CHAPTER II

LITERATURE REVIEW

The purpose of this research was to prepare water dispersible magnetite nanoparticles containing hydrophobic inner shells for efficient entrapment of indomethacin model drug and hydrophilic outer layers for their good dispersibility in an aqueous solution. Hence, this chapter reviews the fundamentals of magnetic materials and magnetism, magnetite nanoparticle, surface modification of magnetite nanoparticles and the background of indomethacin, respectively.

Introduction to magnetic materials and magnetism

1. Types of magnetism

Five basic types of magnetism have been observed and classified on the basis of the magnetic behavior of materials in response to magnetic fields at different temperatures. These types of magnetism are: ferromagnetism, ferrimagnetism, antiferromagnetism, paramagnetism, and diamagnetism [11].

1.1 Ferromagnetism

The magnetic moments in a ferromagnet have the tendency to become aligned parallel to each other under the influence of a magnetic field. However, unlike the moments in a paramagnet, these moments will then remain parallel when a magnetic field is not applied (Figure 1) [11].

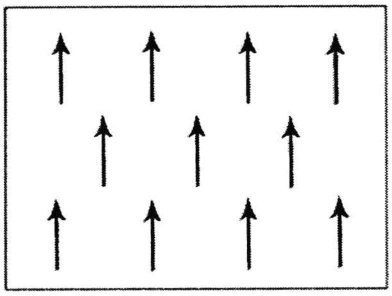

Figure 1 Magnetic dipole moments aligned parallel in a ferromagnetic material [12]

1.2 Ferrimagnetism

The aligned magnetic moments are not of the same size; that is to say there is more than one type of magnetic ion. An overall magnetization is produced but not all the magnetic moments may give a positive contribution to the overall magnetization (Figure 2) [11].

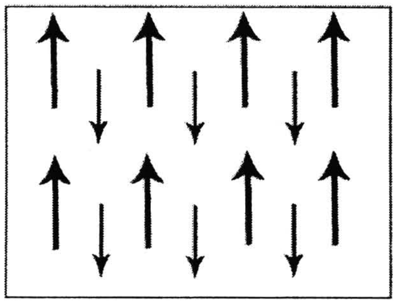

Figure 2 Magnetic moments of different magnitudes aligned anti-parallel in a ferromagnetic material [12]

1.3 Antiferromagnetism

Adjacent magnetic moments from the magnetic ions tend to align anti-parallel to each other without an applied field. In the simplest case, adjacent magnetic moments are opposite in direction and equal in magnitude; therefore there is no overall magnetization (Figure 3) [11].

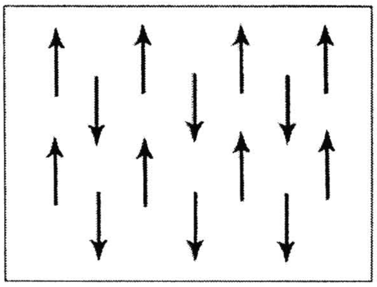

Figure 3 Magnetic dipole moments with equal magnitude aligned anti-parallel in an antiferromagnetic material. This is only one of many possible antiferromagnetic arrangements of magnetic moments [12]

1.4. Paramagnetism

In a paramagnet, the magnetic moments tend to be randomly orientated due to thermal fluctuations when there is no magnetic field. In an applied magnetic field, these moments start to align parallel to the field such that the magnetization of the material is proportional to the applied field (Figure 4) [11].

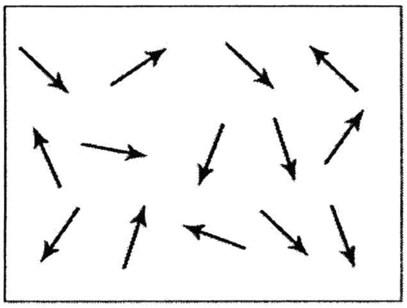

Figure 4 Magnetic dipole moments randomly aligned in a paramagnetic sample [12]

1.5 Diamagnetism

Diamagnetic metals have a very weak and negative susceptibility to magnetic fields. Diamagnetic materials are slightly repelled by a magnetic field and the material does not retain the magnetic properties when the external field is removed. Diamagnetic materials are solids with all paired electrons resulting in no permanent net magnetic moment per atom. Diamagnetic properties arise from the realignment of the electron orbits under the influence of an external magnetic field. Most elements in the periodic table, including copper, silver, and gold, are diamagnetic [11].

2. Superparamagnetic materials

Superparamagnetic materials are defined as those having dipole moments of a single-domain particle with discrete randomly oriented magnetic moments. When placed in an external magnetic field, their moments rapidly rotate into the direction of the magnetic field and enhance the magnetic flux. When the external magnetic field is removed, Brownian motion is sufficient to cause the moments and particles themselves to randomize (Figure 5). Superparamagnetic fluids differ from ordinary paramagnetic fluids in that they have high magnetic susceptibility and are able to be saturated in moderate fields. In addition, they also differ from typical ferromagnetic materials in that they do not exhibit magnetic hysteresis. The advantages of superparamagnetic particles are that they are easy to re-suspend, have large surface area, show slow sedimentation and have uniform distribution of the particles in the suspension media. Once magnetized, the particles behave like small permanent magnets, so that they form aggregates or lattice due to magnetic interaction [13].

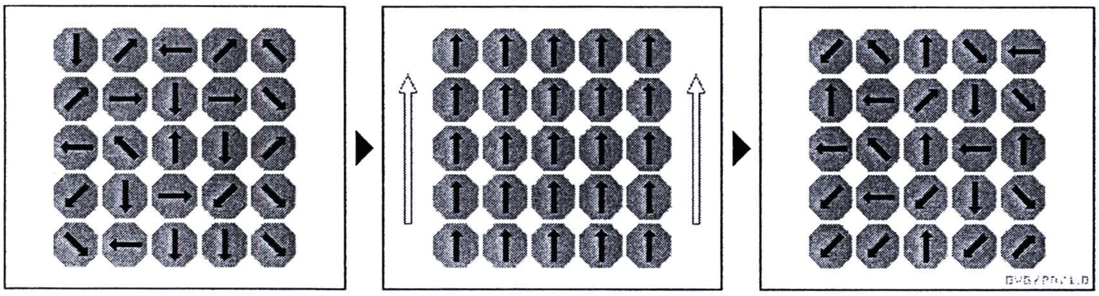
normal
magnetic field applied
magnetic field removed

Figure 5 The effect of magnetic field on the arrangement of magnetic moments of superparamagnetic material [14]

3. Magnetic hysteresis loop

In an unmagnetized ferromagnetic or ferrimagnetic materials, magnetic moments in a domain are randomly oriented throughout the material and therefore the collections of magnetic moments are self-canceled, resulting in a minimal to zero net magnetization (Figure 6 at M ~ 0). When placed in the presence of a sufficiently large external magnetic field, the spins in each domain rotate parallel to the direction of the applied magnetic field until all the dipoles are aligned. The plateau region of the magnetization curve is the saturation magnetization (M_s) (Figure 6). M_s is a measure of the magnetization (M) per gram basis and is given by;

$$\mathrm{Ms} = \frac{\mathrm{M}}{\mathrm{P}}\left(\frac{emu}{g}\right) \tag{1}$$

where ρ is the density of the material (Equation 1) [12].

The arrows in the square boxes in Figure 6 depict the rotation of the vector moments in the domain wall as the magnetic field strength (*H*) is varied. When the applied field is decreased, magnetization decreases. In multi-domain bulk materials, demagnetization occurs primarily *via* spin rotation through the domain walls. If the demagnetization curve, during the removal of the applied field, does not follow the initial magnetization curve, the material displays hysteresis. Remanence (M_R) is the magnetization remaining at zero applied field ($H = 0$). The magnetic field applied in the negative direction required to return the magnetization to zero is the coercive force (H_c) [15].

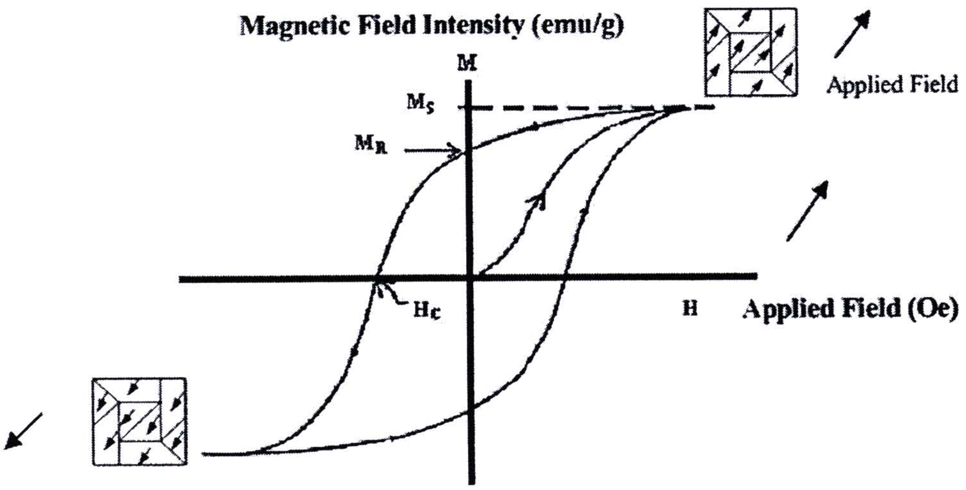

Figure 6Magnetization (*M*) versus magnetic field strength (*H*), where M_s is saturation magnetization, M_R is remanence, and H_C is coercive force [15]

4. Ferrofluids

Superparamagnetic particles dispersed in a carrier fluid are referred to as ferrofluids [15]. They are not found in nature and were first introduced in the early 1960s [16]. Ferrofluids are composed of nanoscale particles (diameter usually 10 nanometers or less) of magnetite, hematite or some other compound containing iron. A stable ferrofluid consists of superparamagnetic nanoparticles, a carrier fluid and a surfactant. The particle is small enough for thermal agitation to disperse them evenly within a carrier fluid, and for them to contribute to the overall magnetic response of the fluid [17].

Introduction to magnetite nanoparticles

In the last decade, nanotechnology has developed to such an extent that it has become possible to fabricate, characterize and specially tailor the functional properties of nanoparticles for biomedical applications and diagnostics. As intermediates between the molecular and the solid states, inorganic nanoparticles combine chemical accessibility in solution with physical properties of the bulk phase. They are thus ideal elements for the construction of nanostructure materials and devices with adjustable physical and chemical properties. The application of small iron oxide particles in *in vitro* diagnostics has been practiced for nearly 40 years. There are several investigations of types of iron oxides have been carried out in the field of nanosized magnetic particles, mostly maghemite (γ-Fe_2O_3), magnetite (Fe_3O_4) and $MO{\cdot}Fe_2O_3$ (where M is Mn, Co, Ni, or Cu) [23], (single domains of about 5–20 nm in diameter), among which magnetite is a very promising candidate since its biocompatibility has already been proven [18].

In recent years, the use of magnetite nanoparticles has attracted significant interest in biomedicine and biomedical engineering for applications including magnetic carriers for drug delivery systems, controlled drug release, contrast enhancement agents in magnetic resonance imaging (MRI) for diagnostics, cellular therapy (such as cell labelling, targeting and as a tool for cell-biology research to separate and purify cell populations), tissue repair and hyperthermia. The motivation for the use of magnetite nanoparticles is related to their superparamagnetic characteristics, high saturation magnetization, good biocompatibility and interactive functions at the surface [19].

1. Synthesis of magnetite nanoparticles

Various synthesis methods of magnetite nanoparticles have been established; co-precipitation, thermal decomposition, microemulsion and aerosol/ vapour methods. The first two methods have been most prevalently attracted much attention and will be profoundly presented herein [20].

1.1 Coprecipitation

Iron oxides (either Fe_3O_4 or γ-Fe_2O_3) can be synthesized through the coprecipitation of Fe^{2+} and Fe^{3+} aqueous salt solutions by addition of a base. Control of size, shape and composition of nanoparticles depends on the type of salts used (e.g. chlorides, sulphates, nitrates and perchlorates), Fe^{2+} and Fe^{3+} ratio, pH and ionic strength of the media. Conventionally, magnetite is prepared by adding a base to an aqueous mixture of Fe^{2+} and Fe^{3+} chlorides at a 1:2 molar ratio. The precipitated magnetite is black in color. The chemical reaction of Fe_3O_4 precipitation is given in Figure 7.

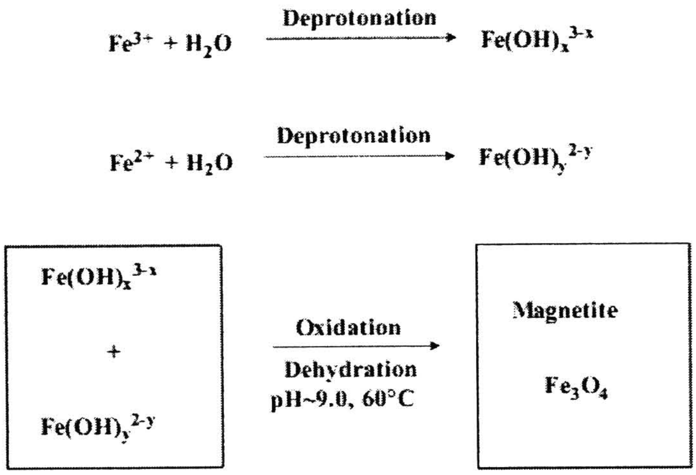

Figure 7 The reaction mechanism of magnetite particle formation from an aqueous mixture of ferrous and ferric chloride by addition of a base [20]

The overall reaction may be written as follow:

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

According to the thermodynamics of this reaction, a complete precipitation of Fe_3O_4 should be expected between pH 9 and 14, while maintaining a 2:1 molar ratio of Fe^{3+}: Fe^{2+} under a non-oxidizing oxygen-free environment. Otherwise, Fe_3O_4 might also be oxidized as the following equation:

$$Fe_3O_4 + 0.25O_2 + 4.5H_2O \longrightarrow 3Fe(OH)_3 \qquad (3)$$

$$2Fe_3O_4 + 1/2O_2 \longrightarrow 3Fe_2O_3 \qquad (4)$$

This would critically affect the physical and chemical properties of the nanosized magnetic particles. In order to prevent them from possible oxidation in air as well as from agglomeration, Fe_3O_4 nanoparticles produced by reaction (1) are usually coated with organic or inorganic molecules during the precipitation process. To control the reaction kinetics, which is strongly related with the oxidation speed of iron species, the synthesis of particles must be done in an oxygen-free environment by passing N_2 gas. Bubbling nitrogen gas through the solution not only protects critical oxidation of the magnetite but also reduces the particle size when compared with methods without removing the oxygen [21, 22].

The reported of magnetic nanoparticles by coprecipitation within spontaneous generated vesicles from the mixtures of single-tailed cationic (cetyltrimethylammonium bromide (CTAB)) and anionic (dodecylbenzene sulphonic acid (DBSA)) surfactants [23]. The CTAB/DBSA molar ratio of 7:3 was used to prepare magnetite particles by gently heating the Fe^{2+} hydroxide precipitate that formed at room temperature conditions. The reported of investigated the dependence of the ionic strength of the reaction solution on the formation of magnetite nanoparticles [24]. The magnetite nanoparticles prepared with the addition of 1.0 M NaCl aqueous solution created the particles having ~1.5 nm smaller than those formed without its presence. In addition, these smaller nanoparticles formed in the higher ionic strength solutions displayed lower saturation magnetization (63 emu/g) than those prepared in NaCl-free solutions (71 emu/g).

1.2 Thermal decomposition

Thermal decomposition is an alternative approach to produce monodisperse magnetite nanoparticles *via* high temperature organic phase decomposition of an iron precursor, such as $FeCup_3$ (Cup: N-nitrosophenylhydroxylamine, $C_6H_5N(NO)O$) and $Fe(acac)_3$ [32]. These two iron precursors produce magnetite (Fe_3O_4) nanoparticles as a resulting product. On the other hand, thermal decomposition of iron pentacarbonyl ($Fe(CO)_5$) followed by oxidation can lead to monodisperse maghemite (γ-Fe_2O_3) nanoparticles [25]. The latter process has recently been extended to the synthesis of monodisperse cobalt ferrite ($CoFe_2O_4$) nanoparticles. Although significant progress in making monodisperse Fe_2O_3 and $CoFe_2O_4$ nanoparticles has been made in organic phase reactions, there is still no general process for producing MFe_2O_4, especially Fe_3O_4 nanoparticles with the desired size and acceptable size distribution.

The synthesis of well-defined nanoparticles with controllable sizes using high-temperature (up to 305°C) reactions of metal acetylacetonate in the presence of 1,2-hexadecanediol, oleic acid, and oleylamine [26]. This work has been a breakthrough discovery on precisely controllable size of nanoparticles by varying the reaction temperatures or changing metal precursors. Alternatively, with the smaller nanoparticles as seeds, larger monodisperse nanoparticles up to 20 nm in diameter were synthesized by seed mediated growth. The speed of nucleation and growth of the nanocrystals were controlled by accurately adjusting the temperature and using abundant surfactants. Therefore, it is feasible to produce magnetic nanoparticles with controllable size, narrow size distribution, and generally a hydrophobic surface. Until now, the precursor decomposition approach has been investigated in many aspects, such as the elucidation of the mechanism, the accurate control of size and shape of the nanoparticles, and even the selection of more economic precursors. Undoubtedly, this method is a nearly perfect way to prepare monodispersed nanoparticles in the laboratory. However, the strict requirement of temperature controlling would be the limitation for implement this approach to industrial fabrication, and the resultant hydrophobic surface to some extent limits their use in biomedical fields [27].

2. Stabilization of magnetite nanoparticles

In general, agglomeration of magnetite nanoparticles arises from three primary attractive forces [15]:

Magnetic attractive forces ; the interaction energy increases as the distance between particles decreases and aggregation may occur unless prevented by a surfactant.

The gravitational forces ; they pull the particles down while the Brownian motion works to keep them dispersed.

London dispersion or van der Waals attractive forces; they are derived from the spontaneously induced polarizability of fluctuating electric dipole-dipole forces, independent of magnetic attractive forces. The attractive forces also promote aggregation in an attempt to lower the large surface energy of nanoparticles that arise from their large surface area to volume ratio.

Electrostatic and steric repulsion forces are two mechanisms used to balance the attractive forces and prevent particle aggregation.

2.1 Electrostatic repulsion

At a neutral pH, the surface of magnetite primarily consists of hydroxyl groups. The surface chemistry of magnetite can be altered by adjusting the pH of the medium. At low pH, surface hydroxyl groups become protonated and at high pH the hydroxyl groups are deprotonated. The positive or negative surface charges at any particular pH are balanced by counterions to maintain electro-neutrality, resulting in an electrostatic double layer. The double layer of surface charge promotes the repulsion of colloidal magnetite particles (Figure 8). The electrostatic double layer repulsive force results from decreased entropy of the counterion distribution as two surfaces approach each other. The reported isoelectric point of magnetite is at pH 6.8. This is the pH at which an equal number of positive and negative surface charges co-exist. At the isoelectric point, there is no electrostatic double layer, no double layer repulsive forces, and so particles begin to aggregate. It is for this reason that surfactants that stabilize *via* a steric mechanism are valuable for preparing stable nanomagnetite fluids at neutral pH [17].

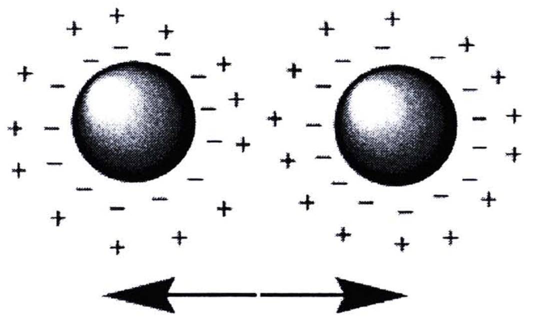

Figure 8 Electrostatic double layer causing repulsive forces between particles [28]

2.2 Steric or entropic repulsion

Magnetite stabilization by steric repulsive forces is achieved by coating a surfactant on the particle surfaces. Normally, the surfactant is an amphiphilic molecule with a nonpolar head that chemically or physically adsorbs onto the surface of magnetite nanoparticles and a hydrophilic tail that extends out into the polar carrier fluid to maintain colloidal stabilization (Figure 9). The steric stabilization mechanism may be described that the surfactant acts to disguise surfaces of the particles using specific surfactants with surface-adsorbable heads and their tails that are chemically similar to the carrier fluid. When the particles approach to each other, the chains of the surfactant interpenetrate to those of other particles, creating an osmotic pressure and a repulsive force due to an increase in entropy as the surfactant chains begin to compress one another [15].

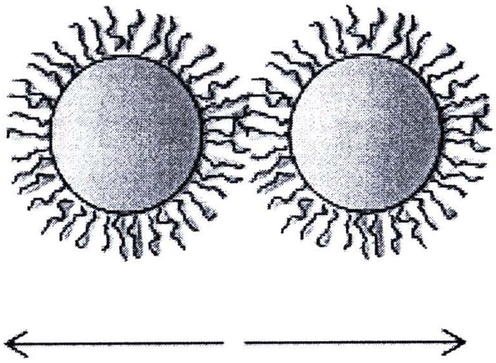

Figure 9 Stabilization of two particles coated with polymeric surfactants by steric or entropic repulsion mechanisms [29]

Introduction to surface modification of magnetite nanoparticles

1. Modification of magnetite nanoparticles by bilayer surface

The studies based on this assumption have been reported [30]. In addition to ionic interaction, physical adsorption of amphiphilic molecules or polymers onto particle surfaces can efficiently yield water dispersible nanoparticles. They have developed water-dispersible magnetite nanoparticles stabilized with bilayer surfactant of oleic acid/Pluronic (poly(ethylene oxide)-poly(propylene oxide), PEO-PPO copolymer) and studied their loading efficiency and releasing behavior of anticancer agents. It was hypothesized that PPO blocks were physically adsorbed onto the particle surfaces coated with oleic acid primary surfactant and PEO blocks provided steric stabilization in water. Similarly, fatty acids can also serve as both primary and secondary surfactants to produce bilayer-stabilized magnetite in aqueous fluids (Figure 10) [31].

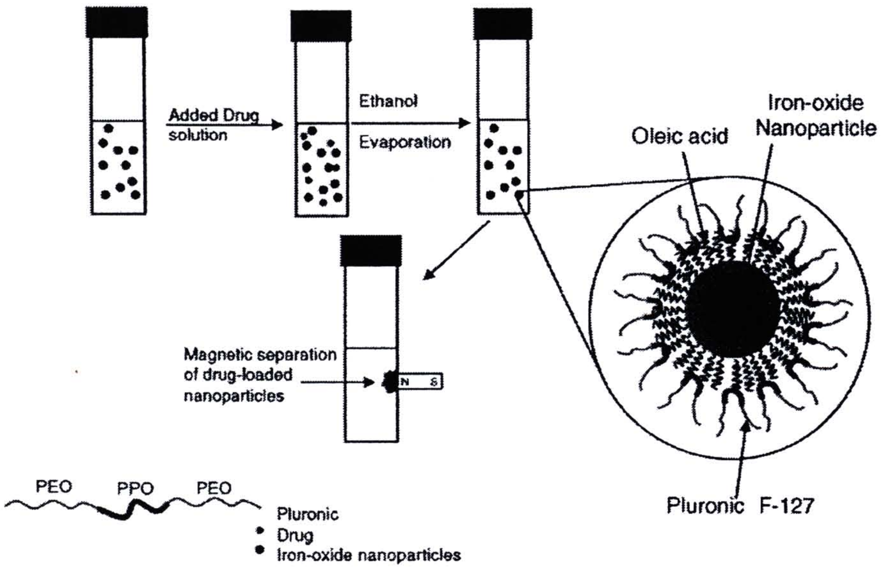

Figure 10 Representing formulation of magnetite nanoparticles and the process for drug loading [30]

Afterwards, research faculty have suggested the preparation and analysis of a highly concentrated hydrophobic oleic acid-coated magnetite gel [29]. By contrast to conventional techniques to prepare magnetic fluids, oleic acid was introduced as a reactant during the initial crystallization phase of magnetite, obtained by the coprecipitation of Fe(II) and Fe(III) salts by addition of ammonium hydroxide (Figure 11). The resulting gelatinous hydrophobic magnetite was characterized in terms of morphology, particle size, magnetic properties, crystal structure, and hydrophobicity /hydrophilicity ratio. This magnetic gel exhibited superparamagnetism with a saturation magnetization of 46.0 emu/g and could be well dispersed both in polar and nonpolar carrier liquids. This protocol produced highly concentrated hydrophobic magnetic gel for biopolymer encapsulations.

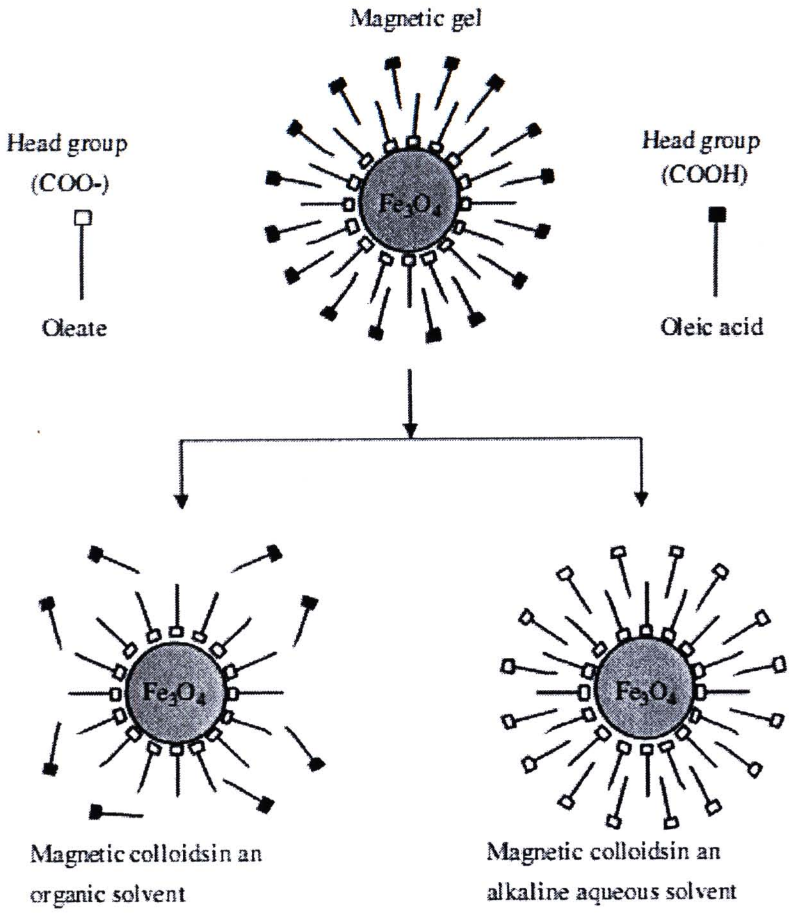

Figure 11 The proposed mechanism of magnetite gels dispersible in polar and nonpolar solvents [29]

Sodium oleate (SO) and sodium dodecyl benzene sulfonate (SDBS) were employed as inner and outer surfactants of magnetite nanoparticles prepared by coprecipitation [32]. Based on this investigation, the mechanisms of adsorption of monolayer and bilayer on the surface of magnetite were investigated by means of determining the adsorption isotherm of surfactants on particles and the dependence of zeta potential on the surfactants concentrations.

With respect to the experimental conclusions above, the adsorption mechanisms of bilayer surfactants on the magnetite particles could be expressed as follows. Firstly, SO monolayer adsorbed on the surface of magnetite particles by the interaction between the particles and the carboxyl groups of SO. Due to the alkyl chain of SO stretching into water, the particles surface was hydrophobic (Figure 12A). This was a chemical adsorption process. Secondly, the SDBS molecules absorbed onto the surface of magnetite particles coated with SO by means of Van der Waals attraction in the SDBS solution. With the polar end of SDBS molecules stretching into water, the particles surface turned into hydrophilic (Figure 12B). This was a physical adsorption process.

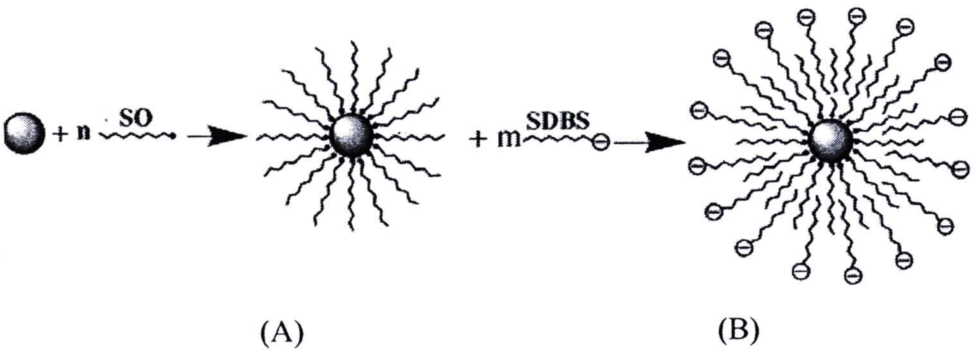

Figure 12 Representation for the adsorption of SO and SDBS on magnetite particles [32]

Many attemps have recently been made on preparing core/shell magnetite nanoparticles possessing polymer-coated surfaces The synthesis of poly(lactide-*b*-siloxane-*b*-lactide) triblock copolymers as magnetite nanoparticle stabilizers [33]. The siloxane central blocks functionalized with certain numbers of carboxylic acid were thought to anchor onto the particle surfaces and form polysiloxane inner shells, while polylactide tail blocks provided steric stabilization in hydrophobic carriers. Using the same concept, water dispersible nanoparticles were successfully achieved when triblock copolymers consisting of COOH-containing polyurethane central blocks and hydrophilic polyether tail blocks were used as dispersants (Figure 13).

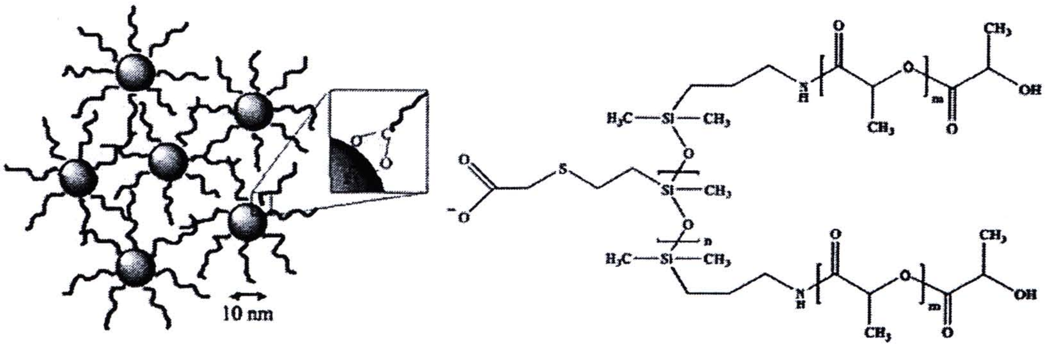

Figure 13 Magnetite nanoparticles complexed with carboxylate-functional poly(lactide-*b*-siloxane-*b*-lactide) block copolymer [33]

Introduction to polyester

Polyesters have always been an attraction from the early days of Carothers [34]. Polyesters that are made by treating a diol (saturated/unsaturated) with saturated and/or unsaturated dicarboxylic acids/anhydrides are valuable materials in polymer technologies [35, 36, 37, 38, 39]. A wide variety of polyesters is available having applications in diverse areas. Saturated polyester, such as poly(ethylene terephthalate) (PET), is well known and is having applications in the areas of fibers, films, bottles, etc. Poly(butylene terephthalate) (PBT) is an engineering thermoplastic material. Unsaturated polyester resins are having extensive use in the fiber reinforced plastics sector. Oligomeric saturated polyester polyols [40-43] is one among the basic building blocks in polyurethanes. Increasing interest exists in the synthesis of new kinds of polyester blocks to impart special properties to the final polyurethanes. Recently, 1,3-bis(2-hydroxyethoxy)benzene (HER)-based polyesters are explored in developing polymers having excellent barrier properties to achieve the desired packaging materials for the highly oxygen sensitive food products and beverages. The chemical structure of HER is shown in Figure 14. Cast and thermoplastic polyurethanes are the other major areas of application of HER-based polyesters [44].

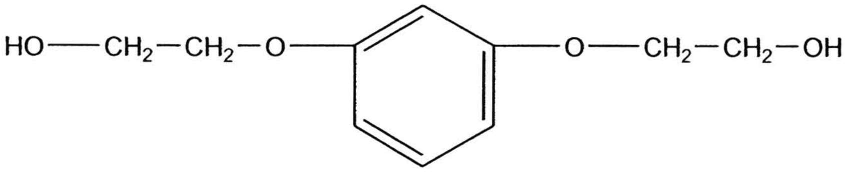

Figure 14 Structure of 1,3-bis(2-hydroxyethoxy)benzene (HER) [44]

Introduction to indomethacin

Indomethacin is a non-steroidal anti-inflammatory drug (NSAID) that reduces fever, pain and inflammation. Because indomethacin may cause or worsen stomach or intestinal bleeding or ulcers and can increase blood pressure and decrease kidney function, it is important to decrease used dosages and side effects at the treatment. One of the possibilities to achieve this is direct delivery of the drug to the target area of one's body [45]. Chemical structure of indomethacin is illustrated in Figure 15.

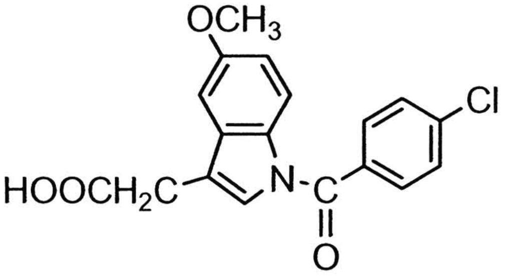

Figure 15 Chemical structure of indomethacin [45]

The preparation and characterization of magnetite nanoparticles loaded with indomethacin as anti-inflammatory drug suitable for magnetic drug targeting [46]. The poorly water-soluble indomethacin was successfully encapsulated in polylactic acid (PLA) magnetic nanospheres by nanoprecipitation method. The evidence of successful entrapment of indomethacin was confirmed by FTIR and spectrophotometric measurements. The as-prepared drug-loaded particles exhibited a response to external magnetic field, indicating their viability for magnetic drug targeting application. A novel complex comprising of poly(ε-caprolactone) (PCL) nanoparticles (good drug loadings) coated with chitosan (good adhesion to the mucosa) [47]. After topical ocular instillation, the nanoparticles of chitosan-coated PCL allowed the bioavailability of indomethacin in the cornea as well as in the aqueous humor to be significantly enhanced.

The synthesized magnetic PCL nanoparticles having a well shaped spherical form *via* an oil-in-water emulsion method [48]. In this study, the influence of some preparative variables on the size and surface properties was investigated. The resulting particles were well individualized, homogeneous in size and exhibited superparamagnetic behavior. An anti-cancer drug was encapsulated in the magnetic nanoparticles during preparation. A typical release behavior was observed for 30 days. *In vitro* experiment of magnetic susceptibility under external magnetic field demonstrated that the magnetic PCL nanoparticles have sufficient magnetic susceptibility for a potential magnetic drug carrier for targeted delivery.

The biodegradable poly(butylene succinate)/poly(ε-caprolactone) (PBS/PCL) microcapsules containing indomethacin which were prepared by an emulsion solvent evaporation method [49]. The microcapsules exhibited porous and spherical form in the presence of gelatin as a surfactant. The release rate of indomethacin from the microcapsules was decreased with increasing the PCL content. Afterwards, research faculties have reported synthesis of indomethacin-loaded bilayer-surface magnetite nanoparticles and their releasing behavior [50]. The particles were first stabilized with oleic acid primary surfactant, followed by poly(ethylene glycol) methyl ether-poly(ε-caprolactone) (mPEG–PCL) amphiphilic block copolymer as a secondary surfactant to form nanoparticles with hydrophobic inner shell and hydrophilic corona (Figure 16). The particles were 9 nm in diameter and exhibited superparamagnetic behavior at room temperature with saturation magnetization (M_s) about 35 emu/g magnetite. Percent release of indomethacin of all complexes reached their equilibrium within 12 h of dialysis.

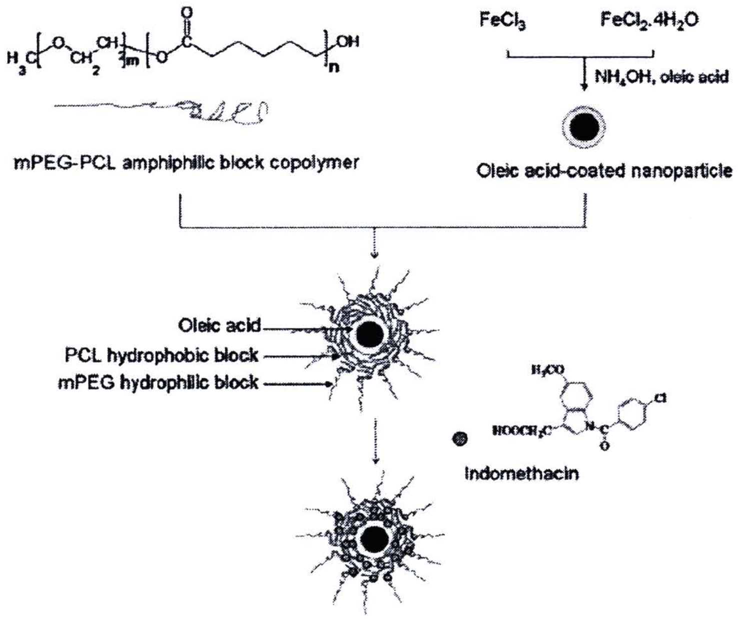

Figure 16 Proposed entrapment mechanism of indomethacin in the hydrophobic inner-layer surfaces of the copolymer-stabilized magnetite nanoparticles [50]