DOKUZ EYLÜL UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

SYNTHESIS AND CHARACTERIZATION OF CHITOSAN BASED MAGNETITE NANOPARTICLES

by
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İZMİR

SYNTHESIS AND CHARACTERIZATION OF CHITOSAN BASED MAGNETITE NANOPARTICLES

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İZMİR

M.SC THESIS EXAMINATION RESULT FORM

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ABSTRACT

Magnetic iron oxide nanoparticles have been used recently for various applications such as separation processes, magnetic resonance imaging (MRI) contrast reagents, tissue healing, immunoassay, detoxification of biological fluids, hyperthermia teraphy, drug delivery and release. These applications in general require that nanoparticles have high magnetization values and low particle size.

In this study, magnetite nanoparticles were synthesized by co-precipitation method using iron(II), iron(III) salts, chitosan and ammonia solutions. The crystal structure of the nanoparticles, their structures, surface morphologies, thermal properties and structural analysis were investigated by X-Ray Diffraction (XRD) Analysis, Scanning Electron Microscopy (SEM), Thermogravimetric Analysis (TGA/DTG) and Fourier Transform Infra-Red (FT-IR) Spectroscopic Methods of Analysis, respectively.

Keywords: Hematite, magnetite, chitosan

KİTOSAN BAZLI MAGNETİT NANOTANECİKLERİN SENTEZ VE KARAKTERİZASYONU

ÖZ

Magnetik demir oksit nanotanecikler ayırma, magnetik rezonans görüntülemede (MRI) kontrast ajanı, doku onarımı, bağışıklık, biyolojik akışkanlarda detoksifikasyon, yüksek ısı ile tedavi, ilaç taşınımı ve hücre ayırımı vb. gibi organizma içinde yaygın bir şekilde kullanılırlar.

Bu çalışmada magnetik nanoparçacıklar, Fe tuzları, kitosan ve amonyak kullanılarak birlikte çöktürme yöntemi ile hazırlanmıştır. Nanotaneciklerin kristal yapıları, şekilleri, yüzey morfolojileri, termal kararlılıkları ve yapı analizleri sırasıyla X-ışını toz kırınımı (XRD), Taramalı elektron mikroskopisi (SEM), Termogravimetrik analiz (TGA/DTG) ve Fourier dönüşümlü kızıl ötesi spektroskopisi (FT-IR) tekniğiyle incelenmiştir.

Anahtar kelimeler: Hematit, magnetit, kitosan

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CHAPTER ONE
INTRODUCTION

1.1 Nanoscience-Nanotechnology and Magnetite Nanoparticles

In recent years, the progress and advancement of a new interdisciplinary field-nanoscience and nanotechnology which cover closely interconnected concepts of physics, chemistry, biology and engineering which are also aimed at the development of new basic knowledge as dealing with objects on a size scale of 1–100 nm.

In the past five years, the development and progress in nanoscience were largely associated with the elaboration of new methods for synthesizing, studying and modifying nanoparticles and nanostructures. Physical and chemical properties of nanoparticles differ from the corresponding bulk materials. A transition from macrosizes to those of naosize gives rise to qualitative changes in physicochemical properties of individual compounds and systems. The dependence of physicochemical properties on the particle size was closely related with optical spectra, magnetic properties, thermodynamics, electrochemistry, conductivity and electron transport (Sergeev, 2006).

The size of the nanoparticles performs best when it is under a critical value and temperature is over the blocking temperature. All nanoparticles become a single magnetic domain and show very high magnetic behavior. That’s why magnetic nanoparticles very striking for applications because of these features.

However, an unavoidable problem that nanoparticle tends to form agglomerates so as to reduce the energy. In addition to uncovered metal nanoparticles are chemically active and are easily oxidized in air. That’s why loss of magnetism and dispersibility.

Iron oxides represent an essential substance due to their widespread natural occurrence. Such oxides are largely used in the production of pigments, gas sensors,

catalysis and magnetic storage devices (Schwertmann & Cornell 1996). Hematite (α-Fe_2O_3) is thermodynamically stable iron oxide, being the most important are of iron oxide. Hence, designing novel iron oxide materials are of great significance and fundamental importance in basic science and relevance to various fields of industrial applications (Kandori, 2008).

Magnetite is a magnetic material, having a cubic inverse spinel structure and has been the subject of increasing interest due to its various applications.

Magnetic nanoparticles and Fe_3O_4 have been the focus on an intensive research for more than 10 years because of the stable under ambient conditions (Kalska et al, 2004; Wang et al, 2004; Jing & Shihua, 2004). It has reported that preparation of monodispersed α-Fe_2O_3 in various shapes such as cubes, rods, wires, tubes, rings, fibers, films, spheres (Hu, Yu & Gong, 2007; Jagadeesan et al, 2008).

1.2 Synthesis of Magnetic Nanoparticles

This part is written partly from the Reviews (Lu, Salabas & Schüth, 2007; Laurent et al, 2008).

In recent years, serious efforts have been spent in the development of magnetic nanoparticles, the understanding of their behavior and the improvement of their applicability in many different areas.

Magnetic nanoparticles have been prepared with a different compositions and phases. Several popular methods can be used such as co-precipitation (Chi, 2006; Pang, Chin & Anderson, 2007), thermal decomposition and/or reduction. (Yu, Falkner, Yavuz & Colvin, 2004; Li, Sun, Gao & Angew, 2005). Micelle synthesis, hydrothermal synthesis (Xuan et al, 2007), polyol methods (Cai & Wan, 2007), sol–gel reaction, aerosol technologies (Laurent, Forge, Port et al, 2008; Sun, Lee & Zhang, 2008; Teja & Koh, 2009), laser pyrolysis techniques etc.

1.2.1 Synthesis by Co-precipitation Method

Co-precipitation is a simple and suitable method to synthesize iron oxides from aqueous Fe^{2+}/Fe^{3+} ionic solutions by the addition of a base under inert atmosphere at room or at elevated temperature. The size, shape and composition of the magnetic nanoparticles depends on the type of salts used, (e.g. chlorides, sulfates, nitrates) the Fe^{2+}/Fe^{3+} ratio, the reaction temperature, the pH value and ionic strength of the media. The magnetic properties of magnetite nanoparticles are in the range of 30–50 emu/ g^{-1} which is lower than the bulk value, 90 emu g^{-1}. Magnetite nanoparticles are not stable under ambient conditions and oxidized to maghemite or dissolved in an acidic medium.

The particles are prepared by this method are polydisperse and the selection of a surfactant is an important procedure for the stabilization of the particles. The particle sizes can be varied from 2 to 8 nm (Yu, Falkner, Yavuz & Colvin, 2004). The effects of organic anions such as carboxylate and hydroxy carboxylate ions, on the formation of iron oxides or oxyhydroxides have been studied (Li, Sun & Gao, 2005; Cai & Wan, 2007; Laurent, Forge, Port, Roch et al, 2008). The studies showed that oleic acid is useful for the stabilization of Fe_3O_4 (Sun, Lee & Zhang 2008; Teja & Koh, 2009).

1.2.2 Thermal Decomposition Method

Magnetic nanoparticles can also be prepared through the thermal decomposition of metal acetylacetonates, metal cupferronates or carbonyls in organic solvents containing stabilizing surfactants (Shi et al, 2009; Li, Sun & Gao, 2005). Fatty acids, oleic acid, and hexadecylamine are common surfactants (Chow & Gang, 2005). In principle, the ratios of the starting reagents, surfactant, solvent, temperature and time are the parameters for the control of the size and morphology of magnetic nanoparticles.

If the metal in the precursor is zerovalent, such as in carbonyls, thermal decomposition proceeds to formation of the metal but two-step procedures can be used to produce oxide nanoparticles as well. For example, iron pentacarbonyl can be

decomposed in a mixture of octyl ether and oleic acid and trimethylamine oxide as a mild oxidant at 100 °C (Langevin & Annu, 1992). Decomposition of precursors with cationic metal centers leads directly to the oxides, Fe_3O_4, if $Fe(acac)_3$ is decomposed in the presence of 1,2-hexadecanediol, oleylamine, and oleic acid in phenol ether (Pu & Jiang, 2005; Liu, Zou, Rondinone & Zhang, 2000). The decomposition approach for the synthesis of size and shape controlled magnetic oxide nanoparticles based on the pyrolysis of metal fatty acid salts in non-aqueous solution. The reaction medium was composed of the metal fatty acid salts such as decanoic acid, lauric acid, myristic acid, palmitic acid, oleic acid and stearic acid, a hydrocarbon solvent (octadecene and tetracosane, or a mixture of them), and activation reagents. Fe_3O_4 nanoparticles, with a size range of 3–50 nm could be synthesized

1.2.3 Chemical Reduction

In the chemical reduction method, the shape of the preparation of nanoparticles was spherically and smaller than 10 nm (Qu, Liu, Wang & Hong, (2010). Chemical reduction involves the reduction of metal salts in the presence of a suitable protecting agent, which is necessary for controlling the growth of metal colloids by preventing agglomeration. In general, synthetic polymers such as gelatin, polyvinylpirrolidone (PVP), polyvinylalcohol (PVA) are used as protecting agents.

1.2.4 Microemulsion Method

Microemulsion (water-in-oil) is a transparent, isotropic and thermodynamically stable medium (Chow & Gang 2005). This microemulsion is composed of dispersion of two unmixable liquids where the crosslinker are dispersed in a continuous oil phase and stabilized by surfactant molecules at the water/oil interface (Langevin & Annu, 1992). The particle size and size distribution are about 4-15 nm with very narrow size disribution (Liu, Zou, Rondinone & Zhang, 2000). This method has the advantages of regular properties and size of the nanoparticles can be oriented. Disadvantages of surfactant are difficult to remove only a small quantity of iron oxide can be synthesised.

The difference between microemulsions and micelles is more than simple but recently these terms have been used interchangeably. The micelle aggregates have sizes of 1–10 nm in diameter, while microemulsions contain aggregates that are 10–100 nm in diameter (Pileni & Duxin, 2000).

1.2.5 Hydrothermal Synthesis Method

It has been reported a hydrothermal method for synthesizing different nanocrystals by a liquid–solid–solution reaction (Wang, Zhuang, Peng & Li, 2005). Size and morphology of the nanoparticles can be controlled in hydrothermal reactions by controlling time and temperature. There are two routes for the formation of ferrites via hydrothermal conditions: hydrolysis, oxidation and neutralisation of mixed metal hydroxides (Makovec, Drofenik & Znidarsic, 1999; Wang, Shih & Lin, 1994). The particle size and size distrubution were about 9-12 nm and monodisperse, hydrophilic and single crystalline ferrite microspheres (Deng, Li et al, 2005).

Hematite (α-Fe_2O_3) ferrimagnetic is the simplest and most efficient method (Fe_3O_4 or γ-Fe_2O_3) from Fe^{2+}/Fe^{3+} salt solutions by the addition of a base under inert atmosphere at room temperature.

$$Fe^{2+} + 2Fe^{3+} + 8OH^- \rightarrow 2\beta\text{-}FeOOH + Fe^{2+} + 2H_2O + 2OH^- \rightarrow Fe_3O_4 + 4H_2O \quad (1.1)$$

According to reaction above, Fe^{2+} and Fe^{3+} ions are first consumed with the formation of β-FeOOH at pH smaller than 5 and second step to raise pH to the values greater than 5. Therefore, the control of pH is very important in controlling the size of magnetite particles. $FeCl_3$, ethylene glycol, sodium acetate and polyethylene glycol mixture was stirred vigorously to form a clear solution. It sealed then in a autoclave and heated to 200 °C for 8–72 h. In this way, monodisperse ferrite spheres were obtained with tunable sizes in the range of 200–800 nm (Deng, Li, Peng et al, 2005).

Magnetite had been generally used in many important technological applications, such as information storage, electronic devices, ferrofluids, refrigeration, biomedical imaging, medical diagnostics and drug delivery (Denkbas, Kilicay, Birlikseven &

Ozturk, 2002). It is important for all these applications to synthesize the nanoparticles with diameter less than 15 nm and a narrow size distribution.

In nanoparticle preparation, each particle must be stabilized against aggregation with a layer of organic or inorganic coatings. For Fe_3O_4 nanoparticles, it is generally difficult to obtain better monodispersity due to its inherent magnetism. Therefore, surfactant/organic coating are necessary for the synthesis of monodispers Fe_3O_4 nanoparticles (Li, Jiang, Huang, Ding & Chen, 2008; Giri, Trewyn, Stellmaker & Angew, 2005). These methods need high temperature, expensive and toxic starting materials, complicated procedure and toxic organic solvents. However, inexpensive and nontoxic starting materials can be chosen and prepared single-crystal Fe_3O_4 nanoparticles with relatively better monodispersity at room temperature co-precipitation method in the present of PVP.

Fe_3O_4 nanoparticles easily attract and aggregate each other due to its inherent magnetism. In this system, the single-crystal Fe_3O_4 nanoparticles are relatively monodisperse by the addition of PVP.

It has been reported that the complexation between the surfactant molecules and ferrites leads to an improvement in the uniformity of crystallite size and morphology (Li, Jiang, Huang, Ding & Chen, 2008; Gupta & Hung, 1993). Furthermore, the small amount of surfactant plays a critical role in modifying the particles and crystalline characteristics. The addition of PVP effectively decreases the coalescence between Fe_3O_4 nanoparticles. Then, Fe_3O_4 nanoparticles show uniform size and relatively better monodispersity.

Fe_3O_4 nanoparticles have superparamagnetic and ferromagnetic properties which depend on temperature. The coercivity of Fe_3O_4 nanoparticles at 10 K is about 178 Oe. It meant that the thermal energy can overcome the anisotropy energy barrier of a single Fe_3O_4 nanoparticle, and the coercivity and remanence of Fe_3O_4 nanoparticles are zero in the absent of external field. This event indicates the transition from ferromagnetic to superparamagnetic behavior with increasing temperature. For instance, the saturation magnetization of single-crystal Fe_3O_4 nanoparticles with the size of 13nm is 68.8 emu/g, which is lower than 83 emu/g (Fe_3O_4 nanoparticles with

the size of 16 nm) (Giri, Trewyn, Stellmaker & Lin, 2005), but is higher than 67.8 emu/g (Fe_3O_4 nanoparticles with the size of 12.4 nm) (Panyam & Labhasetwar, 2003).

1.2.6 Sol-Gel Reactions

Sol–gel processing, in which involves hydrolysis and condensation of metal alkoxides can be used to synthesize various materials such as glasses, powders, films, fibres and monoliths. This process needs to be considered several factors such as solvent, temperature, precursors, catalysts, pH, additives and mechanical agitation. These factors can also affect the kinetics, growth, hydrolysis and condensation reactions (Brinker & Sherrer, 1990). The pH of the solution also influences the isoelectric point and the stability of the sol. These in turn affect the aggregation and particle size. The particle size ranged from 10 to 500 nm at 65 °C and 125 °C respectively.

1.2.7 Polyol Methods

The polyol method in which the polyol acts as reducing agent, surfactant, and solvent is a suitable method for preparing nanophase and micrometre size particles with well defined shapes and controlled particle sizes (Chow, Kurihara & Kemner, 1995; Lewis, Fliflet, Gold, Bruce & Kurihara. 2002). During the reaction, the metal precursors become solubilised in the diol, form an intermediate and then are reduced to form metal nuclei, which form the nanoparticles.

1.2.8 Flow Injection Syntheses (FIS)

Synthesis of magnetite nanoparticles based on a flow injection synthesis technique has been developed and consisted of continuous or segmented mixing of reagents under laminar flow regime in a capillary reactor. The method has advantages, such as a high reproducibility and laminar conditions, a high mixing homogeneity, and an opportunity for a precise external control of the process. The chemical parameters and conditions on the properties of the naoparticles were investigated and 2-7 nm sized magnetite nanoparticles were prepared (Alvarez, Muhammed & Zagorodni, 2006).

1.2.9 Electrochemical Methods

Maghemite particles with particle sizes of 3-8 nm have been prepared from an iron electrode in an aqueous solution of DMF and cationic surfactants by using an electrochemical method. In this method, adjustment of the current density controls the particle size (Reetz, Helbig & Quasick, 1996; Pascal et al, 1999). Electrochemical deposition under oxidizing conditions has been used to prepare nanoparticles of Fe_2O_3 and Fe_3O_4 (Kahn & Petrikowski, 2000).

1.2.10 Aerosol/Vapor Methods

Spray and laser pyrolysis are very novel techniques. These technologies are continuous chemical processes allowing for high rate production. In spray pyrolysis, a solution of ferric salts and a reducing agent in organic solvent is sprayed into a series of reactors, where the aerosol solute condenses and the solvent evaporates (Pecharroman, Gonzalez-Carreno & Iglesias, 1995). The products consist of particles whose size depends upon the initial size of the original droplets. Maghemite particles with size of 5 to 60 nm with different shapes have been prepared by using different iron precursor salts in the solution (Gonzalez-Carreno, Morales, Gracia & Serna, 1993)

1.2.11 Sonolysis

Magnetite can also be prepared by decomposition of organometallic precursors. Polymers, organic agents or structural hosts are used to limit the nanoparticle growth in size (Osuna et al, 1996; Park et al, 2000).

The high temperature hot spot generated by the rapid collapse of sonically generated cavities allows for the conversion of ferrous salts into magnetic nanoparticles. The sonolysis of an aqueous solution is composed of $Fe(CO)_5$ and sodium dodecyl sulfate leads to the formation of Fe_3O_4 nanoparticles (Abu Mukh-Qasem & Gedanken, 2005).

Magnetic iron oxide nanoparticles having high magnetization and crystallinity have been prepared using a sonochemical method. These spherical particles of about 15 nm in diameter showed superparamagnetic behavior. These ferrofluids coated

with oleic acid then dispersed in chitosan can be used as MRI contrast agents and carrier for bioapplications in cell and cancer teraphy for the targeting the tumors. (Kim, Lee, Kwak & Kim, 2005).

Table1.1 Methods of synthesis of magnetic nanoparticles. (Lu, Salabas & Schüth, 2007).

Synthetic Method	**Synthesis**	**Reaction Temp. [°C]**	**Reaction Period**	**Solvent**	**Surface-capping agent**	**Size distribution**	**Shape control**	**Yield**
Co-prepication	Very simple, ambient conditions	20-90	minutes	water	Needed, added during reaction	Relatively narrow	Not good	Higly scable
Thermal decomposition	Complicated, inert atmosphere	100-320	Hours-days	Organic compound	Needed, added during reaction	Very narrow	Very good	Higly scable
microemulsion	Complicated, ambient conditions	20-50	hours	Organic compound	Needed, added during reaction	Relatively narrow	good	Higly scable
Hydrothermal synthesis	Simple-high pressure	220	hours	Water-ethanol	Needed, added during reaction	Very narrow	Very good	medium

1.3 Stabilization of Magnetic Particles

The stabilization of the iron oxide particles is very important to obtain magnetic colloidal ferrofluids that are stable against aggregation in both a biological medium and a magnetic field.

1.3.1 Monomer Stabilizers

It has been reported that functional groups such as carboxylates, phosphates, and sulfates bind to the surface of magnetite nanoparticles (Cornell & Schertmann, 1996; Sahoo, Pizem et al, 2001).

1.3.2 Carboxylates

The magnetite nanoparticle surfaces can be stabilized by the adsorption of citric acid in an aqueous solution (Sahoo et al, 2005). The adsorption mechanism involves coordination of one or two carboxylate functionalitie which depends upon steric factors and surface curvature. This leaves at least one carboxylic acid group exposed to the solvent, which should be responsible for making the surface negatively charged and hydrophilic.

1.3.3 Phosphates

The alkanesulphonic and alkanephosphonic acid were used as surfactants and stabilizers in the preparation of Fe_2O_3 nanoparticles (Portet et al, 2001; Mutin, Guerrero & Vioux, 2003; Kreller, Gibson, Novak, Van Loon & Hugh Horton, 2003).

It has been also proposed two possible bonding mechanism for the phosphonate ions on Fe^{3+}, one O or two O atoms of the phosphonate group (Yee et al, 1999).

It has been suggested that phosphate ions form bidentate complexes with adjacent sites on the iron oxide surface (Tejedor-Tejedor & Anderson, 1990; Persson, Nilsson & Sjöberg, 1996).

The biocompatibility of phosphonate and phosphate ligands may advance them toward the use of encapsulation of magnetic nanoparticles in medical applications, such as MRI and other biophysical purposes (Roberts, Zhu, Fromenn & Rosenzweig, 2000).

1.4 Inorganic Materials

Iron oxide nanoparticles were coated with silica, gold or gadolinium(III) (Zhang, Wangler & Morgenstern, 2007; Mulvaney, Liz-Marzan, Giersig & Ung, 2000; Tartaj, Gonzalez-Carreno & Serna, 2002; Chen, Yamamuro, Farrell & Majetich, 2003; Lin et al, 2001; Xu, Sorensen, Klabunde & Hadjipanayis, 1992).

These coatings provide stability to the nanoparticles in solution and help in binding various biological ligands to the nanoparticle surface. These nanoparticles have an iron oxide core with an outer metallic shell of inorganic materials.

1.5 Polymer Stabilizers

In order to coat the naoparticles, several approaches have been developed. At first approach, nanoparticles are coated during the synthesis. In the literature, the most common coatings are dextran and its derivatives, starch, arabinogalactan, glycosaminoglycan, sulfonated styrene-divinylbenzene, polyethylene glycol (PEG), polyvinyl alcohol (PVA), poloxamers and polyoxamines (Zhang, Kohler & Zhang, 2002; Lacava et al, 2001).

1.5.1 Dextran

Dextran is a polysaccharide composed of R-D-glucopyranosyl units with varying degrees of chain length and branching. Dextran has been used often as a polymer coating mostly because of its biocompatibility (Berry, Wells, Charles & Curtis, 2003; Gamarra et al, 2005).

1.5.2 Polyethylene Glycol (PEG)

PEG is a hydrophilic, water-soluble and biocompatible polymer. It has been reported that the usage of PEG increased the biocompatibility of the iron oxide dispersions (Kim, Zhang et al, 2001; Shultz, Calvin, Fatouros, Morrison & Carpenter, 2007; Butterworth, Illum & Davis, 2001; Ding, Sun, Wan & Jiang, 1998; Kohler, Fryxell & Zhang, 2004; Kang, Josephson, Petrovsky, Weissleder & Bogdanov, 2002).

1.5.3 Polyvinyl Alcohol (PVA)

PVA is also a hydrophilic and biocompatible polymer. PVA coating onto the nanoparticle surface prevents their agglomeration, giving rise to monodisperse particles (Yokoi & Kantoh, 1993; Sairam, Naidu, Nataraj, Sreedhar & Aminabhavi, 2006; Schöpf et al, 2005; Xue & Sun, 2001).

In order to form stable dispersion, the surface of nanoparticles has been modified with PVA by precipitation of iron salts in PVA solution. It has been also suggested that PVA irreversibly binds to the surface of magnetite (Lee, Isobe & Senna, 1996).

PVA coated iron oxide nanoparticles have synthesized. The colloidal stability of the product as well as the resulting particle size distribution was determined for different iron/polymer ratios. The results showed different behavior for iron/polymer mass ratios smaller and larger than 2 (Bee, Massart & Neveu, 1995; Chastellain, Petri & Hofmann, 2004).

PVA-stabilized Fe_3O_4 nanoparticles were synthesized by using chitosan (CS) and an anionic monomer, acrylic acid. PVA stabilizes the Fe_3O_4 nanoparticles in two ways. (1) PVA can form a hydrogen bond between chitosan and poly (acrylic acid) (Dan, Chen, Zhang & Xiang, 2000). (2) The pH and ionic strength have little effect on the PVA due to its acid and basic stability. On the other hand, Fe_3O_4 nanoparticle-loaded CS-PAA spheres were prepared after addition of Fe_3O_4 nanoparticles into CS-AA aqueous solution, followed by polymerization of AA

monomer and cross-linking of chitosan with glutaraldehyde at the end of polymerization (Hu, Chen et al, 2005).

1.5.4 Alginate

Alginate is an electrolytic polysaccharide containing carboxyl groups. The carboxyl groups of alginate and iron ion interact each other due to the electrostatic attraction which may stabilize magnetic iron oxide nanoparticles. Recently, several investigations dealing with the preparation of iron oxide nanoparticles with alginate have been developed (Nishio et al, 2004; Llanes, Ryan & Marchessault, 2000; Finotelli et al, 2004; Kroll, Winnik & Ziolo, 1996).

1.5.5 Chitosan

Chitosan can be produced in various forms such as powder, paste, film, fiber, etc. from various sources (Muzzarelli, Jeuniauk & Gooday, 1986; Sjak-Braek, Anthonsen & Sandford, 1992) and can be used as the materials for the food industry, cosmetics, water treatment, agriculture and biomedicine expecially tissue engineering, artificial skin, wound dressing and drug delivery (Prashanth & Tharanathan, 2007; Rinaudo, 2006). This biopolymer represents an interesting and attractive alternative as an adsorbent due to the presence of chemical reactive groups (hydroxyl, acetamido or amino functions) in polymer chains, physico-chemical characteristics, chemical stability, high reactivity and excellent selectivity towards aromatic compounds and metals (Ciesielski, Lii, Yen & Tomasik, 2000; Polaczek, Starzyk, Malenki & Tomasik, 2000).

Chitosan is nontoxic, hydrophilic, biocompatible and biodegradable partially acetylated glucosamine biopolymer (Hu et al, 2007; Guo, Xia, Wang, Song & Zhang, 2005).

Nowadays, the encapsulation of magnetic nanoparticles in chitosan are of great interest (Jia, Yujun, Yangcheng, Jingyu & Guangsheng, 2006; Sipos, Berkesi, Tombacz, St. Pierre & Webb, 2003; Bhattarai, Bahadur, Aryal, Khill & Kim, 2007; Kim, Ahn & Lee, 2006).

In addition, the amino groups on the chitosan can also be modified for further functionalization with specific components and used to modify the Fe_3O_4 NPs.

Many investigations on chitosan modified magnetic nanoparticles used for biomedical applications have been reported. Superparamagnetic Fe_3O_4 nanoparticles decorated with carboxymethylated chitosan which were used for covalent conjugation of papain was prepared (Liang & Zhang, 2007). Chitosan/polyacrylic acid/ Fe_3O_4 NPs which could be used for MRI was synthesized (Feng et al, 2009).

Iron oxide magnetic particles coated with chitosan was prepared and used for hyperthermia (Donadel et al, 2008).

However, the saturation magnetization (22 emu/g) of the composite nanoparticles is low and the heating effect is not desirable. A Chitosan–Fe_3O_4 nanoparticle in inverse emulsion was prepared and investigated their inductive heating property in AC magnetic field. The problem about this method is that the use of organic solvent is not environment friendly and the dispersity of the chitosan– Fe_3O_4 NPs prepared is poor (Zhao, Wang, Zeng, Xia & Tang, 2009).

For hyperthermia applications, it is required that the Fe_3O_4 nanoparticles have high saturation magnetization, uniform particle size and superparamagnetism (Qu, Liu, Wang & Hong, 2010).

Microspheres composed of superparamagnetic iron oxide nanoparticles and chitosan were developed as a novel MRI detectable embolic material. The nanoparticles about 15 nm in radius have been prepared by sonochemistry and embedded them in chitosan to synthesize a ferrofluid. These microspheres showed a strong enhancement in MRI contrast similar to the ferrofluid (Lee, Kim, Shao & Kwak, 2005).

Chitosan has a unique cationic nature among the other natural polymers (Rinaudo, 2006) and is mucoadhesive (Lehr, Bouwstra, Schacht & Junginger, 1992). Because of the mucoadhesive properties, it can be used to enhance drug penetration across the mucosa. Consequently, in recent years chitosan has also been extensively investigated as a carrier for mucosal drug delivery, an absorption enhancer, suitable biomaterial in ophthalmology and as carrier for gene delivery (Illum, Farraj & Davis, 1994; Alonso, Salve, Remunan-Lopez & Vila-Jato, 1998; Alonso & Sánchez, 2003).

Chitosan has functional groups such as hydroxyl and an amino group on the molecular chain. Reactions involving the NH_2 group at the C-2 position are the most frequently employed which are available for cross-linkages. Chitosan is generally needed to be crosslinked to improve its chemical stability in acid media (Agnihotri, Mallikarjuna & Aminabhavi, 2004).

In spite of these properties and advantages some problems may occur. A limiting factor in the application of chitosan is its low solubility in aqueous media at neutral and alkaline conditions and therefore can not be used as an insoluble sorbent under these conditions, except after physical and chemical modifications (Wurzburg, 1986). The water solubility of chitosan has been improved by different methods such as depolymerization (Nishimura, Kohgo, Kurita & Kuzuhara, 1991), quaternization of the amino group (Fischer, Bieber, Li, Elsaesser & Kissel, 1999), N-carboxymethylation (Muzzarelli, Tanfani & Emanuelli., 1984) and PEGylation (Saito, Wu, Harris & Hoffman, 1997).

Chitosan is soluble in weakly acidic solutions and shows a polycationic character. Usually 1–3% aqueous acetic acid solutions are used to solubilize chitosan. These properties depend on its degree of deacetylation and molecular weight, (Li, Jiang, Huang, Ding & Chen, 2008; Zhao, Wang, Zeng, Xia & Tang, 2009; Fang, Volotinen, Kulkarni, Belova & Rao, 2010).

Chitosan, the commercial material in the form of powder or flakes is not suitable to be used directly as an adsorbent. It is soluble in acidic solutions and has low surface area. Chitosan–magnetite composite particles with high specific surface area, can be used as an adsorbent in the removal of heavy metal ions. After adsorption, it is easy to separate it from the eluent with complexing the heavy metal ions by using a magnetic field (Hu, Bo, Yao, Bo & RongShi, 2009).

1.5.6 Other Polymers

Polymers such as polymethacrylic acid, poly(ethyleneoxide)-*b*-poly(methacrylic acid), polyvinylpyrrolidone (PVP), poly(acrylic acid) (PAA), polyalkylcyanoacrylate, poly(lactic acid), ethylcellulose, poly(*ε*-caprolactone), sulfonated styrene-divinylbenzene or arabinogalactan have been used and studied well (Inouye et al, 1982; Mendenhall, Geng & Hwang, 1996; Wormuth, 2001; Liu et al, 2006; Lee et al, 2005; Iijima, Yonemochi, Tsukada & Kamiya, 2006; Fahlvik, Holtz, Schröder & Klaveness, 1990; Kellar et al, 1999; Kim et al, 2003; Kim, Mikhaylova, Zhang, Tsakalakos & Muhammed, 2003; Arias, Gallardo, Gomez-Lopera & Delgado, 2005; Gomez-Lopera, Arias, Gallardo & Delgado, 2006; Arias, López-Viota, Ruiz, López-Viota & Delgado, 2007; Flesch, Delaite, Dumas, Bourgeaut-Lami & Duguet, 2004; Flesch, Bourgeaut-Lami et al, 2005; Lawaczeck, Menzel & Pietsch, 2004).

1.6 Other Strategies for Stabilization

In order to stabilize magnetic nanoparticles, core/shell magnetic nanoparticles can be synthesized by using synthetic polymers as a matrix (Moeser, Green, Laibinis, Linse & Hatton, 2004; Sun et al, 2002; Gomez-Lopera, Plaza & Delgado, 2001; Deng, Wang, Wang & Fu, 2003; Liao & Chen, 2002; Yu & Chow, 2004

CHAPTER TWO
STRUCTURAL AND PHYSICOCHEMICAL CHARACTERIZATION OF MAGNETITE NANOPARTICLES

The magnetic properties of nanoparticles and their biological behavior depend upon the size and the shape of the particles, their microstructure, and the present chemical phases. Various physicochemical techniques are used to determine these properties.

The size of core of the naoparticle can be determined by Transmission Electron Microscopy (TEM) images (Kaiser & Miskolczy, 1970; Kim, Zhang, Voit, Rao & Muhammed, 2001; Thomas & Hutten, 1997; Ascencio et al, 1998).

TEM reports the total particle size of the core (crystalline and amorphous parts) and gives access to a number-weighted mean value.

XRD analysis can be performed to obtain the crystalline structure of the particles. In a diffraction pattern, the intensity can be used to quantify the proportion of iron oxide formed in a mixture by comparing experimental peak and reference peak intensities (Inouye et al, 1982).

The crystal size can be determined from the line broadening in the XRD pattern using the Scherrer Formula (Rao & Houska, 1986; Calvin, Carpenter & Harris, 2003).

Scanning Electron Microscopy (SEM) images can be investigated to determine the surface morphology and size of the particles in some respect.

The thermal stabilities and also magnetic properties of the magnetite nanoparticle must be determined before usage of these in the applications, especially in the field of biosciences and medicine.

CHAPTER THREE
APPLICATIONS AND USAGE OF MAGNETITE NANOPARTICLES

Superparamagnetic nanoparticles characterized by higher magnetization and good biocompatibility have attracted significant attention as magnetic drug targeting carriers for hyperthermia (Jordan, Scholz, Wust, Hling & Felix, 1999; Babincova, Leszczynsk, Sourivong, Čičmanec & Babinec, 2001; Andreas, Regina & Klaus, 2001; Moroz, Jones, Winter & Bruce, 2001; Kong, Braun & Dewhirst, 2001) and controlled drug release (Detlef & Thomas, 2006; Sonvico, Mornet & Vasseur, 2005; Jain, Morales, Sahoo, Leslie-Pelecky & Labhasetwar, 2005) and as contrast enhancement agents in magnetic resonance imaging (MRI) (Kohler, Sun, Wang & Zhang, 2005; Kangarlu, Ibrahim & Shellock, 2005; Mulder, Strijkers, van Tilborg, Griffioen & Nicolay, 2006). Surface modified superparamagnetic nanoparticles characterized by the absence of magnetism on the removal of a magnetic field can be delivered to the tumor site using an external magnetic field (Ito, Shinkai, Honda & Kobayashi, 2005). However, non-surface-modified superparamagnetic nanoparticles with a large surface area-to-volume ratio tend to agglomerate and form large clusters, with the consequent loss of their superparamagnetic characteristics.

An important disadvantage of the magnetic particles for drug delivery systems is that they are rapidly cleared by macrophages or the reticuloendothelial system before reaching the tumor cells (Gupta & Gupta, 2005).

It is important for biomedical applications that the surface-modified magnetic nanoparticles have water-soluble and specific functionalized groups on the surface to bind the drug. In designing drug delivery systems for cancer therapy, it is preferred to use core-shell type composite nanoparticles. The uncovered anticancer drugs can lead to possible serious side effects during the delivery of the drug (Patil, 2003; Zhang & Misra, 2007; Kohler, Fryxell & Zhang, 2004); Yu, Chang, Sayes, Drezek & Colvin, 2006); Gupta & Curtis, 2004; Gupta & Wells, 2005).

Magnetite (Fe_3O_4) is an important kind of magnetic material having cubic inverse spinel structure which has been attracting increasing attention because of its wide use in magnetic recording (Lu, Salabas & Schüth, 2007), ferrofluid (Raj, Moskowitz & Casciari, 1995), catalyst (Rossi et al, 2007) and biomedical applications, such as magnetic resonance imaging (MRI) contrast agents (Lee, Jun, Yeon, Shin & Cheon, 2006; Hu, Wei, Zhou et al, 2006; Nasongkla et al, 2006; Billotey, Wilhelm et al, 2003), bio-separation (Bao et al, 2007; Shao, Xu et al, 2009), drug targeting (Sonvico et al, 2005); Yang et al, 2008; Chen, Gao & Ni, 2008) and hyperthermia (Shi et al, 2009; Fortin, Wilhelm et al, 2007; Giri, Pradhan, Sriharsha & Bahadur, 2005).

For biomedical applications, Fe_3O_4 NPs are often treated with surface modification. On the one hand the modification could increase the chemical stability of the Fe_3O_4 NPs; on the other hand it could improve the biocompatibility of the NPs. Various materials have been reported to modify Fe_3O_4 NPs, such as precious metals (Wang et al, 2008; Xu, Hou & Sun, 2007), silica (Stjerndahl et al, 2008), carbon (Xu et al, 2004) and biopolymers (Berry, Wells, Charles & Curtis, 2003; Ren, Hong, Ren & Teng, 2006; Zhang, 2005; Laurent et al, 2008).

Magnetic hybrid hollow spheres of about 200 nm were prepared by a core-template-free route. Fe_3O_4 nanoparticles stabilized by poly(vinyl alcohol) (PVA) to an aqueous solution of polymer-monomer pairs composed of chitosan and acrylic acid (Ding, Hu, Zhang, Chen & Jiang, 2006).

Magnetic nanoparticles have a great interest because of their magnetic, electric, biocompatibility (Dzarova et al, 2011; Fang, Volotinen, Kulkarni, Belova & Rao, 2010).

As well as the entire feature, hematite (α-Fe_2O_3) nanoparticles can be used experimentally for various in vivo applications. It is the most important aplication is used to biomedical for example tissue repair, (Giri, Trewyn, Stellmaker, & Lin, 2005), targeted drug delivery (Allen & Cullis, 2004; Prabaharan & Mano, 2005),

magnetic resonance imaging (MRI), magnetic hyperthermia for cancer treatment (Lubbe et al, 1996; Veiseh, Gunn & Zhang, 2010; Singh, Pandey, Tewari & Agarwal. 2011), magnetofection (Arbab et al, 2003), rapid blood detoxification methods (Ito, Shinkai, Honda & Kobayashi, 2005), catalysis, cellular labelling and separation. All these biomedical and bioengineering applications require that these nanoparticles have high magnetization values, biocompatibility and interactive function.

Cellular labelling/cell separation have been developed by Nam et al (Nam, Thaxton & Mirkin, 2003) because of their high separation efficiency (Arbab et al, 2003; Gupta & Hung. 1993).

Tissue repair use of stem cells therapies offers enormous potential for illness treatment and cures for many usual diseases such as cancer, heart disease, Alzheimer's and Parkinson's illness (Bulte, Douglas & Witwer, 2001).

Drug delivery has been developed by Widder et al (Elbert, 1996). This method can be used to reduce drug doses and potential side effects to healthy tissues expecially avoid toxicity and other adverse side effects arising from high drug doses in other parts of the organism (Panyam & Labhasetwar, 2003; Bonnemain, 1998).

In MRI, magnetic nanoparticles serve as contrast enhancement agents (Chen, Gao & Ni, 2008; Shi et al, 2009) due to their high saturation magnetization, low toxicity, and biocompatibility.

Hyperthermia is one of the promising approaches in cancer therapy such as chemotherapy, radiotherapy and surgery in cancer therapy (Gupta & Wells, 2005; Mornet, Vasseur, Grasset & Duguet, 2004).

Diverse methods such as hot water, capacitive heating and induction heating are used in hyperthetmia. This method is used due to the important properties of magnetic particles such as non-toxicity, bio-compatiblilty, injectability, high-level accumulation in the target tumor and effective absorption of the energy of alternating magnetic fields (Cavaliere & Ciocatto, 1967; Stauffer et al, 1984).

Catalysts with the nano-size range can be separated much easier than by filtration and centrifugation especially in a liquid-phase reaction. Small and magnetically separable catalysts have the advantages of high dispersion and reactivity with easy separation

In addition to magnetic nanoparticles can be used in many different application such as data storage (Hyeon, 2003), biolabeling (Jiles, 2003; Huber, 2005), environmental remediation (Elliott, Zhang & Environ, 2001; Takafuji, Ide, Ihara & Xu, 2004), protein and nucleic acid separation (Bao, Yao & Gu, 2009), high-density information storage (Sun, Murray, Weller, Folks & Moser, 2000), electronic devices (Tang, Wang & Zhou, 2001), ferrofluid technology (Pu & Jiang, 2005; Yi, Lee & Ying, 2006), pigments, magnetic separation, detoxification of biological fluids, immunoassay (Wang, He, Rosenzweig & Rosenzweig, 2004), nanoelectronics, chemical sensing, medical diagnostics (Murray, Kagan & Bawendi, 2000) detoxification of biological fluids, immunoassay, cosmetics, sensors, clinical diagnosis, magnetic storage media and treatment, biomedicine and biotechnology, biosensors (Shi et al, 2009).

3.1 The Aim of the Study

In recent years, magnetic nanoparticles have attracted significant attention as magnetic drug targeting carriers for hyperthermia, as contrast enhancement agents in magnetic resonance imaging (MRI), catalysts in the field heterogeneous catalysis, as adsorbents in the separation processes.

The physical and chemical properties of magnetite nanoparticles are greatly affected by the synthesis route. Various approaches such as organic phase decomposition of an iron precursor, hydrothermal route, electrochemical route, DC thermal arc-plasma route, microemulsion route, and solvothermal route have been employed to produce magnetite (Prabaharan & Mano, 2005; Sun, Lee & Zhang, 2008; Lubbe, Bergeman, Riess, Schriever & Possinger, 1996; Veiseh, Gunn & Zhang, 2010; Singh, Pandey, Tewari & Agarwal, 2011; Arbab et al, 2003).

Surface modified magnetic nanoparticles characterized by the absence of magnetism on the removal of a magnetic field can be delivered to the tumor site using an external magnetic field. On the other hand, non-surface-modified magnetic nanoparticles with a large surface area-to-volume ratio tend to agglomerate and form large clusters, with the consequent loss of their magnetic characteristics. Therefore, the surface of the magnetic nanoparticles must be modified properly with inorganic materials and polymer stabilators such as PEG, PVA, Alginate, Chitosan and Dextran because of the stabilization of the nanoparticles in the preparation to prevent the agglomeration. In this study, magnetit nanoparticles will be prepared using iron salts and chitosan and also Na-TPP and ammonia as procersures by co-precipation method. The samples will be then characterized by XRD, SEM, TG/DTG and FTIR measurements.

CHAPTER FOUR
MATERIALS AND METHODS

4.1 Materials

Chitosan (CS) (middle-viscous) was purchased from SIGMA (28191-100G) as a crab shells with a deacetylation degree of 86.7%. Iron (III) chloride hexadydrate ($FeCl_3.6H_2O$) (Cat.-71768) and iron (II) chloride tetrahydrate ($FeCl_2.4H_2O$) (Cat.-220299) were purchased from Sigma-Aldrich. Ammonium hydroxide (NH_4OH) (Cat.-320145), and hydrochloric acid (HCl) (Cat.-258148) were purchased from Sigma. Sodium tripolyphosphate (STPP) (238503) 85% was also purchased from Sigma-Aldrich. Sodium hydroxide (72079-100 ml) (pellets, 97%) was purchased from Fluka. Glacial acetic acid (Cat.-64-19-7) was received from Chemical Company. All the chemicals were used as they received commercially without any further prufication. The solutions were prepared with distilled water.

4.2 Preparation of Magnetite, Chitosan, Magnetite/Chitosan (Core/Shell) and Magnetite/Chitosan/STTP Nanoparticles.

4.2.1 Preparation of Magnetite Nanoparticles

Fe_3O_4 nanoparticles were prepared by co-precipitation method (Panyam, & Labhasetwar, 2003; Bonnemain, 1998). 40 mL of a 1 M $FeCl_3$ solution and 10 mL of a 2 M $FeCl_2$ solution were dissolved in 100 mL distilled water under ultrasonic bath (≈700 rpm) and mechanic stirring at 25 °C. Then 100 mL of 0.7 M ammonia (NH_3) solution were added to this solution. After mixing, the colour of the solution turned to the black. In order to obtain stable suspensions, Fe_3O_4 particles produced were decanted and redispersed in a 10 mL 2M HCI solution. The reaction was continued by stirring for an additional 30 mins. The particles were again magnetically decanted after 12 h and cleaning was achieved by repeated magnetic separation and re-dispersion in water. Particles were then dried in a vacuum oven and stored until their use. Samples were referred to as MNP-U and MNP-M.

4.2.2 Preparation of Magnetite/Chitosan Nanoparticles

A chemical co-precipitation method was followed to prepare chitosan based Fe_3O_4. First of all, chitosan solution was prepared by dissolving 10 mg chitosan in 100 mL 1% (v/v) of acetic acid. This solution was transferred into an erlenmeyer flask containing the 1g $FeCl_2.4H_2O$ and 3g $FeCl_3.6H_2O$ aqueous solution were prepared in 50 mL distilled water. The mixture was homogenized by ultrasonic bath and then precipitated by the addition of 20 mL %25 ammonia solution under ultrasonic stirring. The resulting dark black CS–Fe_3O_4 complex was stirred additionally for an hour in ultrasonic bath. The dark black particles were separated from aqueous phase by centrifugation and washed with distilled water three times. Then, the particles were dried in a vacuum oven. All samples were kept under vacuum, to avoid long-term oxidation by air. The sample was named as CS–Fe_3O_4.



Figure 4.1 The images of the magnetite nanoparticles after formation

4.2.3 Preparation of Magnetite/Chitosan/STPP Nanoparticles

0.1 g Chitosan was dissolved in 100 mL %1 (v/v) aqueous acetic acid. 3g $FeCl_3.6H_2O$ and 1g $FeCl_2.4H_2O$ was dissolved into 50 mL water in another flask. After that this solution was transfered into the chitosan solution under ultrasonic stirring at room temperature. After 30 min stirring, 20 mL of % 25 ammonia solutions was added under ultrasonic bath. After addition, the colour of the solution chanced immediately to dark black and magnetite nanoparticles were formed. This colloidal suspension was stirred about 2 hours until it turned homogeneous. 0.125 g sodium tripolyphosphate (STPP) as a cross-linker was slowly added to the solution in 30 mins. Magnetite/Chitosan/STPP product was decanted from aqueous phase by centrifugation and was washed by distilled water. Then particles were dried in an incubator. All samples were kept in a desicator to avoid oxidation by air and named as CS-Fe_3O_4-TPP.

4.2.4 Effect of pH on the Synthesis of Magnetite Nanoparticles

20 mL of 1 M Na_2SO_3 solution was added to 30 mL of 2 M $FeCl_3.6H_2O$ stock solutions, previously acidified with 2, 1 and 0.5 M HCl solution. The reaction was carried out in after mixing Fe^{3+} and SO_3^{2-}, the color of the solution changed from light yellow to red-brown and after few minutes, the yellow color reappeared. In this time, 75 mL of 0.5 M ammonium hydroxide (NH_3) solution was then quickly poured into the solution under vigorous stirring; then a black precipitate was formed. Final pH of the solution after reaction was 11 and 10. The reaction was continued by stirring for an additional 30 min. The supernatant was discarded and the black precipitate was washed and centrifuged in distilled water and precipitate obtained was centrifuged in an acetone medium and subsequently placed in a desiccator with silica beads to dry at room temperature. Samples were named as MNP-0,5A, MNP-1A and MNP-2A

The possible reaction scheme of magnetite, chitosan and Na-TPP was given in Figure 4.2.

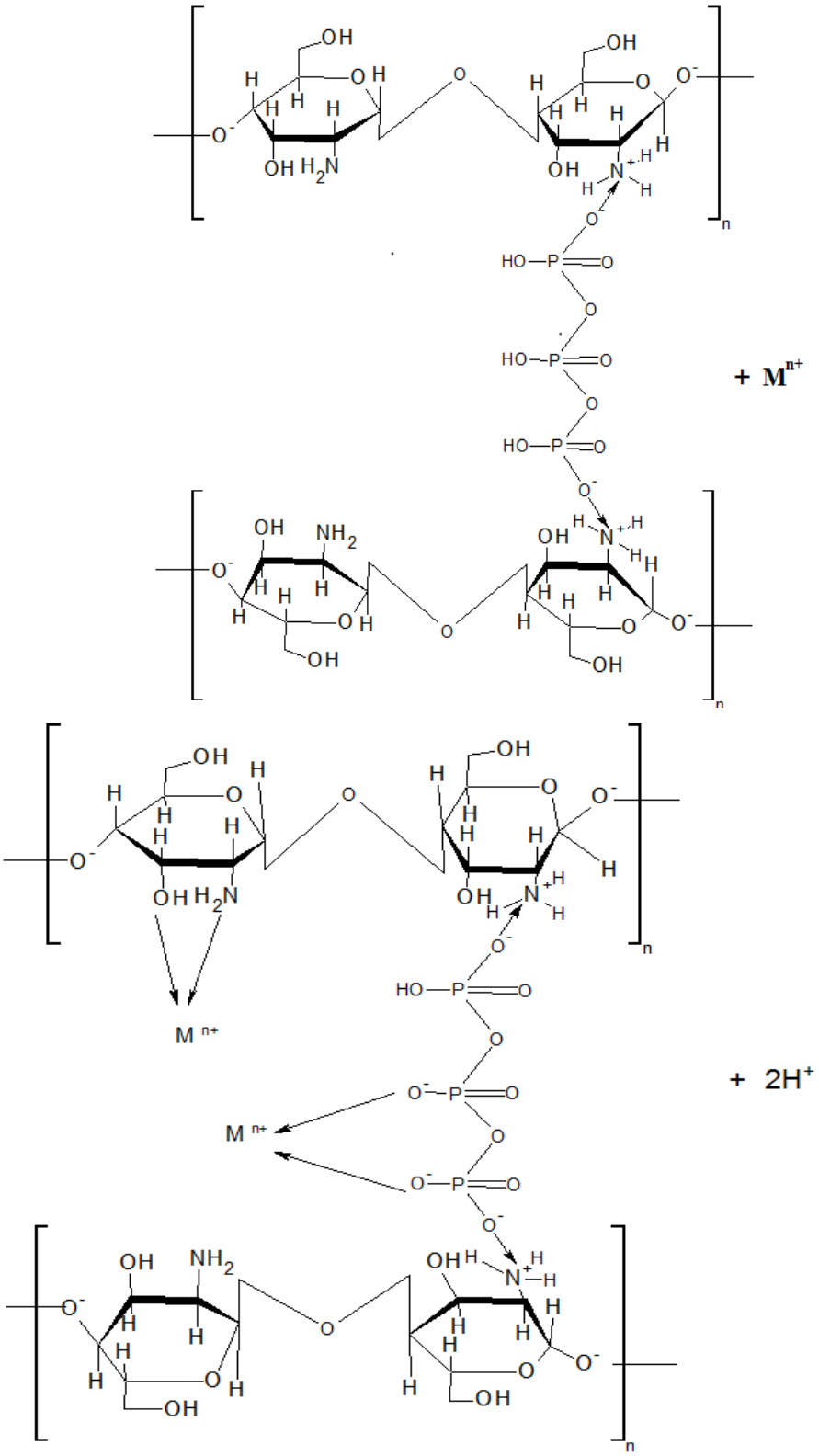


Figure 4.2 Reaction of magnetite, chitosan and Na-TPP

4.3 Characterization Methods

4.3.1 Crystallinity by X-Ray Diffraction (XRD)

X-ray diffraction (XRD) patterns of the nanoparticles were recorded on a Philips X-Pert Pro X-Ray diffractometer using Cu K_α radiation at 45 kV and 40 mA. The scanning scope of 2θ was range from 5 °C to 50 °C. These were carried out at the Laboratories of Metallurgy amd Materials Engineering Department of Engineering Faculty of Dokuz Eylül University.

4.3.2 Fourier Transform Infrared (FTIR) Spectra of the Samples

The nanoparticles were characterized by Fourier Transform Infrared (FTIR) spectroscopy. FTIR was used to investigate the interaction of Chitosan, Na-TPP and Magnetite.

FTIR spectra were measured on a Pelkin-Elmer FTIR spectrophotometer Spectrum BX-II in the range 4000-700 cm^{-1} with the sum of 50 scans at a resolution of 4 cm^{-1}. The samples were prepared with KBr as 1 mg sample/100 mg KBr in the disk form.

4.3.3 Thermogravimetric Analysis (TGA) of the Samples

The thermal properties of the prepared samples were analyzed by thermogravimetric analysis. TG/DTG profiles of the samples were performed from 30°C to 600°C at a rate of 10°C/min with a Perkin Elmer Diamond TG/DTA instrument.

4.3.4 Scanning Electron Microscopy (SEM)

The morphology of the samples was monitored by scanning electron microscopy (SEM). The images were obtained using an accelerating voltage of 10 kV. SEM images were taken at different magnifications (in the range of 1000x and 10000 xs) by using FEI Quanta 250 FEG in the Laboratories of Materials Research Center of İzmir Institute of Technology

CHAPTER FIVE
RESULTS AND DISCUSSION

5.1 Characterization Results of the Samples

XRD patterns of the samples were given in Figures 5.1, 5.2 and 5.3 respectively.

5.1.1 XRD pattern of Fe_3O_4 MNPs

The XRD paterns of Fe_3O_4 can be found, used for comparison and seen in Figure 5.1.

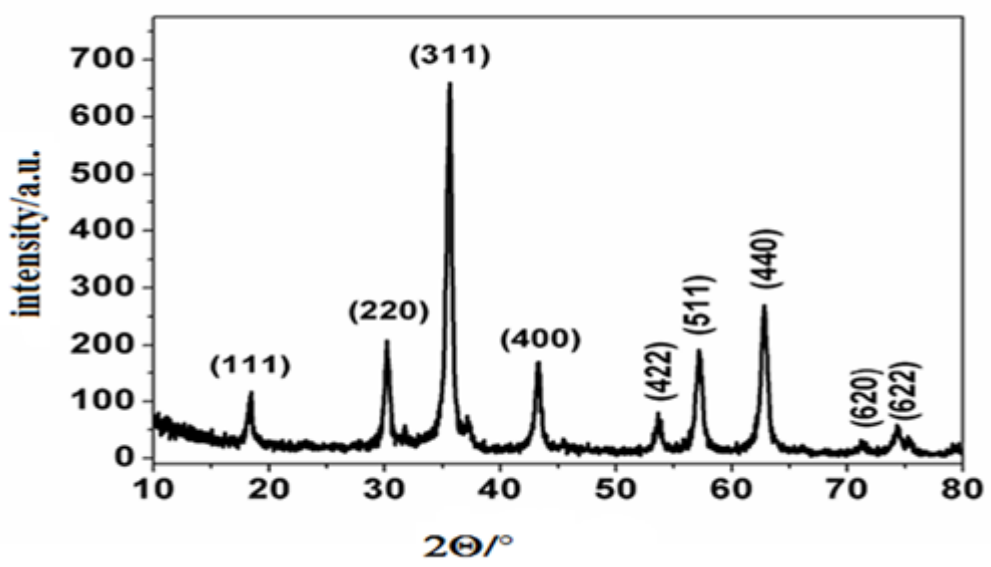


Figure 5.1 XRD pattern of Fe_3O_4 nanoparticles (Ghandoor, Zidan, Mostafa, Khalil & Ismail, 2012)

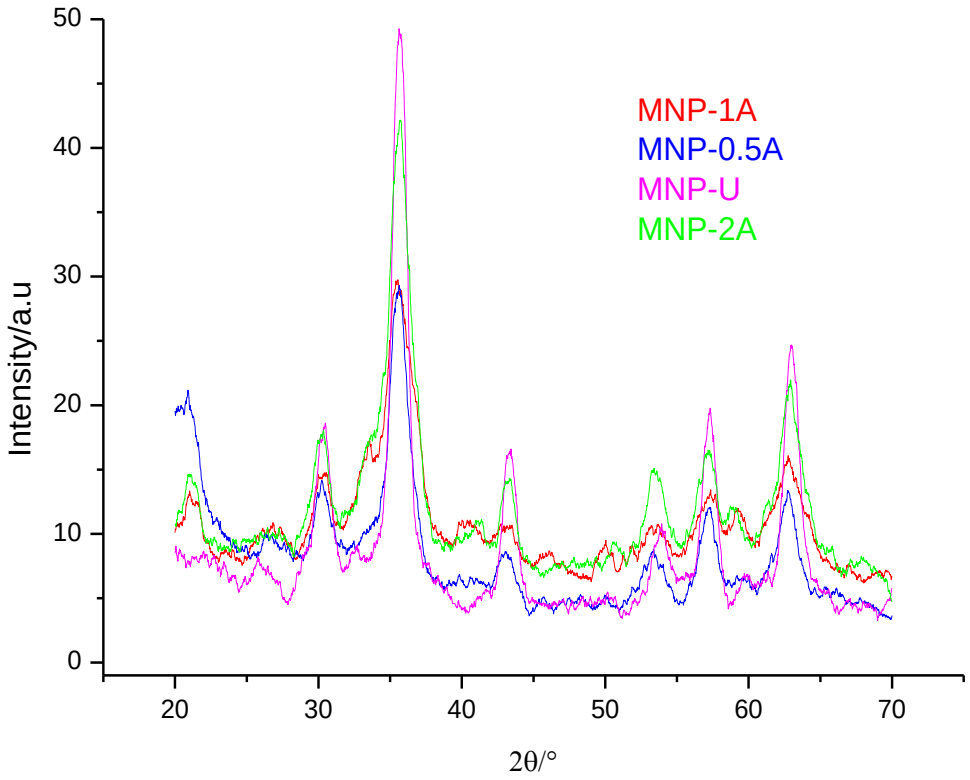


Figure 5.2 XRD patterns of Fe_3O_4 nanoparticles (the pH effect).

The structures of Fe_3O_4 (MNP-U) nanoparticles, prepared with 0.5M HCl (MNP-0.5A), 1M HCl (MNP-1A) and 2M HCl (MNP-2A) are characterized by XRD technique. As illustrated in Figure 5.2, six characteristic peaks of Fe_3O_4 occurred at 2θ/° of 30.1, 35.5, 43.2, 53.5, 57.0 and 62.8, which were marked by their indices (220), (311), (400), (422), (511) and (440), respectively. These peaks revealed that the particles were pure Fe_3O_4 with a spinel structure (JCPDS file, PDF No. 65-3107). For magnetic chitosan microspheres and MNP-0.5A, MNP-1A and MNP-2A the same six characteristic peaks are also observed. It is suggested that nanoparticles are prepared with different pH process did not result in the phase change of Fe_3O_4 particles.

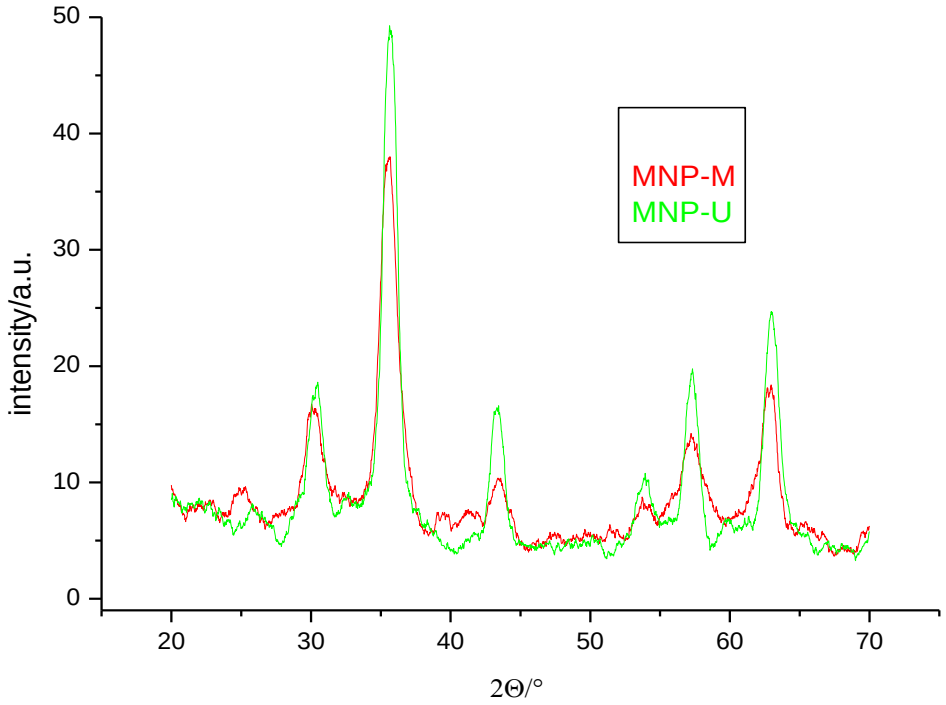


Figure 5.3 XRD pattern of Fe_3O_4 nanoparticles

XRD patterns of MNP-U and MNP-M which were prepared by ultrasonically and mechanically are shown in Figure 5.3, respectively. The XRD analysis results of MNP-U and MNP-M are mostly coincident. Six characteristic peaks for Fe_3O_4 as 2θ/°; 30.1, 35.5, 43.3, 53.4, 57.2 and 62.5 marked by their corresponding to ((220), (311), (400), (422), (511) and (440)), (JCPDS database file, No.79-0418) are observed in two samples. The magnetic particles can preserve their magnetic properties.

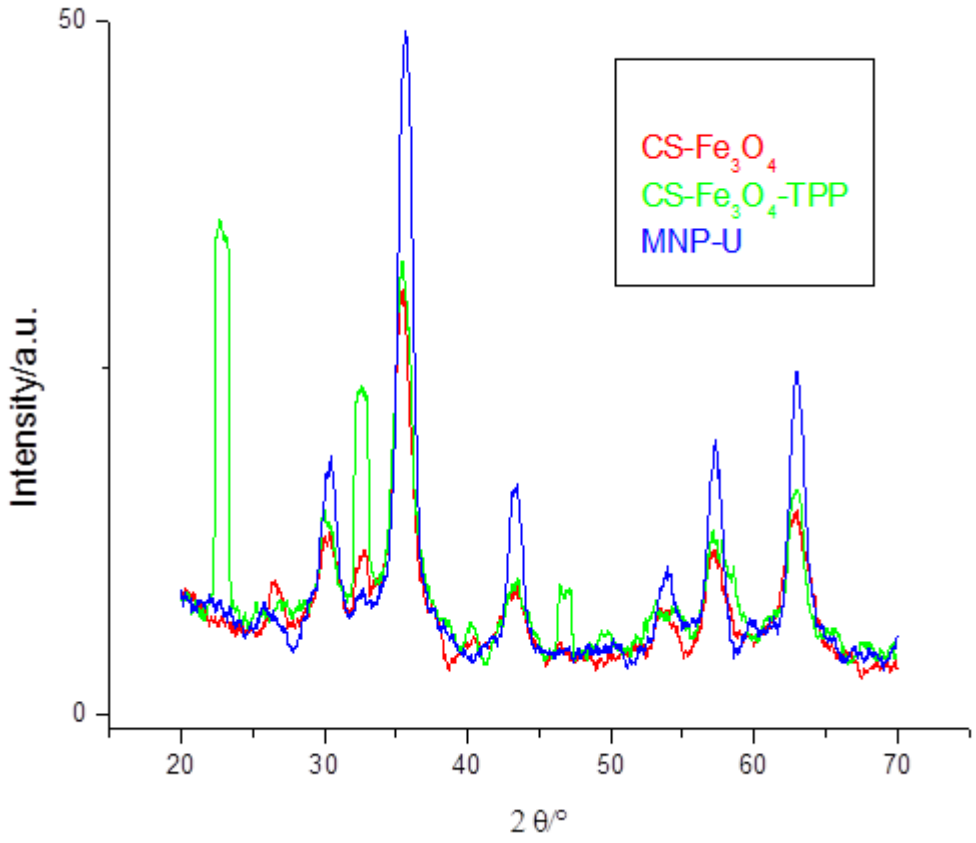


Figure 5.4 XRD pattern of chitosan and Na-TPP coated magnetite nanoparticles

Magnetite-chitosan (CS-Fe_3O_4), magnetite-chitosan-TPP (CS-Fe_3O_4-TPP) and pure Fe_3O_4 (MNP-U) were characterized by XRD technique. As examples in Figure 5.4, for the Fe_3O_4, the six characteristic peaks of Fe_3O_4 (a) occurred at 2θ/° of 30.1, 35.5, 43.2, 53.5, 57.0 and 62.8, which were marked by their corresponding indices (220), (311), (400), (422), (511) and (440), respectively. These peaks revealed that the particles were pure Fe_3O_4 with a spinel structure (JCPDS database file, No.79-0418).For magnetite, magnetite-chitosan and magnetite-chitosan-TPP microparticles, the same six characteristic peaks related with magnetite were clearly observed. The peaks at 22.68° and 46.8° were observed only in the XRD patterns of CS-Fe_3O_4-TPP. These peaks may be due to the crystalline phases of chitosan and Na-TPP. The results indicated that the modification did not change the crystal structure of the Fe_3O_4.

5.1.2 Fourier Transform Infrared FT-IR Spectra of the Samples

FT-IR spectra of the Fe_3O_4 nanoparticles samples were given in Figures of 5.5 and 5.6 respectively.

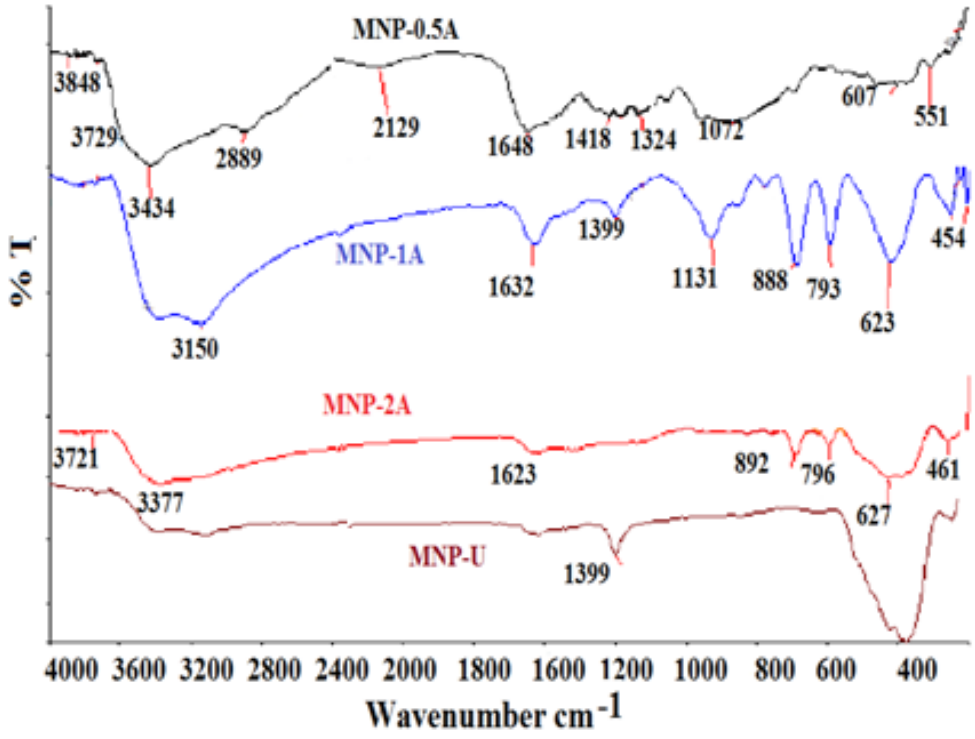


Figure 5.5 FT-IR spectra of the magnetite nanoparticles

Figure 5.5 shows FTIR spectra indicated that magnetite nanoparticles prepared with different pH. The bands at 587, 607 and the 430 cm^{-1}, belonging to the stretch vibration mode and torsional vibration mode of Fe–O bonds in the tetrahedral sites and in the octahedral sites. Two characteristic bands at 3100-3300 cm^{-1} indicated the –OH stretching bands of adsorbed water in the sample. The bands between 590 and 650 cm^{-1} are the characteristics of spectra in the magnetite and maghemite transmission region. Other bands at 900 and 800 cm^{-1} are typical of goethite (α-FeOOH) (Cornell & Schertmann, 1996).

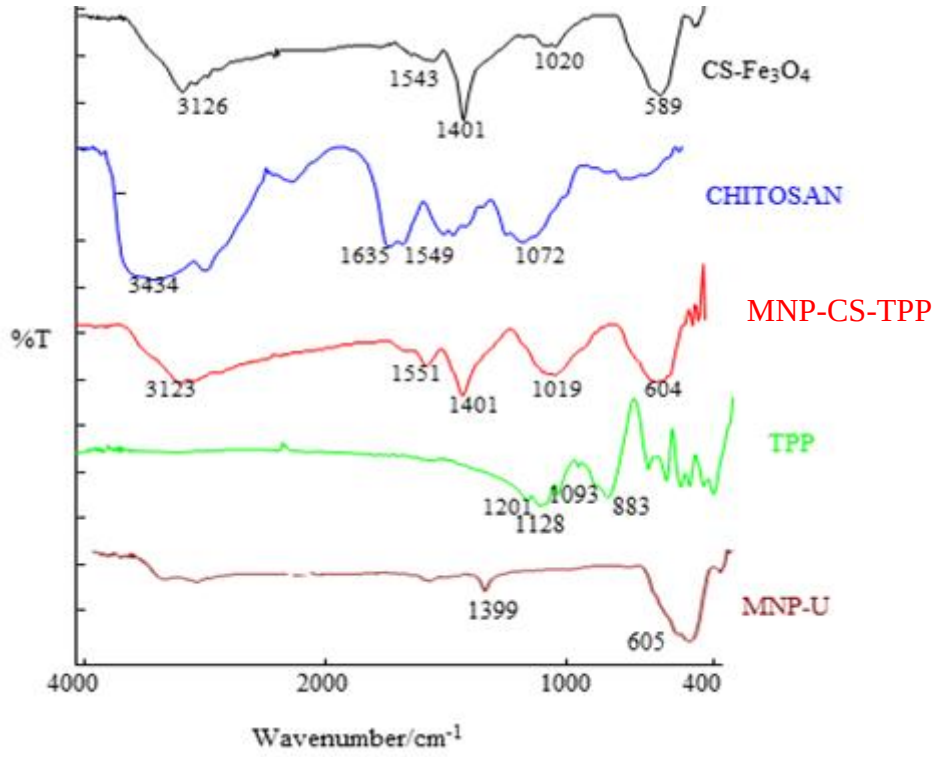


Figure 5.6 FT-IR spectra of chitosan and STPP coated magnetite nanoparticles

The FT-IR spectra of MNP-U, MNP-CS, Chitosan and MNP-CS-TPP are presented in Figure 5.6. The major bands for chitosan were observed around 3434 cm^{-1} attributed to the $–NH_2$ and –OH groups and the band at 1635 cm^{-1} was attributed to the secondary amide C=O band of the remaining acetamido groups. The band at 1549 cm^{-1} was assigned to –NH deformation vibration of $–NH_2$ groups. 1072 cm^{-1} band was attributed to the –CO stretching vibration in –COH.

In the IR spectrum of STPP in Figure 3.4, the following characteristic bands can be observed; 1201 cm^{-1} (P=O stretching), 1128 cm^{-1} (symmetric and antisymmetric stretching vibrations in PO_2 group), 1093 cm^{-1} (symmetric and antisymmetric stretching vibrations in PO_3 group) and 883 cm^{-1} (antisymmetric stretching of the P-O-P bridge) (Corbridge & Lowe 1954).

After cross-linking, the spectra of CS-Fe_3O_4-TPP showed some changes. The band at 1401 cm^{-1} indicating to the -P=O of the -P-OH groups found in the

tripolyphosphate molecule which was shifted from 1201 to 1401 cm^{-1}. The 1128 and 1093 cm^{-1} bands of STPP were not clearly observed in the spectrum of CS-Fe_3O_4-TPP or shifted to 1019 cm^{-1}. The band at 1551 cm^{-1} can be attributed to $-NH_3^+$ in CS-Fe_3O_4-TPP.

The bands at around 587, 607 and the 450 cm^{-1} were belonging to the stretching vibration mode of Fe–O bonds. These bands could be observed in all of the samples. As a consequence, the chitosan was coated successfully on the magnetic Fe_3O_4 nanoparticles.

5.1.3 Thermogravimetric Analysis (TGA)

TGA patterns of the samples were given in Figures of 5.7 and 5.8 respectively. Thermogravimetric analysis is one of the most important techniques used to determine thermal stability and provide qualitative and quantitative information about the volatile components of the nanoparticles. The samples were measured in the condition of nitrogen atmosphere at the heating rate of 10 °C/min until the temperature elevated to 600 °C. Figure 5.8 and 5.9 showed the TGA curves of the nanoparticles, which indicating a two-stage mass losses for MNP-U and MNP-M in the temperature ranges of 29-251°C (2.96%) for MNP-M and (165-317°C) for MNP-U (1.74%) which might be due to the loss of residual water both of the samples. The second mass loss in the temperature range of 266-342 °C (2.37%) might be due to the dehydration, decomposition of chitosan or TPP in both of the samples.

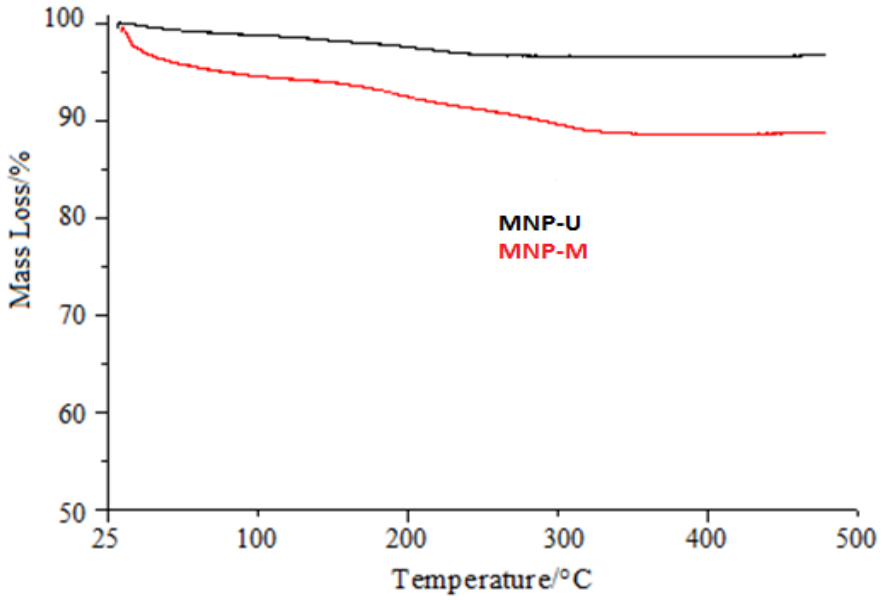


Figure 5.7 TGA of Fe_3O_4 NPs prepared with mechanic and ultrasonic.

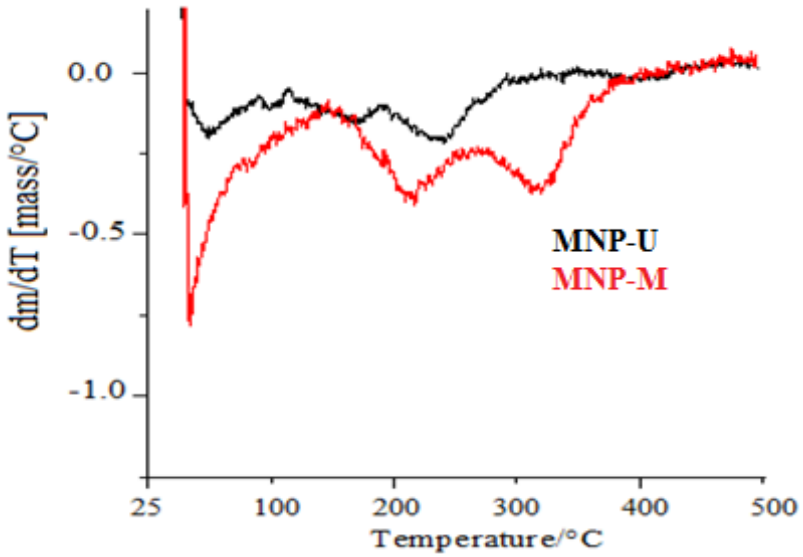


Figure 5.8 DTG curves of Fe_3O_4 NPs prepared with mechanic and ultrasonic nanoparticles

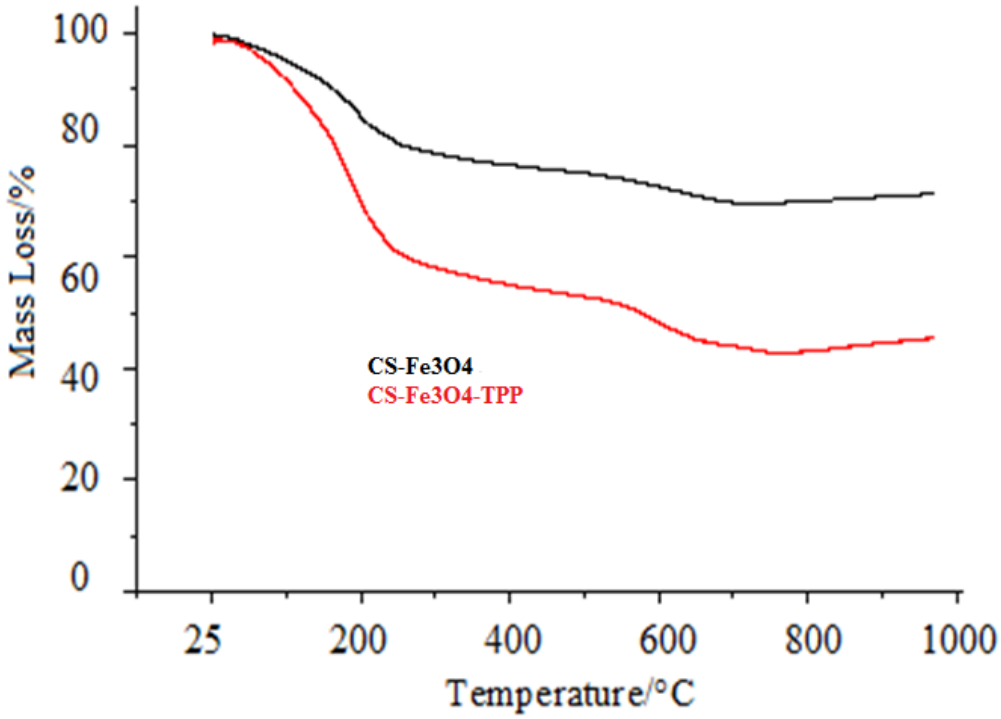


Figure 5.9 TGA curves of Chitosan and Chitosan-TPP coated magnetite nanoparticles

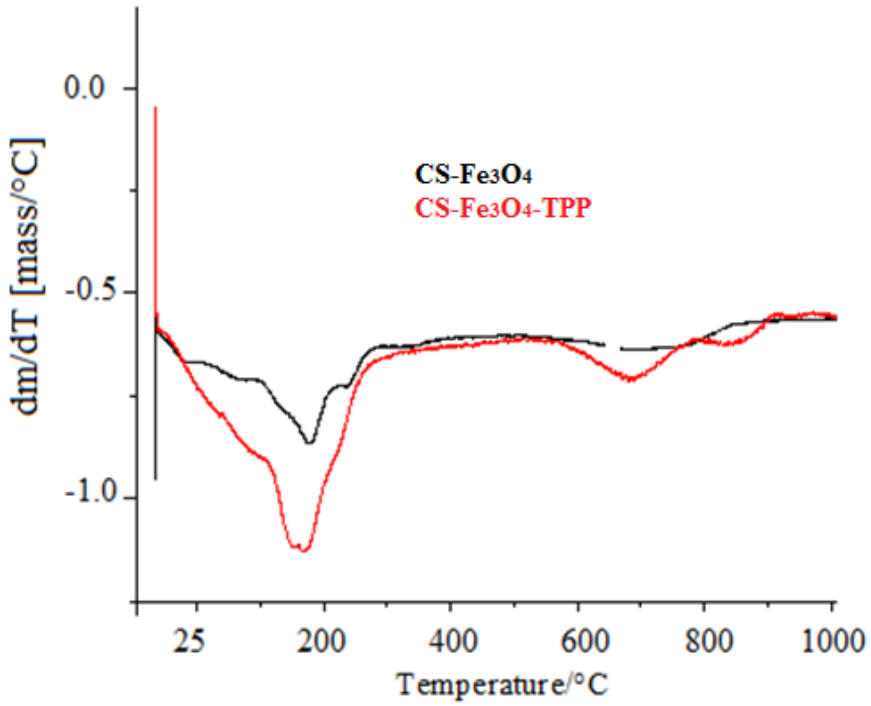


Figure 5.10 DTG curves of Chitosan and Chitosan-TPP coated magnetite nanoparticles

Thermogravimetric analysis of iron oxide nanoparticles coated with chitosan (CS-Fe_3O_4) and chitosan-TPP (CS-Fe_3O_4-TPP) was showed in Figure 5.9 and 5.10. The thermogravimetric analysis curves showed two-stage mass losses for (CS-Fe_3O_4) and third stage for (CS-Fe_3O_4-TPP). In the case of CS-Fe_3O_4-TPP sample, the mass loss is about 36.09% at the temperature range of 176-254 °C. On the other hand, for CS-Fe_3O_4 sample, the mass loss is 21.55 % at the temperatures of 170-247°C. For residual crystalline water both of the samples. The second step of mass loss between 542-872 °C was 6%. It indicates degradation of the principle chains of chitosan. The third step mass loss for CS-Fe_3O_4-TPP between 784-897°C was 8.04 % which may be due to the degradation of the Na-TPP. The TGA report of the samples is given in Table 5.1.

Table 5.1 Result of thermogravimetric analysis

SAMPLE	FIRST STAGE T(°C)	Mass Loss (%)	SECOND STAGE T (°C)	Mass Loss (%)	THIRD STAGE T (°C)	Mass Loss (%)
MNP-U	165-317	1.73	266-342	2.37	---	---
MNP-M	29-251	2.96	266-342	2.37	---	---
CS-Fe_3O_4	176-254	21.55	534-797	6.24	---	---
CS-Fe_3O_4-TPP	170-247	36.09	542-872	6.00	784-897	8.04

5.1.4 Scanning Electron Misroscopy Analysis (SEM)

SEM micrograph of magnetite chitosan particles provides information on their size and morphology. The SEM images of the samples were shown in Figure 5.11 - 5.17. In the SEM images of the MNP-U samples, homogeneous, regular spherical particles with a diameter of about under the 10 nm were observed. On the other hand, coating of the magnetite particles with chitosan and also Na-TPP increased the particle size at about 30 nm which can be seen Figure 12 and 14 for MNP-M.

These SEM images of MNP-U and CS-Fe_3O_4 were taken by Prof. Dr. Mehmet Kadir Yurdakoç and Dr. Pannee Burckel (Chemical Instrumentation Specialist) in Toledo University/Toledo-Ohio/US in JEOL JSM-7500F SEM. Other SEM images were taken in IYTE MAM Laboratories/İzmir-Turkey.

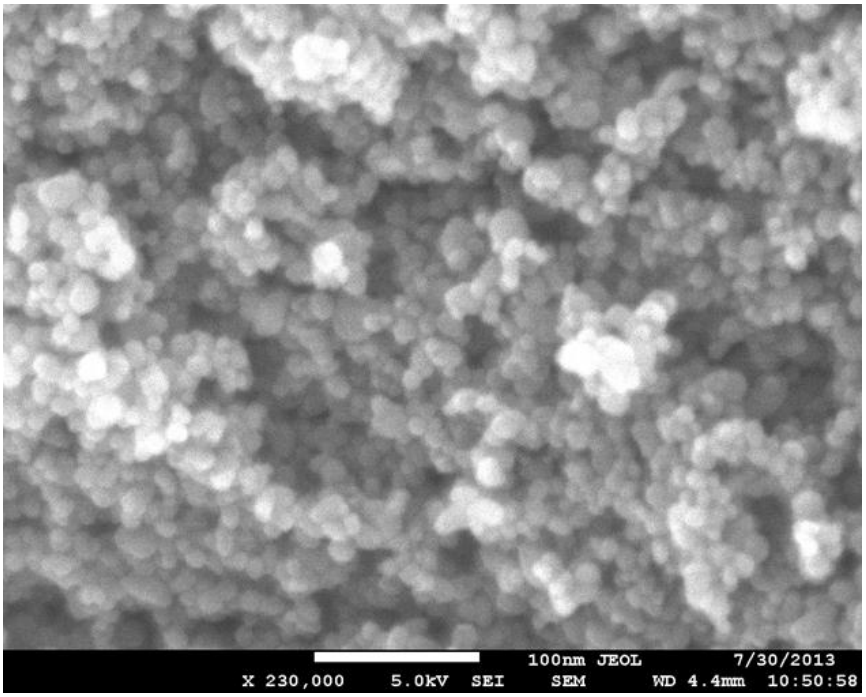


Figure 5.11 SEM images of MNP-U

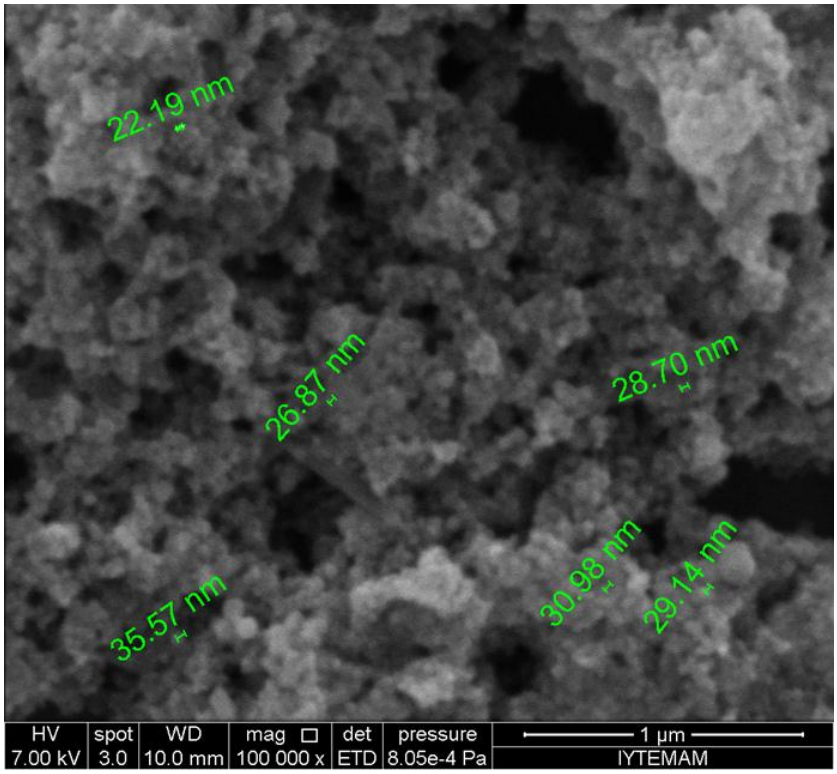


Figure 5.12 SEM images of MNP-M

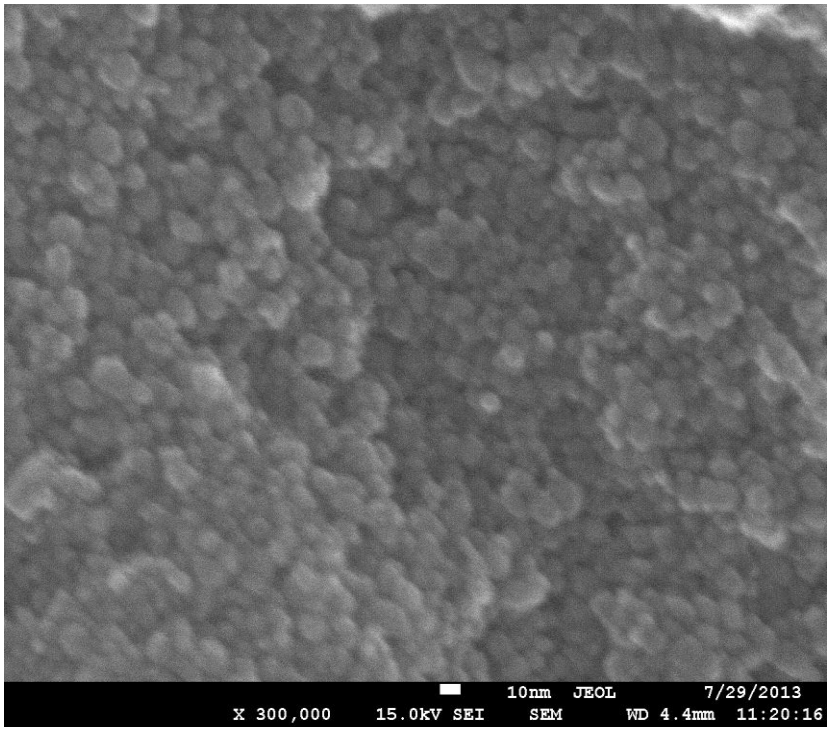


Figure 5.13 SEM images of CS-Fe_3O_4

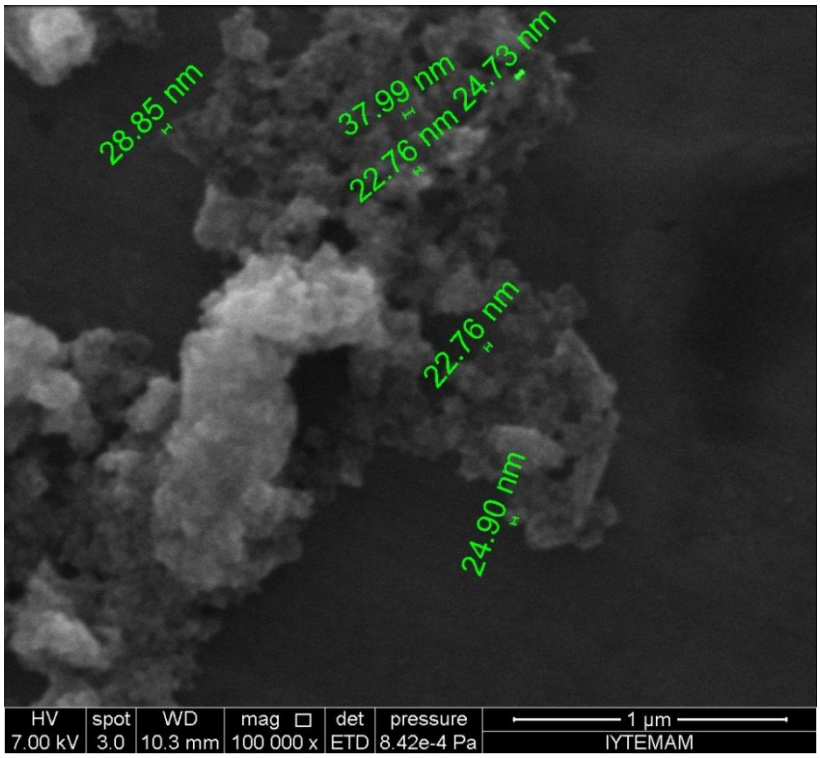


Figure 5.14 SEM images of CS-Fe_3O_4-TPP

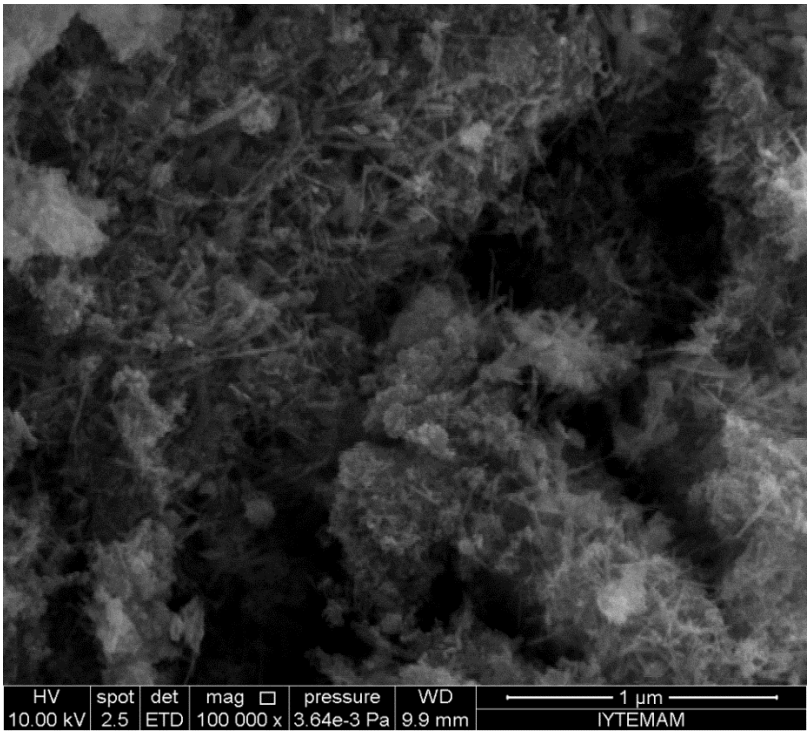


Figure 5.15 SEM images of MNP-1A

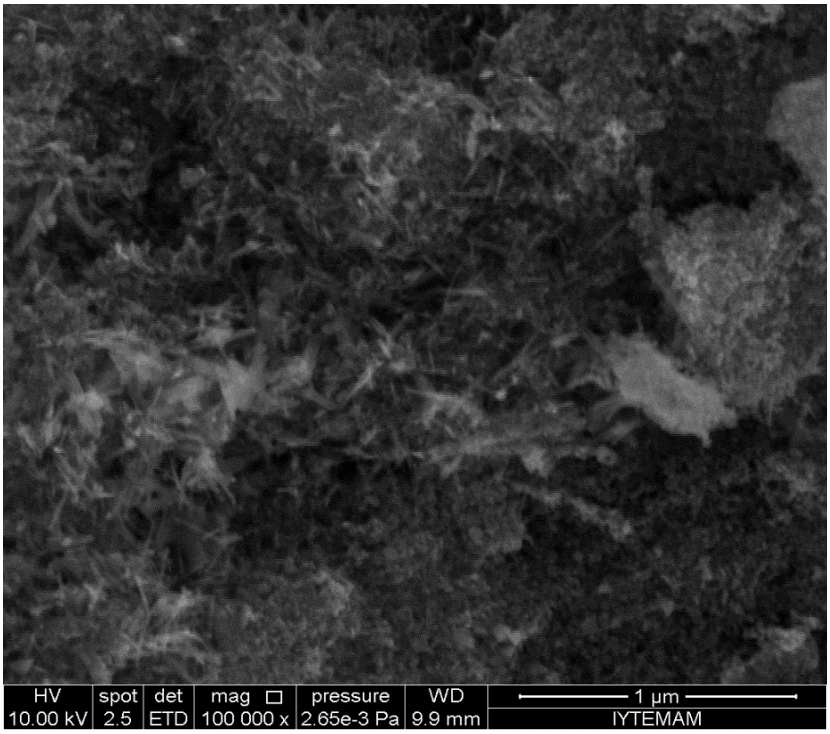


Figure 5.16 SEM images of MNP-2A

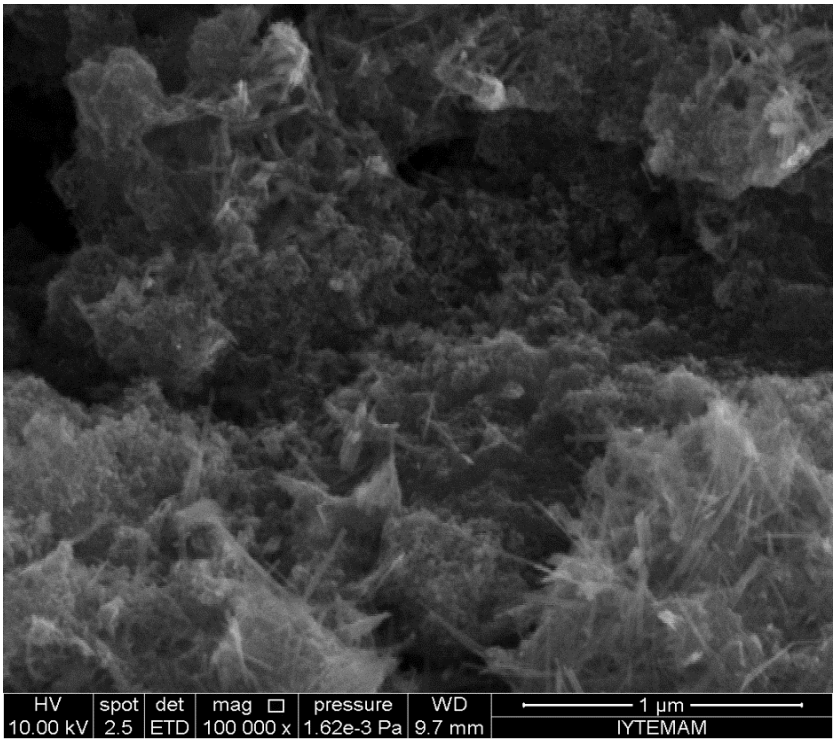


Figure 5.17 SEM image of the MNP-0.5A

Needle-like structures were observed in the SEM images of MNP-0.5A, MNP-1A and MNP-2A samples (Figures 5.15-5.17). These were the results of the HCl treatment at the finishing steps of the preparation of nanoparticles.

CHAPTER SIX
CONCLUSIONS AND PERSPECTIVES

The synthesis of magnetic nanoparticles has made substantial progress, especially over the past decade. The dependence of physicochemical properties on the particle size was closely related with optical spectra, magnetic properties, thermodynamics, electrochemistry, conductivity, and electron transport. Syntheses of oxide or metallic magnetic nanoparticles often require the use of toxic and/or expensive precursors, and the reaction is often performed in an organic phase at high temperature at high dilution. Magnetic nanoparticles have been synthesized with different compositions, the most important method co-precipitation.

Fe_3O_4–chitosan (CS-Fe_3O_4) and chitosan-Fe_3O_4-TPP (CS-Fe_3O_4-TPP) NPs with core-shell structure were prepared by a co-precipatation method. From the XRD patterns of the samples in Figure 5.2 and 5.4, XRD pattern of Fe_3O_4 (Figure 5.1) was conserved. It can be clearly understood that chitosan coating did not change the crystal structure of Fe_3O_4. The magnetite nanoparticles had good crystal structure with a cubic inverse spinel structure. Crystalline phase of chitosan was also observed as in Figure 5.4 with a $2\theta=22.68°$.

Fourier transform infrared spectra showed that the iron oxide nanoparticles were successfully coated by chitosan and cross-linked with TPP. The band at 1551 cm^{-1} can be attributed to $-NH_3^+$ in CS-Fe_3O_4-TPP. The band at around 584 cm^{-1} attributed to the Fe-O bond vibration of Fe_3O_4 was observed in all magnetite samples.

TG/DTG profiles of the samples showed two stages mass losses. These losses are due to the adsorbed water and decomposition of chitosan. On the other hand, in the TG profile of chitosan-magnetite and Na-TPP, third mass loss was observed at 784-897 °C may be due to the degradation of the Na-TPP in Table 1.

SEM images of the samples were given in Figures 5.11-5.17. The spherical shape of magnetite nanoparticles with a size about 15 nm was observed while after coating with chitosan the size of the nanoparticles were increased to 25 nm. Needle-like nanostructures were observed in the SEM images of HCl treated MNP samples.

Magnetic properties of the samples should be studied and the effect of the treatment with chitosan and Na-TPP must be specified in future.

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