ULTRASOUND ASSISTED NON-SEEDED METHOD ON SURFACE MODIFICATION OF SUPERPARAMAGNETIC IRON OXIDE NANOPARTICLES FOR MAGNETIC RESONANCE IMAGING PROBE

by

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ABBREVIATIONS, ACRONYMS AND SYMBOLS

K_{eff}	Anisotropy constant,
B.E.T	Brunauer–Emmett–Teller
K_B	Boltzmann constant
H_c	Coercivity
ω_o	Larmor Frequency
T2	Longitudinal Relaxation Time
H	Magnetic field strength
B	Magnetic induction, or magnetic flux density,
χ_m	Magnetic Susceptibility
γ-Fe_2O_3	Maghemite
Fe_3O_4	Magnetite
M	Magnetization vector
RF	Radio Frequency
μ	Permeability
M_r	Remanence Magnetization
Ms	Saturation Magnetization
T	Temperature
TEVS	Triethoxy Vinyl Silane
T1	Transverse Relaxation Time
V	Volume
ζ	Zeta potential
AC	Alternating Current

APTES	3-Amino Propyl TriethoxySilane
DLS	Dynamic Light Scattering
ESI	Electron Spectroscopy Imaging
FTIR	Fourier Transform Infrared
HRTEM	High Resolution Transmission Electron Microscope
MRI	Magnetic Resonance Imaging
PEG	Polyethylene glycol
SCW	Supercritical water
SPION	Superparamagnetic Iron Oxide Nanoparticles
TEM	Transmission Electron Microscope
USPION	Ultra-small Superparamagnetic Iron Oxide Nanoparticles
TGA	Thermo Gravimetry Analysis
XPS	X-Ray Photoelectron Spectroscopy
XRD	X-Ray Diffraction
VSM	Vibrating Sample Magnetometer

KAEDAH TANPA BENIH DIBANTU ULTRASOND TERHADAP MODIFIKASI PERMUKAAN PARTIKEL BESI TEROKSIDA SUPERPARAMAGNETIK UNTUK PROB PENGIMEJAN MAGNETIK RESONAN

ABSTRAK

Dalam tesis ini, satu kaedah baru tanpa benih dan ultrasonik mudah untuk menggabungkan SPION ke dalam silica telah dibangunkan dan dianalisa. Campuran kedua-dua nanopartikel SPION dan silika yang di sintesis secara berasingan oleh kaedah mendakan-bersama dan sol-gel, masing-masing telah dikenakan pancaran secara-ultrasonik pada julat pH yang berbeza antara 3 dan 5. Daya besar yang dijana daripada peronggaan akustik (pembentukan, pertumbuhan dan keruntuhan buih) dan gelombang-kejut pancaran ultrasonik telah diguna untuk menyebabkan perlanggaran tak kenyal dan pemerbadanan SPION ke dalam nanopartikel silika. Pengukuran elektroforesis pada produk menunjukkan bahawa pada pH yang lebih rendah, SPION ditemui tertanam ke dalam silika. Walau bagaimanapun, pada pH lebih daripada 4, SPION adalah tidak stabil dan tidak dapat menahan aliran gelora dari medan ultrasonik. Keputusan mencadangkan bahawa pembentukan nanopartikel komposit SPION/silika berkait-rapat dengan perlanggaran tak kenyal yang disebabkan oleh pancaran ultrasonik. Tambahan pula, pembentukan nanopartikel komposit bergantung ke pada potensi-zeta dan kestabilan koloid zarah. Pembentukan nanopartikel komposit telah dikaji menggunakan TEM, ESI, XRD, FTIR, XPS, BET, Zeta Sizer dan VSM. Nanopartikel komposit yang dihasilkan oleh kaedah ini dapat mengekalkan hampir 70% dari ketepuan pemagnetan awalannya. Kereaktifan permukaan silika dibuktikan melalui pengikatan lapisan-mono dekanatiol pada nanopartikel komposit. Nanopartikel komposit SPION/silika kemudiannya telah diuji sebagai ejen kontras MRI. Nanopartikel magnet menunjukkan keupayaan untuk memendekkan masa santaian T2 dan T1 lebih dari 99% dan 95%.

ULTRASOUND ASSISTED NON-SEEDED METHOD ON SURFACE MODIFICATION OF SUPERPARAMAGNETIC IRON OXIDE NANOPARTICLES FOR MAGNETIC RESONANCE IMAGING PROBE

ABSTRACT

In this thesis, a new non-seeded and facile ultrasonic method of incorporating SPION into silica was developed and analysed. Mixture of both SPION and silica nanoparticles synthesized separately by co-precipitation and sol-gel method, respectively was ultrasonically irradiated at different pH ranges between 3 and 5. The tremendous force generated from the acoustic cavitation (formation, growth and collapse of bubbles) and shockwave of the ultrasonic irradiation was employed to induce inelastic collision and incorporation of SPION into the silica nanoparticles. Electrophoresis measurement of the as-synthesized SPION/silica demonstrated that at lower pH, SPION was found embedded into the silica. However, at pH greater than 4, SPION was unstable and unable to withstand the turbulence flow from the ultrasonic field. The results suggested that the formation of the SPION/silica composite nanoparticles is strongly related to the inelastic collision induced by ultrasonic irradiation. More so, the formation the composite nanoparticles are dependent on the zeta potential and colloidal stability of the particles. The formation of the composite nanoparticles was analysed using TEM, ESI, XRD, FTIR, XPS, BET, Zeta-Sizer and VSM. The composite nanoparticles developed by this method were able to retain nearly 70% of its initial saturation magnetization. The reactivity of the SPION/silica was demonstrated via binding of decanethiol monolayer on the composite nanoparticles. The SPION/silica nanoparticles were then tested for

MRI contrast agent. The magnetic nanoparticles demonstrate ability to shorten T_2 and T_1 relaxation time by more than 99% and 95% respectively. Importantly, the results demonstrate that all the set objectives of this thesis were achieved.

Chapter 1: Introduction

1.1 Nanotechnology

The history of nanotechnology can be traced back to December 29, 1959 when Nobel Prize winning physicist Richard Feynman gave the lecture "There's Plenty of Room at the Bottom" at an American Physical Society meeting at Caltech (Feynman, 1960). He considered the possibility of direct manipulation of individual atoms as a more powerful tool in the synthetic chemistry than those used at the time. However, it was Norio Taniguchi a Japanese scientist who first used the word “Nanotechnology” (Taniguchi, 1974). Modern nanotechnology began with the development of scanning tunnelling microscope that could see individual atoms. According to the Oxford dictionary nanotechnology can be defined as follows:

> “Nanotechnology is defined as the branch of technology that deals with dimensions and tolerances of less than 100 nanometres, especially the manipulation of individual atoms and molecules”.

One of the most interesting facts in nanotechnology is that the physicochemical properties of materials in the nanometric scale are highly differs from those of the same bulk materials. For example bulk gold material is known to be inert and non-active as a catalyst. However, gold nanoparticles exhibit a surprisingly high catalytic reactivity regarding various reactions such as carbon monoxide and alcohol oxidation in the gas phase (Haruta, Kobayashi, Sano, & Yamada, 1987). Another example of nanomaterials showing variation in their physicochemical properties from their bulk materials is the non-magnetic bulk materials such as Au, Pd or Pt nanomaterials embedded in polymer demonstrated magnetic moments at nanometric size (Nakae et al., 2000; Yamamoto et al., 2003).

The reasons why nanomaterials show much variation in their physicochemical properties from their bulk materials can be related to two reasons: (1) surface effect, the fraction of atoms at the surface have fewer neighbour compare to their bulk material and (2) quantum effects which show discontinuous behaviour due to completion of shells in systems with delocalised electron (Roduner, 2006). Owing to these unique properties, nanomaterials have got wide applications in several fields like electronics, energy, telecommunication and biomedical. The nanomaterials used for biomedical research and development applications include liposomes, polymeric micelles, block ionomer complexes, dendrimers, quantum dots and inorganic nanoparticles such as silica, gold and superparamagnetic iron oxide nanoparticles (Hofmann-Amtenbrink, von Rechenberg, & Hofmann, 2009).

1.2 Superparamagnetic Iron Oxide Nanoparticles

Superparamagnetic iron oxide nanoparticles (SPION) are inorganic nanomaterials of ferromagnetic substances with sizes between 1 – 100 nm. SPION are superparamagnetic (ability to have zero magnetism in the absence of external magnetic field) owing to their small sizes which enable them to have large magnetic susceptibility and single magnetic domain (Di Marco et al., 2007). Superparamagnetism occurs when the size of a ferromagnetic material is so small that the ambient thermal energy is sufficient to induce free rotation of the entire crystallite (Qiao, Yang, & Gao, 2009).

SPION are further classified into SPION with hydrodynamics sizes greater than 50 nm (coating included) and those with sizes less than 50 nm which are called ultra-small superparamagnetic iron oxide nanoparticles (USPION). SPION has got widespread applications in several areas including magnetic fluids, catalysis, data storage, environmental remediation, biotechnology and biomedical (Lu, Salabas, & Schüth, 2007) due to easy synthesis and magnetically controllable behaviour.

1.3 Biomedical applications of SPION

The interplay of nanotechnology with biology and medical sciences has led to the emergence of interdisciplinary field such as nanobiotechnology, nanobiology and nanomedicine. Nanotechnology offers biology and medical sciences new tools while biology and medical sciences offer nanotechnology access to new functional nanosystem (Qiao, Yang, & Gao, 2009). SPION offers several properties that allow its biomedical applications. First, the controllable size (1-100 nm) of SPION, places its dimensions that is smaller than or comparable to biomedical system such as cell (10–100 µm), virus (20–450 nm), protein (5–50 nm) and gene (2 nm wide and 10–100 nm long).

Second, SPION contains single magnetic domain and due to its superparamagnetic features, it can be manipulated and driven by an external magnetic field gradient to a desired body area and target biological entities (Chapa Gonzalez et al., 2014). These properties have enabled SPION to be applied in labelling, sensing, and separation of biomolecules, drug and gene delivery (Gupta, Naregalkar, Vaidya, & Gupta, 2007).

Third, due to the single-domain property of SPION, it possess magnetic moment which can undergo orientational thermal fluctuations because of either Brownian fluctuations or Ne´el fluctuations in the presence of an external alternating magnetic field to generate localized temperature up to 45-47 ℃ (Chastellain, Petri, Gupta, Rao, & Hofmann, 2004; Fortin et al., 2007). This heat is employed in hyperthermia therapy to kill cancer cells (Salas, Veintemillas-Verdaguer, & Morales, 2013).

Fourth, SPION’s superparamagnetic behaviour plus its large magnetic susceptibility cause microscopic field inhomogeneity and activate the dephasing of protons in the presence of external magnetic field. Therefore, SPION can be used as MRI probe (contrast agent) to shorten T2 and T2* relaxation times of the neighbouring regions, and produce a decreased signal intensity in T2- and T2*-weighted MR images (Na, Song, & Hyeon, 2009).

1.4 Limitations of SPION as a Biomedical Probe

The successful biomedical application of SPION depends mainly on the stability of the magnetic nanoparticles under the biological environments. The main drawbacks of SPION are agglomeration and lack of affinity for biomolecules. Agglomeration of the magnetic nanoparticles is caused by the high surface area of the nanoparticles, Van der Waals forces of attraction and dipole to dipole interactions between the particles (Mørup, Hansen, & Frandsen, 2010). Surface modification of SPION with biocompatible materials is one the main strategies used to control or prevent all problems related to the biomedical application of the magnetic nanoparticles.

1.4.1 Surface Modification of SPION

Several materials such as chitosan, dextran, liposome, polymer, hydrogel and inorganic nanoparticles such as gold and silica nanoparticles can be used to modify the surface of the SPION (Kumar & Mohammad, 2011). Silica nanoparticles are one of the inorganic materials used to encapsulate SPION. Silica nanoparticles are biocompatible and dielectric material which can screen the dipole-dipole interactions, and prevent agglomeration of the core SPION. Silica is a hydrophilic material that improves stability, biocompatibility and functionality of the SPION. The silanol (Si-OH) functional group of silica nanoparticles allows the composite nanoparticles to disperse in water or polar solvents. More so, Si-OH can be modified with other functional groups or provide binding site for bio-conjugation. Therefore, this work is aimed at synthesizing surface modified SPION using silica (SPION/silica) through a new novel approach.

1.5 Problem statement

Recently, several wet chemical methods like reverse-micelle (Yang et al., 2004), modified Stöber (Sun et al., 2005), microemulsion (Vogt et al., 2010), Stöber (Gao et al., 2011), in-situ formation of SPION inside mesoporous silica (Zhang et al., 2011) and electro-oxidation (Setyawan et al., 2012) have been deployed to synthesize SPION/silica. However, as shown in Table 1, the saturation magnetization of the SPION is greatly reduced after its surface modification with silica nanoparticles using the conventional method. Due to decrease in the magnetic moment, the silica coated SPION produced from these processes is less sensitivity as MRI probe (Tanaka, et al, 201).

Table 1.1: Magnetization of wet chemical synthesizing of SPION/silica nanoparticles

Methods	Saturation magnetization Ms (emu/g) SPION	SPION/Silica	Ref
Reverse-micelle	-	3.2	(Yang et al., 2004)
Stöber	-	12.7	(Gao et al., 2011)
Modified Stöber	46.3	13.9	(Sun et al., 2005)
Microemulsion	81	17.6	(Vogt et al., 2010)
Mesoporous silica	-	2.7	(Zhang et al., 2011)
Electro-oxidation	-	22.0	(Setyawan et al., 2012)

Most of the conventional wet chemical routes of synthesizing SPION/silica nanoparticles are based on seed growth mediated process. The reasons for decrease in the magnetization of the SPION/silica synthesized by the conventional procedures method can be related to two reasons. First, surface effect, the process of using SPION as template or seed in the production of silica shell can cause surface spin disorder of the core SPION (Yuan et al., 2012). Second, thickness effect, the silica shell produced from the conventional methods is not thin enough. Magnetisation per unit weight of SPION decreases with increase in the thickness of the silica shell (Vogt et al., 2010).

1.6 Aims and Objectives

The aims of this thesis are to conduct a fundamental research on the development of a new and non-seeded protocol of synthesizing highly magnetic SPION/silica nanoparticles which can be highly sensitive for MRI application. Unlike the conventional seed mediated growth method which is based on coating SPION with silica shell, this new non-seeded method is based on incorporating SPION into the framework of silica nanoparticles via in-elastic collision induced by ultrasonic irradiation. To achieve the aforementioned aims of this work, the objectives of this thesis are as follows:

1. To develop an ultrasonic irradiation assisted non-seeded and reproducible process of incorporating SPION into silica nanoparticles.
2. To produce SPION/silica nanoparticles that can retain at least 70% of the initial SPION's saturation magnetization.
3. To evaluate reactivity of the SPION/silica nanoparticles by functionalizing a monolayer molecule such as decanethiol on the SPION/silica composite nanoparticles.
4. To evaluate the sensitivity of the as-synthesized SPION/silica nanoparticles for MRI probe (contrast agent).
5. To show that the unique conditions from the ultrasonic irradiation can also be used to functionalize or modify the surface of SPION directly with organo-silane compound.

1.7 Thesis Outline

This thesis consists of six chapters. The introduction, problem statement, aims and objectives of this thesis are presented in Chapter 1. Literature review on synthesis of SPION and surface modification of SPION with silica nanoparticles using seed mediated methods are presented in Chapter 2. The various theories used to support this research are presented in Chapter 3. The whole experimental procedures, characterisation techniques and application of the as-synthesized SPION/silica nanoparticles for MRI probe are described in chapter 4. Details of the various results observed in this work are highlighted and discussed in Chapter 5. Conclusion and future work of this thesis are presented in Chapter 6.

Chapter 2: Literature Review

The two common forms of SPION are Magnetite (Fe_3O_4) and Maghemite (γ-Fe_2O_3). However, ferrites materials which are mixed oxides of iron and other transition metal ions such as Cu, Co, Mn, and Ni have also been reported to be superparamagnetic (Mahmoudi, Sant, Wang, Laurent, & Sen, 2011). Nevertheless, the scope of this thesis focused only on the superparamagnetic properties of iron oxide nanoparticles and its surface modification with silica nanoparticles.

2.1 Magnetite

Magnetite (Fe_3O_4) is a ferromagnetic material and black in colour. It is a member of spinel group with general formulation $A^{2+}B^{3+}{}_2O^{2-}{}_4$. The oxide anions are arranged in a cubic close-packed lattice while the cations A and B occupied some or all the octahedral and tetrahedral sites in the lattice, respectively (Spiers & Cashion, 2012). Magnetite ($Fe^{2+}Fe^{3+}{}_2O^{2-}{}_4$), sublattices A and B contains Fe^{2+} and Fe^{3+}, respectively at the ratio of 1 to 2. Magnetite unit cell of can be represented as: $[Fe_8^{3+}](Fe_8^{2+}\,Fe_8^{3+})O_{32}^{2-}$.

2.2 Maghemite

Maghemite, γ-Fe2O3, has a spinel structure, and it is closely related to magnetite. Unlike magnetite, maghemite has a vacancy of Fe^{2+} in its structure. It contains only Fe^{3+} O^{2-}. The unit cell of Maghemite can be represented as: $[Fe_8^{3+}]\left(Fe_{40/3}^{3+}\,\text{Ꞙ}_{\frac{8}{3}}\right)O_{32}^{2-}$, where Ꞙ represents vacancy of the Fe^{2+} in maghemite structure and is accepted to be in the octahedral sites (Spiers & Cashion, 2012).

2.3 Magnetic property of SPION

Magnetic moments of a material originated from the orbital and spin motion of the electron. In every atom, orbital and spin moments of electron pairs cancel each other. Therefore, the net magnetic moment for an atom is the sum of the magnetic moments of each of the electron's component. Based on the response to external magnetic field, materials can be classified into three major groups: diamagnetism, paramagnetism and ferromagnetism. Antiferromagnetism and ferrimagnetism are subclasses of ferromagnetism. However, nanoparticles of ferromagnetic and ferrimagnetic materials display superparamagnetic behaviour. Superparamagnetism occurs in nanoparticles with single domain (region whereby the magnetic fields of atoms are grouped together and aligned). Importantly, SPION displayed magnetic anisotropy (show preference to the direction along which their magnetization aligned). Therefore, SPION can randomly flip to the direction of their magnetization.

In the presence of external magnetic field the total magnetic moment of SPION aligned parallel to the field, like a single giant magnetic moment (Kodama, 1999). Due to the magnetic anisotropy of the superparamagnetic nanoparticles they randomly flip to the direction of their magnetization. The average time of flip is termed Relaxation Time (τ) and is given by the Neel Brown's expression (Lu et al., 2007) as equation 2.1:

$$\tau = \tau_o \exp\left(\frac{\mathrm{K}_{eff} V}{\mathrm{K}_\mathrm{B} \mathrm{T}}\right) \tag{2.1}$$

τ_o is the initial time of flip, K_{eff} represents the anisotropy constant, and V is the particle volume. K_B is the Boltzmann constant and T is the temperature. However, a superparamagnetic state is observed when the system flips at a time shorter than the measured experimental time. Otherwise, a blocked state is observed when the magnetic moment flips at a time greater than the experimental time. The behaviour of all classes of magnetic materials can be summarized with the plot of M against H. As shown in Figure 2.1.

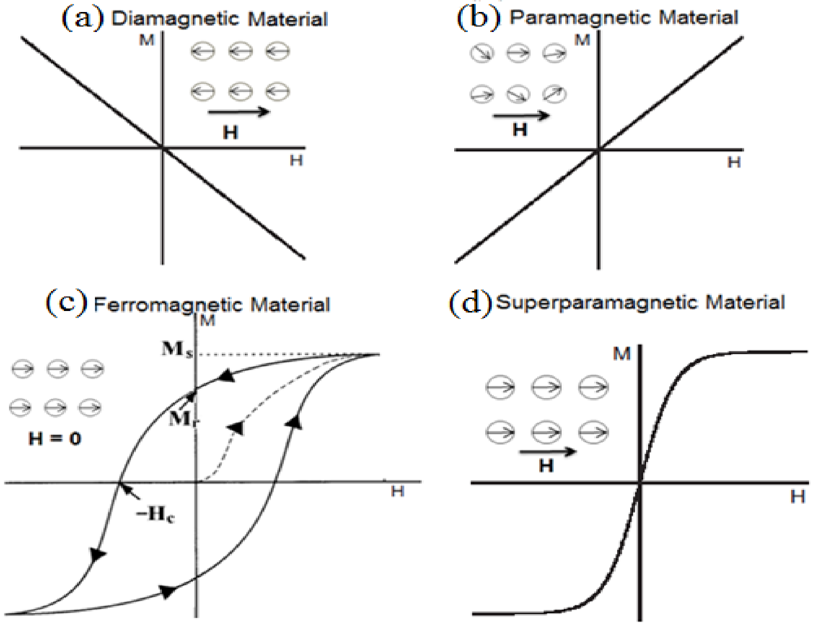

Figure 2.1: Schematic illustration of M-H curves (a) Diamagnetic material, where magnetic moment (M) decreases with increase in external magnetic field (H). (b) Paramagnetic material, M increases with increase in H. (C) Ferromagnetic material, hysteresis loop is observed with remanance (Mr) and coercivity (Mc). (d) Superparamagnetic material which shows similar sigmoid shape with ferromagnetic substance but with the absence of hysteresis loop, remanance and coercivity (Sung & Rudowicz, 2002).

2.4 Synthesis of SPION

Recently, due to superparamagnetic properties and various applications of SPION, extensive research has been focused on preparation and on understanding the magnetic behaviour of SPION. Several methods of synthesizing SPION have been reported. These various synthetic routes can be classified into three: (1) physical (2) chemical and (3) biological methods (Mahmoudi et al., 2011). However, the chemical method is the most adopted method of producing SPION. It involves condensation of atoms or molecular entities of the material components in solution (wet chemical method) or gas phase such as thermal decomposition method. In this thesis SPION is synthesized by a wet chemical method. The wet chemical method of synthesizing SPION includes: (I) co-precipitation, (II) sol-gel, (III) microemulsion, (IV) hydrothermal, (V) solvothermal, (VI) sonochemical and (VII) electrochemical method.

I. Co-precipitation Method

This method of SPION synthesis is one of the most widely used methods. It involves precipitation of SPION by addition of a strong base to a solution of Fe^{3+} and Fe^{2+} salts at ratio of 2 to 1 in water under vigorous stirring (Kang, Risbud, Rabolt, & Stroeve, 1996; Massart, 1981). Several protocols have reported use of strong inorganic and organic base such as NaOH, KOH or NH_4OH and 1,6-hexanediamine, respectively for effective and rapid precipitation of SPION (Chatterjee, Haik, & Chen, 2003; Hong, Li, Wang, & Li, 2007; Iida, Takayanagi, Nakanishi, & Osaka, 2007; Jolivet, Belleville, Tronc, & Livage, 1992). However, when a weak base such sodium carbonate is used as precipitating agent, the reaction proceed sufficiently at a very slow rate (Blanco-Andujar, Ortega, Pankhurst, & Thanh, 2012). Basically, the synthesis of magnetite nanoparticles via this route is carried out in inert gas such as nitrogen environment to prevent oxidation of Fe^{2+}. Nevertheless, synthesis of SPION in air environment where the ratio of Fe^{2+} to Fe^{3+} is less than 2 to 1 has also been reported (Karaagac, Kockar, Beyaz, & Tanrisever, 2010; Maity & Agrawal, 2007). SPION with various size range of 1.5-50 nm can be synthesized by controlling the reaction conditions such as temperature, pH, stirring speed, aging time, and ionic strength of the precipitation medium (Blanco-Andujar et al., 2012; Lee, Jeong, Shin, Kim, & Kim, 2004; Martínez-Mera, Espinosa-Pesqueira, Pérez-Hernández, & Arenas-Alatorre, 2007; Vayssieres, Chanéac, Tronc, & Jolivet, 1998; Zhu & Wu, 1999).

The saturation magnetization of the SPION produced via precipitation decreases with decrease in size of the particles (Gnanaprakash, Philip, Jayakumar, & Raj, 2007). In addition, Goethite (α FeO(OH)) is a common impurity (non-magnetic Fe compound) noticed in SPION synthesized via co-precipitation technique. Their presence can be related to the use of strong alkaline media such as NaOH, KOH and LiOH as hydrolyzing agent and virtually absent when ammonia is used as the precipitating agent (Gribanov, Bibik, Buzunov, & Naumov, 1990). Consequently, the presence of this impurity contributes to a variation drop in the saturation magnetization of the SPION (Gnanaprakash, Mahadevan, et al., 2007). As such, aggregation of the particles is normally observed using this method. Nonetheless, the agglomeration can be controlled via the use of surfactant or surface modification of the SPION. For a complete precipitation of iron oxide, the reaction is normally carried out as demonstrated in equation 2.2.

$$\mathrm{Fe}^{2+} + 2\mathrm{Fe}^{3+} + 8\mathrm{OH}^{-} \rightarrow \mathrm{Fe}_3\mathrm{O}_4 + 4H_2O. \qquad (2.2)$$

Magnetite is highly sensitive to oxygen; it can oxidize and form maghemite, γ-Fe_2O_3 according to equation 2.3:

$$Fe_3O_4 + 2H^+ \rightarrow \gamma Fe_2O_3 + Fe^{2+} + H_2O \qquad (2.3)$$

II. Sol-Gel Method

This is also known as chemical solution deposition method. Sol-gel process is defined to be the conversion of a precursor solution into an inorganic solid by chemical means (Niederberger, 2007). Sol-gel synthesis of SPION occurs in several steps such as: (1) hydrolysis and polycondensation of iron precursor in a solvent to form a colloidal suspension of the particles (sol), (2) gelation of the sol to form gel, (3) aging and (4) drying of the particles through a process called sintering. Several ironic precursors such as iron alkoxide, iron nitrate, iron chloride and metallorganic compounds have been reportedly used to synthesized SPION via sol-gel method (Gun'ko, Pillai, & McInerney, 2001; Kimata, Nakagawa, & Hasegawa, 2003; Qi, Yan, & Lu, 2014; Qi, Yan, Lu, Li, & Yang, 2011). However, based on the solvent used in this route of synthesizing SPION and other metallic oxide nanoparticles, sol-gel can be classified into aqueous and non-aqueous (Akbar, Hasanain, Azmat, & Nadeem, 2004; Bagheri, Chandrappa, & Hamid, 2013; Pinna et al., 2005; X. Zhang, Ren, & Cui, 2011). The former involves the use of water solvent while in the latter, organic solvent is used. The size and saturation magnetization of SPION synthesized by this method increases with increase in the annealing temperature (J. Xu et al., 2007). However, SPION synthesize via wet chemistry are often faced with agglomeration during the washing process. To minimise this effect, Dong et al., used ethylene oxide (EO) with iron precursor as starting materials to prevent agglomeration during washing of the SPION produced by sol-gel process (Dong & Zhu, 2002).

III. Microemulsion Method

Microemulsion is thermodynamically stable systems that consist of two immiscible liquids, usually water and oil plus a surfactant. The surfactant is used to stabilize the droplets of water-in-oil (W/O) or oil-in-water (O/W) when small amounts of water or oil are used, respectively. The microemulsion method is classified into four stages in the work of López-Quintela et al., (2003) as: (1) microemulsion mixing, (2) interchange of reactant among the nanodroplets, (3) reaction nucleation and (4) reaction growth. The microemulsion method of synthesising SPION involves preparation of iron oxide dispersion (microemulsion) and dispersion of a reducing agent. More so, the work of Okoli et al., has shown that SPION can be prepared through either W/O or O/W emulsion process (Okoli et al., 2012). The emulsions are stabilized with a surfactant. Subsequently, the two dispersions are mixed together for the interchange and intermicellar exchange of the reactants. The mixing process can be done in two ways: (1) mixing two microemusions containing the reactants or (2) adding one of the reactants to microemulsion containing the other reactant. The morphology and magnetic property of SPION produced via this route depend mainly on the emulsion (Chin & Yaacob, 2007).

IV. Hydrothermal Method

Hydrothermal method is an environmental friendly route of producing SPION. It is a rapid and simple method which involves heats up of aqueous acidified iron salt precursor solution to a supercritical state of water with temperature and pressure of 400 ^{o}C and 35 MPa, respectively. The particles are synthesized in an apparatus consisting of steel pressure vessel (autoclave). Basically, the solubility of the precipitating cations plays a critical role in attaining the correct product (Cote, Teja, Wilkinson, & Zhang, 2002). The supercritical water (SCW) serves as the reaction medium. In addition to the SCW, a reducing agent is required. Several materials like glycerol, ammonia have been used as reducing agent (Kim et al., 2014; J. Li et al., 2014). The mixture of water and surfactants works as a reaction medium for both hydrothermal synthesis and surface modification (Takami et al., 2007). The presence of surfactant during synthesis hinders particle aggregation, and thereby results in uniform particles and narrow particle size distributions (Xu & Teja, 2008). The magnetic property, morphology and sizes of the SPION formed via this route, is influenced by the concentration of the iron precursor and the composition of the solvent. The higher initial precursor concentration led to smaller particles size. This is due to the formation of a large number of seed nuclei, which provide high particle concentration and yield smaller particles (Ge et al., 2009). However, one of the major limitations of this hydrothermal method is the slow reaction kinetics. Therefore, the use of microwaves for heating during the synthesis of SPION which increase the kinetics of crystallization has been reported (Sreeja & Joy, 2007).

V. Solvothermal Method

Unlike, hydrothermal method, this involves uses of solvent other than water to synthesize iron oxide nanoparticles at moderate to high pressure and temperature of 1-10,000 atm and 100-1000 °C, respectively. Solvents such as methanol, ethylenediamine and hydrazine solution have been reportedly used for the synthesis of SPION (Liu & Kim, 2009; J. Lu, Jiao, Chen, & Li, 2009; Veriansyah, Kim, Min, & Kim, 2010). The complex formation between the surfactant molecules and ferrites leads to the uniformity of crystallite size and morphology of the particles formed by this route (Hou, Yu, & Gao, 2003). Therefore, nucleation, growth and size distribution of the nanoparticles via this method depends on the dispersion of the precursor in suitable solvents, solvothermal temperature and aging time (Si et al., 2005; Thimmaiah et al., 2001).

VI. Sonochemical Method

This is a facile method of synthesising nanostructures. It involves decomposition of organometallic or inorganic iron precursor in double deoxygenated water via ultrasonic irradiation. The irradiation produces acoustic cavitation process (formation, growth and collapse of bubbles). The process generates huge temperature and pressure plus enormous cooling rates of 5000 K, 2000 atm and 10^{10} K/s (Suslick, 1994), respectively. However, for biological application and to synthesize a well dispersed SPION, the ultrasonic irradiation of the ironic solution is carried out in the presence of a suitable stabilizer (Abu Mukh-Qasem & Gedanken, 2005; Shafi et al., 2001). The results of a sonochemical synthesis involving inorganic or organometallic solute are always amorphous nanoparticles (Abu Mukh-Qasem & Gedanken, 2005; Cao et al., 1997). The unique conditions generated from the acoustic cavitation process prevent the crystallization of the nanomaterial during particle formation. Therefore, heat-treatment (sintering) after the synthesis is required to obtain crystalline iron oxide nanoparticles (Hassanjani-Roshan, Vaezi, Shokuhfar, & Rajabali, 2011). However, few works have demonstrated that crystalline SPION can be synthesized at low temperature (Vijayakumar, Koltypin, Felner, & Gedanken, 2000; Zhang et al., 2007). More so, the use of ultrasonic irradiation in the synthesis of SPION has been reported to improve the mono-dispersibility and hydrophilic properties of the magnetic particles (Dang, Enomoto, Hojo, & Enpuku, 2009; Marchegiani et al., 2012). The morphology and size distribution of the particles synthesized via the sonochemical method depends on the ultrasonic irradiation parameters such as temperature and intensity of the ultrasound (Hassanjani-Roshan et al., 2011).

VII. Electrochemical Method

Electrochemical method is a reduction and oxidation reaction with an iron-based electrode in an electrolyte. The anode can be oxidized to metal ion species in the electrolyte and the metal ion is further reduced to metal at the cathode with the assistance of stabilizers. The SPION formed is normally deposited on the electrode in the form of a coating or a thin film (Ramimoghadam, Bagheri, & Hamid, 2014). Several electrolytes such as LiCl solution in de-aerated mixture of water, ethanol and Me_4NCl have been reported (Cabrera, Gutierrez, Menendez, Morales, & Herrasti, 2008; Starowicz et al., 2011). However, amine or other surfactant can be used as supporting electrolytes to produce functionalized and stabilized SPION. The particle size can be controlled by adjusting the imposed current density (Pascal, Pascal, Favier, Elidrissi

Moubtassim, & Payen, 1999). More so, progress related to electrochemical synthesis of SPION has recently been reported (Ramimoghadam et al., 2014).

2.5 Surface Modification of SPION with Silica Nanoparticles

Several, strategies have been reported to modify surface of SPION with silica nanoparticles. Based on their approach, the various methods can be categorized into wet phase and gas phase or flame method. However, the scope of this work only covers wet chemical methods.

2.5.1 Wet Chemical Synthesis of Silica Coated SPION

This involves the synthesis of SPION/silica nanoparticles in liquid phase. Based on the various wet chemical approaches used to produce silica coated SPION, this methods can be classified as seed growth mediated approach. It involves the initial synthesis of SPION and subsequently the SPION is used as a seed or template for the growth of silica coating. From literature the various wet chemical methods used to synthesize SPION/silica nanoparticles are as follows: (1) sol-gel method and (2) microemulsion or reverse micelles method (3) modification of sol-gel and microemulsion method and (4) magnetic mesoporous method.

I. Sol-Gel Synthesis of Silica Coated SPION

This is one of the most adopted methods of coating SPION with silica. This could be due to relatively mild reaction condition, low cost and surfactant-free (Reddy, Arias, Nicolas, & Couvreur, 2012). One of the most cited sol-gel route is known as Stöber method (Barnakov, Yu, & Rosenzweig, 2005). However, other sol-gel methods have been reported by Philipse et al. (Philipse, Van Bruggen, & Pathmamanoharan, 1994, Y. Lu, Yin, Mayers, & Xia, 2002, Andrade, Souza, Pereira, Fabris, & Domingues, 2009 and Im et al., 2005).

The Stöber method involves the hydrolysis and condensation of an alkoxysilane in an alcoholic solution while ammonia is used as a catalyst in the presence of SPION as seed (template). This procedure is normally carried out under mechanical stirring. However, the use of ultrasonic irradiation has also been reported (Dang, Enomoto, Hojo, & Enpuku, 2010; Morel et al., 2008). The ultrasound improves the dispersibility, the rate of hydrolysis and condensation reaction, and prevents agglomeration of the core SPION during the coating procedure (Dang et al., 2010).The size of silica shell generated by this process can be controlled by varying the amount of silica precursor (Deng, Wang, Hu, Yang, & Fu, 2005). Nevertheless, the silica shell is not thin enough. Therefore, drastic reduction in the saturation magnetization (M_s) of the core SPION is recorded. More so, due to direct coating of the silica on the SPION and modality of this procedure (seed mediated), the core SPION might have experienced surface or spin disorder (Yuan et al., 2012).

II. Microemulsion Synthesis of Silica Coated SPION

This involves the preparation of dispersion or emulsion of SPION in water or oil and then the addition of silica precursor under continuous stirring. The core SPION is often disperse in water, oil, and surfactant. The surfactant molecule is used to lower the interfacial tension between water and oil resulting in the formation of a transparent solution (Santra et al., 2001).The initial dispersion or emulsion of SPION in cyclohexane with Triton-X100 or Igepal CO-520, hexanol, water, and $NH_3.H_2O$ have been reported

(Carmen Vogt et al., 2010; Zhang, Cushing, & O’Connor, 2008). Subsequently, silica shell is coated on the SPION via interaction of the SPION’s emulsion and silica source. The thickness of the silica can be controlled by regulating the ratio of water to silica precursor (Caruana et al., 2012). Similar to sol-gel process, this approach also led to drastic reduction in the saturation magnetization of the core SPION.

III. Modification of Sol-Gel and Microemulsion Method

Recently, modifications of the seed growth mediated approach have been reported to produce thin silica shell and highly magnetic silica coated SPION. In other to preserve the spin order, control the thickness of the silica shell and retain the high M_s Property of the core SPION barriers such as polymer and ligand are introduced in-between the shell and core (Arizaga, Millán, Schubert, & Palacio, 2013; Wang, Guo, Li, Sun, & He, 2008). Therefore, the barrier enhanced the production of ultra-thin and highly magnetic silica coated SPION nanoparticles (Kralj, Makovec, Čampelj, & Drofenik, 2010; H. Xu, Cui, Tong, & Gu, 2006). However, this procedure led to high increase in the final size of the composite nanoparticles (He et al., 2009). Consequently, it can limit the in vivo applications of the magnetic core-shell nanoparticles.

IV. Magnetic Mesoporous Silica Nanoparticles

This process involves the coating of SPION with mesoporous MCM-41 silica nanoparticles. This procedure involves different routes such as: the deposition of an iron precursor or nanoparticles within the mesopores of MCM-41 followed by other reduction reaction such solvothermal conversion, aerosol-assisted route, sol-gel that produces the encapsulated nanoparticles (Arruebo et al., 2008; Y. Deng, Qi, Deng, Zhang, & Zhao, 2007; J. Kim et al., 2005). Alternatively, core/shell magnetic mesoporous silica nanoparticles can be synthesized via co-condensation of magnetite nanoparticles with silica precursors in basic aqueous solution of surfactant and pore swelling agent like CTAB and mesithylene, respectively (Knežević, 2014). Similarly, the use of 1,3,5-triisopropylbenzene (TMB)/decane has also been used as a pore swelling agents (Zhang et al., 2011). The CTAB serves as both surfactant which stabilized the SPION and also as organic template for the formation of the mesoporous silica nanoparticles (Kim et al., 2008). Consequently, via calcined process at high temperature the CTAB is removed from the composite nanoparticles. Detail on the synthesis and biomedical application of magnetic mesoporous silica-based core/shell nanoparticles has been reported (Knezevic, Ruiz-Hernandez, Hennink, & Vallet-Regi, 2013). However, due to large thickness of the mesoporous silica shell, there is drastic reduction in the saturation magnetization of the composites nanoparticles developed via this routes magnetization.

2.6 Summary

Research on synthesis of SPION is of intense interest due to its superparamagnetic properties. However, its major drawback is agglomeration. The use of silica to coat surface of SPION has not only prevented agglomeration but also enhanced the applications of the magnetic nanoparticles. Although, several wet chemical routes based on seed mediated growth approach have been employed to synthesize silica coated SPION, preservation of saturation magnetization of the core SPION is still a big challenge.

Chapter 3: Theory

3.1 Theory of Sonochemistry

Sonochemistry is the use of ultrasonic field (sound with a frequency above human hearing threshold) in chemical reactions and processes. As illustrated in Figure 3.1, ultrasonic field (~20 kHz – 10 MHz) can be classified into three main regions: (1) ultrasonic frequency from 20 kHz – 100 kH are ultrasound with low frequency and high power, (2) 100 kHz – 1 MHz are ultrasound with high frequency and medium power, and (3) frequency between 1 – 10 MHz are ultrasound with high frequency and low power. However, only sound wave in the range of 20 kHz to 1 MHz is used in sonochemistry. Ultrasonic frequencies above 1 MHz are normally used for medical purposes (diagnostic ultrasound).

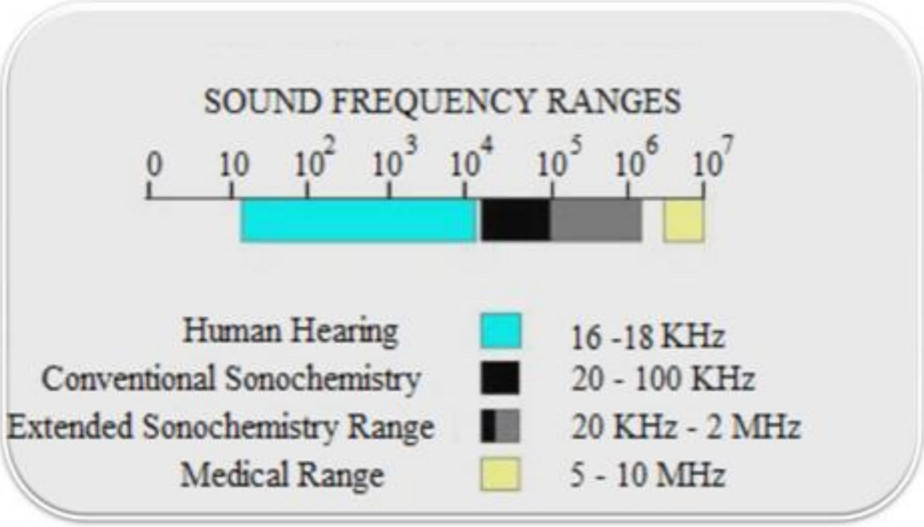

Figure 3.1: Illustrating sound frequency ranges and their importance.

The fundamental of sonochemistry is known as Acoustic Cavitation Process. The term “cavitation” is from a Latin word “cavus”, which means hollow. Cavitation is the formation of cavities (compressed gasses) inside a liquid, as a result of abrupt decrease in the cohesive or intramolecular forces between the liquid, due to rapid pressure drop (Stricker, 2013).

Ultrasonic irradiation of liquid generates oscillatory motion in the molecules which are transmitted through the liquid via pressure waves. Therefore, it induces rarefaction and compression waves to the molecular structure of the liquid. This causes the liquid molecules to vibrate around their position. Increased in the ultrasonic intensity within the liquid can cause increase in the distances between the molecules and lost of intramolecular forces within the molecular structures. The liquid molecules break down and cavity known as Cavitation Bubble is formed. These bubbles respond to the ultrasonic field within the liquid via expansion and contraction, and finally collapse. This process of formation, growth and collapse of bubbles are known as Acoustic Cavitation.

The cavitation can be classified as Stable and Transient. In the stable cavitation, a bubble can vibrate for many refraction and compression cycles before collapse. While in the transient cavitation, bubbles grow double its size in one complete acoustic cycle and eventually collapse. As illustrated in Figure 3.2, the collapse of bubbles are often violent with the generation of hot spot of temperature, pressure and cooling rate of ~5000 K, 1000 atmosphere and 10^{10} Ks^{-1}, respectively (Suslick & Price, 1999). However, transient cavitation is more energetic than the stable cavitation (Flynn, 1964). The dynamics of