water, and were proposed to be used as adsorbents of metal ions in water [26].

Phosphorylated chitosan membranes were prepared from the reaction of ortophosphoric acid and urea on the surface of chitosan membranes in N,N-dimethylformamide, and the ionic conductivity of phosphorylated chitosan in the swollen state was investigated. These chemical modifications contributed to improve ionic conductivity of the chitosan membranes and also crystallinity of the

phosphorylated chitosan membranes and the corresponding swelling indices were changed [27].

1.4 Adsorption

It is known that adsorption is the accumulation of atoms or molecules on the surface of a material. Atomic or molecular species which are adsorbed onto the surface are called adsorbates and the materials that are capable of adsorbing atomic or molecular species are called adsorbent.

Adsorption of molecules may be either physical (physisorption) or chemical (chemisorption), although sometimes both occur simultaneously. Physisorption is related to polarity and molecular mass. In physical adsorption, bonding is realized by intermolecular forces; there is no redistribution of electrons between adsorbate and adsorbent. Chemisorption is related to chemical bond strength. In chemical adsorption, a chemical bond (ionic/covalent) is formed between adsorbate and adsorbent. Physical adsorption occurs on all surfaces if temperature and pressure conditions are favourable. However, chemisorption, occurs only between certain adsorbents and adsorptive species.

A characteristic of physical adsorption is that the adsorbed molecules can be removed at the same temperature at which adsorption occurred. Heating speeds up desorption by providing the required energy to the adsorbed species to escape adsorption site. Compared to the physically adsorbed species, a chemically adsorbed one is strongly bound to the surface and cannot escape without large quantitiy of energy; hence adsorption enthalpy of chemisorptions is much higher than that of

physisorption. Physisorption tends to occur at lower temperatures than chemisorptions [28, 29].

1.4.1 Factors Influencing Adsorption

Surface area, particle size and pore size of the adsorbent, solubility, affinity and the ionic charge of the adsorbate, contact time, pH of the solution, and temperature are important factors affecting the adsorption process. Large surface area provides large adsorption capacity. Smaller particle size reduces internal diffusion and mass transfer limitation to the penetration of the adsorbate inside the adsorbent and it provides a large specific surface area. Longer contact time is needed for complete adsorption. Adsorption of a solute (adsorbate) is inversely proportional to its solubility in the solvent. As solubility of solute increases, the extent of adsorption decreases. Polarity plays an important role in affecting the adsorption capacity. Non-polar adsorbents are ineffective in adsorbing polar compounds. The inversion is also true. But, non-polar adsorbents are effective in adsorbing non-polar adsorbates. Large molecules may be too large to enter small pores. This may reduce adsorption independently from all other parameters. Depending on the nature of adsorbate and adsorbent, pH should be adjusted. As long as the compounds are structurally simple, adsorption is at minimum for the charged species and at maximum for the neutral species. Thus, adsorption decreases with increasing charge. Adsorption reactions are typically exothermic, so adsorption increases with decreasing temperature [4].

1.4.2 Metal Ion Adsorption onto Chitosan and Phosphorylated Chitosan

"The use of adsorbents containing natural polymers has received great attention, in particular polysaccharides such as chitin and its derivative chitosan. The adsorption studies carried out on chitosan and its derivatives show that amino groups are important binding sites on the chitosan backbone" [5]. "It has been reported that the pH of the medium, concentration of the ions and size of the chitosan particles play a role in the adsorption process" [30]. "Flake and powder forms of chitosan are not suitable for use as adsorbents due to their low surface area and no porosity" [31].

Burke et. al. investigated the adsorption of iron (III) ion from a Jectofer solution onto different forms of chitosan.. The equilibrium studies showed that chitosan powder has the highest sorption capacity for the iron (III) ion when compared to chitosan flakes and microspheres. The amount of iron (III) adsorbed onto chitosan was found to increase with the contact time to reach equilibrium. Also a higher initial concentration of a Jectofer solution and higher pH value, in the range 2-7, resulted in a higher amount of iron (III) adsorbed [2].

"Several methods have been used to modify natural chitosan either physically or chemically in order to improve the adsorption capacity. For example; crosslinking with glutaraldehyde (GLA) or epichlorohydrin (EPI) are done in order to prevent dissolution of chitosan in acidic solutions or to improve metal sorption properties" [32]. "The adsorption capacity of chitosan varies with porosity, crystallinity, affinity for water, percent deacetylation and related amino group content" [33].

"Also metal chelating agents for removal of metallic impurities in wastewaters is an excellent application for large-scale use of chitosan. It is reported that chitosan has

the highest chelating ability in comparison to other natural polymers obtained from seafood wastes and natural substances and the synthetic polymer which is used in commercial chelating ion-exchange resins" [34].

"The ability of chitosan to bind transition metals in the presence of alkali and alkaline earth metals is well investigated" [35,36]. "The adsorption of Cu^{2+}, Hg^{2+}, Ni^{2+} and Zn^{2+} on chitosan with various particle sizes (210–1000 μm) and as a function of temperature was studied at neutral pH" [37]. Particle size does not have any great influence on saturation adsorption capacity and an increase in temperature decreases the saturation adsorption capacity of chitosan.

"The adsorption capacity depends on the extent of crosslinking and generally decreases with increase in the extent of crosslinking. Crosslinking in homogenous condition leads to enhanced metal binding capacity as a result of increased hydrophilicity caused by partial destruction of crystallinity as compared to heterogeneous crosslinking. It was found that the binding capacity of chitosan for copper increased when it was crosslinked by glutaraldehyde in homogenous conditions" [38].

"Chitosan beads were crosslinked with glutaraldehyde under heterogeneous conditions; it was found that the saturation adsorption capacity of Cd^{2+} on crosslinked chitosan decreased exponentially as the extent of crosslinking increased" [39].

Glutaraldehyde crosslinking has been used for Cd^{2+} recovery from industrial waste streams on porous magnetic chitosan beads of different diameters [40]. Molybdenum

and vanadium sorption on glutaraldehyde crosslinked chitosan has been studied by Guibal and Milot [41].

"The phosphorylated chitin and chitosan are found to have strong metal-binding ability. It was found that their adsorption of uranium is much greater than that of the other heavy metal ions" [42]. " For example, phosphorylated chitin and chitosan cross-linked with adipoyl chloride in methanesulphonic acid were found to have metal binding ability to $Mg^{2+,}$ $Ca^{2+,}$, $Sr^{2+,}$, $Ba^{2+,}$, $Mn^{2+,}$, $Ni^{2+,}$, $Cu^{2+,}$, $Zn^{2+,}$ and $Cd^{2+"}$ [43]. "Conversion of chitin to insoluble phosphorylated chitin resulted in the increase of the binding ability to all metal ions and this was tested. The results indicated that the alkaline earth metals and $Mn^{2+,}$ were adsorbed strongly to these derivatives than to chitin or chitosan" [26].

CHAPTER 2

EXPERIMENTAL

2.1 Materials

All reagents, listed in Table 2.1, were commercially available, and were used as received.

Table 2.1 Materials and manufacturers.

Material	Manufacturer
Chitosan (Medium MW)	Fluka Biomedicals, Switzerland
DMF (N,N Dimethyl formamide)	Aldrich, Germany
Sodium Triphosphate penta basic (TPP) ($Na_5P_3O_{10}$)	Aldrich, Germany
Ethyl alcohol (C_2H_5OH)	Selim&Sons, Famagusta, Cyprus
Phosphoric acid (H_3PO_4)	Aldrich, Germany
Urea	Sigma-Aldrich, USA
Hydrochloric acid (%37)	Riedel-de Haen, Germany
Acetone	Millennium Nails, Great Britain
Argon gas	Linde-Hadjikyriakos Gas Ltd, Cyprus
Hydrazine Monohydrate	Aldrich, Germany
Iron (III) Chloride ($FeCl_3$)	Aldrich, Germany
Potassium Chloride (KCl)	Sigma-Aldrich, USA
5-sulfosalicylic acid dehydrate	Riedel-de Haen, Germany
Sodium Hydroxide (NaOH)	Aldrich, Germany
Potassium Hydrogen Phthalate (KHP)	Analar, British Drug Houses Ltd, UK
Ethylene glycol diglycidyl ether (EGDE)	Aldrich, Germany
Phthalic anhydride	Aldrich, Germany
Potassium Bromide	Sigma-Aldrich, Germany

2.2 Methods

2.2.1 Synthesis

2.2.1.1 Preparation of N-Phthaloylchitosan

The preparation of N-phthaloylchitosan was carried out by dispersing 5g of chitosan (Mr=400000, DD=85%) in 100 ml DMF. After that 5g phthalic anhydride was added at 130 °C under argon atmosphere. The product dissolved in DMF as it formed. The reaction was continued for seven hours. At the end of seven hours, the product was precipitated in ethanol, washed with ethanol and dried at 60°C.

2.2.1.2 Phosphorylation of N-Phthaloylchitosan

2.2.1.2.1 Phosphorylation of N-Phthaloylchitosan with phosphoric acid

The preparation of phosphorylated N-phthaloylchitosan was carried out with phosphoric acid by dissolving 2.93g N-phthaloylated chitosan in 30 ml DMF and heating to 70°C; at this temperature 12g urea was added. After dissolving the urea, 13.1 ml phosphoric acid was added into the mixture. A white gelatinous precipitate formed as soon as the acid was added followed by some foaming. The reaction was continued for two hours. The product was precipitated and washed in ethanol and distilled water. The precipitate was brown colored containing some white particles. A water insoluble product was obtained after drying at 60 °C.

2.2.1.2.2 Phosphorylation of N-Phthaloylchitosan with sodium triphosphate ($Na_5P_3O_{10}$)

To prepare phosphorylated N-Phthaloylchitosan using sodium triphosphate ($Na_5P_3O_{10}$) 2.93 g N-phthaloylated chitosan was dissolved in 30 ml DMF and heated to 70°C. Meanwhile, TPP solution was prepared and heated to 70°C as well. Then this TPP solution was added into the N-phthaloylated chitosan solution in DMF slowly at 70°C and the reaction was continued for two hours. A white gelatinous precipitate formed instantaneously. The product was precipitated in ethanol after 2 hours of reaction time allowed. Then it was filtered and washed with ethanol and distilled water. A mixture of brown and white colored particles was obtained. Brown part was insoluble in water while the white part was water soluble after drying.

2.2.1.2.3 Phosphorylation of N-Phthaloylchitosan with sodium triphosphate ($Na_5P_3O_{10}$) in the HCl medium

The same procedure as above was followed except for using a TPP solution adjusted to pH=2. A similar product, a mixture of brown and white particles formed. However, white fraction was much higher when compared to the product obtained without using HCl.

2.2.1.2.4 Phosphorylation of N-Phthaloylchitosan with sodium triphosphate ($Na_5P_3O_{10}$) in the presence of urea

The preparation of phosphorylated N-phthaloylchitosan with sodium triphosphate ($Na_5P_3O_{10}$) in the presence of urea was carried out by dissolving 0.8 g N-phthaloylated chitosan in 8.19 ml DMF and heating to 70°C. At this temperature 3.43 g urea was added. Meanwhile TPP solution (2.86g TPP in 28.6 ml pure water) was prepared and heated to 70ºC. Then 21ml TPP solution was added into the N-phthaloylated chitosan-DMF-urea solution slowly at 70°C and the reaction was continued for two hours. As TPP solution added into the N-phthaloylated chitosan-DMF-urea solution, gelatinous white parts were observed. After one hour, besides the gelatinous white parts, a white precipitate was observed. After completing the reaction, the mixture was washed in ethanol and dried. After drying, white and small, brown coloured parts were obtained.

2.2.1.2.5 Phosphorylation of N-Phthaloylchitosan with sodium triphosphate ($Na_5P_3O_{10}$) in the presence of urea and HCl medium

The preparation of phosphorylated N-protected chitosan with sodium triphosphate ($Na_5P_3O_{10}$) in HCl and urea medium was carried out by dissolving 0.75g N-protected chitosan in 12 ml DMF and heated to 70 ºC. At this temperature 3.43g urea was added. About 5 min. was waited for supplying heat balance after dissolving the urea. Meanwhile, TPP solution (solving 2.86g TPP in 28.6 ml pure water) was prepared. Adding concentrated HCl, pH was adjusted to 2. After arranging the TPP solution pH 2, TPP solution was heated to 70ºC. Finally, TPP solution with pH 2 was added

into the N- phthaloylated chitosan – urea - DMF solution at 70 ºC and the reaction was continued for two hours. As TPP solution was added into the N-phthaloylated chitosan-DMF-urea solution, except the yellowish view, gelatinous white parts were observed.

2.2.1.2.6 Phosphorylation of N-Phthaloylchitosan with sodium triphosphate ($Na_5P_3O_{10}$) in the urea - EGDE medium

The preparation of phosphorylated N-protected chitosan with sodium triphosphate ($Na_5P_3O_{10}$) in urea-EGDE medium was carried out by dispersing 0.8g N-protected chitosan in 50 ml DMF for two weeks for maximum dissolution and heating to 70 ºC. Calculated (~0.3 ml) EGDE was added and the reaction was carried out for two hours. At the end of these two hours 0.6g urea was added. Meanwhile TPP solution (solving 0.5g TPP in 5ml pure water) was prepared and heated to 70ºC. Then all of the TPP solution prepared was added into the N-phthaloylated chitosan-DMF-urea-EGDE solution slowly at 70°C and the reaction was continued for four hours. After half an hour, a white precipitate was observed. At the end of the first hour, the solution turned cloudy and remained as it were until the end of the experiment. After completing the reaction, the product was left overnight for complete precipitation of the product. Then it was washed in ethyl alcohol and dried.

2.2.1.3 Deprotection of the Graft Product

Phosphorylated phthaloylchitosan (0.3g) was stirred in 50 ml of DMF and heated to 100 ºC under argon. Calculated hydrazine monohydrate (excess 1 ml) was added and the reaction was continued for 3h to deprotect the phthaloyl group. The yellow solution containing the precipitate was allowed to cool to room temperature. Then the precipitate was collected, washed thoroughly with water and ethanol and dried to obtain the final product.

2.2.2 Characterization of the Products

2.2.2.1 FTIR Analysis

FTIR spectrum of samples in KBr pellets had been taken by using Mattson Satellite 5000 FTIR Spectrophotometer.

2.2.2.2 Quantitative UV analysis for Determination of Fe^{3+}

2.2.2.2.1 UV Spectrophotometer

Quantitative studies for Fe^{3+} adsorption analysis had been done by using Shimadzu UV-1201 V visible spectrophotometer at 505 nm.

2.2.2.2.2 Calibration Curve for pH 1.2 and pH 5

A calibration curve was prepared to determine Fe^{3+} in solution using visible spectrophotometry. To draw the calibration curve first a buffer solution with pH=1.2, (by mixing 0.2 M, 250 ml KCl solution and 0.2 M, 425 ml HCl solution and completed to 1000 ml with distilled water) was prepared. Then, 10 % (w/v) 5-sulfosalicylic acid dehydrate was prepared by weighing 10g 5-sulfosalicylic acid

dehydrate dissolving in some distilled water and completing to 100 ml with distilled water. $5*10^{-3}$ M $FeCl_3$ solution was prepared by weighing 0.2g $FeCl_3$ in 250 ml and solving with pH 1.2 buffer solution and completed to 250 ml. Then $4*10^{-3}$ M, $3*10^{-3}$ M, $2*10^{-3}$ M, $1*10^{-3}$ M , $0.8*10^{-3}$ M, $0.6*10^{-3}$ M, $0.5*10^{-3}$ M, $0.4*10^{-3}$ M, $0.3*10^{-3}$ M, $0.2*10^{-3}$ M, $1*10^{-3}$ M, $FeCl_3$ solutions were analyzed by adding an appropriate amount from $5*10^{-3}$ M $FeCl_3$ solution in a 10 ml volumetric flask, and then mixing with 1 ml 5-sulfosalicylic acid dehydrate solution. The mixture was diluted to 10 ml with pH 1.2 buffer solution. Then the absorbances were measured by visible spectrophotometry at 505 nm. The same procedure was applied by using pH 5 buffer solution (by mixing 0.1 M, 250 ml NaOH solution and 0.1 M, 500 ml KHP solution). The calibration curves obtained at pH 1.2 and pH 5 are given in the Appendix section, Figure A1 and A2 respectively.

2.2.2.2.3. Adsorption experiments

Testing Fe^{3+} adsorption capacities of different samples were done by UV analysis.

Different chitosan products were added to $1x10^{-2}$ or $1x10^{-3}$ M Fe^{3+} solutions, respectively, and stirred 60 rpm at 20°C for 6 hours. 1 ml aliquots had been taken in predetermined time intervals and they had been analyzed for their Fe^{3+} content by transferring each one to 10 mL graduated test tubes followed by addition of 1 mL complex forming agent to colour the solution (5-sulfosalycilic acid) and dilution with buffer solution to 10 mL before the absorbance measurements. The amount of Fe^{3+} adsorbed was calculated from the difference between the concentrations of the initial and final solution. All adsorption experiments were done in duplicate.

2.2.2.3 Quantitative Determination of Degree of Phthaloylation by FTIR Analysis

The distinctive IR peaks shown in Figure B.1 in the Appendix section had been utilized in determination of percent phthaloylation:

2980-2823 cm^{-1} for chitosan content

1830-1713 cm^{-1} for phthalic anhydride content

A series of solid mixtures with varying chitosan/phthalic anhydride content had been prepared. FTIR spectra of these samples were obtained. Above mentioned peak areas were calculated automatically by using the software WinFirst Lite Version 1.02. The calibration curve was then prepared by drawing % Phthaloylchitosan the peak ratios.

The peak ratios were calculated by using the equation:

$$T_{2980\text{-}2823\ cm\text{-}1} / T_{1830\text{-}1713\ cm\text{-}1}$$

where T is the area of the designated peak.

The calibration curve shown in Figure B.2 in the Appendix was obtained and used for rough estimation of the degree of phthaloylation of the phthaloylated products.

2.3 Calculations

2.3.1 The Adsorption Capacity:

The adsorption capacity of chitosan at pH = 1.2 were calculated by using the formulas as follows:

*A= 1.37 * C + 0.000222*, where C is concentration.

And pH=5

A = 1.91 C + 0.0618*

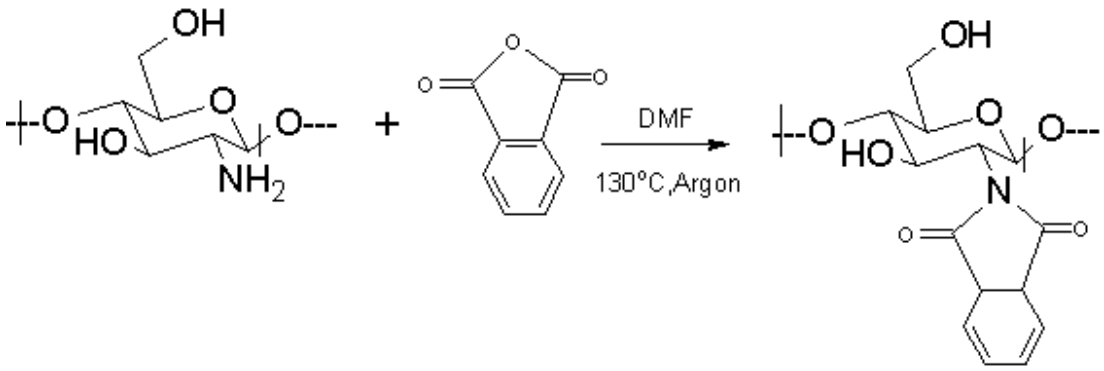
CHAPTER 3

RESULTS AND DISCUSSION

3.1 Formation and Characterization of N-Phthaloylchitosan

N-phthaloylchitosan is prepared by reacting chitosan with phthalic anhydride as shown in Scheme 3.1. Free amine group of chitosan reacts with the carbonyl group of phthalic anhydride. In this way, the amine group is protected.

The product was characterized by FTIR spectrometry. The FTIR spectrum of (a) chitosan (b) phthaloylchitosan is given in Figure 3.1.



Scheme 3.1: Phthaloylation of chitosan

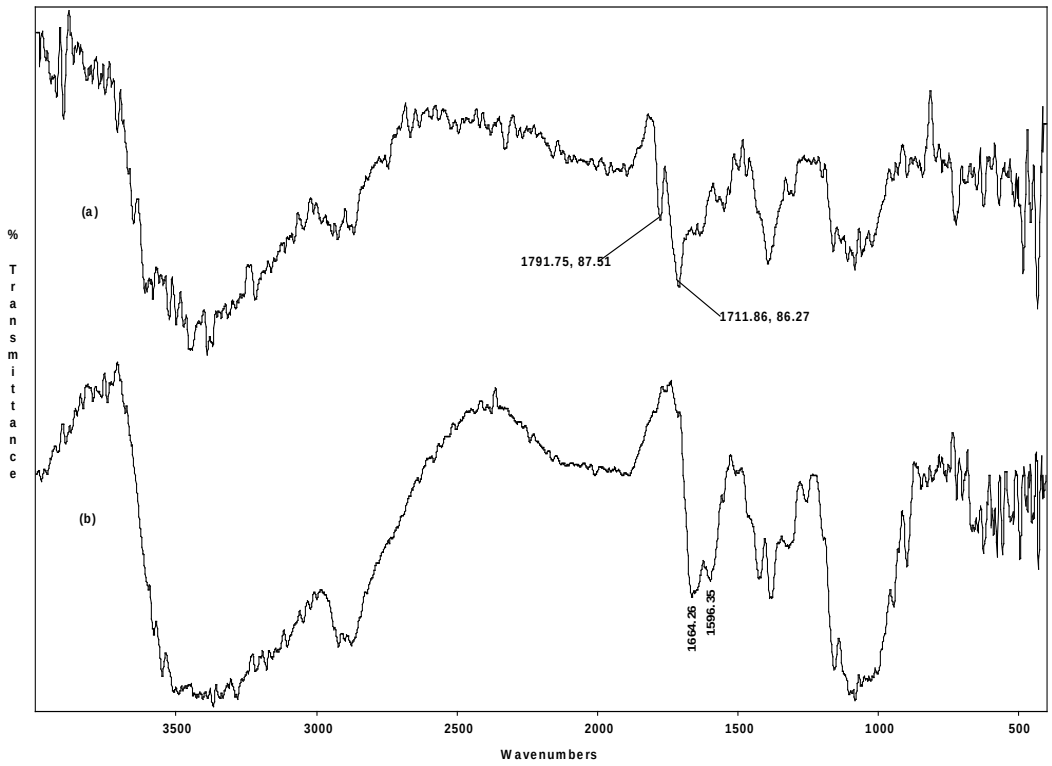


Figure 3.1: (a) Chitosan (b) phthaloylchitosan

The product is examined by FTIR spectroscopy method. Distinct carbonyl peak (ex: 1664, 1596 cm^{-1}) can be observed at chitosan spectrum (Figure 3.1a). In addition, anhydride peaks (ex: 1776, 1708 cm^{-1}), cyclic (5 rings) peaks (ex: 1860, 1773 cm^{-1}) and cyclic (5 rings conjugated) peaks (ex: 1830, 1763 cm^{-1}) that represent phthaloyl group can be observed at product spectrum (Figure 3.1b).

The degree of phthaloylation of the phthaloylated product was found to be 46% (w/w) by using the FTIR method and FTIR calibration curve given in Appendix B. This corresponds to 48 mole percent of phthaloyl groups meaning that almost 1:1 phthaloylation of every chitosan ring was achieved.

3.2 Phosphorylation of N-Phthaloylchitosan

Phosphorylation of N-phthaloylchitosan was studied by changing the reaction conditions. The results have been summarized in Table 3.1.

Table 3.1: Optimization of Phosphorylation Conditions of N-Phthaloylchitosan

Reagents	**Products**
Phosphoric acid	The greatest part of the product consists of brown particles which are not soluble in water and the rest are white particles which are not soluble in water either. (Figure 3.2.a)
Penta sodium tripoly phosphate	Water insoluble cream colored particles were obtained. (Figure 3.2.b)
Penta sodium tripoly phosphate at pH:2	A lot of brown particles which are not soluble in water and a lot of water soluble white particles have been observed. (Figure 3.2.c)
Penta sodium tripoly phosphate and urea	A great white solid bulk that is water soluble and a lot of brown particles that are not soluble in water have been observed. (Figure 3.2.d)
Penta sodium tripoly phosphate and urea at pH:2	A lot of water insoluble brown particles that contain a lot of water soluble white particles have been observed. (Figure 3.2.e)
Penta sodium tripoly phosphate, urea and EGDE	Insoluble powder particles have been obtained. (Figure 3.2.f)

The optical pictures of the products obtained are shown in Figure 3.2. The white precipitate obtained in all cases has been identified as phosphate or polyphosphate bearing products not bound to chitosan whereas the brown powder obtained is the phosphorylated N-Phthaloylchitosan as will be described below. The FTIR spectra of TPP and the white precipitate are compared to each other in Figure 3.3 (a) and (b) respectively. The FTIR spectrum of the white precipitate is almost identical with that of TPP.

(a) Phosphorylation of phthaloylchitosan with phosphoric acid

(b) Phosphorylation of phthaloylchitosan with sodium triphosphate ($Na_5P_3O_{10}$)

(c) Phosphorylation of phthaloylchitosan with sodium triphosphate ($Na_5P_3O_{10}$) at pH 2

(d-1) Phosphorylation of phthaloylchitosan with sodium triphosphate ($Na_5P_3O_{10}$) at pH 2 in urea medium (brown colour particles)

(d-2) Phosphorylation of phthaloylchitosan with sodium triphosphate ($Na_5P_3O_{10}$) at pH 2 in urea medium (white particles)

(e) Phosphorylation of crosslinked phthaloylchitosan with sodium triphosphate ($Na_5P_3O_{10}$) in urea medium

Figure 3.2: The optical pictures of the products

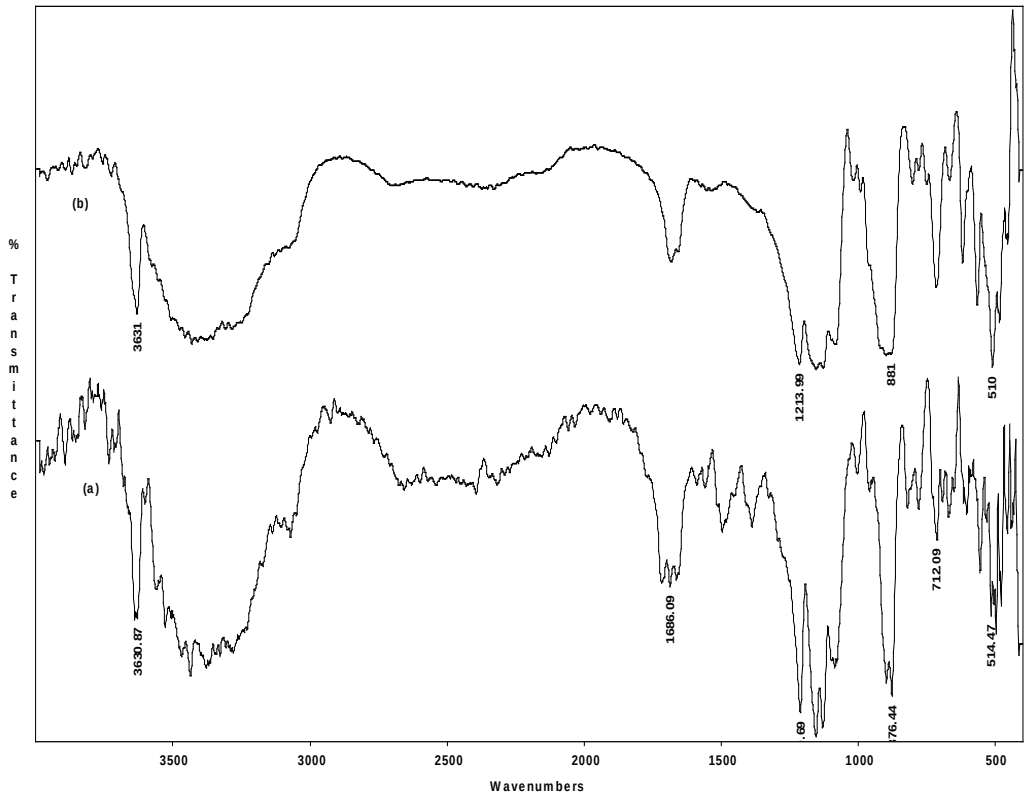
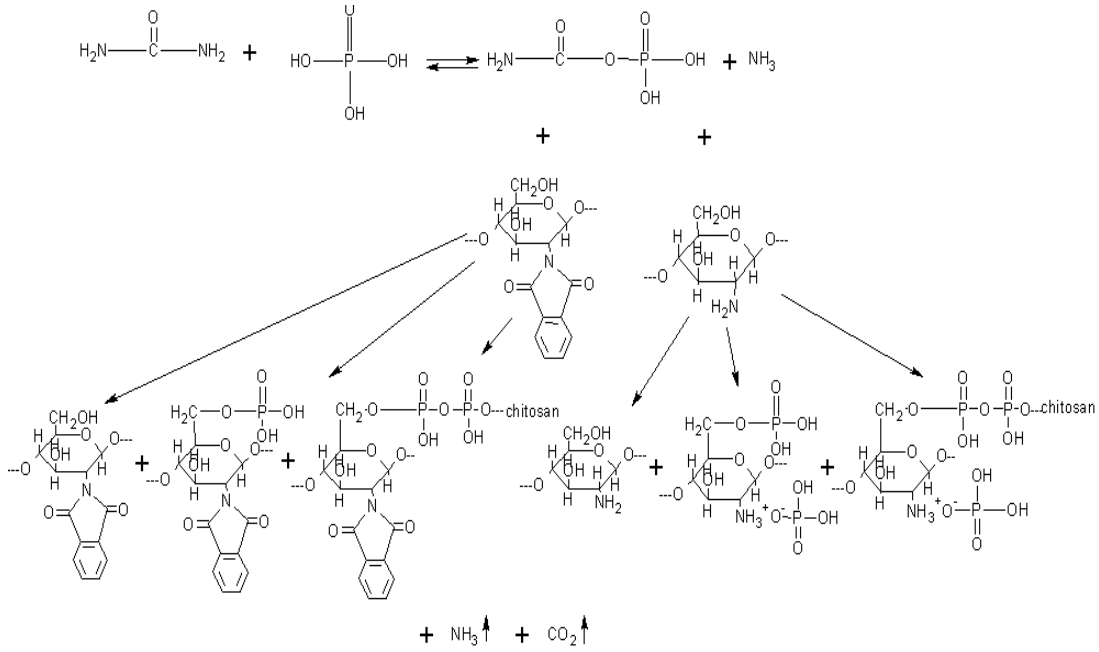


Figure 3.3: (a) TPP (b) white precipitate obtained in all cases

3.2.1 N-Phthaloylchitosan phosphate Ester

The preparation of phosphorylated N-phthaloylchitosan was carried out using phosphoric acid as the phosphorylating agent in the presence of urea. The reaction scheme is shown in Scheme 3.2. Water insoluble white and brown colored particles were obtained and this product is examined by FTIR spectroscopy method. The FTIR spectrum of (a) N-Phthaloylchitosan (b) N-Phthaloylchitosan phosphate are given in Figure 3.4. In addition to characteristic phthaloylchitosan peaks, the phosphate group exhibits itself by the vibrations at 872 cm^{-1} and 722 cm^{-1}.



Scheme 3.2: Phosphorylation of N-phthaloylchitosan and chitosan by using phosphoric acid

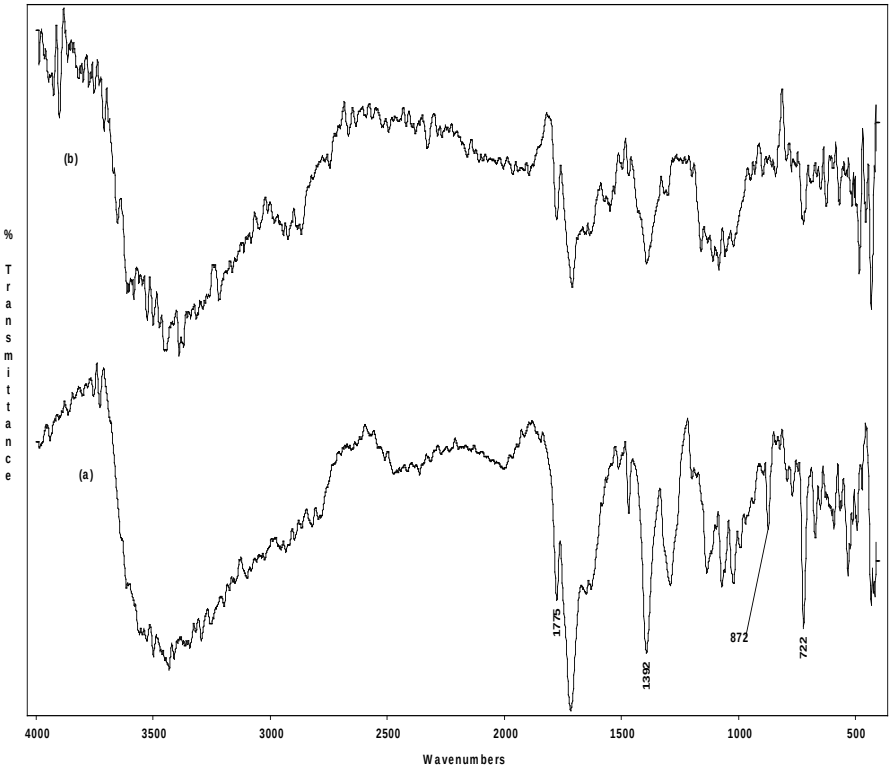


Figure 3.4: (a) N-Phthaloylchitosan (b) N-Phthaloylchitosan phosphate

3.2.2 N-Phthaloylchitosan – Triphosphate Ester

The preparation of phosphorylated N-protected chitosan with sodium triphosphate ($Na_5P_3O_{10}$) was carried out by reacting N-phthaloylchitosan with TPP in DMF at 70 C in the presence or in the absence of urea. The reaction proceeds as shown in Scheme 3.2. except that the phosphorylating agent is TPP instead of phosphoric acid. The FTIR spectrum of (a) N-phthaloylchitosan triphosphate and (b) N-phthaloylchitosan obtained in the presence of urea are given in Figure 3.5. The product is examined by FTIR spectroscopy method.

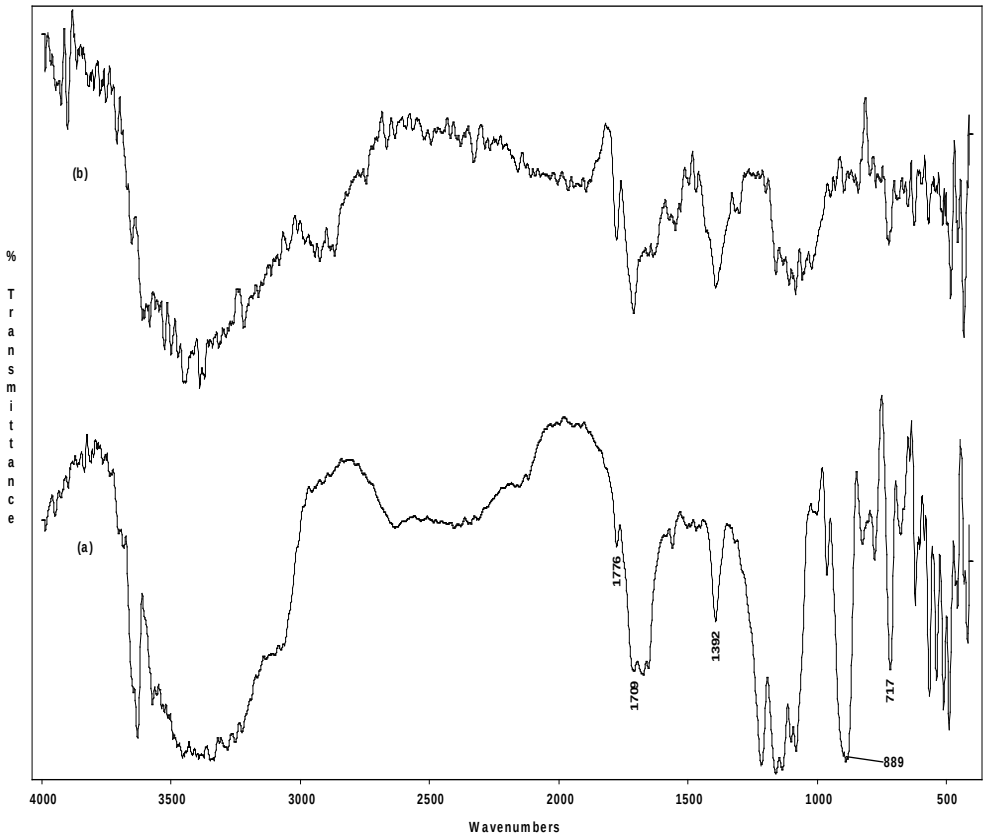


Figure 3.5: (a) N-phthaloylchitosan triphosphate (b) N-phthaloylchitosan

In Figure 3.5 phosphate peaks (ex: a broad, strong peak at 889 cm^{-1} and 717 cm^{-1}) can be observed. This indicates that chitosan was successfully phosphorylated by the above described method.

3.2.3 N-Phthaloylchitosan with sodium triphosphate ($Na_5P_3O_{10}$) in the HCl medium

Similar results to those explained in Section 3.2.2 were obtained. However, polyphosphates were formed rather than phosphate esters of chitosan. This was attributed to the protonation of $H_3P_3O_{10}^{2-}$ ion of triphosphate at pH=2. Similar products were obtained either in the presence or absence of urea.

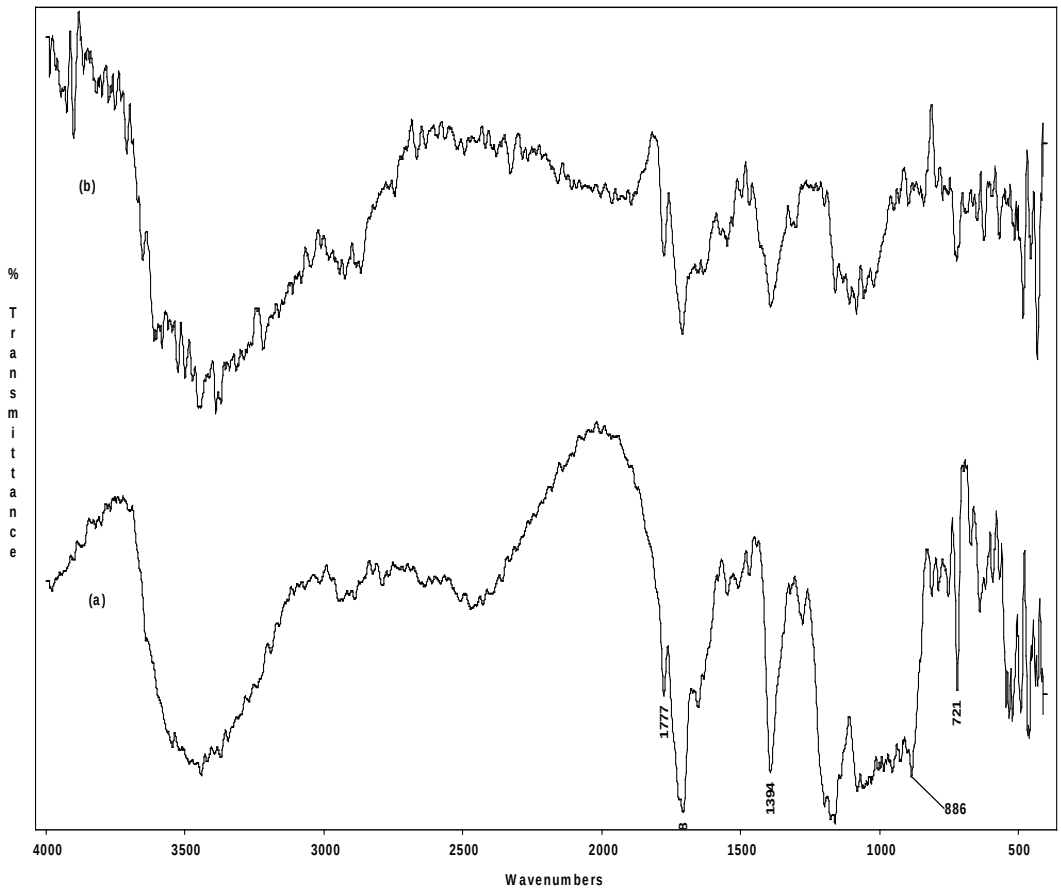


Figure 3.6: (a) N-phthaloylchitosan triphosphate in HCl medium

(b) N-phthaloylchitosan

3.2.4 N-Phthaloylchitosan - Sodium Triphosphate ($Na_5P_3O_{10}$) - Urea – EGDE Ester

In order to improve the stability of the phosphorylated product in aqueous medium, crosslinking with EGDE was applied. The preparation of phosphorylated N-protected chitosan with sodium triphosphate ($Na_5P_3O_{10}$) in urea-EGDE medium was carried out by dispersing N-protected chitosan in DMF and heated to 70 ºC. Calculated amount of EGDE was added and dispersed for two hours. At the end of this duration

urea was added. Meanwhile TPP solution was prepared and heated to 70ºC. After that all of the TPP solution was added into the N-phthaloylated chitosan-DMF-urea-EGDE solution slowly at 70°C and the reaction was continued for four hours. After completing the reaction, the mixture was washed in ethyl alcohol and dried. The product is examined by FTIR spectroscopy method.

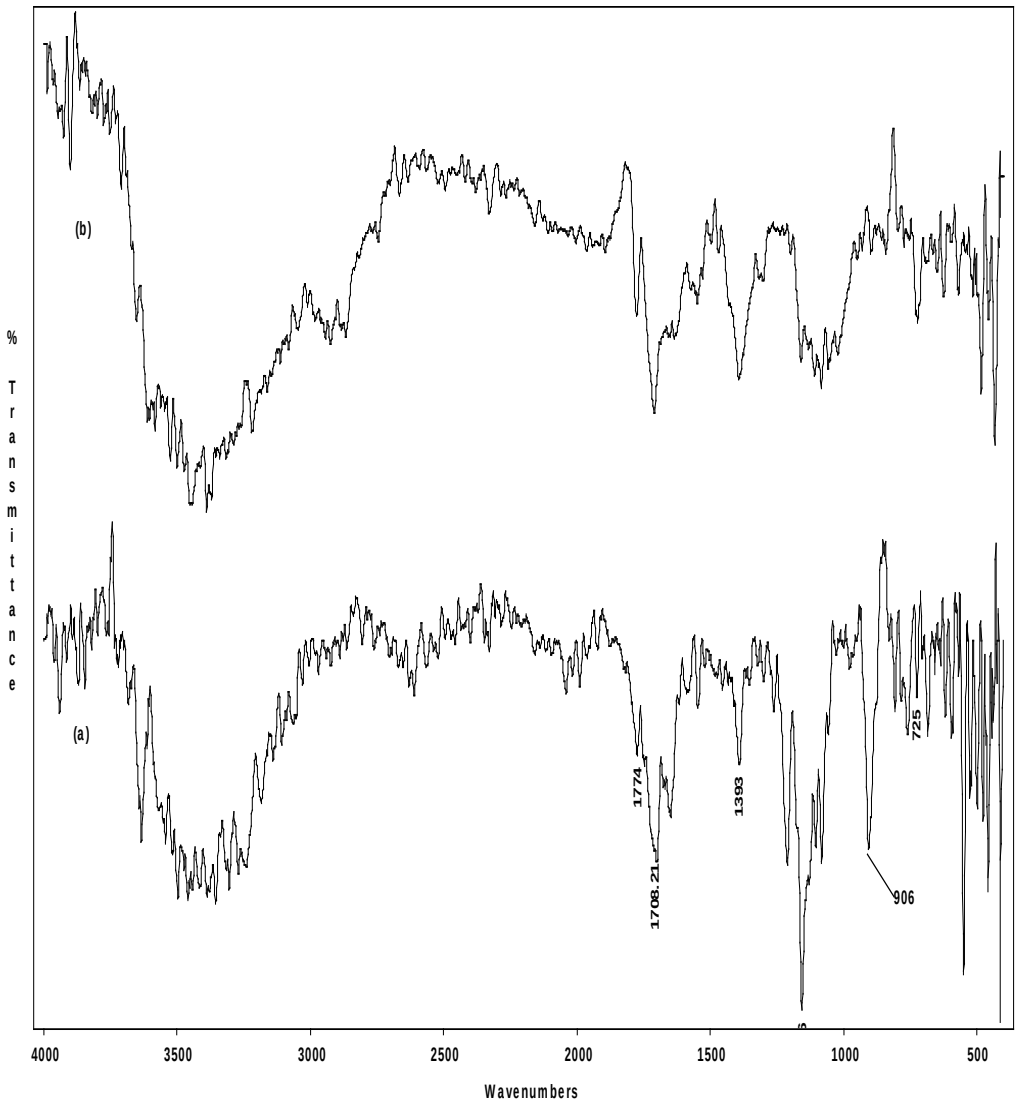


Figure 3.7: (a) N-Phthaloylchitosan - TPP - Urea – EGDE Ester

(b) N-Phthaloylchitosan

In Figure 3.7 phosphate peaks (906 cm^{-1} and 726 cm^{-1}) can be observed. This indicates that chitosan was successfully phosphorylated by the above described method. The decrease in the transmittance of the –OH peak at 1393 cm^{-1} is an evidence of the crosslinking reaction between the –OH groups of chitosan and the ether groups of EGDE. As the amine groups of chitosan are protected, the crosslinking reaction proceeds via the alcohol groups of chitosan.

3.2.5 Dephthaloylation of N-Phthaloylchitosan - Sodium Triphosphate ($Na_5P_3O_{10}$) - Urea - EGDE Ester

Phthaloyl-protected graft copolymer was stirred in DMF and heated to 100 ºC under argon atmosphere. Calculated hydrazine monohydrate was added and the reaction was continued for 3h to deprotect the phthaloyl group. The yellow solution was allowed to cool to room temperature. Then the precipitate was collected, washed thoroughly with ethanol and dried to obtain the final product.

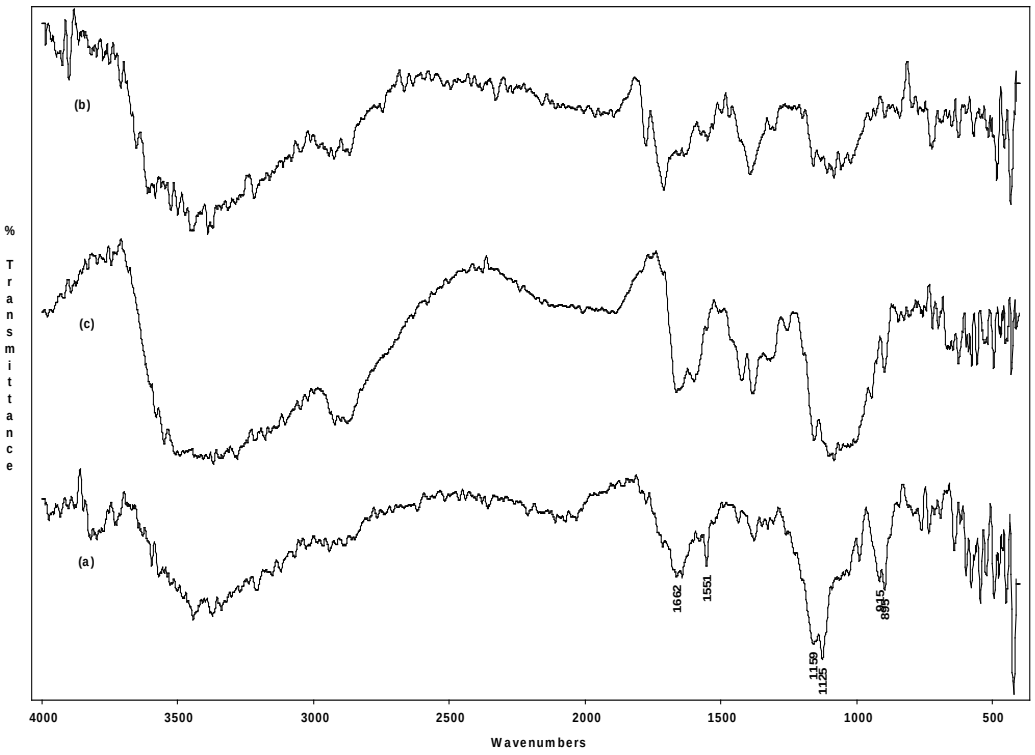


Figure 3.8: (a) Dephthaloylated chitosan - TPP - Urea - EGDE

(b) N-Phthaloylchitosan (c) Chitosan

In the spectrum of the dephthaloylated product, at 1662 and 1590 cm^{-1} the amide groups of chitosan can be observed. The amine groups at 1551 cm^{-1} are available indicating that the deprotection reaction has successfully occurred. The phosphate groups are also observable at 849 cm^{-1} and 890 cm^{-1}.

3.3 Fe^{3+} Adsorption Experiments

3.3.1 Fe^{3+} Adsorption on Chitosan at pH 1.2

A 1g of medium (MM_w) molecular weight chitosan sample (powder form) was placed in a $1x10^{-2}$ M 250 ml aqueous $FeCl_3$ solution at pH 1.2 and stirred 60 rpm at 20°C for 7 hours. 1 mL aliquots were taken in a 10 mL flask, added 1 mL 5-sulfosalicylic acid dehydrate and completed to 10 mL with pH 1.2 buffer solution in predetermined time intervals and analyzed for Fe^{3+} concentration by visible spectrophotometry. At the end of this analysis, the graph on Figure 3.9 was obtained. Figure 3.9 shows absorbance at 505 nm versus contact time. According to this graph, the absorbance values of the aliquots taken from the solution in contact with chitosan remain constant at an average value of 1.0 throughout the experiment which corresponds to an Fe^{3+} concentration of 0.8×10^{-3}. This result indicates that there is an almost instantaneous adsorption of Fe^{3+} onto chitosan that reaches equilibrium fast. This is due to the high concentration of the Fe^{3+} solution used. The adsorption sites on chitosan become fully saturated rapidly under these conditions.

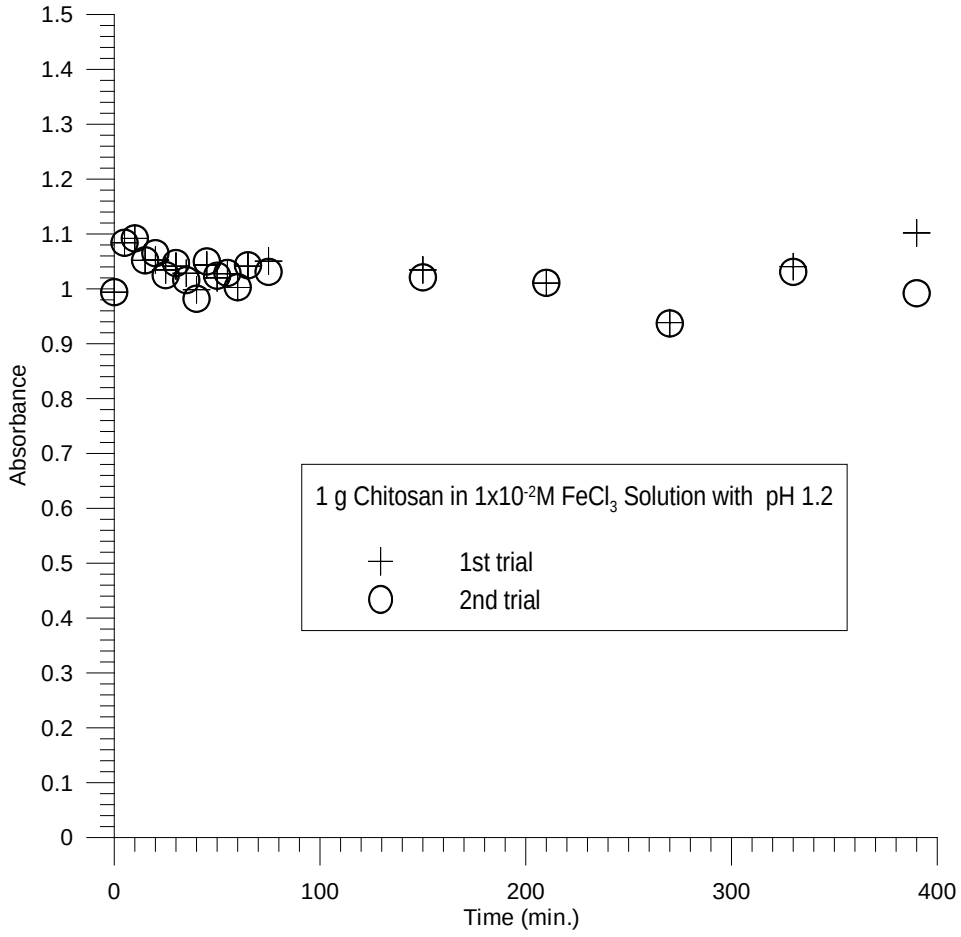


Figure 3.9: Fe^{3+} Adsorption on Chitosan at pH 1.2

At longer contact times chitosan dissolves in the pH 1.2 medium and becomes ineffective as an adsorbent. Since the solution concentration was too high and the solution pH was too low, the experiment was repeated in $1x10^{-3}$ M solution at a pH of 5.

3.3.2 Fe^{3+} Adsorption on Chitosan at pH 5

1g of medium (MM_w) molecular weight chitosan sample (powder form) was placed in a $1x10^{-3}$ M 250 ml aqueous $FeCl_3$ solution at pH 5 and stirred 60 rpm at 20°C for 4 hours. 1 mL aliquots were taken in a 10 mL flask, added 1 mL 5-sulfosalicylic acid dehydrate and completed to 10 mL with pH 5 buffer solution in predetermined time intervals and analyzed for Fe^{3+} concentration by visible spectrophotometry. At the end of this analysis, the graph on Figure 3.10 was obtained. As seen on this graph, an increase in Fe^{3+} adsorption, measured as a decrease in solution absorbance, was observed. As the result obtained in this experiment is as desired, all products were analyzed under these conditions. It is important to note that at 250 minutes contact time desorption occurs leading to an increase in the absorbance value of the sample solution. The equilibrium is reached at an absorbance value of 0.08 which corresponds to 0.02×10^{-3} M. The equilibrium adsorption capacity of chitosan under these conditions was calculated to be 14 mg Fe^{3+}/gchitosan. The equation $Y = 1.91 X 10^3 * X + 0.0619$ was used for this calculation.

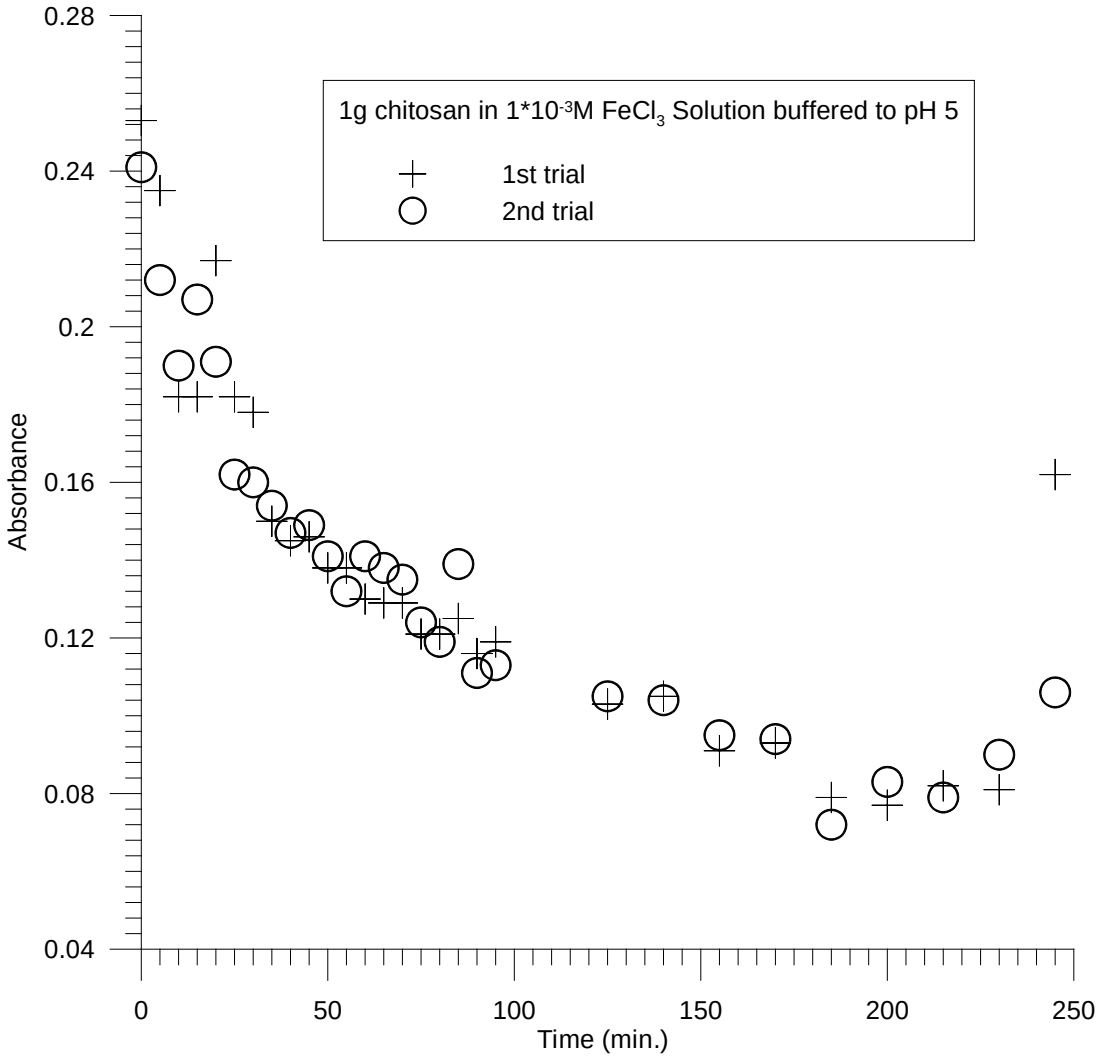


Figure 3.10: Fe^{3+} Adsorption on Chitosan at pH 5

3.3.3 Fe^{3+} Adsorption on N-Phthaloyl Phosphorylated Chitosan at pH 5

0.25g of phosphorylated phthaloylchitosan sample (powder form) was placed in a $1x10^{-3}$ M 250 ml aqueous $FeCl_3$ solution at pH 5 and stirred to 60 rpm at 20°C for 4 hours. 1 mL aliquots were taken in a 10 mL flask, 1 mL 5-sulfosalicylic acid dehydrate was added and completed to 10 mL with pH 5 buffer solution in predetermined time intervals and analyzed for Fe^{3+} concentration by visible spectrophotometry. The graph of Fe^{3+} adsorption on phosphorylated phthaloyl chitosan was shown on Figure 3.11 below. In this experiment, phosphorylated phthaloylchitosan was dissolved instantly in $1x10^{-3}$ M at pH 5 $FeCl_3$ solution. So, the absorbances measured were not taken as reliable indication of Fe^{3+} adsorption. Complex formation between the dissolved sample and Fe^{3+} in solution might have taken place which interfered with the absorbance measurements.

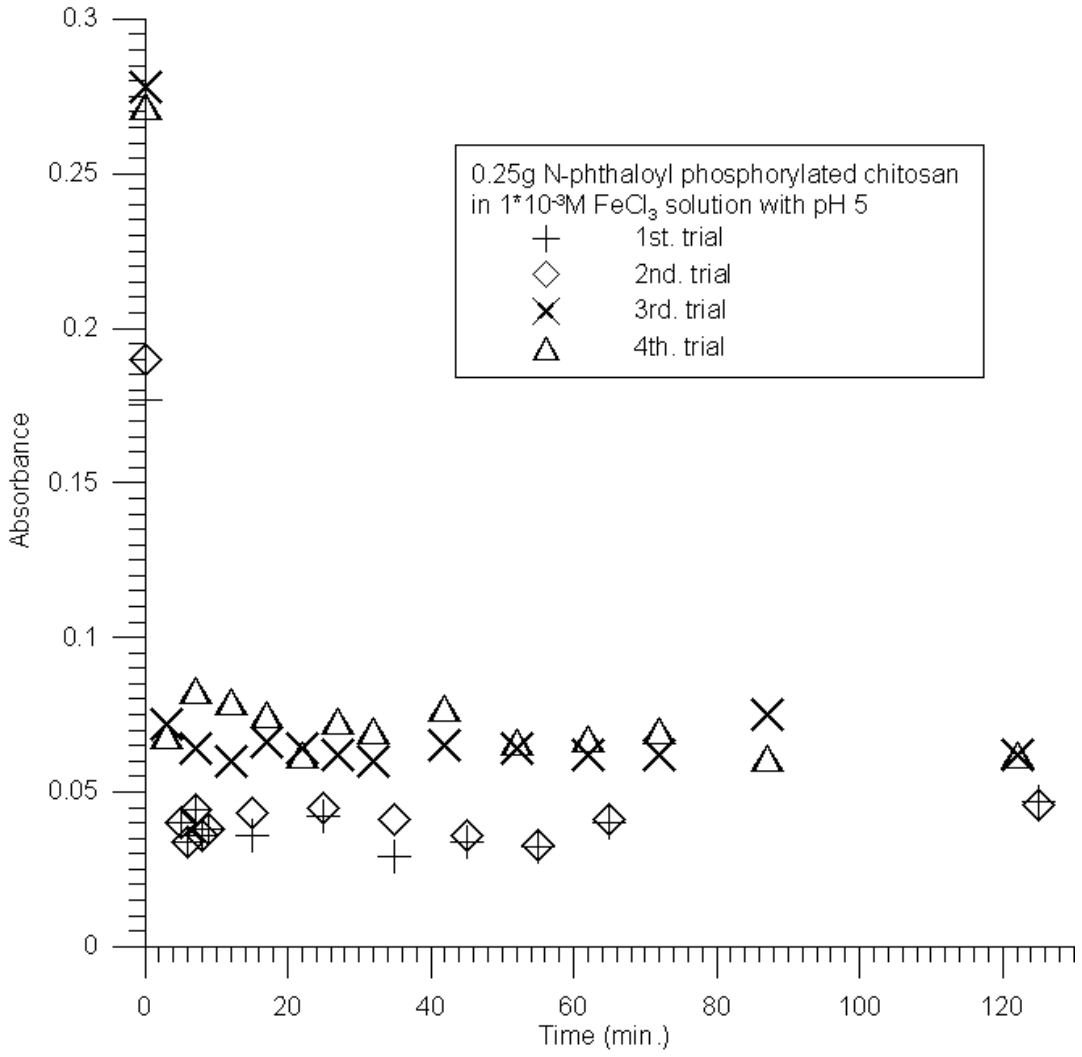


Figure 3.11: Fe^{3+} Adsorption on N-phthaloyl Phosphorylated Chitosan at pH 5

3.3.4 Fe^{3+} Adsorption on Phosphorylated Chitosan Crosslinked with EGDE at pH 5

0.02g sample of phosphorylated chitosan crosslinked with EGDE was placed in a $1x10^{-3}$ M 50 ml aqueous $FeCl_3$ solution at pH 5 and stirred at 60 rpm at 20°C for four and half hours. 1 mL aliquots were taken in a 10 mL flask, 1 mL 5-sulfosalicylic acid dehydrate was added and volume completed to 10 mL with pH 5 buffer solution at one hour intervals and analyzed for Fe^{3+} concentration by visible spectrophotometry. The graph of Fe^{3+} adsorption on phosphorylated and crosslinked phthaloylchitosan was shown on Figure 3.12 below. Because of the dissolution of N-phthaloyl phosphorylated chitosan in $1x10^{-3}$ M 250 ml aqueous $FeCl_3$ solution at pH 5, the product was crosslinked with EGDE and analyzed again. As seen on this graph, a regular decrease in the absorbance value is observed indicating increased adsorption of Fe^{3+} on to the adsorbent. The equilibrium is reached at an absorbance value of 0.12 which corresponds to 2.5×10^{-5} M (0.025×10^{-3} M). The equilibrium adsorption capacity of the product was calculated to be 140 mg Fe^{3+}/g adsorbent.

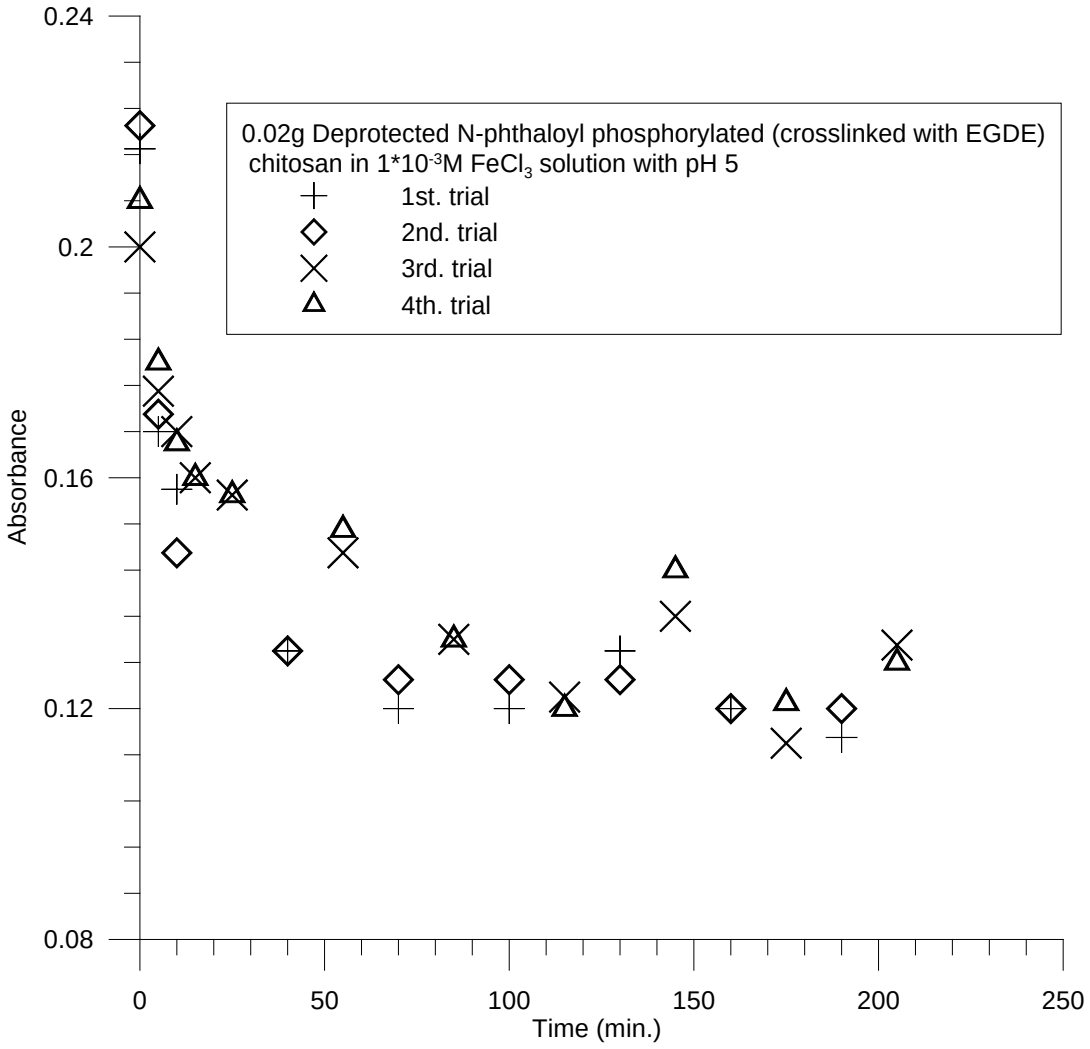


Figure 3.12: Fe^{3+} Adsorption on Phosphorylated Chitosan Crosslinked with EGDE at pH 5

CHAPTER 4

CONCLUSIONS

1) Amine groups of chitosan can successfully be protected by N-phthaloylation.
2) N-phthaloylchitosan can succesfully be crosslinked with calculated EGDE at 70 ºC in order to improve the stability of the product in aqueous medium.
3) Crosslinked N-phthaloylated chitosan can succesfully be phosphorylated by using sodium triphosphate ($Na_5P_3O_{10}$) solution at 70 ºC in the presence of urea.
4) The deprotection can succesfully be carried out on EGDE-crosslinked N-phthaloylated chitosan by using hydrazine monohydrate. Deprotection could not be achieved on the other products. In those products, deprotection conditions also resulted in dephosphorylation.
5) EGDE–crosslinked phosphorylated chitosan rich in amine groups can succesfully be applied as an Fe^{3+} adsorbent in aqueous solution at pH=5.
6) The equilibrium adsorption capacity of the EGDE-crosslinked phosphorylated chitosan is 140 mg Fe^{3+}/g adsorbent at pH=5. This value is ten times better than the equilibrium adsorption capacity of chitosan which is 14 mg Fe^{3+}/g chitosan under the same conditions.

REFERENCES

[1] Burke A., "Chitosan: An Iron – Chelating Agent and a Controlled Release System for Iron – Chelating Drugs, Desferal and Deferiprone", Ph.D. Thesis, Eastern Mediterranean University, (2002)

[2] A. Burke, E.Yilmaz, N.Hasirci, O. Yilmaz, "Iron (III) Ion Removal from Solution through Adsorption onto Chitosan" *Journal of Applied Polymer Science*, 84, 1185-1192 (2002)

[3] Semsarilar M., "*Preparation and Characterization of Chitosan Gel Beads*", M.S. Thesis, Eastern Mediterranean University, (2006)

[4] Yalınca Z., "Iron (III) Adsorption onto Chitosan Gel Beads", MSc thesis, Department of Chemistry, Eastern Mediterranean University / Turkey

[5] Yılmaz, E.. "Chitosan: A versatile biomaterial" *Advances in experimental medicine and biology* ISSN 0065-2598, vol. 553, pp. 59-68, (2004)

[6] R.A.A. muzzarelli, Human enzymatic activities related to the therapeutical administration of chitin derivatives, Cell. Mol. Biol. Life Sci. 53, 1997, 131

[7] H.F. Mark, N.M. Bikales, C.G.Overberger, G. Menges (Eds.), Encyclopedia of Polymer Science and Engineering, vol.1, Wiley, New York, 1985, 20

[8] S. Miyazaki, K.Ishii, T.Nadai, The use of chitin and chitosan as drug carriers, Chem. Pharm. Bull. 33, 1985, 3986

[9] Borchard, G., 2001, Chitosan for gene delivery, *Advanced Drug Delivery Reviews*, 52: 145-150

[10] Liu, W., G., and Yao, K.D., 2002, Chitosan and its derivatives-a promising non-viral vector for gene transfection, *J.Controlled Release*, 83:1-11

[11] Van der Lubben, I.M, Verhoef, J.C., Borchard, G., and Junginger, H.E., 2001, Chitosan for mucosal vaccination, *Adv. Drug.Delivery Reviews*, 52: 139-144.

[12] Illum, L., Jabbal-Gill, I., Hinchcliffe,, M,. Fisher, A.N, Davis, and M.S.S., 2001, Chitosan as a novel nasal delivery system for vaccines, Adv. Drug.Delivery Reviews, 51: 81-96.

[13] Suh, J.K.F., and Matthew, H.W.T., 2000, Application of chitosan-based polysaccharide biomaterials in cartilage tissue engineering: a review. Biomaterials, 21:2589-2598.

[14] M.L. Markey, M.L. Bowman, M.V.W. Bergamini (Eds.), Chitin and chitosan, Elsevier Appl. Sci., London, 1989, 713

[15] G. Mckay, H.S. Blair, J.R. Gardner, Adsorption of dyes on chitin. I. Equilibrium studies, J.Appl. Polym. Sci. 27, 1989, 3043

[16] T.C. Yang, R.R.Zall, I & EC Product R & D 23, 1984, 68

[17] G. Allan, G.D. Crospy, J.H. Lee, in: Proceedings of a Symposium on man-made polymers in Paper Making, Helsinki, Finland, 1972.

[18] Nishimura, S. I., Kohgo, O., Kurita, K., & Kuzuhara, H. (1991). Chemospecific manipulations of a rigid polysaccharide: Synthesis of novel chitosan derivatives

with excellent solubility in common organic solvents by regioselective chemical modifications. Macromolecules, 24(17), 4745–4748.

[19] Nishimura, S.; Kohgo, O.; Kurita, K. & Kuzuhara H. Macromolecules 1991, 24, 4745–4748.

[20] Kurita, K.; Ikeda, H.; Yoshida, Y.; Shimojoh, M.; Harata, M. Biomacromolecules 2002, 3, 1–4.

[21] Amaral I. F., Granja P. L., Melo L. V., Saramago B., Barbosa M. A., "Functionalization of chitosan membranes through phosphorylation: Atomic force microscopy, wettability, and cytotoxicity studies", *Journal of Applied Polymer Science*, Volume 102 Issue 1, Pages 276 – 284, (2006)

[22] Sakaguchi, T.; Hirokoshi, T.; Nakajima, A.; *Agric. Biol. Chem.* 1981, 45, 2191.

[23] Yalpani, M. *Carbohydr. Polym*. 1992, 19, 35.

[24] Jung, B. O.; Kim, C. H.; Choi, K. S.; Lee, Y. M.; Kim, J. J.; *J. Appl. Polym. Sci*. 1999, 72, 1713.

[25] Heras, A.; Rodriguez, N. M.; Ramos, V. M.; Agullo, E.; *Carbohydr. Polym*.2001, 44, 1.

[26] Nishi, N.; Maekita, Y.; Nishimura, S.; Hasegawa, O.; Tokura, S.; *Int. J. Biol.Macromol*. 1987, 9, 109.

[27] Wan Y., Creber K. A. M., Peppley B., Bui V. T., "Synthesis, Characterization and Ionic Conductive Properties of Phosphorylated Chitosan Membranes", *Macromolecular Chemistry and Physics*. Volume 204 Issue 5-6, Pages 850 – 858, (2003).

[28] Atkins, P. W., Physical Chemistry; Process at solid surfaces, third edition (1986), 771-781.

[29] Dabrowski, A., Adsorption-From theory to practise, Colloid and İnterface Science, 93 (2001), 135-224.

[30] Piron, E., III; Domard, A. Int J Biol Macromol 1997, 21, 327

[31] Kawamura, Y., Mitsuhashi, M., Tanibe, H., & Yoshida, H. (1993). Industrial and *Engineeering Chemistry Research*, 32, 386

[32] E. Guibal, Interactions of metal ions with chitosan-based sorbents: a review, Sep. Purif. Technol. 38 (2004) 43-74.

[33] K. Kurita, T. Sannan, Y. Iwakura, Studies on chitin. VI. Binding of metal cations, J.Appl. Polymer. Sci. 23 (1979) 511-515.

[34] Varma A.J., Deshpande S.V., Kennedy J.F. "Metal complexation by chitosan and its derivatives: A review" *Carbohydrate Polymers*, 55 (1), pp. 77-93 (2004).

[35] Deans J. R., & Dixon, B. G. (1992) Water Research, 26 469-472.

[36] Peniche-Covas, C. Alvarez, L. W., & Arguelles-Monal, W. (1992). *Journal of Applied Polymer Science*, 46, 1147-1150

[37] McKay, G., Blair, H.S., & Findon, A. (1989). Indian Journal of Chemistry, 28(A), 356-360

[38] Koyama, Y., & Taniguchi, A. (1986). *Journal of Applied Polymer Science*, 31(6), 1951-1954.

[39] Hsien, T.Y., & Rorrer, G.L.(1997). *Industrial and Engineering Chemistry Research*, 36(9), 3631-3638.

[40] Rorrer, G.L., Hsien, T. Y., & Way, J.D. (1993). *Industrial and Engineering Chemistry Research*, 32(9), 2170-2178.

[41] Guibal, E., & Milot, C. (1998). *Industrial and Engineering Chemistry Research*, 37, 1454.

[42] Tikhonov, V. E., Radignina, L. A., & Yamskov, A. (1996). *Carbohydrate Research*, 290(1), 33-41.

[43] Sakaguchi, T.; Hirokoshi, T.; Nakajima, A.; *Agric. Biol. Chem.* 1981, 45, 2191
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APPENDICES

Appendix A: Visible spectrophotometric calibration curve for determination of Iron (III) solution at pH 1.2 and pH 5.

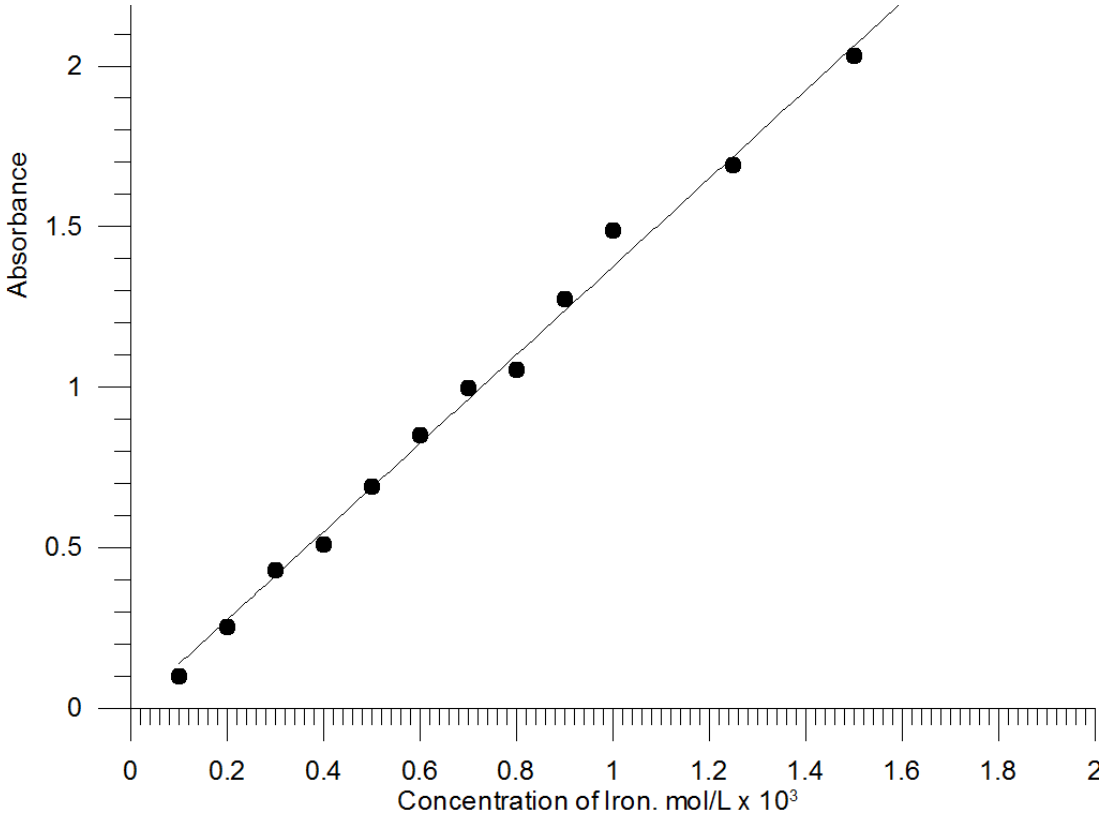

Figure A.1: The calibration curve for determination of Fe^{3+} at pH 1.2

Equation $Y = 1.37 X 10^{3} * X + 0.000222$

Correlation coefficient of determination, R-squared = 0.996404

Wavelength = 505 nm

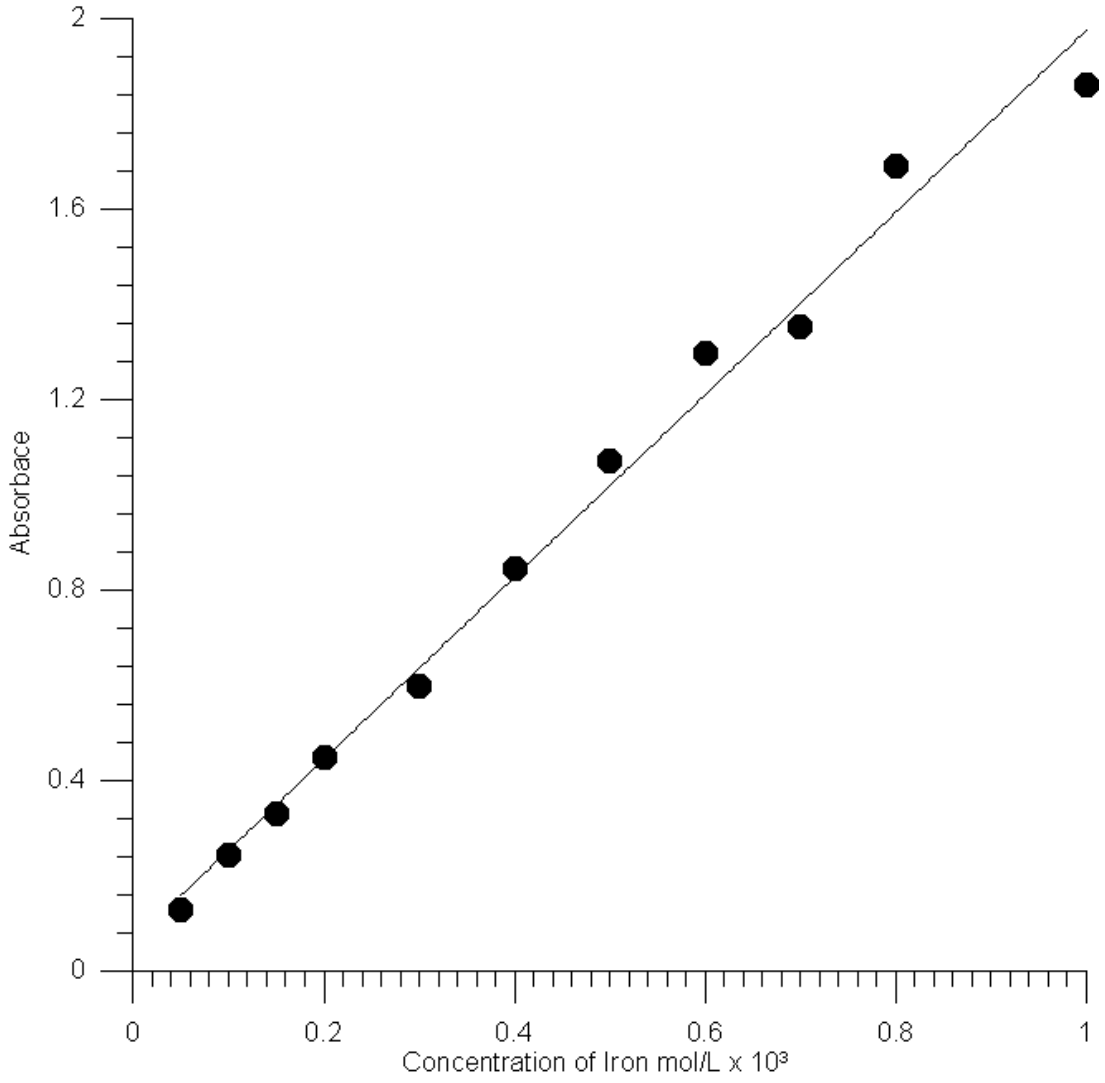

Figure A.2: The calibration curve for determination of Fe^{3+} at pH 5

Equation Y = 1.91 X 10^3 * X + 0.0619

Correlation coefficient, R-squared = 0.989299

Wavelength = 505 nm

Appendix B: Quantitative Determination of Degree of Phthaloylation by FTIR Analysis

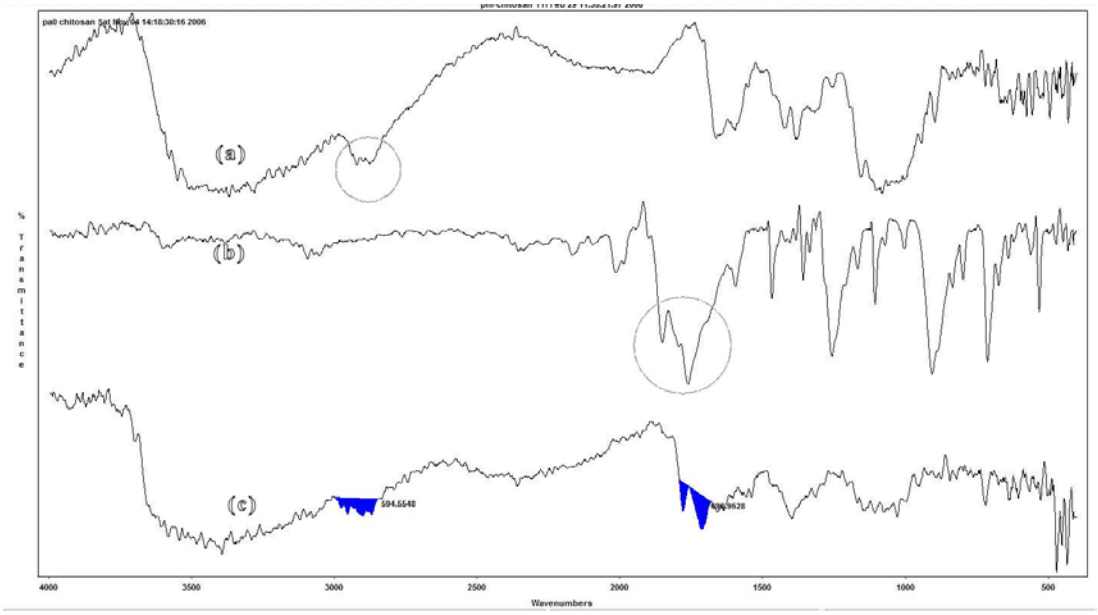

Figure B.1: FTIR Spectra of a) chitosan, b) phthalic anhydride and c) a sample phthaloylated chitosan.

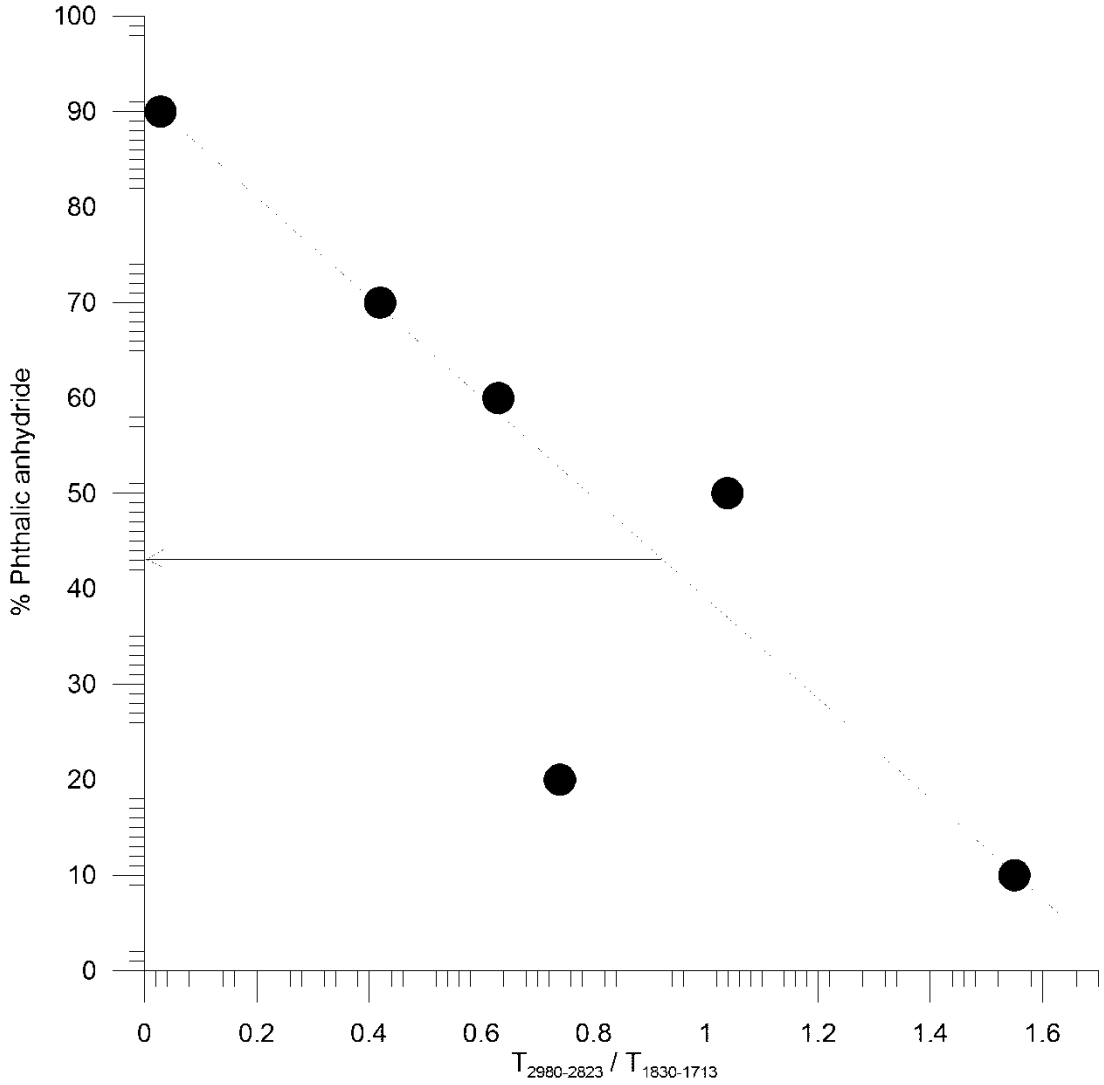

Figure B.2: FTIR Callibration curve for estimation of degree of Phthaloylation