Grafting of Poly (4-Vinyl Pyridine)
onto Chitin under Heterogeneous Conditions

Jihad Mohammed Hadi

Submitted to the
Institute of Graduate Studies and Research
in partial fulfillment of the requirements for the Degree of

Master of Science
in
Chemistry

Eastern Mediterranean University
August 2014
Gazimağusa, North Cyprus

Approval of the Institute of Graduate Studies and Research

<div align="right">

————————————————
Prof. Dr. Elvan Yılmaz
Director

</div>

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

<div align="right">

————————————————
Prof. Dr. Mustafa Halilsoy
Chair, Department of Chemistry

</div>

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.

<div align="right">

————————————————
Prof. Dr. Elvan Yılmaz
Supervisor

</div>

Examining Committee
————————————————

1. Prof. Dr. Elvan Yılmaz   ————————————————

2. Assoc. Prof. Dr. Mustafa Gazi   ————————————————

3. Asst. Prof. Dr. H.Ozan Gülcan   ————————————————

ABSTRACT

Chitin gel beads were prepared, crushed into powder and grafted with poly(4-vinylpyridine) (P4VP) under nitrogen gas by using cerium ammonium nitrate(CAN) as a redox initiator under heterogeneous conditions in toluene. The effect of the amount of chitin, concentration of the monomer (4VP), amount of the initiator (CAN), reaction time and temperature on the grafting percent has been studied. The maximum grafting yield of poly (4-vinylpyridine) on to chitin beads was determined to be 380%. The optimum conditions were 0.10 g of chitin, 10 mL of (4VP) monomer, and 0.50 g of cerium ammonium nitrate (CAN) initiator at 60°C for one hour reaction time. The grafted samples were found to be soluble in aqueous acidic buffer solution (pH=1.2). The grafted and non grafted products were characterized by FTIR spectroscopy, SEM, DSC and C13 NMR analysis.

Keywords: 4-vinylpyridine (4VP), chitin gel, redox initiated grafting.

ÖZ

Kitin jel boncuklar hazırlandıktan sonra ezilerek toz hale getirilmiş ve heterojen ortamda, seryum amonyum nitrat (CAN) başlatıcı kullanılarak azot atmosferinde poli(4-vinil piridin) (P4VP) ile toluen içinde aşılanmıştır. Kitin miktarı, monomer konsantrasyonu, başlatıcı konsantrasyonu, reaksiyon zamanı ve reaksiyon sıcaklığının aşılanma yüzdesi üzerindeki etkisi incelenmiştir. En yüksek aşılanma yüzdesi 0.10 g kitin, 10 mL 4VP, ve 0.50 g CAN kullanılarak 60°C sıcaklıkta bir saatlik reaksiyon süresi sonunda %380 olarak bulunmuştur. P4VP aşılanmış kitin örneklerinin asitli ortamda pH=1.2 tampon çözeltide çözündüğü gözlemlenmiştir. Aşılanmış ve aşılanmamış ürünler FTIR ve C-13 spektroskopisi yöntemleri ile ve DSC ve SEM analizi yapılarak karakterize edilmiştir.

Anahtar Kelimeler: 4-vinilpiridin, kitin jel, redoks başlatıcı, aşılanma.

ACKNOWLEDGEMENT

I would like to express my very great appreciation to my supervisor Prof. Dr. Elvan Yılmaz for her patient, valuable and constructive suggestions, during of this thesis.

I would also like to offer my special thanks to Dr. Zulal Yalinca for her advice and assistance in keeping my progress on schedule.

Finally, I thank almighty to my father, mother, brothers, sisters, and my friends for their caring, support, motivate, and love thank you all.

.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 1

INTRODUCTION

Chitin (β-1,4-linked 2-acetamido-2-deoxy-Dglucose) and its derivative, chitosan (β-1,4-linked 2-amino-2-deoxy-D-glucopyranose) are polysaccharides with ability to form gels, films, fibers, and sponges. These polymers can also be formed into porous matrices for various applications such as controlled drug release systems, enzyme immobilization support, molecular fractionation, and transition-metal adsorption.

Formation of chitin gel beads via coagulation of chitin/DMAc/LiCl system from ethanol has extensively been investigated before.(Yilmaz and Bengisu, 2003).

These gel beads can act as surfaces for polymer grafting and other type of chemical modifications. Formation chitin-*graft*-poly(4-vinylpyridine), Chitin-*graft*-P4VP, gel beads was furthermore studied and synthesis conditions and some properties of these gel beads have been reported (Oylum Hasan; Yilmaz, Elvan,2013). Grafting reaction was carried out in Chitin/DMAc/LiCl solution by redox initiation. Then the grafted product was isolated from solution by coagulation from ethanol. Chitin and chitin-*graft*-poly(4-vinyl pyridine) gel beads obtained in this way proved to act as useful adsorbents for Fe^{3+} removal and cholesterol removal from solution. (Oylum and Yilmaz, 2013)

In this thesis work, a different approach from the previous one has been undertaken to synthesize Chi-*graft*-P4VP gel beads. Chitin gel beads have been obtained and grafting of P4VP has been carried out on these beads. Hence, the aim of this thesis is to optimize grafting conditions of P4VP onto chitin gel beads. The effects of monomer concentration, amount of chitin, initiator concentration, time and temperature on the grafting yield were investigated. The product characterization was carried out using FTIR spectrometry, DSC, C13 NMR and SEM analyses..

1.1 Chitin

1.2 Structure, Properties and Application

Chitin, $(C_8H_{13}O_5N)_n$, is naturally existing polysaccharide of a N-acetylglucosamine derivative as of exoskeletons of arthropods containing crustaceans (crabs, lobsters and shrimps) and moreover as of cell walls of fungi and bug. In addition, seafood diversion industries such as shells of crustaceans, shrimps and crabs are plentiful and applied for the commercial synthesis of chitin. Furthermore, chitin can be produced from krill, insects, clams, jellyfish, algae, oysters, fungus, and crayfish, which between them Krill can be the most useful source in the future (Tamura *et al*., 2004). Cuttle pens also include chitin that is categorized as β-chitin. The difference between α-chitin and β-Chitin lies in the difference between the crystalline structure. β-Chitin has weaker intermolecular forces and the arrangement of polymer layers is parallel in β-Chitin whereas it is antiparralel in α-chitin. β-chitin is receiving increasing attention from the scientists , although it is less abundant and is not yet produced commercially. The chitin as well as chitosan can be found in the cell walls of some fungi (Zygomycetes). Chitin is a linear cationic heteropolymer of randomly distributed N-acetylglucosamine and glucosamine units with β-1,4-linkage. The chemical structure of chitin is shown in Figure 1.
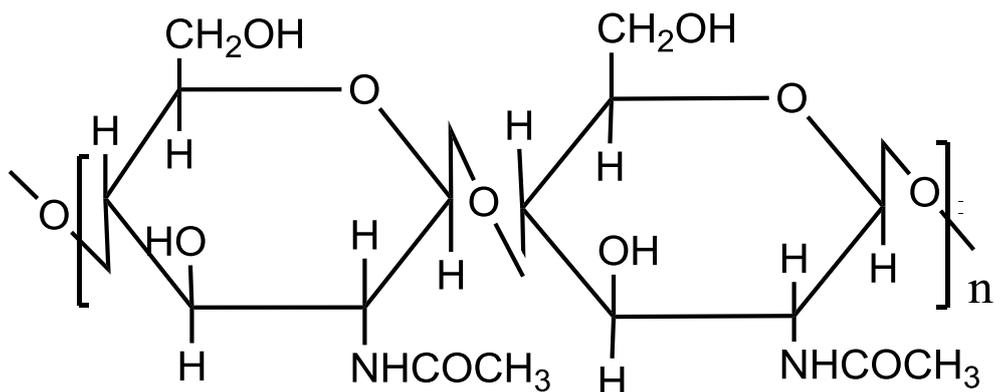
Figure 1. Chemical Structure of Chitin

The solubility of chitin is restricted due the presence of the strong intermolecular hydrogen bonding. It is not soluble in water and common solvents and it is soluble in relatively few special solvents including (hexafluoroacetone, hexaureo-2-propanol) and N,N-dimethylacetamide (DMAc) containing 5–8% LiCl (Rutherford and Austin, 1978). Recently, another solvent system made up of methanol saturated with calcium chloride dihydrate, which is a solvent for nylons, proved to be able to dissolve chitin as well (Tamura *et al.*, 2004).
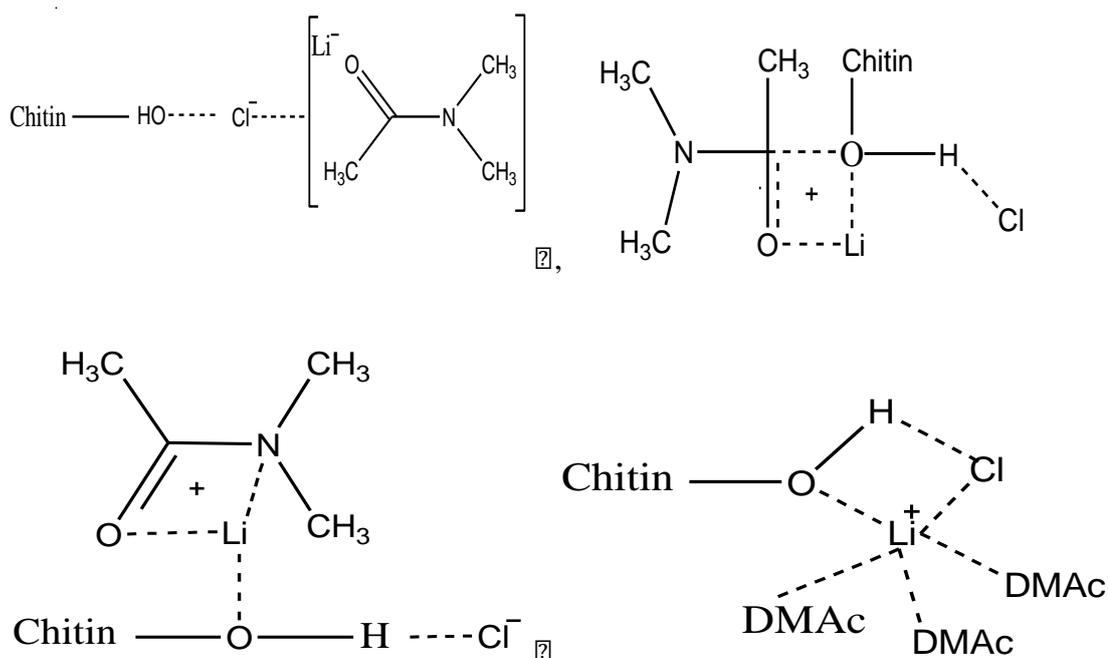
Figure 2. Some Suggested Structures for the Dissolution of Chitin in DMAc-LiCl Solvent System (Oylum, H.,2013).

3

Chitin is a chemically stable biopolymer. The existence in its solid state of both intra- and inter-molecular hydrogen bonds, additionally pose problems inherent to the operating difficulties (Mourya and Inamdar, 2008).

Chitin is a natural multifunctional biopolymer containing hydroxyl and amine groups has considerable properties such as biodegradability, bioactivity, and biocompatibility. On the other hand, owing to an absence of solubility in common solvents some problems arise. For example, if the reactions are done under heterogeneous conditions because of the poor degree of reaction, some problems like structural non uniformity of the products will appear (Kurita, 2001). Subsequently, in several studies the scientist tried to simple modify of chitin that may open an approach to utilize chitin all the more adequately. Therefore, studies on chitin applications are continuing and it can be anticipated that a lot of commercial products will appear on the market soon. However, it is interesting to explore the reactions of chitin under normal conditions. For finding the efficient modifications of chitin, the focus on inflexibility of this polysaccharide will be a basic step. Recently, the scientists reported that chitin can be dissolved in water or organic solvents by proper structural modifications with the demolition of its crystalline structure. Some derivatives of chitin which are soluble reveal remarkable reactivities and can be useful for simplistic modification reactions (Kurita, 2001).

Due to the characteristic crystalline structures with physically powerful intermolecular forces, chitin and chitosan are not accessible as potential reactants like cellulose. Therefore, the modifications of chitin and chitosan often will be done under heterogeneous conditions with some exclusion in solution. It is fundamental to control the reactions of chitin in a well-controlled manner for preparation of

derivatives with well-defined structures and developed greater efficient materials. Therefore, the efficient modification reactions have been done using β-chitin, chitosan, and some soluble derivatives as reaction originators (Watthanaphanit *et al.,* 2008).

Chitin is harmless, antibacterial, and biodegradable, and consequently more environmentally friendly than most macromolecules. Scientist began to study the chitin in 20th century. Biological activity of chitin makes it an attractive material which can be formed into fibers that can be used for biomedical applications. Chitin accelerates healing times of wounds, and chitin sutures give cosmetic seams which dissolve away in a patient's body within the period sufficient for complete therapy of a wound. However, insufficient elasticity of chitin sutures prevents commercial production of resolving chitin based surgical seam materials in world practice (Mikhailov and Lebedeva, 2005).

1.3 Grafting of Polymers onto Chitin

1.3.1 Chitin-*graft*-Poly(Vinyl Acetate)

Vinyl acetate monomer was grafted onto chitin using cerium ammonium nitrate (CAN) as a redox initiator. The emulsion polymerization technique was used for graft copolymerization of pure poly(vinyl acetate) (PVAc)onto chitin. Acetone extraction was used to remove the PVAc homopolymer. The grafting percentage and grafting efficiency were determined by gravimetry. The scanning electronic microscope and also Transmission were used to observe the morphologies of the grafted product. The experimental outcomes detected that graft copolymerization, which occurred on the surface of chitin particles, at initial stage of reaction, dominated over the emulsion polymerization. After that, the rate of emulsion

polymerization increased greatly and became dominant. The rate of polymerization increased by the occurrence of chitin not only provided the stability of the emulsion particles. The grafted product was improved with the techniques of FTIR and DSC. (Don *et al.,* 2002)

1.3.2 Chitin-*graft*-P4VP

(4VP) monomer grafted on to chitin using potassium persulphate ($K_2S_2O_8$) (KPS) with a redox initiator to form chitin-*g*-P4VP. The reaction was carried out under homogeneous conditions. The product was coagulated from solution in the form of beads. The effect of monomer concentration on the extent of grafting (%G) was studied by the gravimetric method. Chitin-*graft*-P4VP was characterized by SEM, TGA, XRD and FTIR analyses. Grafted chitins were coagulated from the non-solvent ethanol in the form of beads. In addition the grafted product improved by iron adsorption capacity (Fe3+), the chitin-*graft*-P4VP were observed to have higher adsorption capacities than chitin beads due to a microporous bead surface and chemical modification. (Oylum *et al.* 2013).

1.3.3 Synthesis, Characterization and Sorption Properties of N-(2-(2-pyridyl) ethyl) Chitosan

PE-chitosan with substitution degrees (DS) up to 1.2 has been developed using the direct addition reaction between chitosan and 2-VP. The maximum sorption capacities of PE-chitosan in 0.1M Hydrochloric acid solution were expected as 5.56 mmol/g, 3.67 mmol/g and 2.75 mmol/g for Au(III), Pd(II) and Pt(IV) respectively. Sorption capacities of the sample for transition metal ions neutral and acid medium were 1.5–2.6 higher than of chitosan with the highest values attained for Cu(II) 1.50 mmol/g and Ag(I) ions 1.53 mmol/g. The produced sample was proved for atomic

absorption spectroscopy analysis application of solutions with low gold content (Bratskaya *et al*., 2011).

1.3.4 Chitin, Chitin–Hydroxyapatite, and Cellulose for Producing Tissue Engineering Scaffolds

Carbohydrate-based open porous structure appropriate for tissue engineering. The pore inducing particles were water-soluble and another set of the particles were water-insoluble PMMA. The water-soluble particulate was adapted in order to retain the porous structure. This approach resulted in a bulk porous structure with low interconnectivity and no surface porosity. This method established also inappropriate for application to chitin–hydroxyapatite composite. To overcome these problems, a novel water-insoluble particulate leaching method is invented which has been successfully applied to chitin, chitin–hydroxyapatite and cellulose (Tsioptsias *et al*., 2009).

1.3.5 Chitosan-*graft*-P4VP

Chitosan-*graft*-poly(4-vinylpyridine)-supported iron(III) complex, [Cs-PVP/Fe(III)], could be prepared in the form of beads of higher catalytic activity, and efficiently used as a controlling recyclable catalyst to oxidize methylpyridazinone in the presence of hydrogen peroxide to afford the corresponding furopyridazinone derivative (Khalil and Al-Matar, 2013).

1.4 4-vinylpyridine (4VP) and Poly(4VP)

1.4.1 Structure and Properties

The 4-vinyl pyridine (IUPAC Name: 4-ethenyl-pyridine), C7H7N, one of the stable

vinyl monomer. The chemical structure is shown in the Table 1.

Table 1.Chemical Identity of (4VP) Monomer

Name	4-vinylpyridine (4VP)
Formula	$(CH_2=CH)C_5H_4N$
structure	CH$_2$ (pyridine ring with vinyl group, N at position 4)
Molecular formula	C_7H_7N
Synonyms	4VP, 4-vinyl-pyridin 4 VINYLPYRIDINE gamma-vinylpyridine 4-Pyridylethylene Pyridine,4-ethenyl- Pyridine, 4-vinyl- 4-ethenyl-pyridin
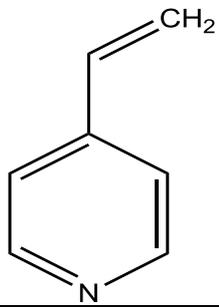
4-Vinylpyridine is a significant monomer and its homopolymer are as identified

catalysts. Copolymers of 4- vinylpyridine is utilized as an efficient dispersant and

flocculants for colloids. The quaternized poly(4-vinylpyridine) acts as bio-acid and

its graft copolymers with poly(vinylalcohol) are utilized as a part of amperometric

bio-sensor. Polymers of vinylpyridine are competent and selective catalytic activities when anchored on hard substrate that gives a steric environment for the reactants. The radiation initiated grafting of 4-vinylpyridine onto cellulose has been attempted in distinctive solvents of diverse dielectric constants and it has been observed that effective grafting of this monomer occurs in solvents which can swell the cellulose to give adequate surface for grafting and give effective action and reaction of 4-vinylpyridine with cellulose. Various studies for the grafting of 4-vinylpyridine onto polyvinylalcohols, polyamides have been carried out (Gupta and Sahoo, 2001).

Table 2. Properties and Value of (4VP) Monomer

property	Value
Molecular weight	105.137 g/mol
Melting point	-39 $°C$
Boiling point	179 $°C$
Density	0.975 g/ml at 25 °C(lit.)
Flash point	48 °C - closed cup
Vapor pressure	0.27 kPa (at 25°C)

P4VP is soluble in a range of solvents, and its modification with carbon nanotubes leads to incredible properties and increased solubility for the same solvents. For these reasons, studies have been made using radical polymerization techniques concerning ''grafting from'' procedures but none has been reported in the literature for the combination of ''living'' anionic polymerization and ''grafting to'' techniques (Katsigiannopoulos *et al*., 2012).

9

Besides, poly(4-vinylpyridine) nanoparticles contain Cu and Ag are useful antimicrobial materials(Sahiner and Ilgin, 2010), (Sahiner and Ozay, 2011).

The effect of solvents with respect to the type of alcohols and composition of binary alcohol/water mixtures on grafting of 4-VP onto FEP film by pre irradiation technique was also estimated . However, the grafting of 4-VP onto hydrocarbon polymer or any fluorinate films has not been reported for an optimization study (Nasef *et al*. 2011).

1.4.2 Copolymers Based on P4VP Properties and Applications

P4VP has good mechanical properties such as high glass transition temperature (Tg). Furthermore, depending to a hard/delicate and acid/base idea, P4VP can form a stable complex with different metal salts and acids. Therefore, P4VP grafted composite materials has ability to apply in catalysts and biomedical applications, strong state electrolytes and metal adsorptions. SiO_2-NP grafted with P4VP brushes was considered as a new cross-linker in proton interchange membranes. It is obvious that the large amount of the protogenic groups in the proton interchange membranes (e.g. sulfonic acid, phosphonic acid) has hydrophilic character, which cause considerable water swelling or solubility. However, for proton change membranes used in fuel cell applications, a degree of water swelling is fundamental. P4VP grafted SiO2-NP could be estimated as an active cross-linker in proton exchange membranes, due to the acid–base interactions between pyridine groups and sulfonic acid or phosphonic acid groups causes the increase in strength of the membranes, dimensional stability and mechanical strength. Matyjaszewski was stated the "free" initiators to ATRP of 4-vinyl pyridine (4VP) in solution (Jiang *et al.,* 2013).

Poly cationic nature of P4VP allows complication with DNA, proteins, and polyanionic drugs via electrostatic interactions (Demirci, *et al.,* 2013). Acid/base complexed membrane obtained by radiation encouraged grafting of 4-vinylpyridine (4-VP) onto poly(ethylene-co- tetraflouroethene) (ETFE) film followed by doping with phosphoric acid was efforted as a PEM for proton conduction in high temperature PEM fuel cells (Nasef *et al.*, 2013).

P4VP is a polyelectrolyte in an aqueous solution due to the presence of ionizable pyridyl groups in its backbone. Therefore, It is easy to expect that surface modification of magnetite nanoparticle (MNP) with P4VP via ATRP would produce a well-defined polyionizable P4VP stabilizer with narrow molecular weight distribution. Furthermore, it exhibit attractive pH-responsive behaviors related with their large electrostatic potentials (pKa of 4-vinylpyridine is 5.62) due to serving as steric and electrostatic stabilizers. The grafting polymerization of P4VP and poly(3-vinylpyridine) (P3VP) brushes onto magnetite surface via NMRP has been investigated before. However, the complexe's pH-responsive properties were not reported in detail. Previously, NMRP (nitroxide-mediated radical polymerization) was applied for surface modification of magnetite particles with poly(3- or 4-vinylpyridin and their copolymerization with other polymers, such as polystyrene, acrylamidoethylamine, and polyethyleneglycol have been offered. In addition, surface modifications of Pd,Si and Au nanoparticles with P4VP or poly(2-vinylpyridine)(P2VP) have also been investigated (Rutnakornpituk *et al.,* 2011)

Poly(4-vinylpyridine-DVP) has ability to prepare three strong base-anion exchange resins with different alkyl such as ($-CH_3$, C_2H_5, $n-C_4H_9$) halides. In the laboratory also the base polymer granular form was prepared. The product were carried out for separation of TcO_4-ions from basic and acidic solutions, by using the column containing Poly(4-vinylpyridine-DVP) removal of (TcO_4-)-Tc-99 ions from waste solutions was examined, it was observed that the resin with n-butyl group on the pyridine nitrogen has high attraction for (TcO_4-)-Tc-99 ions after these resins evaluated for batch uptake of (TcO_4-)-Tc-99 ions from waste solutions. These results indicate the usefulness of this novel resin in the treatment of effluent generated at the back end of nuclear fuel cycle (Banerjee, *et al.,* 2012).

Cross-linking reaction of poly(2-vinylpyridine-co-styrene) and oligo(ethylene oxide) with epoxide functional groups was successfully prepared a gel polymer electrolyte without utilizing initiator. By adding 1% polymer gelator a stable gel polymer electrolyte could be generated. At the room temperature the ionic conductivity of gel polymer conductivity contain 99 wt% of liquid electrolyte was calculated to be ca. 10^{-2} S/cm., the ionic conductivity of the product (GPE) was similar to liquid electrolyte. The electrochemical stability window of the gel polymer electrolyte which produced was measured to be 5.2V. Finally, the resulting of gel polymer electrolyte prepared by thermal cross-linking without polymerization initiator has ability for application on the high power lithium-ion polymer batteries (Oh, *et al*., 2011)

The poly(styrene-block-poly4-vinylpyridene) (PS-b-P4VP) was prepared by utilizing living free radical polymerization. The atomic force microscope (AFM) and gel permeation chromatography (GPC) techniques were specialized of polystyrene and diblock copolymers. Finally, the result observed that the polystyrene could be synthesis with molecular weight distribution in the 1.15 to 1.25 ranges. The living free radical polymerization of 4-vinylpyridine can be continuously initiated by polystyrene with living groups to form the PS-b-P4VP with molecular weight distribution in the 1.08 to 1.35 ranges. The diblock copolymer PS-b-P4VP is a good compatibilizer for PS and P4VP indicated by the atomic force microscope (AFM) diblock (Watthanaphanit, *et al*., 2008).

By using various experimental tools some properties of polymer electrolyte such as electrical, structural, photoelectrochemical, and also manufacture of nonporous TiO_2 electrode have been inspected. The synthesis of a porous nanocrystalline TiO_2 electrode and a new kind of solid polymer electrolyte poly(N-methyl 4-vinylpyridine iodide) PVPI doped with an Ionic Liquid (IL) 1-ethyl-3-methylimidazolium thiocyanate for Dye-sensitized solar cell (DSSC) application were observed (Bhattacharya, *et al*., 2012).

13

Chapter 2

EXPERIMENTAL

2.1 Materials

Chitin (Sigma, Germany) Acetic acid (Aldrich) Toluene (Aldrich-Germany), n-Hexane (Emplura-Germany), Dimethyl formamide (DMF) (Germany), Chloroform (Aldrich), (sodium hydroxide (Aldrich), Ceric ammonium nitrate (CAN) (Aldrich, Germany), LiCl, HCl, (Sigma-Germany), N,N-dimethylacetamide (DMAc) (Aldrich-Germany), ethanol (Sema-Northern Cyprus), Potassium persulfate (KPS) (Aldrich-Germany), 4-vinyl pyridine (4-VP)(Aldrich-Germany), toluene (Sigma-Aldrich), dimethyl sulfoxide (Aldrich-Germany), and Calcium carbonate (Aldrich-Germany).

2.2 Methods

2.2.1 Solubility Properties of 4-vinylpyridine

The monomer solubility was determined in several solvent systems. Solvents used include distilled water, ethanol, DMSO, Acetic Acid, toluene, dimethylformamide, Lithium Chloride/DiMethylAcetamide, chloroform, hexane and acetone. To determine the solubility, 0.50 mL of monomer (4VP) sample was added into 0.50 mL of each solvent/solvent system in a test tube at ambient temperature.

2.2.2 Chitin Purification

This was achieved by treating chitin with 1 M sodium hydroxide for three hours at 80ºC. Water was added to neutralize it and digestion was carried out using 1 M hydrochloric acid solution for 12 hours after which neutralization was repeated using water. The procedure was repeated twice to get a pure chitin.

2.2.3 Preparation of the Solvent System

Molecular sieves of 400 Å were activated at 300°C for about 4 hours. NMP and Dimethylacetamide were then dried to remove water using the activated molecular sieves for 2 days. Lithium Chloride was also dried in the oven at 130 °C. 5 g of the dried Lithium chloride was then dissolved in 100 mL of NMP and Dimethylacetamide. The salt solvent system was stirred for 24 hours to aid the complete dissolution of the lithium chloride in the solvent.

2.2.4 Preparation of Chitin Solution

1 g of chitin was dissolved in 100 mL of the solvent system prepared in section 2.2.3. The solution was stirred constantly for 2 days at ambient temperature so as to completely dissolve the chitin and form a homogeneous mixture.

2.2.5 Preparation of Chitin Beads

5 g of $CaCO_3$ was added to the chitin solution prepared in section 2.2.4 to act as a porogen and stirred constantly for 2 days. The beads were prepared by pouring the solution into a burette and dropping carefully drop wise into ethanol. The beads were formed immediately. The beads formed were left in the ethanol overnight to allow for solvent exchange. After that, the beads were carefully removed by filtration and transferred to a 3 M HCl solution so as to form pores by the evolution of CO_2. The beads were then neutralized with distilled water and dried at room temperature. The experimental set up is illustrated in Figure 3.
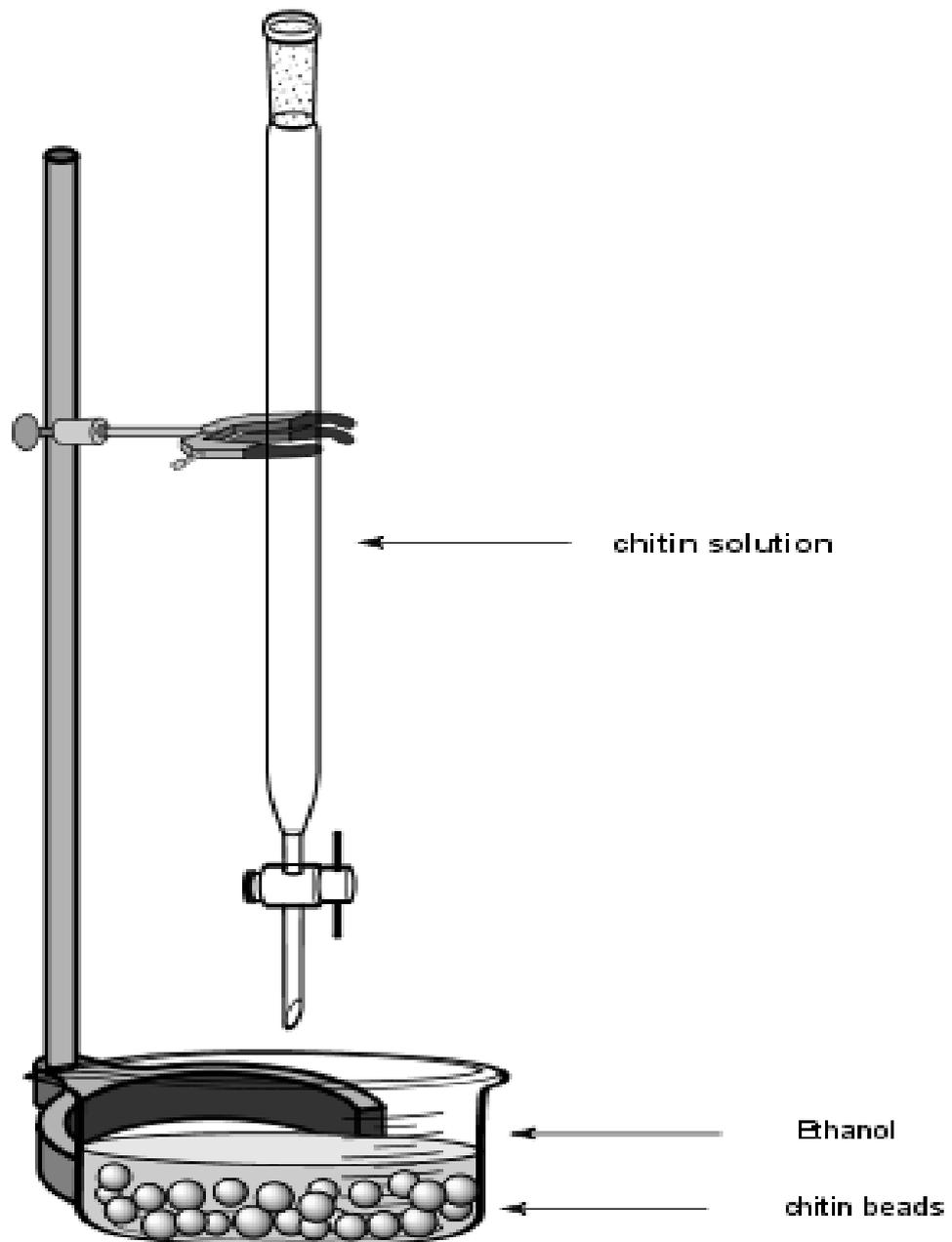
Figure 3. Chitin Bead Formation

2.2.6 Grafting P4VP on to Chitin Gel Beads

A small amount of chitin beads was crushed in to powder. 0.1g of the crushed chitin beads was transferred into a 3 neck round bottom flask; 200 mL of toluene was added to it. The round bottom flask was then put in a water bath at a fixed temperature. The advantage of toluene was that it acted as a solvent for any homopolymer P4VP formed. N_2 gas was passed into the system for about 30 mins for removal of oxygen from the system. The initiator (Cerium ammonium nitrate) was then added so as to start graft copolymerization. After 15 minutes varying amount of 4-VP monomer was added. The system was kept under reflux to avoid evaporation of toluene. Homopolymer removal was achieved by treating the grafted beads with excess ethanol. The experimental set up is shown in Figure 4.
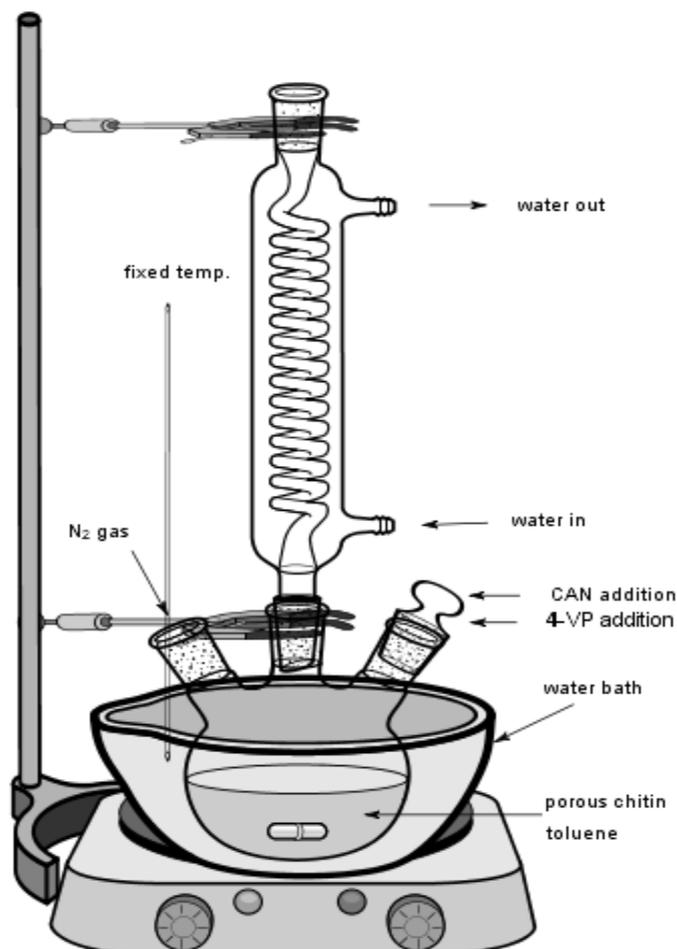

Figure 4. P4VP Grafted Chitin Gel Beads Formation.

2.3 Optimization of Grafting Conditions

Grafting of 4VP unto chitin was optimized by differing one variable and keeping other variables constant. Variables include changing monomer concentration, amount of chitin, initiator concentration reaction duration and temperature. All the variables changed so as to optimize the grafting process are shown in Table 3.

2.3.1 Effect of the Amount of Chitin on Grafting Percent

The effect of varying the mass of chitin on grafting was also determined. To achieve this, 0.1 g and 0.5 g of chitin was used for grafting

2.3.2 Monomer Concentration Effect on Grafting Percent

To observe the effect of varying the amount of (4VP) 1 mL, 5 mL and 10 mL of 4VP was applied and the yield obtained from the grafting process was determined.

2.3.3 Effect of Reaction Time on Grafting Percent

Reaction time was varied (1, 2, 3 , 4 and 5 hours) so as to determine how long the reaction will be required to go on to obtain maximum yield

2.3.4 Effect of CAN Amount on Grafting Percent

0.1 g, 0.25 g, 0.5 g of initiator CAN was used to determine the effect of initiator concentration on grating yield of 4VP so as to determine the appropriate amount of initiator that must be added to obtain maximum yield.

2.3.5 Effect of Reaction Temperature on Grafting Percent

The temperature of the grafting procedure was varied accordingly (40°C, 60°C and 70°C) to determine the best temperature needed to carry out the grafting process.

Table 3. The Sample Preparation Conditions.

Chitin (g)	4VP (mL)	CAN (g)	Temperature (°C)	Time (hours)
0.10	1.0	0.50	60	1
0.10	5.0	0.50	60	1
0.10	10	0.50	60	1
0.10	1.0	0.10	60	1
0.10	5.0	0.10	60	1
0.10	10	0.10	60	1
0.10	10	0.25	60	1
0.10	10	0.50	60	2
0.10	10	0.50	60	3
0.10	10	0.50	60	4
0.10	10	0.50	60	5
0.50	10	0.50	60	1
0.10	10	0.50	40	1
0.10	10	0.50	70	1

2.4 Analysis of the Products

2.4.1 Gravimetric Analysis

% grafting yield was determined according to equation 1.

$$\% \, \text{Grafting} = \frac{\text{Graft Copolymer (g)} - \text{Chitin (g)}}{\text{Chitin (g)}} \times 100$$

(1)

2.4.2 FTIR Analysis

FTIR spectrum of chitin beads was taken using a Perkin Elmer spectrum-65 FTIR Spectrophotometer. Chitin bead was ground with KBr to form pellets.

2.4.3 C-13 NMR Analysis

The 13 CPMAS samples were analyzed in ODTU Markez Laboraturan using a Bruker Super conducting FTNMR Spectrometer Arance TM 300 MHz WB.

2.4.4 DSC Analysis

Thermal behavior of the samples was analyzed using a Perkin Elmer Jade calorimeter in TUBITAK-MAM, Gebze. The samples were studied under nitrogen atmosphere. The heating rate was 10 °C /minute and the temperature range was 10-300 °C.

2.4.5 SEM Analysis

JEOL/JSM-6510 LV scanning electron microscope was used to obtain the SEM pictures in TUBITAK-MAM.

2.4.6 Weight Loss

The soluble fraction was determined by immersing and keeping a sample of beads (approximately 0.025 g) in the buffer solution (pH 1.2) for 6 hours. The swollen hydrogel samples were taken and dried in the oven at 50°C to constant weight. The fraction that was dispresed in solution as powder was determined using equation 2.

$$\text{Weight Loss (\%)} = \left(\frac{m_{d_1} - m_{d_2}}{m_{d_1}} \right) \times 100 \qquad (2)$$

m_{d2} is the weight of the beads after removing rom buffer and drying.

Chapter 3

RESULTS AND DISCUSSION

Chitin and P4-VP grafted chitin were prepared. The grafting reaction was carried out with redox initiator CAN (cerium ammonium nitrate). The characterization of obtained samples and optimum grafting conditions were examined. The effect of the amount of chitin, 4VP, initiator CAN, reaction temperature and reaction duration on the grafting yield (%G) was investigated by the gravimetric method. The dissolution of monomer and prepared samples was studied.

3.1 Solvents for 4VP

4VP is soluble in hexane, toluene, ethanol, chloroform whereas insoluble in water and acetic acid as shown in Table 4.

Table 4. Solvents for 4VP.

solvent	solubility
Toluene	+
Ethanol	+
dd H2O	-
Acetic acid	-
Chloroform	+
Hexane	+

3.2 Preparation of Chitin Beads

The beads formed from 1% (w/v) chitin solution were used for the grafting study carried out in this thesis as aforesaid in section (2.2.5). The beads were crushed in to powder using pestle and mortar after drying. The optical pictures of the beads gained from 1% (wt/vol) chitin solution are shown in Figure 5.

Figure 5.(A)Chitin beads with $CaCO_3$, (B) Chitin beads in 3M HCl, (C) Porous Chitin Beads before dry, (D) Porous Chitin Beads after drying

3.3 Investigation of the Effect of Grafting Conditions on the Grafting Yield

Optimum condition for grafting of (4VP) monomer on to chitin beads using cerium ammonium nitrate (CAN) as redox initiator was investigated. The products were analysed by FTIR, SEM, DSC and C13 NMR, analysis of chitin and chitin-*graft*-P4VP were studied.
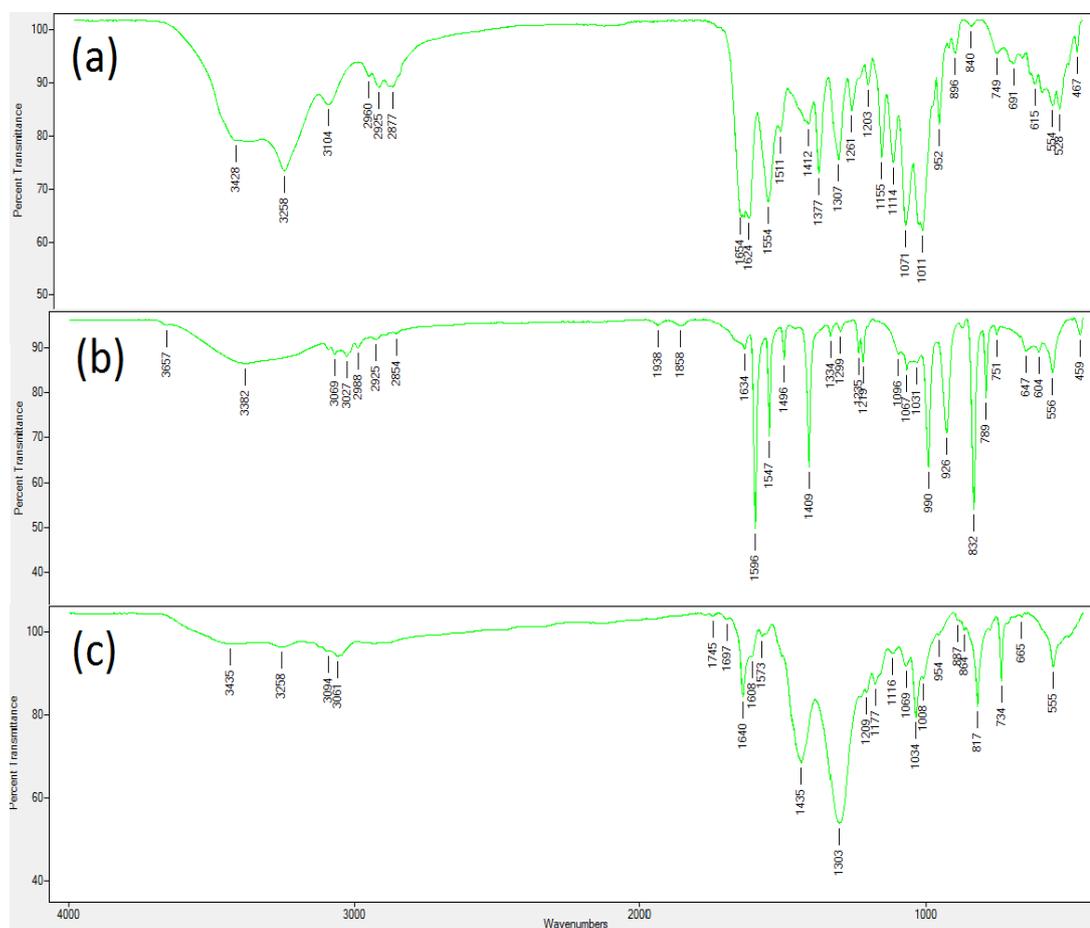
3.4 FT-IR Analysis

Figure 6. FTIR Spectrum of (a) Chitin, (b) (4VP) and (c) Chitin-*graft*-P4VP Respectively.

Chitin-*graft*-P4VP sample was analyzed by FTIR spectroscopy. FTIR spectrum of (a) chitin, (b) (4VP) and (c) chitin-*graft*-P4VP are given in Figure (6a), (6b) and (6c) respectively. In the spectrum of chitin, the following main bands can be observed: a broad O-H stretching band at 3428 cm^{-1} ,asymmetric N-H stretching at 3258 cm^{-1},

symmetric N-H stretching and 3104 cm^{-1}. Stretchings of –CH$_3$ and –CH$_2$ groups are found at 2960 cm^{-1}, 2925 cm^{-1} and 2877 cm^{-1} . Amide I bands is at 1654 cm^{-1} and 1624 cm^{-1}. The amide II band is at 1554 cm^{-1}. Amide III appears at 1307 cm^{-1}. Methylene carbons (-CH$_2$) stretching and C-CH$_3$ stretching appear at 1412 cm^{-1} and 1377 cm^{-1} respectively. C-O stretching of the repeat unit is at 1155 cm^{-1} and C-O stretchings of O-H group is in the 1011-1114 cm^{-1} region (Figure 6a).

An examination of the spectrum of the monomer (4-VP) shows (Figure 6b) (C-H) stretching bands at (3070-3000) cm^{-1}. Ring stretching vibration of the pyridine group appears at 1634 cm^{-1}, 1596 cm^{-1}, 1547 cm^{-1} and at 1409 cm^{-1}. (C-H) out of plane bending vibrations are observed at 990 cm^{-1}, 926 cm^{-1} and at 832 cm^{-1}.

The spectrum of the chitin-*graft*-poly(4-VP) includes a broad O-H stretching at 3435 cm^{-1}. (N-H) stretching of chitin (3258 cm^{-1}) for grafted sample. A more intense –CH$_2$ stretching band is observed at (3061-3094) cm^{-1} compared to the chitin spectrum indicating the contribution of methylene groups of (4VP). The aromatic stretching bands of (4VP) together with amide, (C-C) and (C-H) stretching bands of chitin appear in the region 1640-1300 cm^{-1}. Two strong bands at 1435 cm^{-1} and 1303 cm^{-1} are particularly noticeable. The (C-H) out of plane of the aromatic ring is observable at 817 cm^{-1} (Figure 6c).

3.5 C-13 NMR Analysis

C-13 NMR spectrum of chitin and chitin-*graft*-P4VP are shown in Figure 7 and Figure 8 respectively.
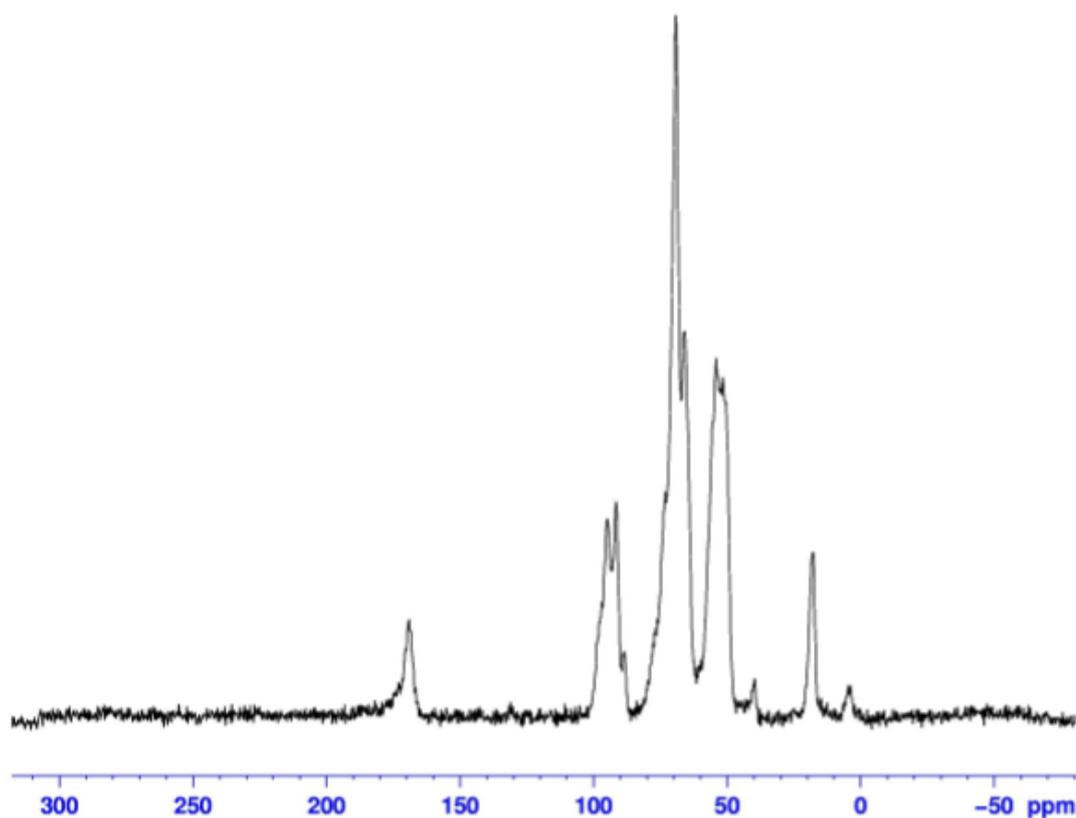

Figure 7. C-13 NMR Spectrum for Chitin

The characteristic peaks of chitin can be listed as follows from Figure 7: amide carbonyl at 169 ppm, C-1 and C-4 at 100 and 90 ppm respectively, C-3 and C-5 at 70 and 60 ppm, and C-2 and C-6 at 60-50 ppm. The peak at 20 ppm belongs to the methyl of the acetamide group.
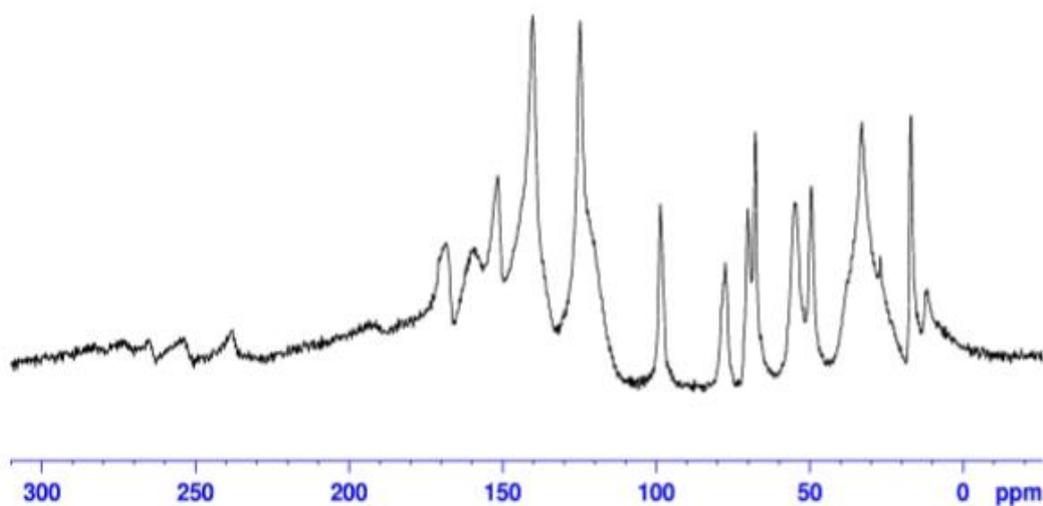
Figure 8. C-13 NMR Spectrum for Chitin-*graft*-poly(4VP)

From Figure(8) in addition to the characteristic peaks of chitin as identified above, the methyl group of 4VP appears at 8-12 ppm, the C-2 and C-6 peaks of chitin in the region 50-60 ppm appear , also the C-3 and C-5 appear in the region 65-70 ppm, the C atoms of the aromatic ring of the pyridine group can be observed in the region 125-150 ppm. The –CH, CH_2 and –CH_3 carbons appear at 20-40 ppm range. New peaks appear at 235, and 255 ppm indicating some oxidation reactions that occur on the chitin backbone during redox initiation.

3.6 Gravimetric Analysis

The effect of the amount of 4VP, chitin, CAN, temperature, reaction duration was investigated by the gravimetric method (on the degree of grafting percent) in order to find the optimum grafting conditions to achieve the highest grafting yield. The sample preparation conditions and grafting yield (%G) are shown in Table 5. As described in section 2.2.6 chitin beads were dispersed in toluene whereas the monomer, 4VP and the initiator (CAN) were dissolved in toluene. The advantage of this system is that the homopolymer that may form during reaction is kept in solution as P4VP is soluble in toluene. Hence, P4VP grafted chitin beads obtained are relatively free from homopolymer impurity. Further treatment with ethanol ensures complete removal of any P4VP homopolymer adhered on the bead surface.

Table 5. The Sample Preparation Conditions and %G Yield.

Chitin (g)	4VP (mL)	CAN (g)	Temperature (°C)	Time (hours)	Grafting%
0.10	1.0	0.50	60	1	148
0.10	5.0	0.50	60	1	296
0.10	10	0.50	60	1	380
0.10	1.0	0.10	60	1	3.1
0.10	5.0	0.10	60	1	52.4
0.10	10	0.10	60	1	65
0.10	10	0.25	60	1	99.5
0.10	10	0.50	60	2	316
0.10	10	0.50	60	3	304
0.10	10	0.50	60	4	311
0.10	10	0.50	60	5	400
0.50	10	0.50	60	1	48
0.10	10	0.50	40	1	368
0.10	10	0.50	70	1	278

3.6.1 Grafting Yield with Respect to the Amount of Monomer

There is a steady increase in the grafting yield with increasing amount of monomer, 4VP, as shown in Figure 9. This observation indicates availability of active sites on chitin backbone for grafting of P4VP, within the concentration are studied.
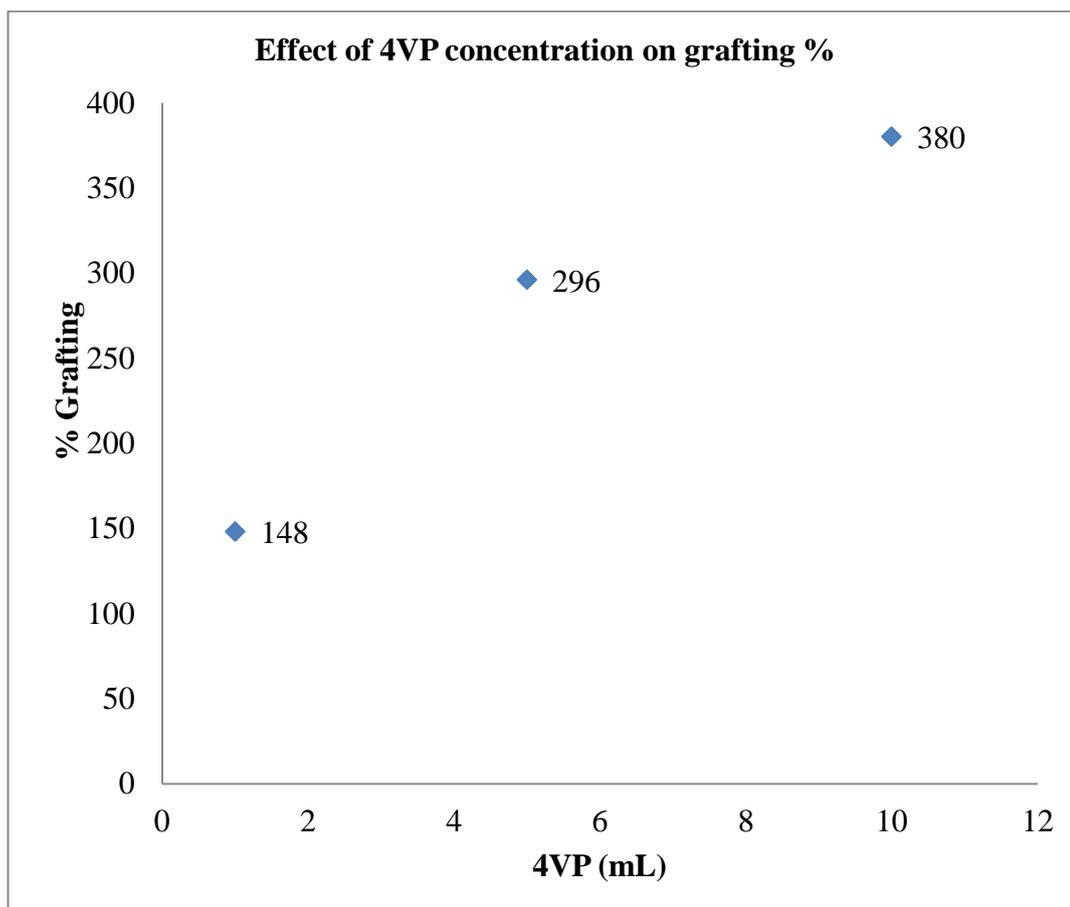

Figure 9. Effect of 4VP Concentration on Grafting

3.6.2 Grafting Yield with Respect to the Amount of Chitin

As the amount of chitin is increased, the grafting% decreases. Different amount of chitin (0.10g and 0.50g) were treated under the same conditions namely 10 mL 4VP, 0.50g CAN at 60 ^{0}C for 1hour grafting time. The results as given in Table 5, and Figure 10, show that a greater amount of chitin beads causes a decrease in the grafting yield. The %G value is 380 for 0.10g chitin whereas it's only 48 for 0.50g chitin under the same conditions. The reason for this behavior could be that a higher amount of chitin loaded in the system restricts the availability of the active sites on the surface, in other words, available free surface area decrease.
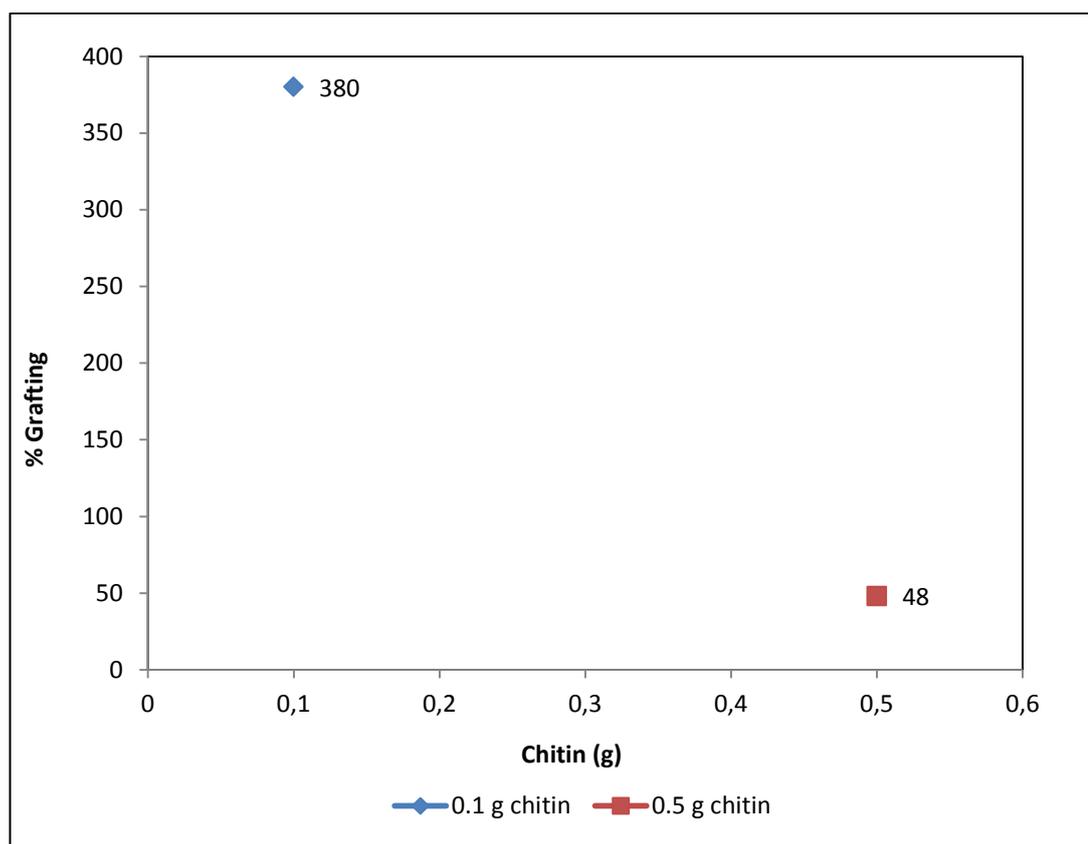

Figure 10. Effect of the Amount of Chitin on % Grafting

31

3.6.3 Grafting Yield with Respect to the Amount of Initiator

An increase in the amount of the initiator resulted in an increase in % grafting yield. Because the highest increasing active sites on the backbone the maximum grafting yield was obtained with 0.5 g CAN as shown in Figure 11.
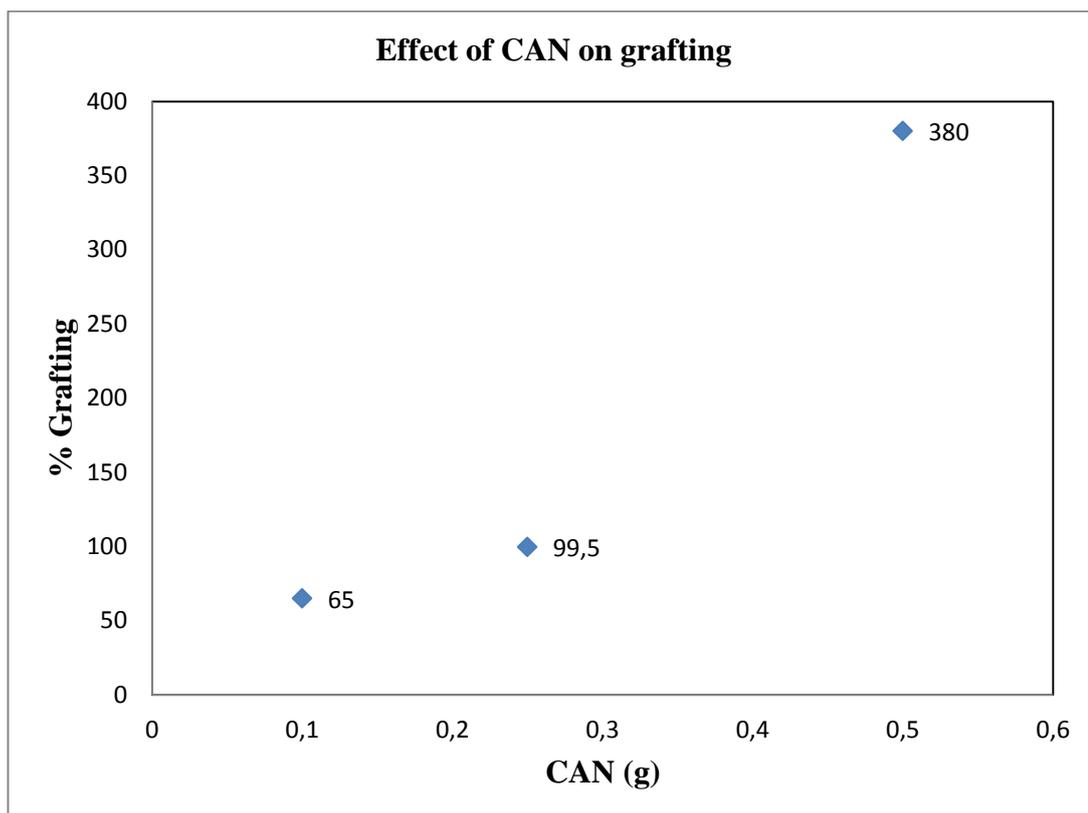
Effect of CAN on grafting

Figure 11. The Effect of CAN Amount on Grafting

3.6.4 Grafting Yield with Respect to Temperature

The temperature range studied was (40-70) °C. The highest value of percent grafting is observed at 60°C and then decreased at 70°C as shown in Figure 12. Additionally increase in temperature resulted in a leveling off in the grafting yield with a tendency to decrease at high temperature value. This can be attributed to increased rate of termination reaction and decrease stability of CAN at high temperature.
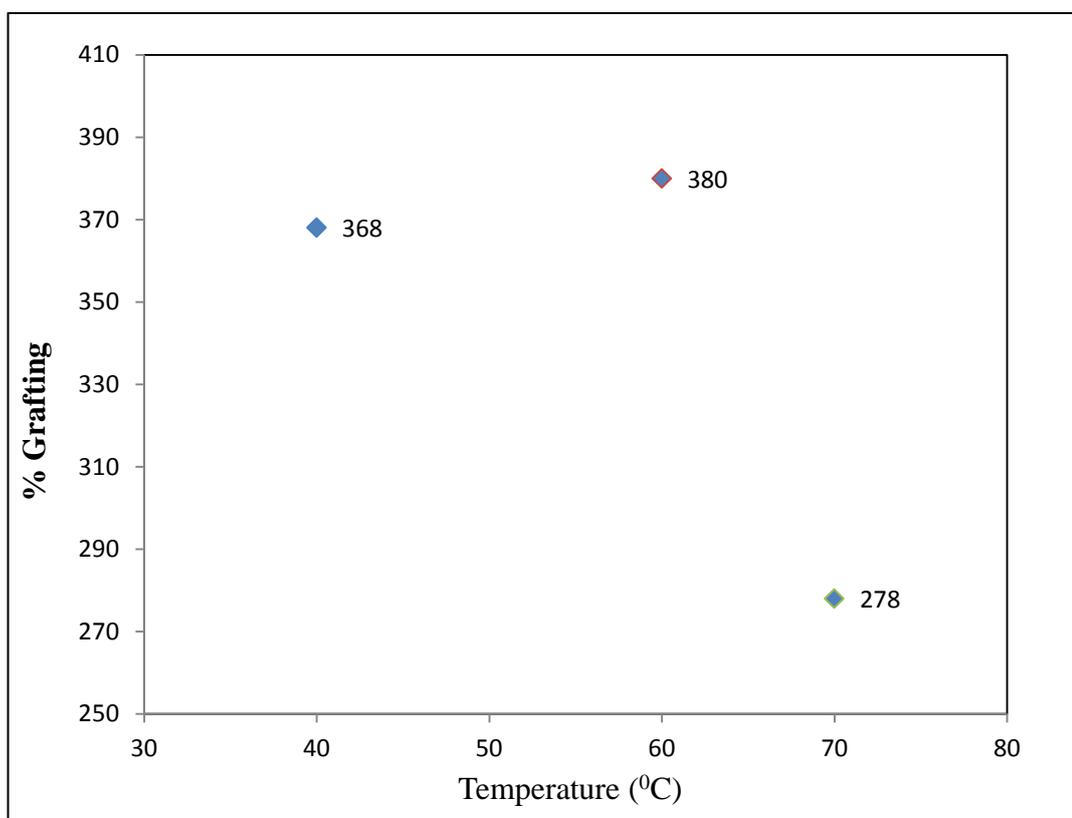

Figure 12. Effect of Temperature on Grafting

3.6.5 Grafting Yield with Respect to Time

Reaction time was varied (1, 2, 3, 4 and 5 hours) so as to determine the effect of reaction duration on the percent grafting and the results are given in the Figure 13. The highest grafting was obtained at 1 hour reaction time. %Grafting yield is independent with reaction time the first 5 hours. The reaction should be due to the heterogeneous nature of the reaction system. The grafting reaction should be taking place on the surface of the beads, hence a maximum amount of grafting of the order of 300% occurs within 1.0 hour; an increase in reaction time dose not make a significant change on the grafting yield as the surface is already modified within an hour using the monomer available in the system.
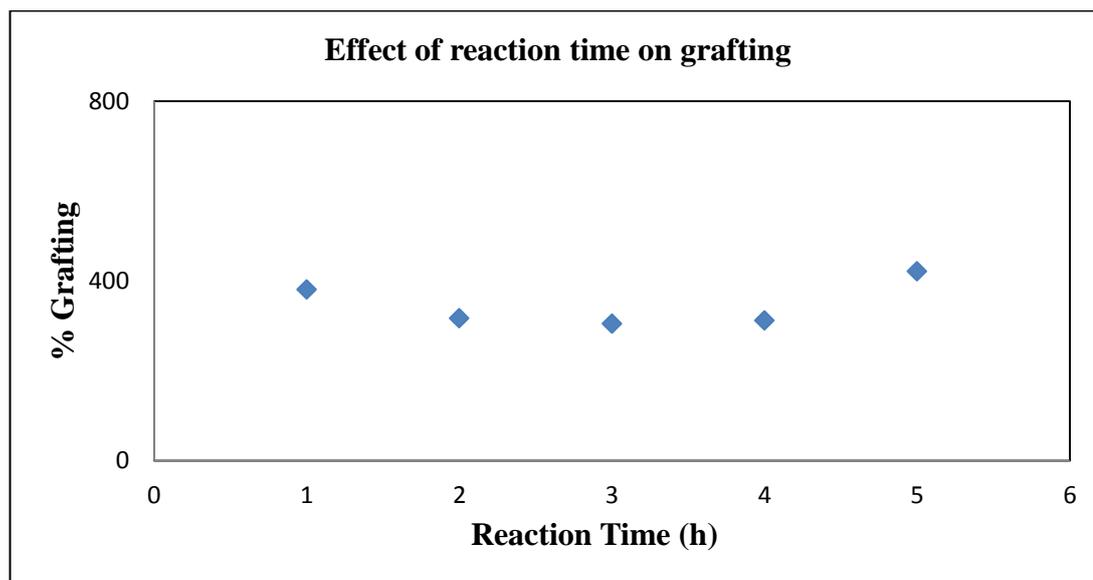

Figure 13. Effect of Reaction Time on Grafting

3.7. SEM analysis

SEM micrographs of the non grafted, chitin-*graft*-P4VP (G%=48) and chitin-*graft*-P4VP (G%=380) are given in Figure 14, Figure 15, and Figure 16, respectively. It can be estimated from the SEM pictures that the average size of the beads is of the

order of 100-200 μm. it should be noted that the initial sizes of the beads are of the

order of 550 μm as reported by (Kader.D, 2014).

Hence, the crushing power has caused a 5 times decrease in the bead size. The non

grafted beads have a very heterogeneous surface when modified by grafting.
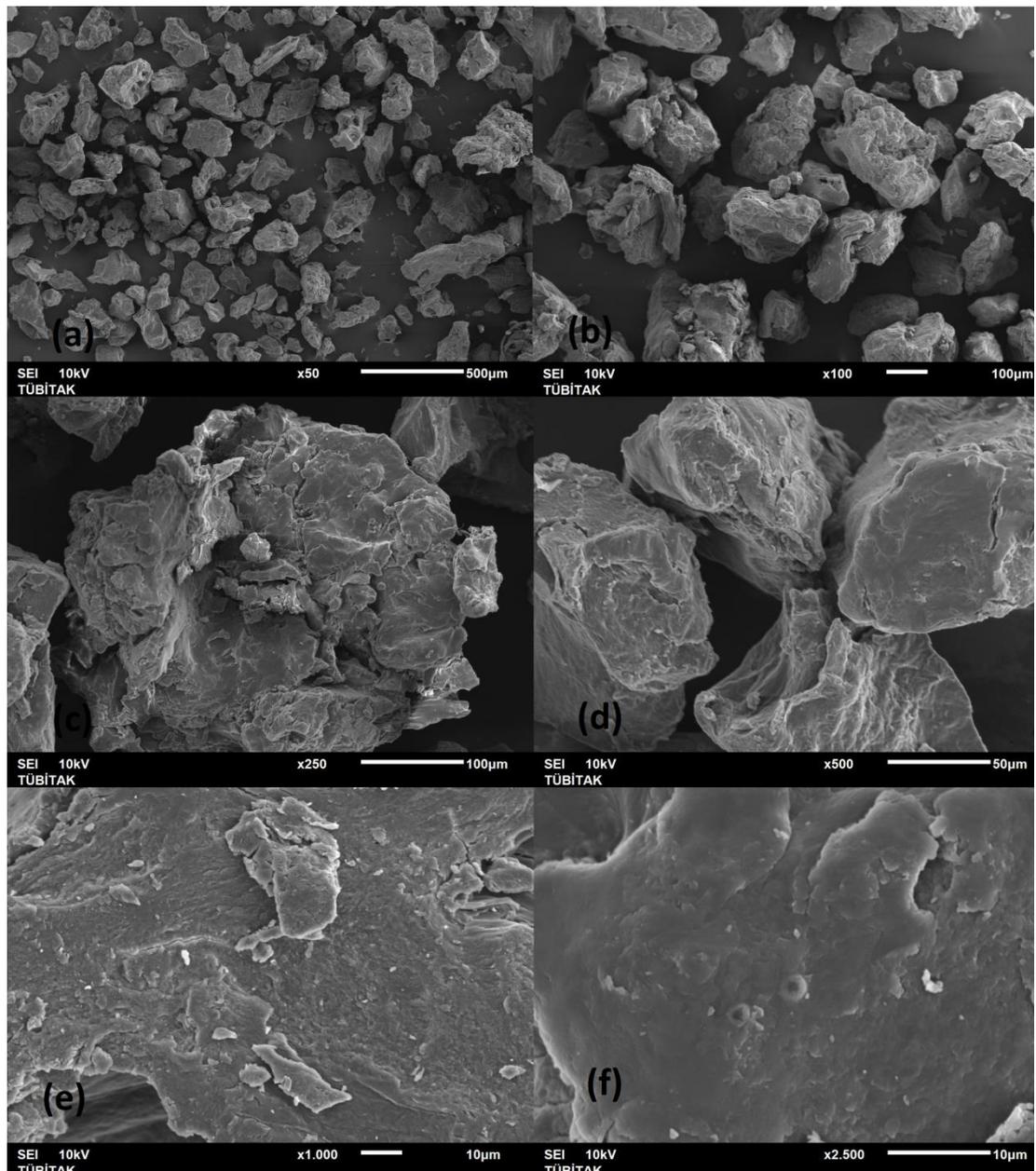

Figure 14. SEM Micrograph of the Chitin, (a) is X 50, (b) is X 100, (c) is X 250, (d) is X 500, (e) is X 1000, (f) is X 2500
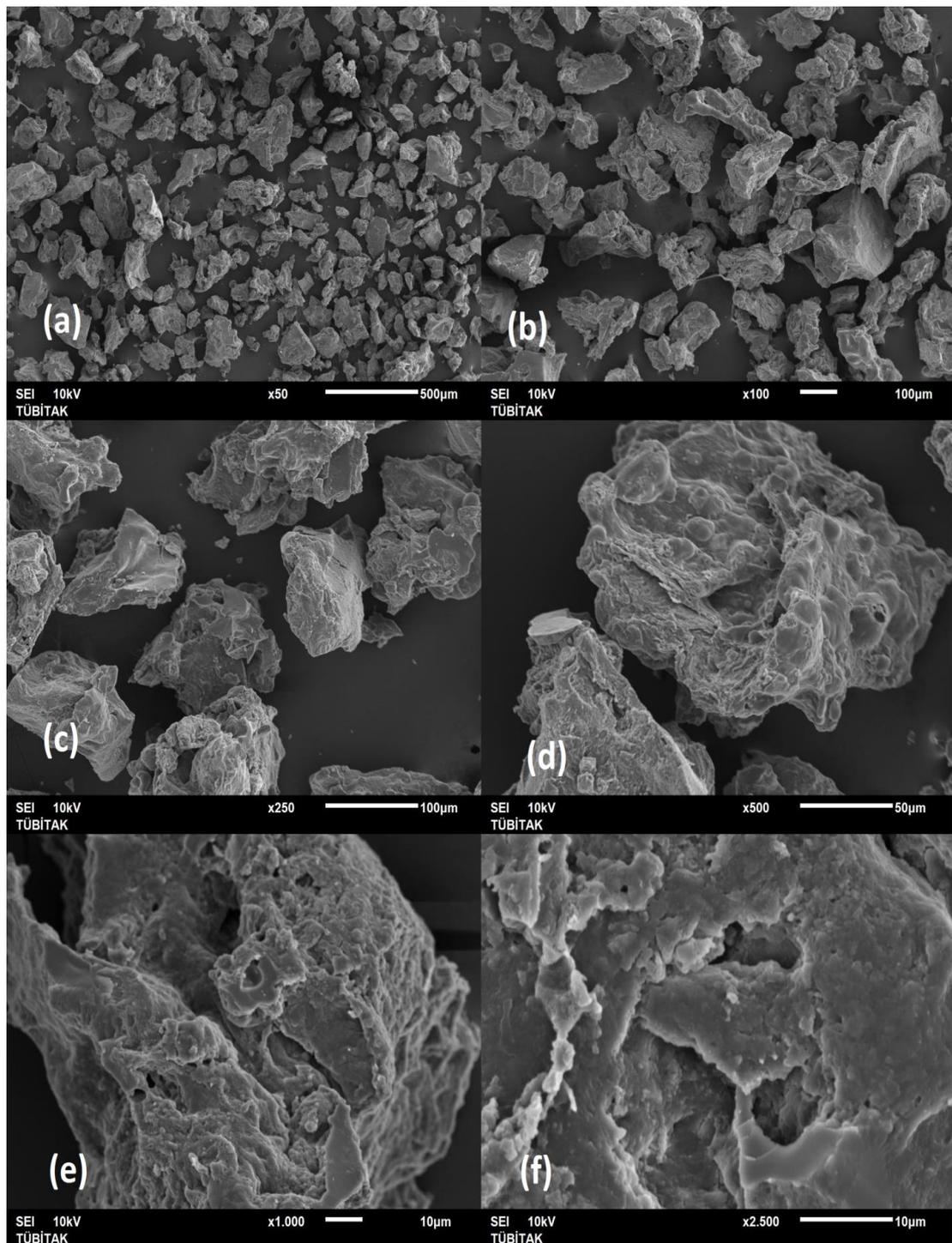
Figure 15. SEM Micrograph of the Grafted Chitin-*graft*-P4VP (48%), (a) is X 50,
(b) is X 100, (c) is X 250, (d) is X 500, (e) is X 1000, (f) is X 2500
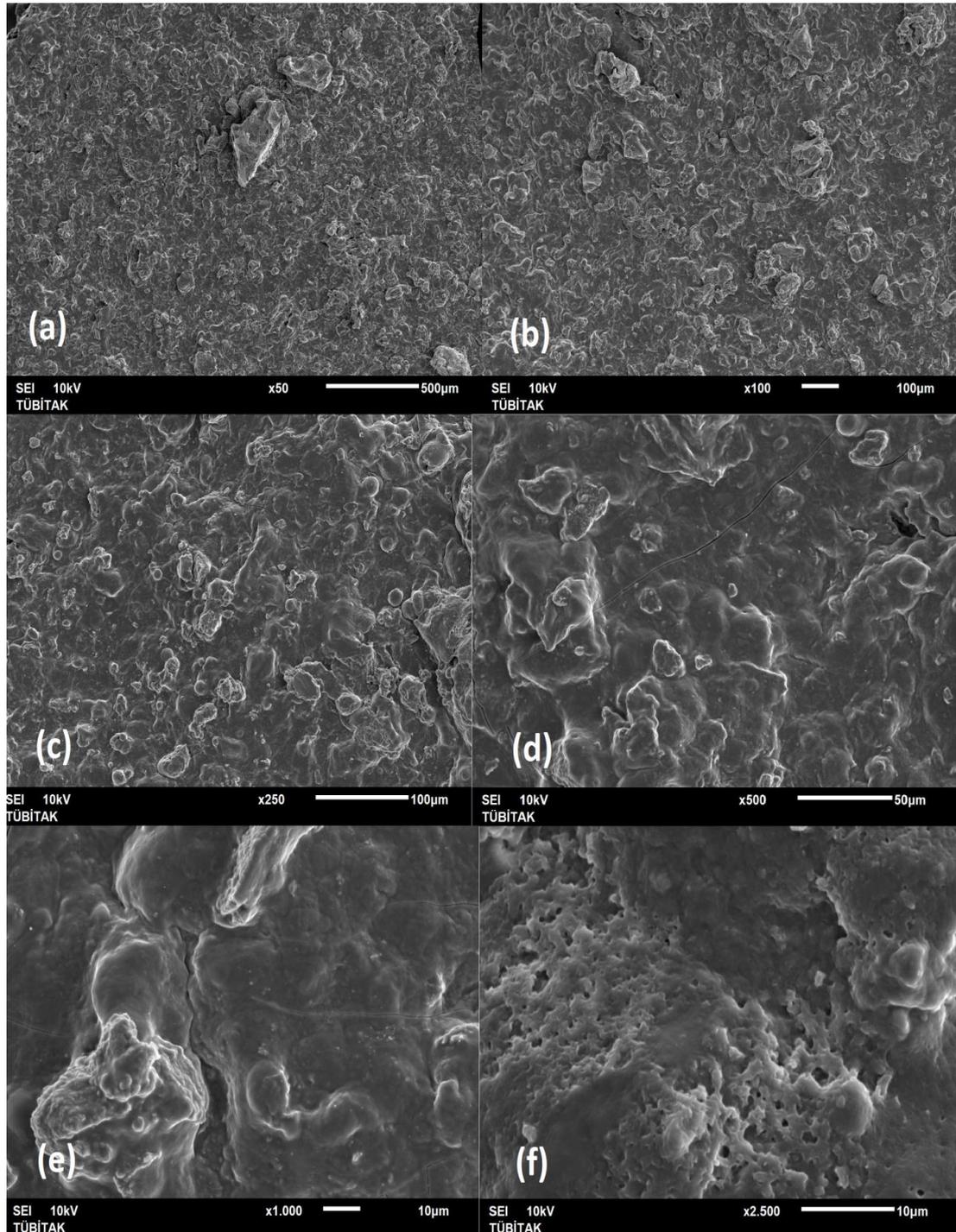
Figure 16. SEM Micrograph of the Grafted Chitin-*graft*-P4VP (380%), (a) is X 50, (b) is X 100, (c) is X 250, (d) is X 500, (e) is X 1000, (f) is X 2500

3.8 DSC Analysis

The DSC thermograms of chitin non grafted and chitin-g-P4VP (G=380) are shown in Figure 17 and Figure 18, respectively.
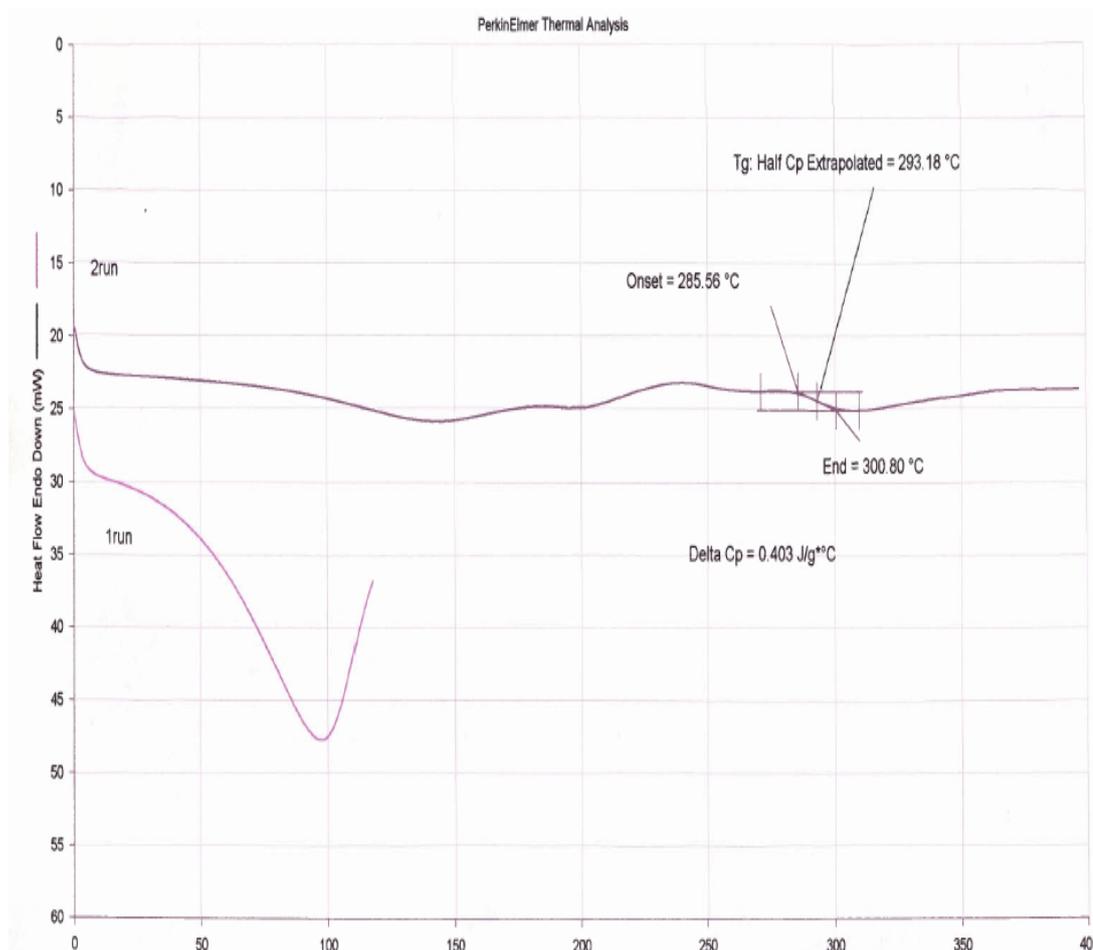
Figure 17. DSC Thermo Gram of Chitin

In the first run carried out up to 120 ^{o}C, water loss at 90 ^{o}C is observed. In the second run studied up to 400 ^{o}C, the glass transition temperature of chitin is observed at 293 ^{o}C. In the DSC thermo gram of chitin-*graft*-P4VP, two exothermic decomposition peaks are observed at 164 ^{o}C and 174 ^{o}C. Hence, thermal stability of chitin has been decreased significantly up on grafting with P4VP.

Figure 18. DSC Thermo Gram of Chitin-*graft*-P4VP (G=380%)

3.9 Dissolution Properties of Chitin and Grafted Chitin

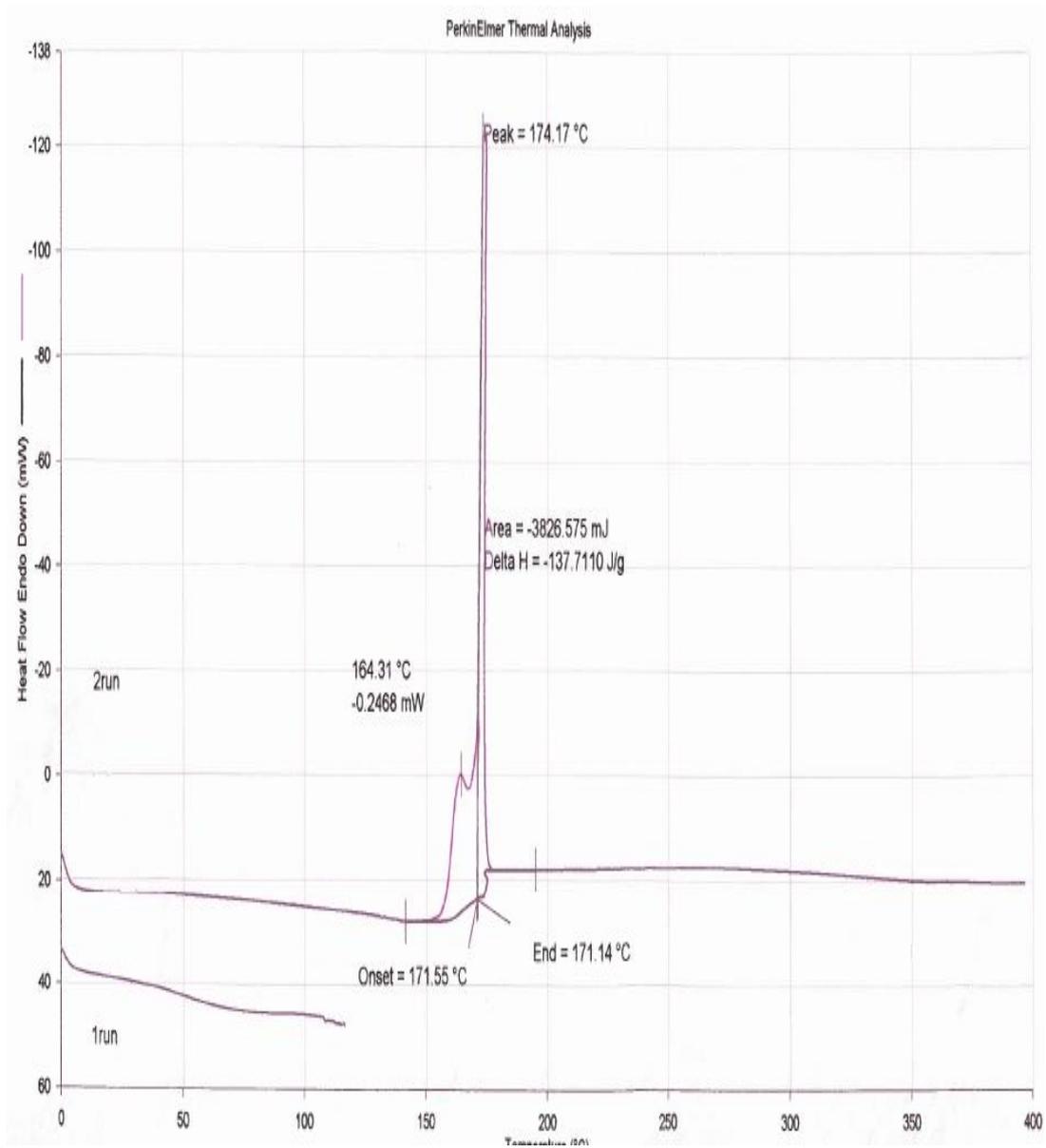All samples were mixed with pH=1.2 buffer solution. After 6 hour contact with the acid buffer solution, it was observed that the color of the solution turned yellowish and the grafted products partially dissolved in the acid solution. The color of the solution became darker with increasing %grafting value. The dispersion was filtered % weight loss was calculated. The results shown in Figure 19 were obtained. After filtration clear transparent yellowish solution were obtained. The polymer dissolved in solution could be re precipitated in ethanol.
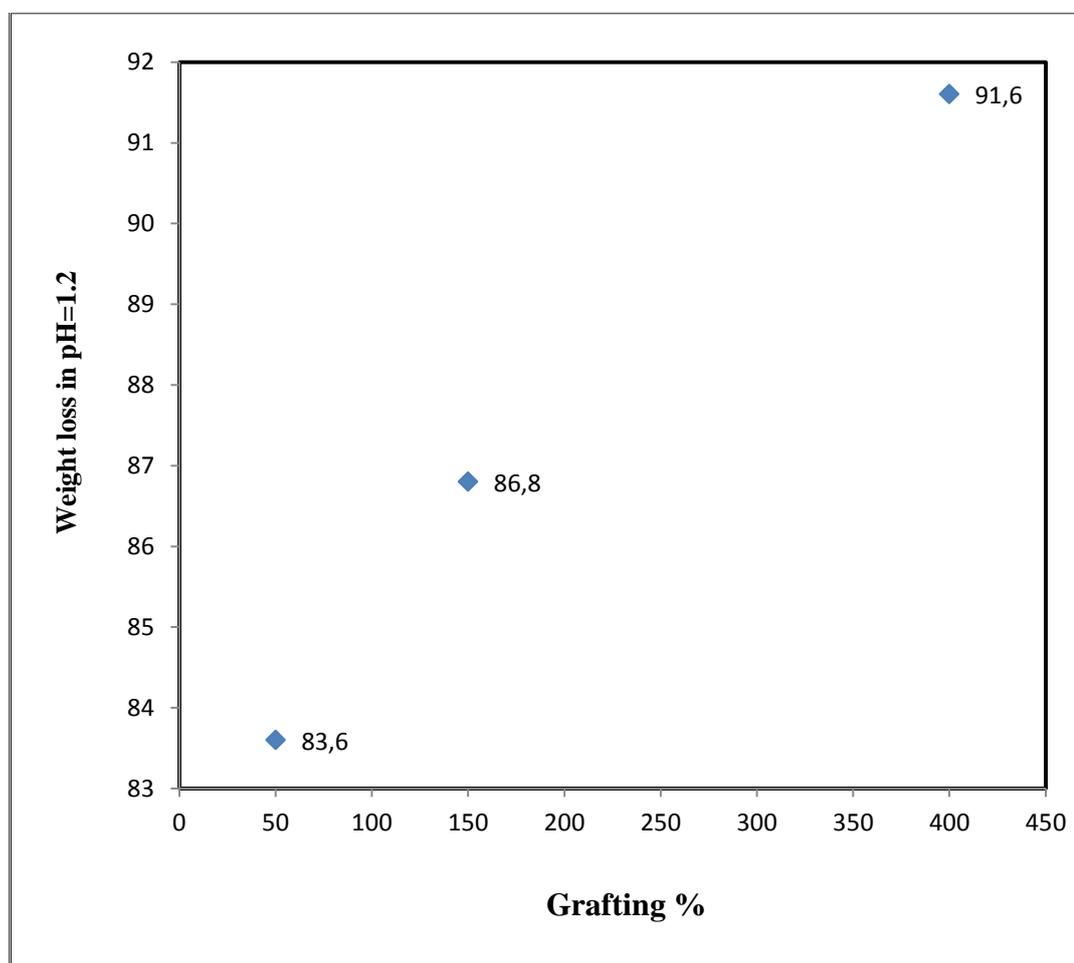

Figure 19. Weight Loss% in Acidic Buffer.

Chapter 4

CONCLUSION

Poly(4-vinylpyridene) can be grafted onto chitin to form chitin-*graft*-P4VP by using cerium ammonium nitrate (CAN) as a redox initiator and toluene as a solvent, the advantage of toluene was that it acted as a solvent for the homopolymer, P4VP. The excess 4VP and CAN have been removed by using ethanol.

The maximum grafting percent of chitin-*graft*-P4VP is 380% produced from the optimum grafting conditions are 0.10 g of chitin, 10 mL of (4VP) monomer, 0.50 g of cerium ammonium nitrate(CAN) initiator at 60°C for one hour reaction time.

In the FTIR analysis for chitin-*graft*-P4VP two strong bands appear at 1435 cm^{-1} and 1303 cm^{-1}, also the (C-H) out of plane of the aromatic ring is observable at 817 cm^{-1} while this bands does not exist in the chitin non grafted. This is got evidence of poly(4-vinylpyridene) grafted onto chitin beads.

In the C-13 NMR analysis for chitin-*graft*-P4VP in the region 125-150 ppm the C atoms of the aromatic ring of the pyridine group can be observed and also new peaks appear at 235, 255 ppm indicated some oxidation reaction during redox reaction that occur on the chitin backbone.

According to DSC thermograms the glass transition temperature of chitin is observed at 293 ^{o}C. In the DSC thermo gram of chitin-*graft*-P4VP, two exothermic decomposition peaks are observed at 164 ^{o}C and 174 ^{o}C. Hence, thermal stability of chitin has been decreased significantly up on grafting with P4VP.

SEM micrographs of the non grafted and chitin-*graft*-P4VP (G%=380) are shown that the non grafted beads have a very heterogeneous surface when modified by grafting.

The dissolution of the grafted samples in aqueous acidic buffer solution (pH=1.2) was tested. The swollen hydrogel samples were taken after dry in oven the % weight loss was calculated. Solubility in acid solution has significantly been improved by P4VP grafting.

REFERENCES

Adali, T. (2013). "Synthesis and characterization of noncytotoxic and biodegradable polymethacrylates-grafted chitosan gels." Bio-Medical Materials and Engineering 23(5): 349-359.

Adali, T. (2007). "Grafting of polymethacrylates onto chitosan and characterization of the products." (Doctoral thesis). Eastern Mediterranean University, north Cyprus.

Banerjee, D., et al. (2012). "Preparation of poly (4-vinylpyridine-DVB) based anion exchangers and its applications on the separation of (TcO4-)-Tc-99 ions from reprocessing waste solution." Desalination and Water Treatment 38(1-3): 254-258.

Bhattacharya, B., et al. (2012). "Porous nanocrystalline TiO2 electrode and poly(N-methyl 4-vinylpyridine iodide): ionic liquid solid polymer electrolyte for device application." *International Journal of Nanotechnology* 9(10-12): 1030-1039.

Bratskaya, S. Y., et al. (2012). "N-(2-(2-pyridyl)ethyl)chitosan: Synthesis, characterization and sorption properties." Carbohydrate Polymers 87(1): 869-875.

Demirci, S., et al. (2013). "A new selenium-based RAFT agent for surface-initiated RAFT polymerization of 4-vinylpyridine." Polymer 54(20): 5345-5350.

Don, T. M., et al. (2002). "The synthesis of chitin-g-poly(vinyl acetate) copolymers with a redox initiator." *Journal of Polymer Research-Taiwan* 9(4): 257-263.

Gupta, K. C. and S. Sahoo (2001). "Graft copolymerization of 4-vinylpyridine onto cellulose using Co (III) acetylacetonate comlex in aqueous medium." Cellulose 8(3): 233-242.

Hsu, S. T., et al. (2013). "Preparation of methacrylic acid-grafted chitin using cerium (IV) ion and its application in adsorbing paraquat." *Journal of Applied Polymer Science* 127(1): 760-764.

Jiang, F. J., et al. (2013). "Dense poly(4-vinyl pyridine) brushes grafting from silica nanoparticles via atom transfer radical polymerization." Colloids and Surfaces a-Physicochemical and Engineering Aspects 436: 302-308.

Kader. D. A. (2014). "Grafting of poly(2-hydroxy ethyl methacrylate) onto chitin beads. Eastern Mediterranean University, north Cyprus.

Khalil, K. D. and H. M. Al-Matar (2013). "Chitosan Based Heterogeneous Catalyses: Chitosan-Grafted-Poly(4-Vinylpyridne) as an Efficient Catalyst for Michael Additions and Alkylpyridazinyl Carbonitrile Oxidation." Molecules 18(5): 5288-5305.

Katsigiannopoulos, D., et al. (2012). "Nanohybrids based on polymeric ionic liquid prepared from functionalized MWCNTs by modification of anionically synthesized poly(4-vinylpyridine)." *Journal of Polymer Science Part a-Polymer Chemistry* 50(6): 1181-1186.

Kurita, K. (2001). "Controlled functionalization of the polysaccharide chitin." Progress in Polymer Science 26(9): 1921-1971.

Mikhailov, G. M. and M. F. Lebedeva (2005). "Preparation and modification of chitin-based fibers." *Russian Journal of Applied Chemistry* 78(9): 1479-1485.

Mourya, V. K. and N. N. Inamdar (2008). "Chitosan-modifications and applications: Opportunities galore." Reactive & Functional Polymers 68(6): 1013-1051.

Nasef, M. M., et al. (2013). "Preparation and Characterization of Phosphoric Acid Composite Membrane by Radiation Induced Grafting of 4-Vinylpyridine onto Poly(ethylene-co-tetrafluoroethylene) Followed by Phosphoric Acid Doping." *Journal of Applied Polymer Science* 128(1): 549-557.

Nasef, M. M., et al. (2012). "Optimization strategies for radiation induced grafting of 4-vinylpyridine onto poly(ethylene-co-tetraflouroethene) film using Box-Behnken design." Radiation Physics and Chemistry 81(4): 437-444.

Nasef, M. M., et al. (2013). "Modeling, prediction, and multifactorial optimization of radiation-induced grafting of 4-vinylpyridine onto poly(vinylidene fluoride) films using statistical simulator." *Journal of Applied Polymer Science* 127(3): 1659-1666.

Oh, S., et al. (2011)."Poly(vinylpyridine-co-styrene) based in situ cross-linked gel polymer electrolyte for lithium-ion polymer batteries." Electrochimica Acta 57: 46-51.

Oylum Hasan; Yilmaz, Elvan; Yilmaz, Osman, J.Macromolec.Chem. Part A: Volume:5 Issue:2, Pages: 221-229 DOI: 10.1080/10601325.2013.742815 Published: JAN 1 2013.

Oylum, H., et al. (2013)."Preparation of Chitin-g-poly(4-vinylpyridine) Beads." *Journal of Macromolecular Science Part a-Pure and Applied Chemistry* 50(2): 221-229.

Oylum, H. (2013). " Preparation and characterization of poly(4-vinylpyridine) grafted chitin beads. (Doctoral thesis). Eastern Mediterranean University, north Cyprus.

Rutnakornpituk, B., et al. (2011). "Surface-initiated atom transfer radical polymerization of poly(4-vinylpyridine) from magnetite nanoparticle." *Journal of Nanoparticle Research* 13(12):6847-6857.

Sahiner, N. and O. Ozay (2011). "Highly charged p(4-vinylpyridine-co-vinylimidazole) particles for versatile applications: Biomedical, catalysis and environmental." Reactive & Functional Polymers 71(6): 607-615.

Sahiner, N. and P. Ilgin (2010). "Multiresponsive polymeric particles with tunable morphology and properties based on acrylonitrile (AN) and 4-vinylpyridine (4-VP)." Polymer 51(14): 3156-3163.

Watthanaphanit, A., et al. (2008). "Fabrication, structure, and properties of chitin whisker-reinforced alginate nanocomposite fibers." *Journal of Applied Polymer Science* 110(2): 890-899.

Yilmaz, E. and M. Bengisu (2003). "Preparation and characterization of physical gels and beads from chitin solutions." Carbohydrate Polymers 54(4): 479-488.