CHAPTER II
LITERATURE REVIEW

2.1 Magnetic Nanoparticles (MAG)

The studies of magnetic nanoparticles, MAG, rapidly grow during the past few decades due to the their properties such as ultrafine size, biocompatibility, functional group availability for further chemical modification, and most important characteristic property, responsiveness to external magnetic field or so called superparamagnetic properties .

2.1.1 Synthesis and General Properties

Synthesis of MAG

To synthesize MAG, there are several scientific and technological challenges to synthesize MAG of customized size and shape. Physical method such as gas phase deposition and electron beam lithography are elaborate procedures that suffer from the inability to control the size of particles in the nanometer size range. The wet chemical routes to synthesize MAG are simpler, more trace able and more efficient in controlling size, composition and shape of the nanoparticles (A.K. Gupta and M. Gupta, 2005).

Iron oxide can be synthesized through co-precipitation of Fe^{2+} and Fe^{3+} aqueous salt solution (Mahmoudi *et al.*, 2008). Conventionally, magnetite is prepared by adding a base to an aqueous mixture of Fe^{2+} and Fe^{3+} at a 1: 2 molar ratio. The precipitated magnetite is black in color. The chemical reaction of Fe_3O_4 precipitation is as following;

$$Fe^{2+} + 2Fe^{3+} + 8OH^- \longrightarrow Fe_3O_4 + 4H_2O. \quad (1)$$

According to the thermodynamics of this reaction, a complete precipitation of Fe_3O_4 should be ecpected between pH 9 and pH 14, while

maintaining a molar ratio of Fe^{2+}: Fe^{3+} at 1: 2 under a non-oxidizing oxygen-free environment. Otherwise, Fe_3O_4 might also be oxidized as

$$Fe_3O_4 + 0.25O_2 + 4.5H_2O \longrightarrow 3Fe(OH)_3. \quad (2)$$

In order to prevent MAG from oxidation in air as well as from agglomeration, MAG produced by reaction (1) are usually coated with organic or inorganic molecules during the precipitation process (A.K. Gupta and M. Gupta, 2005).

Type of magnetization

Iron oxide particle materials are classified by their response to an externally applied magnetic field. Description of orientations of the magnetic moments in a particle helps to identify different types of magnetism observed in nature. The magnetic properties of these particles can be described by the dependence of the magnetic induction B on the magnetic field H. Some materials such as iron exhibit ferromagnetism, in that they can be permanently magnetized. In most materials the relation between B and H is linear: $B = \mu H$; where μ is the magnetic permeability of the particles. Iron oxide particles exhibit paramagnetism if $\mu > 1$, and diamagnetism if $\mu < 1$. In vacuum, $\mu = 1$. Alternatively, the magnetic susceptibility $\chi = \mu - 1$ is used. Hence, paramagnetic nanoparticles have $\chi > 0$, diamagnetic particles $\chi < 0$, and in vacuum $\chi = 1$ (Chen, 1986).

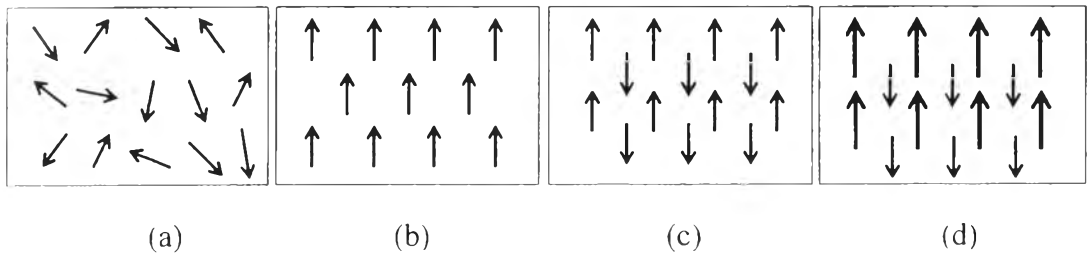


Figure 2.1 Types of magnetism: paramagnetism (a), ferromagnetism (b), antiferromagnetism (c), and (d) ferrimagnetism.

A type of magnetism in which the magnetic moments of atoms in a solid are aligned within domains, which can in turn be aligned with each other by a weak magnetic field. The total magnetic moment of a sample of the substance is the vector sum of the magnetic moments of the component domains. In a diamagnetic material, there are no unpaired electrons, so the intrinsic electron magnetic moments cannot produce any bulk effect.

There are four main types of magnetic behaviour apart from diamagnetism; paramagnetism (Figure 2.1(a)), in which the unpaired electrons are randomly arranged; ferromagnetism (Figure 2.1(b)), in which the unpaired electrons are all aligned; and antiferromagnetism (Figure 2.1(c)), in which the unpaired electrons line up in opposite directions to one another; ferrimagnetism (Figure 2.1(d)), in which their magnetization remain in the absence of a field. Ferromagnetic materials have an overall magnetic moment, whereas antiferromagnetic materials have a magnetic moment of zero. A substance is said to be ferrimagnetic if the electron spins are orientated antiparallel to one another but, due to an inequality in the number of spins in each orientation, there exists an overall magnetic moment. When the ferromagnetic particles are removed from the field, they exhibit permanent magnetization. Ferromagnetic materials, which are ground down to particle dimensions smaller than a particular domain, are no longer ferromagnetic but exhibit superparamagnetism (Elloitt, 1998).

Physical and Chemical Properties

Iron oxide is a widely used and well-recognized compound, which exists in 16 identified forms (Cornell and Schwertmann, 2003). In this work, the focus will be placed on three forms of iron oxide, hematite (α-Fe_2O_3), magnetite (Fe_3O_4), and maghemite (γ-Fe_2O_3), because they are the principal forms used in industrial applications. Some of their physical and magnetic properties are summarized in Table 2.1.

Table 2.1 Physical and chemical properties of hematite, magnetite, and maghemite *

property	value/ remark		
	hematite	magnetite	maghemite
molecular formular	α-Fe_2O_3	Fe_3O_4	γ-Fe_2O_3
crystal struture	rhombohedral, hexagonal	cubic	cubic or tetrahedral
density (g/cm^3)	5.26	5.18	4.87
melting point (°C)	1350	1583-1597	
boiling point (°C)		2623	
color	red	black	reddish - brown
hardness	6.5	5.5	5
type of magnetization	weakly ferromagnetic or antiferromagnetic	ferrimagnetic	ferrimagnetic
Curie temperature (K)	956	850	820-986
saturation magnetization, σ_s, at 300 K (Am^2/kg)	0.3	92-100	
standard free energy of formation, ΔG^0_f (kJ/mol)	-742.7	-1012.6	-711.1
heat of decomposition (kJ/mol)	461.4	605	457.6

* Adaped from Cornell and Schwertmann (2003).

2.1.2 Magnetic Nanoparticles Coating

In the preparation and storage of nanoparticles in colloidal form, the stability of the colloid is very important. In the absence of any surface coating, MAG have hydrophobic surfaces with a large surface area to volume ratio. Due to hydrophobic interactions between the particles, these particles agglomerate and form large clusters, resulting in increased particle size. These clusters exhibit strong magnetic dipole–dipole attractions between them and show ferromagnetic behaviour.

When two large-particle clusters approach one another, each of them comes into the magnetic field of the neighbour (Hamley, 2003).

The size, charge and surface chemistry of the magnetic particles are particularly important and strongly affect both the blood circulation time as well as bioavailability of the particles within the body (Chouly *et al.*, 1996). In addition, magnetic properties and internalization of particles depend strongly on the size of the magnetic particles (Chatterji *et al.*, 2003). For example, following systemic administration, larger particles with diameters greater than 200 nm are usually sequestered by the spleen as a result of mechanical filtration and are eventually removed by the cells of the phagocyte system, resulting in decreased blood circulation times. On the other hand, smaller particles with diameters of less than 10 nm are rapidly removed through extravasations and renal clearance. Particles ranging from 10 nm to 100 nm are optimal for intravenous injection and demonstrate the most prolonged blood circulation times. The particles in this size range are small enough both to penetrate the very small capillaries within the body tissues and therefore may offer the most effective distribution in certain tissues (Pratsinis, 1996).

To make MAG that practical for using in typical application, especially biomedical application, coating MAG surface is very necessary.

2.1.2.1 Coating via Non-covalent Bond

Non-polymeric Small molecules as a Stabilizer

In order to stabilize the colloidal dispersion, several studies about the surfactant adsorption on the surfaces of MAG were proposed. Tadmore *et al.* (2000) reported the surface modification of MAG by oleic acid and stearic acid to stabilize the nanoparticles in organic solvents. They found that only oleic acid could be used for obtaining thermodynamically stable dispersions of MAG. Although oleic acid and stearic acid are similar surfactants, the ferrofluid dispersions were stabilized only by oleic acid. Oleic acid was found to form highly organized surfactant bilayers on MAG surface (Shen *et al.*, 1999). Other several combinations of surfactants with different chain lengths (lauric acid (LA), myristic acid (MA), oleic acid (OA) and dodecyl-benzene-sulphonic acid (DBS)) were used. It was found that MA+MA or

LA+LA biocompatible double layer covered magnetite nanoparticles are the most stable colloidal systems among the investigated samples (Bica *et al.*, 2007). Moreover, a mixture of dodecyltrimethylammonium bromide/ didodecyl dimethylammonium bromide (Loo *et al.*, 2008), sodium dodecylsulfonate (SDS), sodium dodecylbenzene sulphonate (DBS), cetyltrimethyl ammonium bromide (CTAB) and hexadecyipyridinium chloride (HPC) (Jing and Wu, 2004), sodium dodecyl sulfate (SDS) (Figure 2.2) (Zargar *et al.*, 2009), 4-methylcatechol (Xie *et al.*, 2008) were also reported to be effectively used as a stabilizer for MAG.

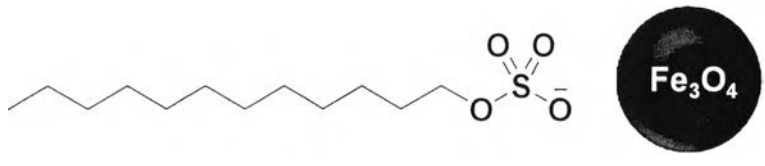


Figure 2.2 Suggested mechanism for sodium dodecyl sulfate adsorption on MAG surface (Zargar *et al.*, 2009).

Polymeric Stabilizer

Polymeric coatings on magnetic nanoparticles offer a high potential in several areas of applications. Precipitation of inorganic particles in a cross-linked polymer matrix or network of gel often prevents coagulation of particles, giving rise to monodisperse particles. For better dispersion, magnetite particles are often modified after precipitation. A copolymer of poly(ethylene oxide), PEO and poly(propylene oxide), PPO grafted poly(acrylic acid) coated on MAG was successfully prepared by co-precipitation of MAG in polymer solution (Figure 2.3). PEO played an important role of layer for stearic stabilization whereas PPO provided the layer for organic extraction (Moeser *et al.*, 2002).

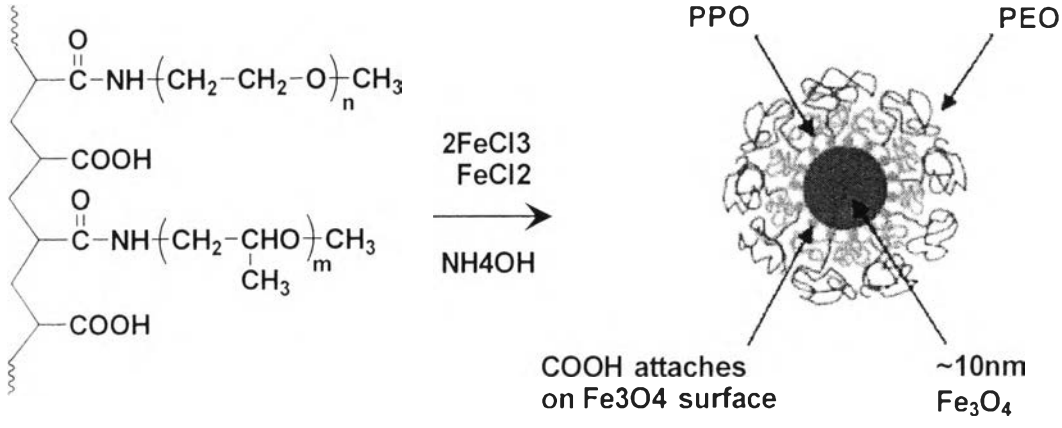


Figure 2.3 Co-precipitation of MAG in PEO-PPO grafted copolymer with poly(acrylic acid) solution.

2.1.2.2 *Coating via Covalent Bond*

Coupling Reaction with Silane Coupling Agent

Coating MAG surface via covalent bond provides many advantages over non-covalent bond type. Coated molecules might be detached during suspending in solution or in a severe case like intravenous system. Covalently surface functionalized MAG by silane coupling reaction followed by polymerization has been widely reported. Surface modifying of MAG with 3-[2-(2-aminoethyl)-ethylamino]-propyltrimethoxysilane followed by functionalization with methyl acrylate and ethylene diamine was reported by Yoza *et al.* (2003) (Figure 2.4).

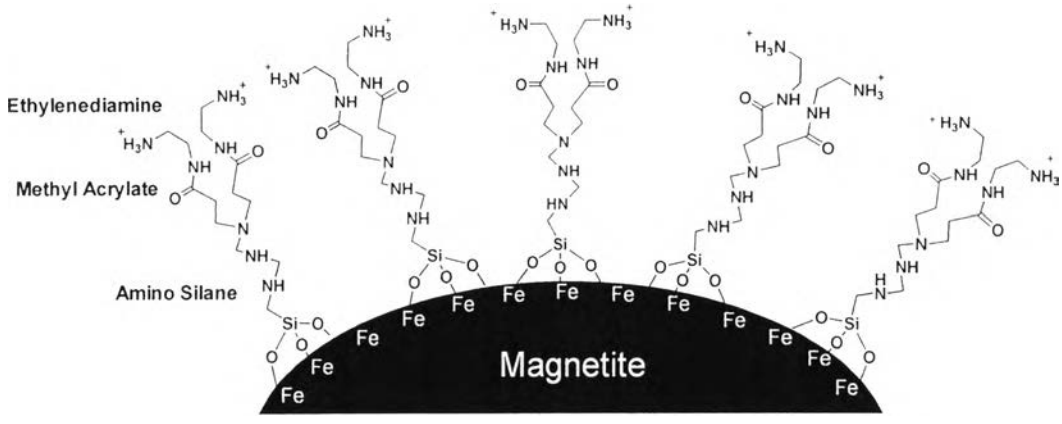


Figure 2.4 Dendrimer generation on surface of amino silane modified MAG (Yoza *et al.* 2003).

In case of small molecule coating on MAG, antibodies were immobilized on iron oxide nanoparticles using aminopropyl trimethoxy silane and subsequent activation by glutaraldehyde was purposed. Silane coupling agents alone are frequently used to stabilize MAG. Three silane compounds (3-Aminopropyltriethoxysilane, *N*-(trimethoxysilylpropyl) isothiouronium chloride, and 3-[2-(2-aminoethyl)-ethylamino]-propyltrimethoxysilane) were coupled to naked MAG to extract DNA from bacteria (Koh *etal.*, 2006). Recently, surface functionalization of hydrophobic surface MAG via ligand exchange was reported. The authors reported that amino- , carboxylic-, and poly(ethylene glycol)-terminated silane were found to provide a highly stable and dispersible MAG in water due to electrostatic and/or stearic repulsion (De Palma *et.al.*, 2007) (Figure 2.5).

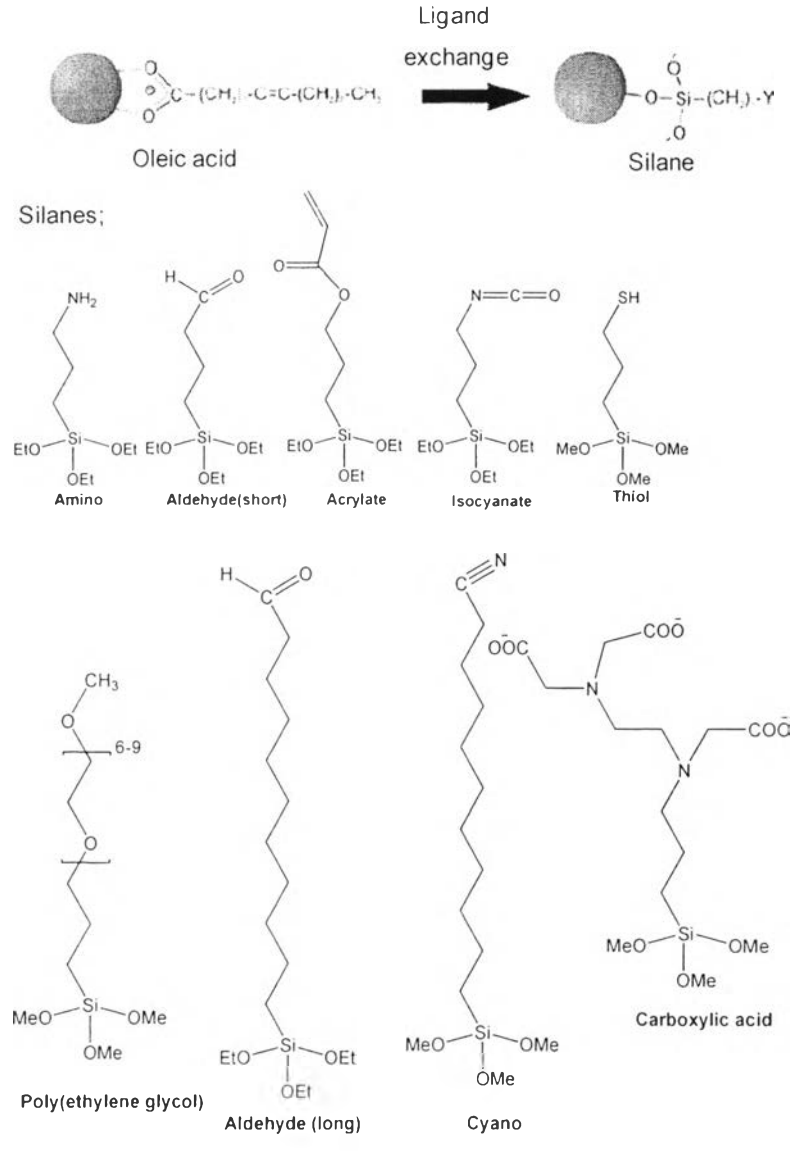


Figure 2.5 Ligand exchanging of chemisorbed oleic acid on MAG surface by silanes. Purposed different silanes with wide variety of end groups.

2.1.3 Applications of Magnetic Nanoparticles

2.1.3.1 Contrast Agent for Magnetic Resonance Imaging (MRI)

When using MAG as contrast agents in MRI, higher quality images with better differentiation between the pathogenic cells and the healthy cells

are produced, compared to conventional contrast agents as shown in Figure 2.6 (Xie *et al.*, 2008).

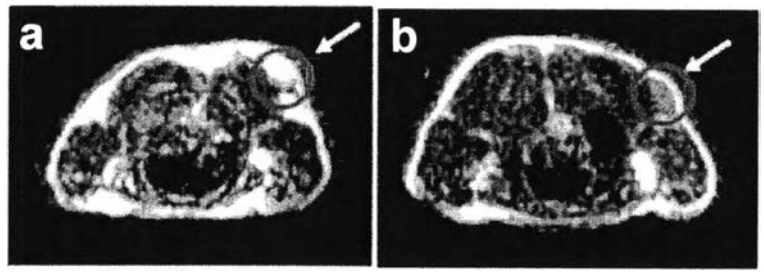


Figure 2.6 MRI cross section of the U87MG tumors implanted in mice: (a) without MAG, and (b) with the injection of 300 μg of peptide c(RGDyK)-modified MAG (Xie *et al.*, 2008).

2.1.3.2 Cancer Treatment by Hyperthermia

Hyperthermia is a technique for cancer therapy that destroys cancer cells by heat. Research has shown that heating to temperature between 41 °C–46 °C for 30 min with careful monitoring is able to kill cancer cells successfully without damaging the healthy tissues. Heat will be generated when an alternating magnetic field is applied to MAG which were placed at the tumor site. MAG and poly(vinyl alcohol) hydrogel composite materials were purposed for hyperthermia applications by Lao and Ramanujan (2004). They found that the amount of heat generated from an alternating magnetic field at 375 kHz gave a stable maximum temperature ranging from 43 °C to 47 °C and a specific absorption rate was found to depend on the magnetic field strength.

2.1.3.3 Drug Delivery/ Drug Targeting

In magnetic drug targeting, the combination of polymeric materials and SPIONs with a therapeutic agent can create an effective controlled-release system, in addition to the targeting achieved by the external magnetic guidance. The attachment of drugs to magnetic nanoparticles can be used to reduce drug doses and potential side effects to healthy tissues and the costs associated with drug treatment. (Zhang *et al.*, 2007).

2.1.3.4 Separation Approach

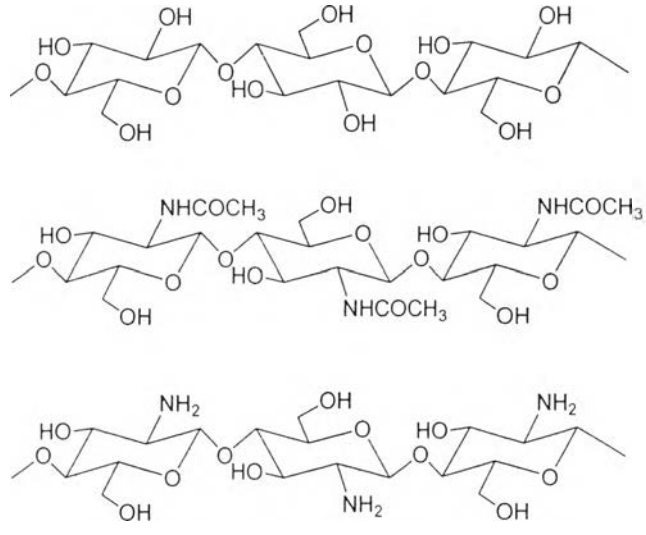
Magnetic separation is a process used to separate magnetic materials from a nonmagnetic surrounding. In the past decade, magnetic separation has been applied to a complex separation depending on functionalized magnetic particles that are selectively tailored to target applications. Many studies have been reported that magnetic separation can be used to remove cells (Scarberry *et al.*, 2008), environmental contaminants such as toxic dye (Zarkar *et al.*, 2008), heavy metal ions (Liu *et al.*, 2008), and nonpolar organic contaminants in waste water (Moeser *et al.*, 2002).

2.2 Chitin-chitosan

Chitin is the second most abundant naturally occurring amino polysaccharide next to cellulose. The chemical structure is similar to cellulose, but it is an amino polysaccharide having acetamide groups at the C-2 positions is place of hydroxyl groups. It is widely found in the shells of crustacean and insects. Chitosan is highly advantages for providing biological functions and for conducting modified reactions resulting from the presence of amino groups more than 70% in chitin. In general, chitin and chitosan units are distributed in random (Scheme 2.1).

Utilization of the materials and derivatives prepared from chitin and chitosan is in various fields including medicine, pharmacology and the food industry as a result of their biological activity, biocompatibility and biodegradability in combination with its low toxicity.

Scheme 2.1 Chemical structures of (a) cellulose, (b) chitin, and (c) chitosan



2.2.1 Functionalization of Chitin-chitosan

Considering the chemical structure of chitin-chitosan, the high molecular weight and strong inter- and intra-molecular hydrogen bond network provide the limitation of chitin-chitosan applications due to the lack of solubility and chemically inert. However, chitosan (degree of deacetylation more than 70%) can be solublized in dilute acid such as 1% of acetic acid. For chitin, it can be dissolved in N,N-dimethylacetamide (DMAC)-LiCl (Cho *et al.*, 2000 and Einbu *et al.*, 2004), hexafluoro-acetone, and hexafluoro-2-propanol (Kurita, 2001). The developments of chitin-chitosan by either nanomerterization to provide materials in nano-scale or functionalization on chitin-chitosan to obtain water-soluble chitin-chitosan were studied to improve for using in many advance applications such as medicine, pharmacology, the food industry.

2.2.1.1 Chitin Whisker (CTWK)/ Chitosan Nanoscaffold (CSSF)

In 2001, Paillet and Dufresne found that the purification step of chitin with 3N hydrochloric acid has to be optimized in order to remove remaining proteins and to take the advantage by favoring the formation of a rigid chitin formation, so-called "chitin whiskers".

2.2.1.2 Chitosan Nanosphere (CSNS)

In recent decades there has been increased interest in the use of nanoparticles for drug delivery applications. From literature, preparation method for chitosan nanoparticles/ nanospheres can be categorized into 4 types.

Covalently Crosslinked Nanoparticles.

Ohya *et al.*, (1994) reported preparation of chitosan nanospheres by water-in-oil (W/O) emulsion method followed by glutaraldehyde crosslinking of the chitosan amino group. However, the later discovery of the negative effects of glutaraldehyde crosslinking on cell viability and the integrity of macromolecular drug shifted general interest to less harsh procedures for the synthesis of nanospheres.

Ionically Crosslinked Nanoparticles

The cationic nature of chitosan has been conveniently exploited for the development of particulate drug delivery systems. Chitosan is not only complex formation with negative charge polyanion but also gel spontaneously on contact with specific polyanion. Among some polyanion investigated, tri-polyphosphate is mostly widely used because of non-toxic property and quick gelling property. Gan *et al.*, (2007) studied chitosan nanoparticles as protein carrier by using polyanion tripolyphosphate (TPP) as the coacervation crosslinking agent. BSA, bovine serum albumin, was applied as model protein to incorporate with nanoparticles. As a crosslink and condensing agent, TPP form further hydrogen bonds with free amine groups on both protein and chitosan molecules, resulting in more compact protein-chitosan nanoparticles. However, the difficulty of chitosan nanoparticle is controlling initial burst effect in releasing large quantities of protein molecules.

Dissolvation of Nanoparticles

This method was proposed to introduce the simple process to prepare chitosan microsphere by using sodium sulfate as precipitant (Berthold *et al.*,

1996) Sodium sulfate was added dropwisely into chitosan solution under stirring and ultrasonication to dissolvate chitosan in a particular form. The amount of sodium sulfate required for microsphere formation increased with an increasing in molecular weight of chitosan. The reason is probably dependent on the number of positive charges on chitosan surface.

Amphiphilic Chitosan Nanoparticles via Self-assembly Process

This process has recently attracted increasing interest in pharmaceutical areas. The nanoparticles consist of hydrophobic inner core and hydrophilic outer shell in aqueous media. Compared with others method as mention previously, preparing nanoparticles through the self-assembly of amphiphilic chitosan is more simple and effective method, as it needs no additives.

Yuan *et al.*, (2006) proposed chitosan nanoparticles by grafting cholesterol as hydrophobic part. The nanoparticles were formed by self-aggregated through sonication or filtration methods. Shape of chitosan-cholesterol nanoparticles was mostly spherical as observed by TEM and diameter size was 50-200 nm. In addition, the diameter was decreased as degree substitution of cholesterol increases.

Wu *et al.*, (2005) synthesized water soluble chitosan (CS) derivatives containing polylactide (DLLA) unit by reacting DL-lactide with chitosan in DMSO solution in the presence of triethylamine. Sphere diameter from DLS measurement demonstrates that the diameter increased with an increase in DLLA/CS molar ratio, suggesting the elongation of hydrophobic polylactide side chain facilitates the growth of the hydrophobic core of polymeric micelles.

Huang *et al.*, (2006) introduced facile preparation of chitosan nanoparticles consisted of poly(butylenes glycol adipate) (PBGA) as hydrophilic side chain. The graft copolymers of chitosan with PBGA were prepared due to the esterification reaction between PBGA and 6-O-succinate-N-phthaloyl-chitosan (PHCSSA) in the presence of toluene as a swelling agent. The copolymers particles are nanoparticles with the size of a few hundred nanometers.

Kulkarni *et al.*, (2006) studied chitosan nanoaggregates by linking methoxy polyethyleneglycol (MPEG) to chitosan (PLC) in the presence of formaldehyde in a solvent of formic acid and dimethylsulfoxide (DMSO). Size of particles and zeta potential are decreased as degree substitution of MPEG increases.

2.2.1.3 Water-soluble Chitosan (WSCS)

The $-NH_2$ group at the C-2 positions gives the specific reaction to chitosan (Rinaudo, 2006). As chitin-chitosan is lacking of the solubility in common solvents, the functionalization at either $-NH_2$ or -OH group is an effective way to obstruct inter and intra hydrogen bond resulting in solubility for bioactivity applications.

2.3 Chitosan as a Coating Material and/ or Stabilizer for MAG

Numerous studies have been performed on preparation of iron oxide-chitosan composite materials during the past decade. There are several ways to fabricate composite between the two particles ranging from a very simple method to advanced and complicated technique of synthesis. Here, techniques fabricate MAG-chitosan nanocomposites were classified into 3 categories; (i) MAG suspension in polymeric solutions: MAG was incorporated with chitosan-based materials via non-covalent bond; (ii) Conjugating or coupling reaction and covalently modification on MAG' surface; (iii) In situ co-precipitation of MAG in chitosan hydrogel matrix: MAG was grown after or together with matrix formation. The details are as follow;

MAG suspension in polymeric solutions

The first step to make a composite between chitosan-based materials and MAG is synthesizing MAG. Following by simply dispersing MAG in acidic chitosan solution without any crosslinker added, MAG-chitosan hybrids were formed (Bhattarai *et al.*, 2008). The MAG-chitosan hybrid formed by reprecipitation of MAG-chitosan solution in alcohol was reported. The hybrids were stated as a full capping core-shell structure due to DTA data and TEM observations (Belessi *et al.*, 2008). Xie *et al.* (2006) also reported a MAG-CMCS hybrid formation by dispersing

MAG in CMCS solution. In many cases, crosslinker was added to provide a strong covalent bond network structure of chitosan hydrogel. MAG was suspended in chitosan solution and acrylic acid monomer with the presence of glutaraldehyde. The obtained chitosan-poly(acrylic acid) magnetic nanospheres were found to have highly ordered spherical aggregates. The formation of aggregates depended on crosslinked acrylic acid monomer and chitosan (Ge *et al.*, 2009). Barium ferrite coated with chitosan, modifying with glutaraldehyde, was crosslinked by epichlorohydrin to obtain magnetic composite for enzyme purification (An, *et al.*, 2003). Dispersing MAG and chitosan solution in microemulsion followed by glutaraldehyde crosslinking were frequently reported (Zhang *et al.*, 2007, Qui *et al.*, 2009, Zhi *et al.*, 2006 and Fang *et al.*, 2009) (Figure 2.7). Surfactants were also reported as a stabilizer for MAG dispersion in chitosan solution. Then, the chitosan coated MAG were obtained after crosslinking by sodium tripolyphosphate (Hritcu *et al.*, 2009) or glutaraldehyde (Wang, D.S., *et al.*, 2009).

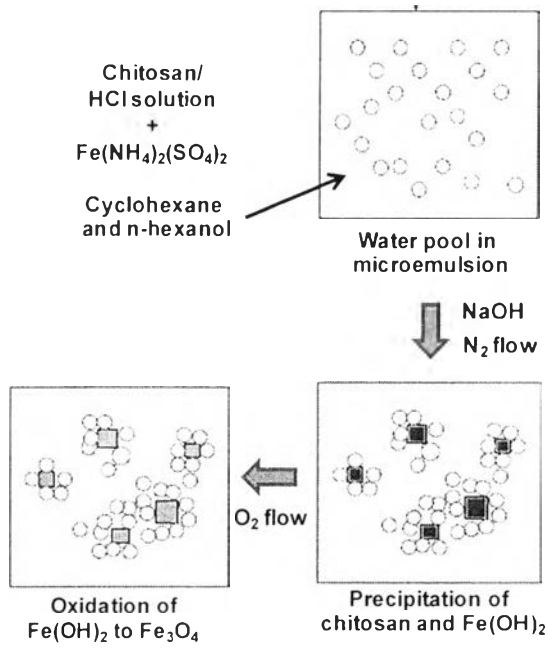


Figure 2.7 Preparation procedure of water-in-oil microemultion (Jia *et al.*, 2006).

Conjugating or coupling reaction and covalently modification on MAG' surface

Amine functionalization on MAG surface followed by establishing a covalent linkage with chitosan is also purposed (Liu, *et al.*, 2009). In the same way, chitosan chains were grafted from MAG via recombination of chitosan free radical. Then, chitosan molecules might act as a crosslinker to connect with other MAG molecules (Zhang, *et al.*, 2010). Carboxymethyl chitosan (CMCS), a water-soluble chitosan, was bound to MAG via a carbodiimide conjugating agent, cyanamide. The reaction was conducted in a mild reaction and complete in a short time (1 h) (Chang, *et al.*, 2006). Water dispersible MAG coated with covalently linked chitosan was purposed. MAG were first synthesized and coated with oleic acid followed by ligand exchanging with *N*-[(3-Trimethoxysilyl)propyl] EDTA trisodium salt. Chitosan was then crosslinked with COOH containing silane modified MAG via amide linkage by conjugation reaction with *N*-(3-Dimethyl aminopropyl)-*N*-ethyl carbodiimide hydrochloride (Figure 2.8) (Lopéz-Cruz, *et al.*, 2009).

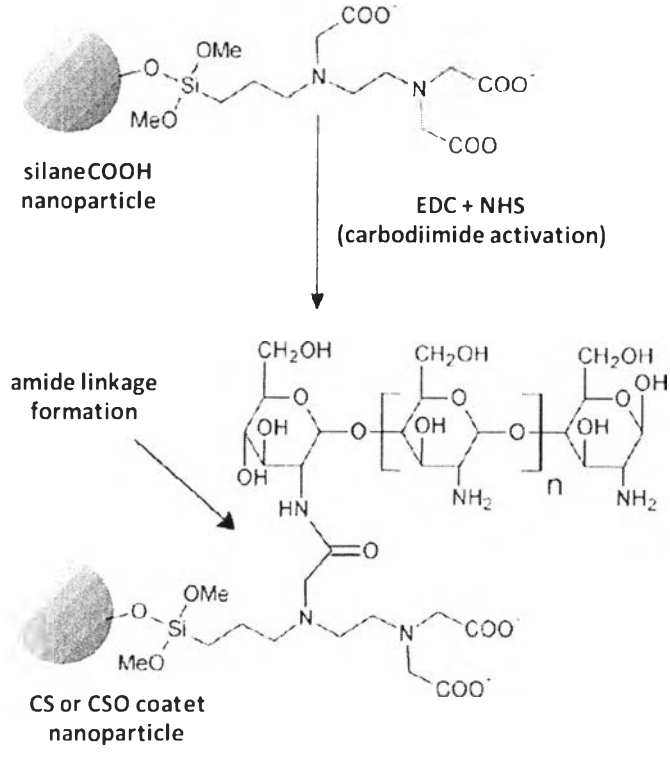


Figure 2.8 Scheme of amide linkage formation between the carboxylic group on the particle surface and the amino group found in chitosan, via carbodiimide activation (López-Cruz, *et al.*, 2009).

In situ co-precipitation of MAG in chitosan hydrogel matrix

Differently from the above techniques, this way, MAG were grown after or during matrix formation. A ferrite solution of Fe^{2+} and Fe^{3+} at a stoichiometric ratio of 1: 2 was added into chitosan in acetic acid solution. Ammonium hydroxide was gradually added to form a co-precipitated chitosan-MAG until the pH reaches 10–11. The chitosan-conjugated MAG were collected by a strong magnet (Yang, *et al.*, 2007). The hybridization of chitosan and MAG induced by magnetic field was reported. Chitosan in acetic acid solution was mixed with magnetic precursor solution (Fe^{2+}: Fe^{3+} = 1:2) then cast on a mold. Casted mold was then soaked in NaOH solution in a presence of external magnetic field and allowed MAG formation. It was found that MAG formed a chain-like structure under the influence of the external magnetic field (Li, *et al.*, 2006). An in situ MAG-chitosan composite

preparation in microemulsion was studied. Chitosan in hydrochloric acid solution was formed a water pool in water-in-oil emulsion system. A surfactant was used to stabilized the water droplets. NaOH solution was then introduced to precipitate chitosan and $Fe(OH)_2$. Oxidation of $Fe(OH)_2$ was conducted by a controlled oxygen flow (Zhi *et al.*, 2006). Chitosan-induced synthesis of MAG via ions assembly was reported by Wang, Y.L., *et al.* (2008 and 2009). The interaction between . Chitosan hydrogel was firstly fabricated by crosslinking with glutaraldehyde. The hydrogel was then soaked in $FeCl_3$ solution alternating with $FeCl_2$ solution. The soaking process was repeated 3 times before treating with NaOH. MAG were found to disperse uniformly all over the obtained composite hydrogel. The authors purposed that amino group from chitosan caused a chelation effect with iron ions during precursor formation as shown in Figure 2.9.

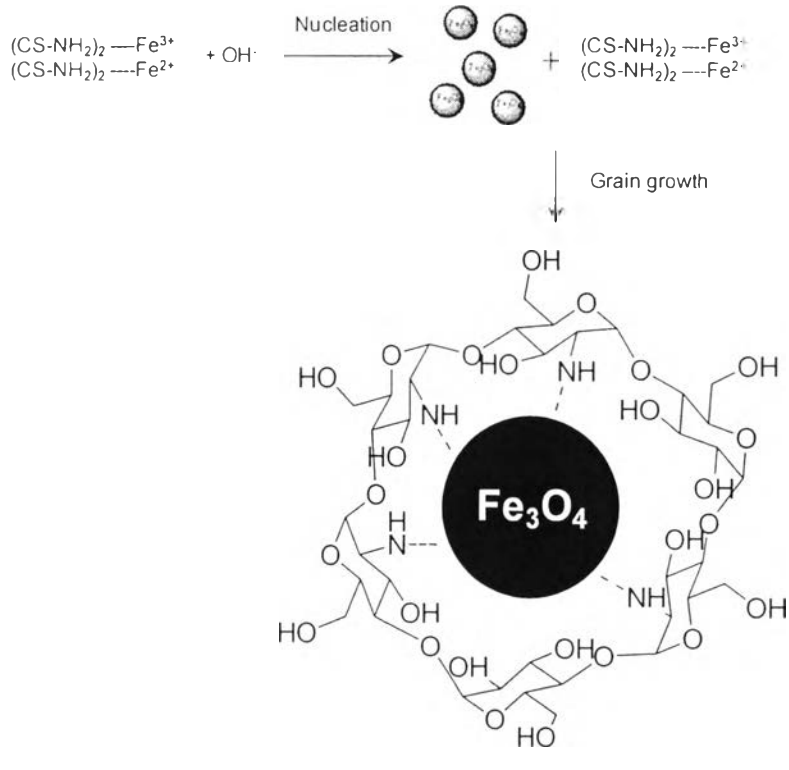


Figure 2.9 Principle of in situ mineralization of MAG in chitosan hydrogel (Wang, Y.L., 2009).

2.4 Point of Study

The present work focuses on fabricating nanocomposite between MAG and chitosan-based materials and stabilizing MAG in aqueous system. In the past, most studies have shown that the MAG were bound in coating materials successfully by both covalent bond and non-covalent bond. In the case of non-covalent bond, the explanation about the interactions between magnetic nanoparticles is limited, which is very important in in-depth study. Only few groups reported about electrostatic interaction between positive surface charge on chitosan and negative surface charge of MAG (Yang, *et al.*, 2007). Some report explained the chelating effect between iron ions and amino group of chitosan in chitosan-Fe complex (Wang, Y.L., *et al.*, 2009).

In Chapter III, we focus on stabilizing naked MAG by chitosan nanosphere. Chitosan is a good material to use as a stabilizing material due to the reactive functional groups (amino group and hydroxyl group) in the main chain. Previously, our group succeeded in preparing amphiphilic chitosan nanospheres with the core-corona structure and studying the hydrophobic drug incorporation (Choochottiros *et al.*, 2010). The core is found to be hydrophobic part due to the phthaloyl group functionalization and the corona represents the hydrophilic part resulting from poly (ethylene glycol) grafted chain (Yoksan *et al.*, 2004).

The work extends to study the DNA isolation to investigate the efficiency of bacterial DNA extraction both quantitatively and qualitatively studies comparing with commercially available extraction kits.