SYNTHESIS, PROPERTIES AND CYTOTOXICITY OF
NANOSTRUCTURED NICKEL FERRITE PARTICLES

YIN HONG
(B. Eng., BUCT)
(M. Sc., NUS)

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Summary

Motivated by using nickel ferrite magnetic nanoparticles as potential drug carrier, this thesis studied their synthesis and characteristics, their composites with biodegradable polymer, and their cytotoxicity.

Using mechanochemical method, nickel ferrite particles without oleic acid coating were obtained. Using nonhydrolytic sol-gel method, by applying surfactants, as-prepared nickel ferrite particles were successfully coated with oleic acid. By varying the composition of metal precursors, two microstructures could be achieved, i.e individual nanocrystals and aggregates of many nanocrystals. Because oleic acid could complex with iron (III) ions, but not with nickel (II) ions, the variation of precursor composition contributed to the difference in microstructures. Increasing the concentration of iron precursor thus consumed more oleic acid and led to insufficient oleic acid coating on particle surface. Strong inter-crystallite interactions were induced from less protected surface and were possibly the driving force of aggregation.

Nickel ferrite nanoparticles with or without oleic acid surface coating formed composite with poly (D, L-lactide) (PLA). If the nanoparticles were prepared without any coating, they attached on PLA surface. For the nanoparticles coated with oleic acid, they were encapsulated by PLA microspheres. No detectable new chemical bond was found between nanoparticles and PLA. A slight decrease in $T_g$ could be related to the difference in interfacial energies between the two components. The optimally mixed composite was achieved by reducing the interfacial energy. However, the loading capacity was limited in this composite. Increasing the amount of nickel ferrite nanoparticles was not useful to increase the loading capacity.
The cytotoxicity of nickel ferrite nanoparticles significantly depended on the surface coating and surface characteristics, which in turn depended on the type of synthesis and processing used. When 1 layer or 2 layers of oleic acid were coated on the surface, larger particles were more cytotoxic than smaller ones. The size effect could be related to the surface energies and surface interaction areas that were size-dependent. The cytotoxicity of oleic acid varied dramatically among randomly distributed oleic acid monomers, their organized assemblies (micelles) and coating of oleic acid on nickel ferrite particles. Oleic acid monomers were not cytotoxic. However, if they developed micelles or coated on the ferrite particles, i.e. when their functional groups were spatially aligned, cytotoxicity was observed.
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<tr>
<td>CMC</td>
<td>Critical Micelle Concentration</td>
</tr>
<tr>
<td>DCM</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>DMEM</td>
<td>Dulbecco's Modification of Eagle's Medium</td>
</tr>
<tr>
<td>DPE</td>
<td>Diphenyl Ether</td>
</tr>
<tr>
<td>DSC</td>
<td>Differential Scanning Calorimetry</td>
</tr>
<tr>
<td>EDX</td>
<td>Energy Dispersive X-ray Spectroscopy</td>
</tr>
<tr>
<td>FBS</td>
<td>Fetal Bovine Serum</td>
</tr>
<tr>
<td>FC</td>
<td>Field Cooling</td>
</tr>
<tr>
<td>FTIR</td>
<td>Fourier Transition Infrared Spectrometer</td>
</tr>
<tr>
<td>HRTEM</td>
<td>High Resolution Transmission Electron Microscopy</td>
</tr>
<tr>
<td>IEP</td>
<td>Isoelectric Point</td>
</tr>
<tr>
<td>MTT</td>
<td>3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide</td>
</tr>
<tr>
<td>PLA</td>
<td>Poly (Lactic Acid)</td>
</tr>
<tr>
<td>PVA</td>
<td>Poly(Vinyl Alcohol)</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning Electron Microscopy</td>
</tr>
<tr>
<td>SQUID</td>
<td>Superconducting Quantum Interference Device</td>
</tr>
<tr>
<td>TEM</td>
<td>Transmission Electron Microscopy</td>
</tr>
<tr>
<td>TGA</td>
<td>Thermogravimetry Analysis</td>
</tr>
<tr>
<td>TOP</td>
<td>Trioctylphosphine</td>
</tr>
<tr>
<td>XPS</td>
<td>X-ray Photoelectron Spectroscopy</td>
</tr>
<tr>
<td>XRD</td>
<td>X-ray Diffraction</td>
</tr>
<tr>
<td>VSM</td>
<td>Vibrating Sample Magnetometer</td>
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<td>ZFC</td>
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Chapter 1 Introduction

Nanoparticles exhibit different behaviors from bulk materials because a significant number of atoms are located at the surface or interface. The surface effect may dominate material properties. In magnetic nanoparticles, this difference becomes more obvious because the normal macroscopic domain structure transforms into a single domain state when grain sizes are reduced to nanoscale. Unique magnetic properties, such as giant magnetoresistance\(^1\), quantum tunneling of magnetization\(^2\) and superparamagnetism\(^3\), can be found in many types of magnetic nanoparticles. Associated with the above scientific uniqueness, many applications have been developed, for example, high density magnetic recording media\(^4\), ferrofluid\(^5\), catalysis\(^6\) and biomedical applications\(^7\) etc.

1.1 Magnetic nanoparticles for bio-applications

Recently bio-applications of magnetic nanoparticles have been intensified because of their properties. First, they have controllable sizes ranging from a few nanometers up to tens of nanometers, which places them at dimensions that are smaller than or comparable to those of a cell (10–100 μm), a virus (20–450 nm), a protein (5–50 nm) or a gene (2 nm wide and 10–100 nm long)\(^8\). With such dimensions, nanoparticles can easily approach the above bio-molecules with little spatial obstacles. Second, if the nanoparticles are magnetic, they can be manipulated by an external magnetic field gradient. This ‘action at a distance’, combined with the intrinsic penetrability of magnetic fields into human tissue, opens up many applications involving transport and/or
immobilization of magnetic nanoparticles, or of magnetically tagged biological entities. Applications also include delivery of packages such as anticancer drugs, or a cohort of radionuclide atoms, to a targeted region of the body. Third, magnetic nanoparticles can resonantly respond to a time-varying magnetic field, transferring energy from the exciting field to the nanoparticles. In this way, the nanoparticles can be heated up, which lead to applications such as hyperthermia agents or as chemotherapy and radiotherapy enhancement agents. A moderate degree of tissue warming results in more effective malignant cell destruction. To fully exploit these attractive possibilities, a clearer understanding of the physical phenomena of magnetic nanoparticles is essential.

### 1.1.1 Basic concepts

If a material is placed in an external magnetic field of strength $H$, the magnetic moment of individual atom in the material contribute to its overall response. At room temperature the magnetization $M$ induced by $H$ can be characterized as $M = \chi H$, where $\chi$ is the magnetic susceptibility of the material.

Most materials display little magnetism and can be classified as diamagnets or paramagnets. Diamagnets without any atomic magnetic moment have no magnetization in zero fields. When a field is applied, a small and negative moment is induced on the diamagnetic atoms proportional to the applied field strength. The typical $M-H$ curve of a diamagnet is shown in Fig.1-1. In paramagnets, the atoms have a net magnetic moment which is oriented randomly throughout the material due to thermal agitation, giving no magnetization in a zero field. As a field is applied the moments tend towards alignment.
along the field, giving a net magnetization which increases with applied field as the moments become more ordered\textsuperscript{11}. Its $M-H$ curve is also shown in Fig. 1-1.

![M-H curves of diamagnetic and paramagnetic materials.](image)

Some materials exhibit ordered magnetic states and are magnetic even without an applied field. They are classified as ferromagnets, ferrimagnets or antiferromagnets, where the prefix refers to the nature of coupling interaction between the electrons within the material\textsuperscript{12}. This coupling can give rise to spontaneous magnetizations. Due to the relatively small susceptibility, antiferromagnets are less favorable than ferromagnets or ferrimagnets in bio-applications. The atomic magnetic moments in a ferromagnetic substance interact strongly with one another and tend to align themselves parallel to each other, as shown in Fig. 1-2(a). A nearly perfect alignment of the spins is expected in spite of the thermal agitation at room temperature. The most common ferromagnets are metallic iron, cobalt and nickel. Ferrimagnetism describes the magnetism of ferrites. In these substances, magnetic ions occupy two kinds of lattice sites, $A$ and $B$. Spins on $A$ sites align antiparallel to those on $B$ sites because of the strong negative interaction.
between the two spin systems. The number of magnetic ions and the magnitude of spins of individual ion are different on \( A \) and \( B \) sites. An ordered arrangement of spins gives rise to a resultant magnetization, as shown in Fig. 1-2(b). Ferrimagnetic materials show magnetization without the action of any external magnetic field. With an increase of temperature, the arrangement of the spins is disturbed by thermal agitation, which is accompanied by a decrease of spontaneous magnetization. At a certain temperature, called the Curie point \( (T_c) \), the arrangement of the spins becomes completely random, and the spontaneous magnetization vanishes\(^{13}\).

![Diagram of spin arrangements](image)

Figure 1-2 Arrangements of spins of (a) ferromagnetic and (b) ferrimagnetic materials.

When an external magnetic field is applied to the biological circulatory system, the magnetic response of the blood vessel will include a paramagnetic response (for example, from the iron-containing haemoglobin molecules) and a diamagnetic response (for example, from those intra-vessel proteins that comprise only carbon, hydrogen, nitrogen and oxygen atoms). If ferromagnetic or ferrimagnetic nanoparticles are injected into the same circulatory system, the magnetic signals from the injected nanoparticles far exceed those from the blood vessel itself\(^8\).
For ferromagnetic or ferrimagnetic materials, irreversibility in the magnetization process is observed. This gives rise to an open $M-H$ curve, called hysteresis loop. Figure 1-3 shows a typical hysteresis loop. Various parameters such as saturation magnetization, coercivity and remanence can be determined from it. Saturation magnetization is the maximum magnetization of the material where further increase of $H$ cannot produce further enhancement of $M$. When a ferromagnetic or ferrimagnetic material is magnetized in one direction, it will not relax back to zero magnetization when the imposed magnetizing field is removed. The amount of magnetization it retains at zero applied field is called its remanence. Coercivity is the amplitude of reverse field required to reduce the magnetization to zero. In addition, the area enclosed by the hysteresis loop is characterized as the energy delivered by the applied field. The physical origin of hysteresis loop is related to the pinning of magnetic domain walls at impurities or grain boundaries. It also can be caused by the intrinsic effects such as magnetic anisotropy of the crystalline lattice\textsuperscript{12}. 
Figure 1-3 Typical hysteresis loop of ferromagnetic or ferrimagnetic materials.

As shown in Fig. 1-4, depending on the particle size, ferromagnetic and ferrimagnetic materials exhibit either multi-domain, single-domain or superparamagnetic hysteresis loop. For large particles in the order of micron size or more, there is a multi-domain ground state. It leads to a narrow hysteresis loop since relatively little field energy is required to move the domain walls. While in smaller particles there is a single domain ground state which leads to a broad hysteresis loop involving more energy. At even smaller sizes in the order of tens of nanometers or less, superparamagnetism is observed. Superparamagnetism refers to materials which become magnetic in the presence of an external magnet, but revert to a non magnetic state when the external magnet is removed. It occurs in small length scale, where the energy required to change the direction of the magnetic moment of a particle is comparable to the ambient thermal energy. The magnetic moment of the particle as a whole is free to fluctuate in response to
thermal energy. In the hysteresis loop, zero remanence and coercivity are observed. Consequently, when the superparamagnetic particles are placed in an external magnetic field, their magnetic moments align rapidly in the direction of the field and the particles display a net magnetization. When the magnetic field is removed, thermal energy is again sufficient to cause individual moment to fluctuate randomly and magnetization disappears.

Figure 1-4 Hysteresis loops of particles with (a) multi-domain, (b) single-domain or (c) superparamagnetism, depending on the particle size.

1.1.2 Magnetic drug delivery

The major disadvantage of most chemotherapies is that they are relatively non-specific in their targets. The therapeutic drugs are administered intravenously leading to general systemic distribution, resulting in deleterious side-effects as the drug attacks normal, healthy cells in addition to the targeted cells. Recognition of this led researchers in the late 1970s to propose the use of magnetic particles to target specific sites (generally cancerous tumors) within the body\textsuperscript{14}. The objectives are two-fold: (i) to reduce the amount of systemic distribution of the cytotoxic drug, thus reducing the associated side-
effects; and (ii) to reduce the overall dosage required by more efficient, localized targeting of the drug.

In magnetically targeted therapy, a cytotoxic drug is attached to a magnetic carrier. These drug/carrier complexes are injected into a patient via the circulatory system. When the complexes have entered the bloodstream, external, high-gradient magnetic fields are used to concentrate them at a specific target site within the body, as shown in Fig. 1-5. Once the drug/carrier is concentrated at the target, the drug can be released either via enzymatic activity or changes in physiological conditions such as pH, osmolality, or temperature\textsuperscript{15}. This system, in theory, has major advantages over the normal, non-targeted chemotherapies. Since the first magnetic polymer carrier was introduced in the late 1970s, a variety of magnetic nanoparticle and microparticle carriers have been developed\textsuperscript{16}. The optimization of these carriers continues today\textsuperscript{17}.

Figure 1-5 A hypothetical magnetic drug delivery system. The magnetic field gradient can capture magnetic drug carrier/drug complexes in the circulatory system.
1.1.3 Nickel ferrite magnetic nanoparticles

Because of the generally appropriate magnetic properties and biological compatibility, iron oxides magnetite (Fe$_3$O$_4$) and maghemite (γ-Fe$_2$O$_3$) are most commonly studied in vitro$^{18}$. However, the ultimate objective of magnetic-field controlled delivery is in vivo implementation. In this case, the well-studied iron oxide becomes undesirable because their iron atoms are poorly distinguishable from those of hemoglobin. A conceivable solution is to use ferrites with another metal to lower the iron content$^{19}$.

Nickel ferrite is a good candidate for in vivo applications because the biodistributions of such nanoparticles can be easily determined by nickel titration of blood probes$^{20}$. Consequently, nickel ferrite nanoparticles have been considered for magnetic resonance imaging, magnetic extraction and targeted drug delivery. After surface functionalization, nickel ferrite nanoparticles could be specifically coupled to a medical agent and employed as in vivo drug carriers$^{21}$.

Applications in drug carrier impose strict requirements on the physical, chemical and pharmacological properties of magnetic nanoparticles. A number of publications studied the possibility of modifying the properties of iron oxides to suit such applications$^{22}$. Compared with iron oxide, nickel ferrite has not been well studied. However, the major concerns on iron oxides also apply for nickel ferrites. In summary, the following three concerns are crucial for nickel ferrite nanoparticles.

1. Certain physical properties, such as particle size and surface coating, are essentially required for the drug carrier application. Can they be controlled in a simple and efficient way during the synthesis of nickel ferrite nanoparticles?
2. What are the properties of drug carriers consisting of nickel ferrite nanoparticles and polymer?

3. What is the cytotoxicity of nickel ferrite nanoparticles?

1.2 Research work of this thesis

Motivated by using nickel ferrite magnetic nanoparticles as potential drug carrier, the research work in this thesis focused on studying the above three crucial concerns.

1.2.1 Synthesis and characterization of nickel ferrite nanoparticles

Application of nickel ferrite nanoparticles as drug carrier requires good control of particle size. First, superparamagnetic particles are desirable as magnetic drug carriers because they do not retain any magnetism after removal of external magnetic field. It is well known that superparamagnetism only occurs below a critical particle size which is \(~15\, \text{nm}\) for common magnetic materials. Blocking temperature, which is the transition temperature between the ferrimagnetic (or ferromagnetic) and superparamagnetic state, is directly proportional to the size of the particles\(^{23}\). Second, nanoparticles with uniform size have overall homogeneous physical and chemical properties, such as effective surface areas, sedimentation rate and reactivity. Third, control of particle size and distribution will ensure uniform physiological interaction between magnetic nanoparticles and biological entities. Thus better circular distribution and targeting efficiency can be achieved.

The preparation method and conditions will determine the size of obtained particles. Various preparation methods, such as chemical co-precipitation\(^{24}\), hydrothermal
synthesis, hydrolysis of metal carboxylate in organic solvent, and mechanical milling have been developed to fabricate nickel ferrite nanoparticles. However, these methods are unable to control the particle size efficiently and effectively.

By applying various surfactants, monodisperse and size-controlled magnetic nanoparticles could be synthesized. The dominant size-control factor was the molar ratio of precursors to surfactants. In addition to controlling particle size, surfactant coating can also increase the stability of nanoparticles. In the absence of surface coating, nickel ferrite nanoparticles have hydrophilic surfaces with a large surface area to mass ratio. Due to hydrophilic interactions between the particles, they agglomerate and form large clusters. These clusters can be attracted in a magnetic field, forming even larger aggregates. Such aggregation has to be prevented because large aggregates are usually sequestered by the spleen and removed by the phagocyte systems before they could be concentrated to the target.

Biocompatible surfactants, such as fatty acids are desirable in biomedical applications. They could be added at the time of preparation to prevent aggregation of magnetic nanoparticles. Not all fatty acid are useful in this aspect. It was reported that similar fatty acids (oleic acid and stearic acid) had different effects on stabilizing iron oxide nanoparticles. Only the oleic acid layers were solvated by the suspension medium and stabilized the nanoparticles. As a result, oleic acid was used as a popular surfactant in preparation of magnetic particles for bio-application.

It is challenging to find a simple synthesis method which can control particle size and introduce desirable surface coating (i.e. oleic acid) in the same process. Recently monodisperse maghemite nanoparticles with oleic acid surface coating were synthesized...
and reported\textsuperscript{34}. The main reaction of the synthesis was thermal decomposition of iron oleate produced by heating a mixture of iron pentacarbonyl and oleic acid. Using similar procedures, highly crystalline and monodisperse cobalt ferrite (CoFe\textsubscript{2}O\textsubscript{4}) nanocrystals were also synthesized\textsuperscript{35}. In this synthesis, three essential steps were involved. First, a mixed-metal–oleate complex was produced from the reaction of a single molecular precursor, (η\textsubscript{5}-C\textsubscript{5}H\textsubscript{5})CoFe\textsubscript{2}(CO)\textsubscript{9} and oleic acid. Second, the mixed-metal–oleate complex was thermally decomposed to produce crystalline iron–cobalt alloy nanoparticles. Third, the metallic nanoparticles were oxidized by a mild oxidant, trimethylamine N-oxide. Cobalt ferrite nanoparticles with particle size of 6 nm were synthesized when the molar ratio of metal precursor to oleic acid was 1:3. However, this method has not been applied to the synthesis of nickel ferrite nanoparticles. The difficulties may be described as follows. First, it is hard to find the single molecular precursor, i.e. nickel-iron mixed carbonyl. Second, the carbonyl precursor is toxic and not environmentally friendly. Third, the three-step reaction is complicated and difficult to control. More recently, aiming to control shape and particle size, nonhydrolytic sol-gel method has been used to synthesize TiO\textsubscript{2} \textsuperscript{36} and ZnO nanocrystals\textsuperscript{37}. Oleic acid was used as surfactant and was successfully introduced onto the surface of nanocrystals.

In nonhydrolytic sol-gel method, formation of an oxo-bridge is provided by the condensation reaction between two different functional groups bonded to two different metal centers (M\textsubscript{1} and M\textsubscript{2}), by eliminating a small organic molecule. As featured below, ester elimination and alkyl halide elimination are two basic condensation reactions\textsuperscript{38}.

Ester Elimination:

\[
\begin{align*}
\overset{\rightarrow}{M_1-O-C\overset{\rightarrow}{R}} + R-O-M_2^\overset{\rightarrow}{\textcircled{\text{O}}} \rightarrow M_1-O-M_2^\overset{\rightarrow}{\textcircled{\text{O}}} + R-O-C\overset{\rightarrow}{R'}
\end{align*}
\]
Alkyl Halide Elimination:

\[
\begin{align*}
&\text{M}_1\text{Cl} + R\text{O-M}_2 \rightarrow \text{M}_1\text{O-M}_2 + \text{RCI}
\end{align*}
\]

Nonhydrolytic sol-gel method is very suitable for the preparation of homogeneous bi-component oxide because condensation reactions can reduce the differences in reactivity of the two precursors. A number of mixed transition metal oxides with a high level of homogeneity have been prepared, such as SiO$_2$-TiO$_2^{39}$, ZrTiO$_4^{40}$ and Hf$_x$Zr$_{1-x}$O$_2^{41}$.

To our knowledge, nonhydrolytic sol-gel method has not been used in the synthesis of magnetic ferrite nanoparticles, which are an important group of transition metal oxides. Considering its advantages of preparing mixed oxide and providing proper surface coating, we applied this method to synthesize nickel ferrite nanoparticles with oleic acid coating. Two very different particle sizes (approximately 10 nm and 150 nm respectively) were obtained by changing the composition of metal precursors. Detailed characterization was carried out and the results are discussed in Chapter 3.

To facilitate the studies in Chapter 4 and Chapter 5, i.e. how does oleic acid surface coating affect the properties of composites and what is the cytotoxicity of nickel ferrite particles, bare particles without oleic acid coating were synthesized using a reported mechanochemical method$^{42}$. Various particle sizes were obtained by changing heat treatment temperature. It was known that the difference in particle size could result in different surface areas, which complicates the study of surface coating effect on the properties. For this purpose, the effects of particle size had to be separated from that of surface coating. We aimed to synthesize bare nickel ferrite particles with similar sizes as
what were obtained in the nonhydrolytic sol-gel method. The characterization of obtained particles was carried out and the results are also briefly presented in Chapter 3.

1.2.2 Composite of nickel ferrite nanoparticles and polymer

For in vivo applications, such as drug carrier, it is necessary for nickel ferrite nanoparticles to form composite with suitable polymers. The major functions of the polymer are (i) to improve biocompatibility of the nanoparticles and (ii) to allow effective binding of drugs. Magnetic nanoparticles/polymer composite may be advantageous if the polymer chosen is properly functionalized. For example, if iron oxide nanoparticles are encapsulated by a temperature-responsive polymer, N-isopropyl acrylamide (NIPAAM), magnetic-thermosensitive drug delivery can be achieved\textsuperscript{43}. If biodegradable polymer, such as poly (lactide-co-glycolide) or poly (D, L-lactide) is used, the composite may exhibit minimum toxicity and minimum immunological response. By controlling the degradation rate of the polymer, drugs embedded within the biodegradable polymer matrix could be released gradually\textsuperscript{44}.

A number of works have addressed the composites of magnetic nanoparticles and biodegradable polymers\textsuperscript{45}. Double emulsion method was most commonly used to prepare such composite although covalent grafting\textsuperscript{46} and in-situ polymerization\textsuperscript{47} were also reported. The obvious advantage of double emulsion method is that it could be generalized to various polymers or copolymers, such as poly (lactic acid) (PLA), poly (D, L-lactide-co-polyethylene glycol) (PLA-PEG) or poly (D,L-lactide-co-glycolide) (PLGA). The magnetic components in the reported systems were confined to iron oxides, i.e. magnetite, maghaemite or a mixture of them\textsuperscript{45}. The preliminary studies on the stability,
magnetic properties and formulation suggested that above iron oxide/biodegradable polymer composite could constitute a promising candidate for targeted drug carrier. To date nickel ferrite particles have not been studied as a magnetic component in such composites.

In Chapter 4, the possibilities of fabricating nickel ferrite particles/biodegradable polymer composites were explored. Particles with or without oleic acid coating were used to prepare composites by double emulsion method. Investigations of microstructures, surface charges, surface energies, interface energies and interactions between the two components were carried out to evaluate the effects of surface coating on the properties of the composites.

1.2.3 Cytotoxicity of nickel ferrite nanoparticles

With increasing applications of nanoparticles in biomedical fields, recent publications on their potential environmental and health impacts have been raised. It was demonstrated that surface properties could dramatically affect biocompatibility and cytotoxicity of studied particles. Some typical examples are given below. First, surface charge was found to be one of key features to influence hemolytic activity. Decreasing surface charge could prevent the electrostatic interaction between particles and positively charged lipids on the cell surface. The presence of poly-(ethylene oxide) (PEO) significantly mitigated the effect of surface charge. Second, cell cytotoxicity/adhesion studies of superparamagnetic iron oxides (SPION) on human dermal fibroblasts showed that the particles were toxic and their internalization resulted in disruption of cytoskeleton organization of cells. On the other hand, pullulan coated SPIONs (Pn-SPION) were non-
toxic and induced different changes in cytoskeleton organization. Results of transmission electron microscopy indicated that SPION and Pn-SPION were internalized into cells via different mechanisms, thereby suggesting that the particle endocytosis behavior was dependent on the surface characteristics of the nanoparticles. Third, a range of hemocompatibility studies was carried out on surface-modified NiTi. No differences among surface modifications were found in relation to fibroblast cytotoxicity and cytocompatibility. However, significant reductions in thrombogenicity were observed for heat-treated NiTi compared to mechanical-treated and electrochemical-treated NiTi. The differences were attributed to both the surface concentration and the oxidative state of nickel at the surface. Finally, cationic PAMAM dendrimers (new class of macromolecular architecture called "dense star" polymers) exhibited cytotoxicity toward Caco-2 cells, surface modification led to a marked reduction in cytotoxicity. In our current work, it was not clear whether oleic acid coating could affect the cytotoxicity of nickel ferrite particles. Therefore, Chapter 5 focused on the cytotoxicity study on nickel ferrite particles with or without oleic acid surface coating.

In addition to surface coating, it was also demonstrated that reduction of particle size changed the toxicological properties of a material. For example, bulk iron oxide is generally recognized as safe by the Food and Drug Administration (FDA) and its application in cosmetics has also been approved. However, nanoparticles of iron oxide showed considerable cytotoxicity in human fibroblast cell line, but showed little cytotoxicity in Cos-7 monkey kidney cell line. Moreover, the effects of particle size on the cytotoxicity of some other particles were also investigated. It was reported that cell mortality on J774 macrophages was decreased when the size of ceramic particles (Al₂O₃,
ZrO₂) decreased from micron to sub-micron\textsuperscript{56}. Tamura et al.\textsuperscript{57} reported that no significant difference was found in the viabilities of human neutrophils when the size of titanium particle was increased from 2 µm to 150 µm. However, using the same cell line, distinctly lower viability was found in nickel particles with the size of 0.4 µm compared to larger particles. Liu et al.\textsuperscript{58} studied the cytotoxicity of fly ash particles on rat alveolar macrophages. The particle sizes varied between 10 µm and several hundred nanometers. It was found that cytotoxicity of the minuscule particles was augmented in comparison to the larger ones. The above reported conclusions greatly depended on the materials and cell line used and they could not be generalized to our current work. In Chapter 5, nickel ferrite particles with same surface coating but with different sizes were therefore used to study the particle size effects on cytotoxicity.

1.3 Outline of the thesis

The outline of this thesis was described as below.

1. Synthesis and characterization of nickel ferrite nanostructured particles (Chapter 3);

2. Preparation of composites containing nickel ferrite particles and biodegradable polymer and investigation of their properties. (Chapter 4).

3. Study on cytotoxicity of nickel ferrite particles with different surface coatings and particle sizes. (Chapter 5).
53 Code of Federal Regulations - Title 21 - Food and Drugs Title 21—Food and Drug Chapter I—Food and Drug Administration Department of Health and Human Services SubChapter E
Chapter 2 Characterization techniques

2.1 Phase identification--X-ray Diffraction (XRD)

When a beam of monochromated X-ray impinges on a crystal composed of atoms arranged periodically in three dimensions, it will be scattered by the atoms within the crystal. The coherently scattered X-rays, having the same frequency and definite phase difference, interfere with one another, constructively or destructively. A diffraction pattern is generated when the scattered rays mutually reinforce one another.

The principle of X-ray diffraction is governed by the well-known Bragg’s law\(^1\).

\[
2d \sin \theta = n\lambda
\]

where \( \theta \): angle between the incident X-ray and the diffraction plane,

\( d \): interplanar (diffraction plane) distance,

\( \lambda \): wavelength of the incident X-ray, and

\( n \): an integer corresponding to the order of diffraction.

The above equation relates the diffraction angle (\( \theta \)) to the crystal interplanar spacing (\( d \)) and the wavelength (\( \lambda \)) of incident X-ray. The unique set of \( d \) spacings for a particular material can be found in the powder diffraction files (PDF) compiled by the Joint Committee of Powder Diffraction Standards (JCPDS).

In this project, XRD phase analyses were performed using Bruker D8 Advance Diffractometer (Cu K\( \alpha \) radiation, \( \lambda \sim 1.5418 \) Å), operated at 40 mA and 40 kV.
2.2 Investigation of particle morphology

2.2.1 Transmission Electron Microscopy (TEM)

TEM is a very powerful tool for obtaining morphology and crystallographic information of materials. Equipped with Energy Dispersive X-ray (EDX) analysis system, it can also be used to obtain compositional information in the area of a few nanometers in diameter.

The main components of a TEM include an electron gun, a condenser lens system, a specimen chamber, objective and intermediate lenses, projector systems for producing images & diffraction patterns, and vacuum/computer systems. For the most common types of TEM, the electron energy employed is in the range of 60-300 kV. The appropriate electron energy depends on the nature of the specimen and the information required. Much higher electron energy is suggested if the specimen is relatively thick or very high resolution is required. To satisfy different research interests, two types of TEM image are usually taken.

A. Bright field image

In a bright field image, only the undeflected electrons contribute to the formation of image. In the absence of a specimen, a bright background is observed. Regions of the specimen that are thicker or of higher density will scatter electrons more strongly (i.e. more electrons will be deflected) and will appear darker in the image. Bright field image is suitable for all specimens (amorphous or crystalline, biological or metallic), provided the specimen is thin enough.
B. Dark field image

In dark field image, only the part of the specimen that diffracts electrons contributes to the image. It has the reverse contrast to the bright field image. The background appears dark in the absence of a specimen. To obtain a dark field image of better resolution, the primary electron beam is tilted so that the chosen diffracted beam travels along the optical axis and pass through the centered aperture. It is always used to detect the crystalline grains and defects in specimen, which cannot be clearly observed in bright field image\(^2\).

In this work, nickel ferrite particles were studied using High Resolution TEM (HRTEM, JEOL3010) operated at 300 kV.

2.2.2 Scanning Electron Microscopy (SEM)

In an SEM, an electron gun emits a beam of electrons, which passes through a condenser lens and is refined into a thin stream. From there the objective lens focus the electron beam onto the specimen. These objective lenses contain a set of energized coils. The coils create an electromagnetic field that exerts a force upon the electrons in the electron beam, which in turn redirects the electrons to scan the specimen in a controlled pattern. The electromagnetic field of the coils also causes a spot of light on a cathode-ray tube to move along at the same rate as the scanning electron beam. When the electrons from the beam hit the specimen, a series of interactions deflect secondary electrons to a detector, which then converts the signal to voltage and amplifies it. This voltage is then applied to the cathode-ray tube and converted to an image. The intensity of the image (brightness) is determined by the number of secondary electrons that hit the cathode-ray
tube, which is dependent upon the angle that the electrons bounce off the specimen. Thus the image of the specimen depends on the topography of the specimen in question\textsuperscript{3}.

SEM imaging involves bombarding a material with an electron beam. The surface of the sample will accumulate charge if the electrons are not allowed to escape from the surface via a conductive path. Charging can also lead to excessive heating of the sample, causing material degradation. Non-conducting samples should be coated with a conductive material to prevent surface charging.

A Philips XL30 SEM was used to study the morphologies of nickel ferrite particles/biodegradable polymer composites. A gold layer of 20 nm was sputtered on the specimen surface before SEM observation.

2.3 Thermal analysis

2.3.1 Thermogravimetry Analysis (TGA)

TGA is a technique that involves continuously measuring the mass of a sample as a function of temperature. The thermogravimetric curves provide information concerning the thermal stability and the composition of the initial sample, any intermediate compounds formed and the residue. It can be used to qualitatively determine the stabilities of compounds, the rate of reaction and the composition of sample\textsuperscript{4}.

The shape of TGA curves and the temperatures at which mass changes occur are of interest. The magnitude of mass changes is directly related to the specific stoichiometries of the occurring reactions. In this project, a TGA 2950 (Dupont Instruments) was used to study the thermal stability of nickel ferrite particles with surface coating.
2.3.2 Differential Scanning Calorimetry (DSC)

DSC is a thermal analysis technique which is used to measure the heat flow associated with phase transitions in a material as a function of time or temperature. Such measurements provide qualitative and quantitative information about the physical and chemical changes that involve endothermic and exothermic processes, or changes in heat capacity.\(^4\)

In this project, Dupont TA DSC 2910 was utilized to study the glass transition of the polymer and its composites with nickel ferrite particles.

2.4 Composition analysis

2.4.1 Surface composition---X-ray Photoelectron Spectroscopy (XPS)

XPS is a surface sensitive analytic tool to study the surface composition and electronic state of a sample. A sample maintained under ultra high vacuum is bombarded with X-rays. The X-rays penetrate a substantial distance into the sample and excites electrons (referred to as photoelectrons). A small fraction of these photoelectrons (from the top ~ 5 nm on the surface) escape from the sample. The kinetic energy of these photoelectrons is measured by an analyzer.

When using monochromatic X-rays, the energy that one photon imparts on an electron is a known quantity. The binding energy of the ejected electron can then be determined from: \(E_{\text{binding}} = E_{\text{photon}} - E_{\text{kinetic}} - \Phi\), where \(\Phi\) is the work function of the material. In XPS, because the bonding energy of core electrons is very specific for the element that the atom belongs to, the spectrum gives information on the elemental composition of the shallow surface region.\(^5\)
In this project, XPS, ESCALAB 220i-XL spectrometer was used to analyze the surface of magnetic nanoparticles. XPS is a surface analysis technique ideal for thin film or bulk materials. When XPS is used on nanoparticles, the emitted photoelectrons could originate from either the top layer of particles or the internal counterpart because the surface of each particle was not at the same level. In our XPS analysis, powder samples were put on carbon tape and the surface were flattened. Spectra were collected without removal of any surface layer by sputtering. It is assumed that the average signal predominantly arose from the surface coating, and to a lesser extent the interior of particles.

2.4.2 Bulk composition--Energy Dispersive X-ray Spectroscopy (EDX)

When an electron beam bombards the sample, the excess energy of the electron, i.e. the difference between states in the two shells, is radiated during the transition as an X-ray photon. Such X-ray photons created electron-hole pairs in the Si(Li) detector. Under an applied bias, these electrons and holes are swept apart and collected on the electrodes forming a charge pulse, which is then converted to a voltage pulse. Each voltage pulse is proportional to the energy of the incoming X-ray photons. Every atomic species exhibits a characteristic X-ray emission spectrum. The fact that the spectrum of interest, from 0.1 keV to the beam energy (e.g. 20 keV) can be acquired in a short time (10~100 s) allows for a rapid evaluation of the sample. The bulk compositions of nickel ferrite particles were obtained by EDX accessories installed in the Philips SEM.
2.5 Measurement of Zeta potentials

A charged particle will move in an applied electric field. The mobility of the particle is related to some exterior factors, such as dielectric constant and viscosity of the suspending liquid. It also depends on the electrical potential at the boundary between the moving particle and the liquid. This boundary is called slip plane and is usually defined as the point where Stern layer and diffuse layer meet. Stern layer consists of ions tightly bound to the particle. Diffuse layer consists of free ions that rapidly exchange with ions in Stern layer and it can be visualized as a charged atmosphere surrounding the particle. Stern layer and diffuse layer together are called double layer. The electrical potential at the slip plane is related to the mobility of the particle and is called Zeta potential. Zeta potential is derived from measuring the mobility distribution of a dispersion of charged particles as they are subjected to an electric field. It is the measurable potential of a solid surface and is a function of particle surface charge density. Zeta potentials of nickel ferrite particles and their composites were measured by an Malvern Zeta Sizer 2000.

2.6 Magnetic characterization

2.6.1 Superconducting Quantum Interference Device (SQUID)

SQUID is an instrument which converts magnetic flux (which is hard to measure) into voltage (which is easy to measure). As a result, SQUID magnetometer can then be used to detect and measure very small magnetic fields.

Quantum Design MPMS-XL SQUID was used in this study. Magnetic particles were investigated by standard zero-field-cooling (ZFC) and field cooling (FC) procedures. The applied magnetic field was 100 Oe and the temperature range was from 5 K to 300 K.
2.6.2 Vibrating Sample Magnetometer (VSM)

An Oxford Instruments VSM was used for measuring the magnetic properties of magnetic particles and their composites. It is based on vibrating a sample in a magnetic field to produce an alternating electromagnetic field in the pickup coils.

Magnetic sample placed in a magnetic field of 50 kOe (generated by superconductor) with the longitudinal side of the sample parallel to the direction of magnetic field. The sample vibrated vertically and the oscillation will induce an AC signal in a set of suitably placed pickup coils. The amplitude of this signal is proportional to the magnetic moment of the sample and hence the magnetic moment at different magnetic field is obtained. A nickel film of similar geometry was used for calibration.

2.7 Other techniques

2.7.1 Fourier Transition Infrared Spectrometer (FTIR)

Molecular bonds vibrate at various frequencies depending on the elements and the type of bonds. For any given bond, there are several specific frequencies at which it can vibrate. According to quantum mechanics, these frequencies correspond to the ground state (lowest frequency) and several excited states (higher frequencies). One way to cause the frequency of a molecular vibration to increase is to excite the bond by absorbing light energy. For any given transition between two states the light energy (determined by the wavelength) must exactly equal the energy difference between the two states (usually ground state ($E_0$) and the first excited state ($E_1$)), as shown in the following equation:

$$E_1 - E_0 = \frac{hc}{\lambda}$$

where $h = $ Planck’s constant, $c = $ speed of light, and $\lambda = $ the wavelength of light.
The energy corresponding to these transitions between molecular vibration states is generally 1-10 kilocalories/mole that corresponds to the infrared portion of the electromagnetic spectrum.

FTIR is a powerful tool for identifying types of chemical bonds (functional groups). The wavelength of light absorbed is characteristic for the chemical bond. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined. FTIR spectra of pure compounds are generally so unique that they are like a molecular "fingerprint". FTIR spectra of the nickel ferrite particles and their composites were recorded using transmittance mode on a Bio-Rad FTS 135 FT-IR spectrometer.

2.7.2 Measurement of contact angle-- Surface Analysis System

Contact angle is the angle at which a liquid/vapor interface meets the solid surface. It is specific for any given system and is determined by the interactions across the three interfaces.

In this thesis, VCA OPTIMA Surface Analysis System was used to measure contact angles of a liquid against a solid pellet made of a tested material. Immediately after the liquid formed a droplet on the pellet surface, the drop profile was recorded using a video camera. On the digital image, the angle between the baseline of the drop and the tangent at the drop boundary was measured as contact angle. Surface energy of the test solid can be estimated from a series of contact angle values measured using different liquids with known surface energies. The calculation details will be discussed in Chapter 4.
2.7.3 Measurement of surface tension--Advanced Surface Tensiometer System

The polar liquid molecules in the bulk liquid arrange themselves so that the cohesive forces between the molecules are shared by the neighboring molecules, thus leading to zero net force. This, of course is the most favorable energetic situation. On the surface, however, the molecules lack the screening from the above thus leading to non-zero net force. This force divided by the length along the liquid surface is called surface tension. Dimensional analysis shows that the units of surface tension (N·m\(^{-1}\)) are equivalent to joules per square meter (J·m\(^{-2}\)). This means that for liquids surface tension can also be considered as surface energy which is defined as the work required increasing surface area by one unit area. In nature, water is the liquid with the highest surface tension (excluding mercury). At 20 °C the surface tension of water is 72.8 mN/m.

If surface active compound (e.g. surfactant, detergent, or oil) is added to the liquid, it tends to partition into the surface thus decreasing the surface tension. The most common method for measuring the surface tension of liquids is the Wilhelmy Plate Method. In the standard method, a small platinum coupon is attached to a force-measuring sensor and precisely dipped into a liquid and withdrawn to the point where the bottom edge of the coupon is exactly parallel with the surface of the liquid. At this point, the surface tension is the downward force exerted by the liquid divided by the wetted length of the platinum coupon\(^9\).

In this project, surface tension was measured using DCAT 21 Advanced Surface Tensiometer System to determine the critical micelle concentration (CMC) of the surfactant.
Chapter 3 Synthesis and characterization of nickel ferrite particles

In this chapter, nickel ferrite particles with oleic acid surface coating were prepared by nonhydrolytic sol-gel method. To date, this method has not been applied to synthesize magnetic oxides. Mechanochemical method was used to synthesize nickel ferrite particles without surface coating. The experimental procedures reported by Shi et al.\(^1\) were adopted.

3.1 Nonhydrolytic sol-gel method

Traditional sol-gel process is based on hydrolysis and condensation of molecular precursors, such as metal alkoxides\(^2\). The major problem is to control the reaction rates which are generally too fast, resulting in a loss of microstructural control of the final oxide products\(^3\). Recently, nonhydrolytic sol-gel processes have been successfully applied to the synthesis of various nanocrystals of transition metal oxides with a good control of size and shape\(^4\). The formation of an oxo bridge (-M-O-M-) is achieved by condensation of two different metal centers after eliminating a small organic molecule. Esters and alkyl halide eliminations have been postulated\(^5\). Trentler et al. synthesized TiO\(_2\) nanocrystals based on an alkyl halide elimination reaction\(^6\). Joo et al. synthesized ZnO nanocrystals with cone, hexagonal cone, and rod shapes via nonhydrolytic ester elimination sol-gel reaction\(^7\). This method has not been used for the synthesis of magnetic nanoparticles. In this part, nickel ferrite particles were prepared via ester elimination using nickel acetate tetrahydrate, iron acetate and 1, 12-dodecanediol in diphenyl ether (DPE) solution. Surface coating was achieved using oleic acid as surfactant. The particle size was varied by changing the composition of precursors.
3.1.1 Experimental method

Unless noted, the following procedures were followed.

4 mmol nickel (II) acetate tetrahydrate and 8 mmol iron (III) acetate were combined with 3.84 ml (12 mmol) oleic acid in a flask with 120 ml of DPE and heated to 80°C. In another flask, 6.3 g of 1, 12-dodecanediol was dissolved in 30 ml of DPE. The solution was heated to 80°C under vacuum for 15 min, and added to the contents of the first flask. The mixture was heated to 200°C. 2.2 ml trioctylphosphine (TOP) was introduced and maintained at the same temperature for 10 min. Then the mixture was further heated to 250°C, and this temperature was held for 30 min to complete the reaction. The obtained particles were dispersed in alcohol and isolated by magnetic separation, followed by re-dispersion in alcohol. This cycle was repeated three times to completely remove DPE. The alcohol was finally evaporated at 40°C. The chemical structures of the reactants are shown as below.

Nickel acetate tetrahydrate: \( \text{Ni(OCH}_2\text{CH}_3\text{)}_2\cdot 4 \text{H}_2\text{O} \)

Iron acetate: \( \text{Fe(OCH}_2\text{CH}_3\text{)}_3 \)

Oleic acid:

Trioctylphosphine (TOP):

Diphenyl ether (DPE):

1, 12-dodecanediol:
The basic reactions occurred in the nonhydrolytic sol-gel reaction are proposed as below.

(1)

\[
\begin{align*}
\text{Ni} & \quad \text{O} \\
\text{H} & \quad \text{O} \\
\text{OH} & \\
\end{align*}
\quad \rightarrow \quad \text{Ni} \quad \text{OH} \quad + \quad \text{OH}
\]

(2)

\[
\begin{align*}
\text{Fe} & \quad \text{O} \\
\text{H} & \quad \text{O} \\
\text{OH} & \\
\end{align*}
\quad \rightarrow \quad \text{Fe} \quad \text{OH} \quad + \quad \text{OH}
\]

(3)

\[
\begin{align*}
\text{Ni} \quad \text{OH} \quad + \quad \text{HO} \quad \text{Fe} \quad \rightarrow \quad \text{Ni} \quad \text{O} \quad \text{Fe}
\end{align*}
\]

3.1.2 Compositional analysis

In the above preparation method, the molar ratio of nickel acetate tetrahydrate to iron acetate was 1.0:2.0. We denoted the particles obtained using this composition as C1. The XRD pattern of C1 is shown in Fig. 3-1. The pattern matched well with the diffraction peaks of fcc nickel ferrite and no other phase was detected. The atomic surface and bulk compositions were determined as Ni:Fe=1.0:1.4 using XPS and Ni:Fe=1.0:1.5 using EDX, respectively. It is worth noting that the obtained composition was different from that of metal precursor, i.e. Ni:Fe =1.0:2.0.
Figure 3-1 XRD pattern of C1. The molar ratio of nickel acetate tetrahydrate to iron acetate was 1.0:2.0.

To explore the effects of precursor composition, another synthesis was carried out by changing the molar ratio of nickel acetate tetrahydrate to iron acetate to 1.0:3.5. The rest of experimental conditions were kept the same. The particles obtained using this composition were denoted as C2. Similar to the XRD pattern of C1, the only detectable phase was fcc nickel ferrite in C2, as shown in Fig. 3-2. The surface and bulk compositions of C2 were measured as Ni:Fe=1.0:2.6 and 1.0:3.0 respectively. Similarly, the obtained composition was different from that of metal precursor, i.e. Ni:Fe =1.0:3.5.
The molar ratio of nickel acetate tetrahydrate to iron acetate was 1.0:3.5.

Compared to stoichiometric NiFe$_2$O$_4$, it was noted that C1 was rich in Ni and C2 was rich in Fe. There were no observed diffraction peaks for excess segregated Ni in C1 or excess segregated Fe in C2. This could be due to that they existed as amorphous state or the crystallite order of segregated phase was smaller than the detectable X-ray coherence length. They could also exist as single phase Ni$_x$Fe$_y$O$_4$, which also had an fcc structure with lattice parameter (a=8.335Å when x=1.3, y=1.8 $^8$ and a=8.354Å when x=0.6, y=2.4 $^9$) very similar to that of NiFe$_2$O$_4$ (a=8.337Å).

FTIR is an established way to identify the amorphous state of oxides which could not be detected by XRD$^{10}$. Figure 3-3 shows FTIR spectra of C1, C2 before and after heat treatment in air at 700°C. The peak at 586 cm$^{-1}$ was found in all four spectra and could be attributed to Fe-O stretching mode in nickel ferrite. Ni-O stretching mode in nickel ferrite was located below 400 cm$^{-1}$ which was out of the measurement range$^{11}$. Nickel oxide should give a single broad band at approximately 466-470 cm$^{-1}$ wave number$^{12}$. Iron
oxides should show strong bands at 400-600 cm\(^{-1}\) depending on their crystallization types\(^{13}\). None of these bands could be found in C1 or C2 spectra before heat treatment. It indicated that except nickel ferrite, there was no nickel oxide or iron oxides in as-synthesized C1 or C2. In the spectra after heat treatment in air, the bands of nickel oxide or iron oxides still could not be detected, suggesting that there was no metallic nickel or iron in as-synthesized C1 or C2.

![Figure 3-3 FTIR spectra of C1, C2 before and after heat treatment in air at 700\(^{\circ}\)C.](image)

Based on the above FTIR results, it was suggested that both C1 and C2 were most likely to be single phase Ni\(_{x}\)Fe\(_{y}\)O\(_{4}\). For easier comparison, a summary of the precursor composition, bulk composition, surface composition and x, y values in Ni\(_{x}\)Fe\(_{y}\)O\(_{4}\) of C1 and C2 is presented in Table 3-1. The x, y values were calculated based on electron balance in Ni\(_{x}\)Fe\(_{y}\)O\(_{4}\) molecule. It suggested that our nonhydrolytic sol-gel reaction did not proceed as normal sol-gel reactions in which the composition of precursor was similar to
that of product. In our case, Fe composition in products (both C1 and C2) was lower than the Fe composition in precursors. The reason will be discussed in 3.1.7.

Table 3-1 Summary of the atomic compositions of precursors and products.

<table>
<thead>
<tr>
<th></th>
<th>Precursor (Ni:Fe)</th>
<th>Product, Bulk (Ni:Fe)</th>
<th>Product, Surface (Ni:Fe)</th>
<th>x, y values in Ni$_x$Fe$_y$O$_4$*</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>1.0:2.0</td>
<td>1.0:1.4</td>
<td>1.0:1.5</td>
<td>x=1.26, y=1.83</td>
</tr>
<tr>
<td>C2</td>
<td>1.0:3.5</td>
<td>1.0:2.6</td>
<td>1.0:3.0</td>
<td>x=0.79, y=2.21</td>
</tr>
</tbody>
</table>

*The average of surface and bulk composition was used for calculation.

The crystal structure of stoichiometric NiFe$_2$O$_4$ is illustrated in Fig. 3-4. The structure is formed by a cubic close-packed array of oxygen ions, in which one eighth of the tetrahedral and one half of the octahedral interstitial sites are occupied by cations. Those two interstitial sites are referred to as A site and B site, respectively. Nickel ferrite is an inverse ferrite, in which divalent ions (Ni$^{2+}$) are on B sites, and trivalent ions (Fe$^{3+}$) are equally divided between A and B sites.

To maintain the electron balance in a nickel ferrite cell unit, different x, y values in Ni$_x$Fe$_y$O$_4$ could lead to different ion substitution compared with NiFe$_2$O$_4$. In Ni-rich C1, some Fe$^{3+}$ ions were missing and some extra Ni$^{2+}$ ions were present. For C2 which was Fe rich, some Ni$^{2+}$ ions were replaced by Fe$^{2+}$. The distribution of substituted ions on A and B sites may be illustrated in detailed Mössbauer and neutron diffraction studies, which is beyond the scope of this thesis.
Figure 3-4 The spinel structure. The unit cell can be divided into octants. Tetrahedral cations A, octahedral cations B, and O atoms (large circles) are shown in the two octants.\textsuperscript{14}

### 3.1.3 Size analysis

Scherer’s formula, as shown below, was used to estimate crystallite size.

\[
L = \frac{k\lambda}{\delta 2\theta \cos\theta_o}
\]

where \(\delta 2\theta\) is the breadth at half-maximum intensity of the diffraction peak; \(\theta_o\) is the position of the peak maximum; \(k\) is particle shape factor, for spherical particles, \(k = 0.9\); \(L\) is crystallite size.

The calculated crystallite sizes for C1 and C2 are listed in Table 3-2.

<table>
<thead>
<tr>
<th>HKL</th>
<th>2 Theta (°)</th>
<th>Crystalline size (Å)</th>
<th>Average crystallite size (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(h k l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C1</td>
<td>(220)</td>
<td>30.14</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>(311)</td>
<td>35.68</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>(400)</td>
<td>43.22</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>(440)</td>
<td>62.69</td>
<td>74</td>
</tr>
<tr>
<td>C2</td>
<td>(220)</td>
<td>30.07</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>(311)</td>
<td>35.36</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>(400)</td>
<td>43.11</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>(422)</td>
<td>54.37</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td>(511)</td>
<td>57.15</td>
<td>82</td>
</tr>
</tbody>
</table>

The calculated crystallite sizes for C1 and C2 are listed in Table 3-2.
The average crystallite size of C1 and C2 was ~7 nm and ~8 nm, respectively. The TEM and HRTEM images of C1 are shown in Fig. 3-5. The particles were well separated and the average particle size was 9.7 nm which was larger than the crystallite size estimated by XRD line broadening. Such difference was also observed in monodisperse iron oxide nanoparticles\(^\text{15}\). It was probably due to phase segregation at surface area, as suggested by Li et al.\(^\text{16}\). The phase at the surface might be amorphous and could not contribute to X-ray line broadening. The HRTEM image indicated that each particle was single crystal with average size around 9.5 nm.

![Image](image_url)

Figure 3-5 (a) Low magnification TEM image of C1, (b) corresponding histogram of particle size distribution (total counts: 30), (c) HRTEM image of C1. The particles were well separated and each particle was single crystal.
Figure 3-6(a) shows the morphology of C2. It was interesting to find that particles of C2 were spherical with average size of 152 nm. To investigate the sub-structure of C2 particles, dark field image focusing on one particular particle were taken and is shown in Fig. 3-6 (c). It was found that the large particle consisted of many small crystallites (bright contrast in dark field image). The corresponding histogram of crystallite size distribution in Fig. 3-6 (d) suggested a mean diameter of 8.1 nm. It was consistent with the average crystallite size estimated from XRD line broadening (~8 nm). The HRTEM image focusing on the edge of this particular particle is shown in Fig. 3-6 (e). It also indicated existence of small crystallites about 8 nm. Consequently, C2 were sub-micron aggregates of nanocrystals.
Figure 3-6 (a) Low magnification TEM image of C2, (b) corresponding histogram of particle size distribution (total counts: 30), (c) dark field image focusing on one particular particle, (d) corresponding histogram of crystallite size distribution (total counts: 30), (e) HRTEM image focusing on the edge of one specific particle of C2.
3.1.4 Zeta potential analysis

Figure 3-7 shows the relationship of zeta-potential vs. pH for C1 and C2. Isoelectric point (IEP) is the pH value at which the net surface charge of a particle is zero. For bare nickel ferrite particles in water, the dependence of surface charge on pH values could be explained by hydroxyl groups (-OH) adsorbed on the surface. In this case, the IEP should be close to 7 because dissociation of -OH occurred at the intrinsic acidity constant of water (pK_a=7). In C1 and C2, their IEPs were found in pH=3-4, which suggested that their surface charges were different from bare particles. With the coating of oleic acid, the origin of surface charge changed to carboxylic acid groups, which were neutral (COOH) and dissociated to COO^-. The IEP was the dissociation point of carboxylic group (pK_a=3-4) \(^{17}\). The lower IEPs in both C1 and C2 suggested that oleic acid molecules had been successfully coated on the nickel ferrite surface. The increase of zeta potentials for C2 at higher pH was possibly because free oleic acid left particle surface and the hydrophobic ends were exposed to the solution.

![Figure 3-7 Relationship between zeta-potential and pH for C1 and C2.](image)
3.1.5 Thermal analysis

The surfactants on particle surface have less thermal stability than nickel ferrite itself and are supposed to decompose at lower temperatures. C1 and C2 were heated to 700°C in air with the heating rate of 10°C/min. Their TGA results are shown in Fig. 3-8. Both C1 and C2 were thermally stable beyond ~380°C. Below this temperature, there were two stages of decomposition in both C1 and C2. The first stage showed a low rate of weight loss due to evaporation of adsorbed water. It involved about 5 wt% in both C1 and C2. The second stage whose onset temperatures were represented by arrows in Fig. 3-8 showed sharp gradients and suggested the loss of adsorbed surfactant on particle surface. Such coating provided steric protection over nanocrystals and sufficient coating allowed nanocrystals to separate from each other, despite strong magnetic attraction. The higher percentage of second weight loss in C1 implied that more surfactants were adsorbed on the particle surface.

![Figure 3-8 TGA results of C1 and C2. The arrows represent the onset points of the second stage of weight loss.](image-url)
3.1.6 Oleic acid on particle surface

Oleic acid and TOP are surfactants used to control particle growth and stabilize particles against agglomeration. The zeta-potential measurement (shown in Fig. 3-7) already suggested the presence of oleic acid on particle surface by the observed lower IEPs. XPS analysis which will be discussed later confirmed the chemisorptions of oleic acid. However, the XPS results indicated that there was no TOP on the particle surface of both C1 and C2. As shown in Fig. 3-9, no peaks at the bonding energies of 189.6 eV (P2s) and 130.5 eV (P2p3/2) were observed. Both of them were characteristic peaks of surface containing phosphorus. The only observed peak in Fig. 3-9 belonged to C1s at bonding energy of 284.2 eV.

![Figure 3-9 XPS spectra of C1 and C2. No peak was observed at bonding energies of 189.6 eV (P2s) and 130.5 eV (P2p3/2). The only observed peak was from C1s at bonding energy of 284.2 eV.](image)

It was reported that TOP coordinated with neutral surface sites, slowing but not stopping the particle growth, i.e. when TOP was used alone it could lead to undispersible
aggregates\textsuperscript{18}. Oleic acid, when employed alone, was an excellent stabilizing agent, but it bound tightly to the particle surface during synthesis and greatly impeded the particle growth. The combination of TOP and oleic acid produced a tight ligand shell which allowed the particles to grow steadily while protecting them from aggregation. This was the reason why TOP and oleic acid were always used in pair\textsuperscript{19}. With the process of reaction, oleic acid replaced TOP on the surface because it could form a stronger bond with particles\textsuperscript{20}. This explained that oleic acid was the only surfactant present on the surface in our final products.

Table 3-3 indicates the vibration mode assignments of oleic acid in FTIR\textsuperscript{21}. Figure 3-10 shows the FTIR results of C1 and C2.

<table>
<thead>
<tr>
<th>Peak (cm\textsuperscript{-1})</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>3500-2500</td>
<td>O-H stretch</td>
</tr>
<tr>
<td>3005</td>
<td>C-H stretch in C=C-H</td>
</tr>
<tr>
<td>2963</td>
<td>Asymmetric CH\textsubscript{3} stretch</td>
</tr>
<tr>
<td>2924</td>
<td>Asymmetric CH\textsubscript{2} stretch</td>
</tr>
<tr>
<td>2854</td>
<td>Symmetric CH\textsubscript{2} stretch</td>
</tr>
<tr>
<td>1710</td>
<td>C=O stretch</td>
</tr>
<tr>
<td>1462</td>
<td>In plane O-H stretch</td>
</tr>
<tr>
<td>1409</td>
<td>CH\textsubscript{3} umbrella mode</td>
</tr>
<tr>
<td>1285</td>
<td>C-O stretch</td>
</tr>
<tr>
<td>937</td>
<td>Out-of-plane O-H stretch</td>
</tr>
</tbody>
</table>
The peaks at 2924, and 2854 cm\(^{-1}\) were assigned to the asymmetric methylene stretch \(\nu_{as}(\text{CH}_2)\), and the symmetric methylene stretch \(\nu_s(\text{CH}_2)\), of oleic acid molecule, respectively. The strong and sharp peak at 586 cm\(^{-1}\) was assigned to the Fe-O stretch mode in nickel ferrite. The C=O stretch mode of carboxyl group present at 1710 cm\(^{-1}\) in the IR spectrum of pure oleic acid, was absent in that of coated nickel ferrite particles. It indicated that free oleic acid was not detected on the particle surface.

To further examine the chemical structure of oleic acid coated nickel ferrite particles, the XPS spectra of O1s and C1s core levels of C1 and C2 were obtained. The results are shown in Fig. 3-11 and Fig. 3-12, respectively. In Fig. 3-11, the O1s peak at 531.5 eV found in both C1 and C2 was assigned to the carboxylate on particle surface. In C1, in addition to the peak at 531.5 eV, a high binding energy O1s peak at 536.9 eV was also observed. This peak could not be attributed to C=O in oleic acid or nickel ferrite because these two peaks had to be related to a lower binding energy than that of
carboxylate. Most probably, the peak corresponded to adsorbed oxygen species on the particle surface. It is reasonable that the oxygen peak was not observed in the spectrum of C2. Compared to the smaller particle size of C1, C2 had much less surface area and less oxygen could adsorb onto the C2 surface. Similarly, in Fig. 3-12 (a), the XPS spectra of C1 at C1s core level, the C1s peak at highest binding energy 292.9 eV indicated the presence of adsorbed CO$_2$ on C1 surface. In addition, two characteristic peaks at 284.2 eV and 288.9 eV were found in both C1 and C2. They were ascribed to the carbon atoms in the aliphatic chain (C-C) and the carboxylate moiety (-COO$^-$), respectively. This result was consistent with literature report $^{21}$. No C1s peak corresponding to carboxylic carbon (-COOH) was found in the spectrum, indicating the absence of free acid on nickel ferrite surface. This agreed with the single O1s peak from both C1 and C2 in Fig. 3-11, i.e. no C=O bond in free acid was detected. Based on the above discussion on XPS, the presence of chemisorbed oleic acid on particle surface and the formation of carboxylate groups were confirmed.

![Figure 3-11 XPS spectra of O1s core level obtained from oleic acid coated nickel ferrite particles (a) C1 and (b) C2.](image)
Two binding modes have been suggested for the surface carboxylate bonding\(^{22}\). In one mode, the carboxylate is bound symmetrically to the surface as shown in Fig. 3-13 (a). Only the symmetrical C-O stretch band appeared around 1400 cm\(^{-1}\) in this mode. In the other mode, as shown in Fig. 3-13(b), the carboxylate was connected to the surface through one oxygen atom. Both symmetric C-O stretch (~1420 cm\(^{-1}\)) and asymmetric stretch (~1580 cm\(^{-1}\)) should be observed in this mode. In Fig. 3-10, both C1 and C2 showed two bands. In C1, the two bands were located at 1415 cm\(^{-1}\) and 1565 cm\(^{-1}\), respectively. In C2 the two bands absorbed at 1415 cm\(^{-1}\) and 1590 cm\(^{-1}\), respectively. Therefore, oleic acid was supposed to connect to nickel ferrite particles (both C1 and C2) through one oxygen atom. From the wave number separation ($\Delta$) between the symmetric $\gamma_s$(COO\(^-\)) and asymmetric $\gamma_{as}$(COO\(^-\)) stretches, the coordination types (from monodentate, bridging, to bidentate ligand) could be interpreted\(^{23}\). For $\Delta>200$ cm\(^{-1}\), a monodentate coordination was suggested. For a bidentate ligand, $\Delta$ laid below 110 cm\(^{-1}\). While for $\Delta$ between 140 and 200 cm\(^{-1}\), a bridging ligand was expected. In our case, both C1 ($\Delta=150$) and C2 ($\Delta=175$) implied bridging ligands. However, the differences in $\Delta$ might suggest
the coordination environment was not identical between C1 and C2. The higher $\Delta$ in C2 suggested more asymmetric carboxylate coordination.

![Diagram](image)

Figure 3-13 Two binding modes for the surface carboxylate group. (a) carboxylate is bound symmetrically to the surface, and (b) carboxylate is connected to the surface through one oxygen atom.

3.1.7 Effects of precursor composition on particle size

In TEM images, C1 and C2 showed significant difference in particle size. C1 were nanocrystals of ~10 nm. C2 were sub-micron aggregates of nanocrystals. The common method to control particle size is to vary the ratio of precursors to surfactants\textsuperscript{24}. However, when preparing C1 and C2, the total concentration of precursors (nickel acetate tetrahydrate and iron acetate) and the total concentration of surfactants remained the same. The only difference was the ratio of nickel acetate tetrahydrate to iron acetate. It was 1.0:2.0 for C1 and 1.0:3.5 for C2. An intermediate ratio (1.0:2.7) was discussed in the supporting information-1 at the end of this chapter. For the product synthesized using this ratio, both small particles and large aggregates were found. Therefore, the precursor composition should be the key factor affecting the changes in particle size.

In the reaction involving iron acetate and oleic acid, formation of iron oleate complex was highly possible\textsuperscript{25}. The complex was derived due to coordination of iron
cations and carboxylate (RCOO⁻) ligands in oleic acid. The oxygen atom possessed an
electron pair that was readily able to form dative bond with iron cations. The complex
could be obtained at ~100°C and decomposed to produce iron oxide at 350°C. As shown
in the thermal analysis result of iron oleate complex, only 30% weight loss was observed
at 250°C which was the reaction temperature of our nonhydrolytic method. It suggested
that 70% of iron oleate complexes remained in the reaction system. Such complex
consumed certain amount of iron precursor which led to iron deficiency in both C1 and
C2 (as discussed in 3.1.2).

Compared with C1, with the increase of iron acetate in C2, the oleic acid used for
complexing also increased. Thus the remaining amount of oleic acid which could act as
surfactant decreased. The smaller concentration of oleic acid in C2 could not provide
sufficient steric and electrostatic separation between nanocrystals and thus allowed them
to agglomerate and form larger particles.

To support the above argument, one possibility i.e. nickel acetate could form
oleate as efficiently as iron acetate had to be experimentally ruled out. Because of the
difficulty to differentiate nickel oleate and iron oleate, the following experimental
procedures were followed to evaluate the likelihood of formation of nickel oleate. 12
mmol nickel acetate tetrahydrate was combined with 3.84 ml (12 mmol) oleic acid and
120 ml of DPE. The mixture was heated to 300°C. Its color remained light green,
indicating complete dissolution of nickel acetate in DPE. Yet, no further reaction was
observed. In a typical formation of oleate complex, obvious color change was observed.

For example, if 12 mmol nickel acetate tetrahydrate was replaced with 12 mmol iron
acetate in the above experiment, a color change from light brown to dark reddish was
observed at 150°C. It indicated that iron acetate easily reacted with oleic acid to form
oleate complex. However, it was difficult for nickel acetate to react with oleic acid in the above conditions. It was reported that Ni-oleylamine complex was used to synthesize nickel and nickel oxide as reported by Park and coworkers. Iron oleate and manganese oleate were used for synthesis of iron oxide and manganese ferrite. This could be due to the difficulty if not the impossibility to obtain nickel oleate.

3.1.8 Magnetic characterization

Magnetization as a function of temperature for both zero-field-cooled (ZFC) and field cooled (FC) processes are shown in Fig. 3-14. When a sample is cooled in zero field the total magnetization will be minimized since the magnetic moments of the particles are randomly oriented. An external magnetic field energetically favors the orientation of the moments of individual particles along the applied field at low temperatures. As the temperature is increased, more thermal energy is available to disturb the system. Therefore, more moments will align with external direction. Eventually the net magnetization of the system reaches the maximum where the greatest population of moments has aligned with the external field. The peak temperature is called blocking temperature, $T_B$. It is the temperature, at which the magnetic anisotropy energy barrier of a nanoparticles is overcome by thermal activation and the nanoparticle becomes superparamagnetically relaxed.

Above $T_B$, the strong thermal vibrations randomize the moments, thus decreasing the net magnetization. As shown in Fig. 3-14, the $T_B$ of C1 and C2 (measured as the peak temperature of ZFC curve) were 150 K and 125 K, respectively. According to the equation $K = 25k_BT_BV^{-1}$, (where $K$ is anisotropy constant, $k_B$ is Boltzmann’s constant, and $V$ is the volume of a single nanocrystal), anisotropy constants of C1 and C2 were
calculated as shown in Table 3-4. Higher K in C2 could imply a stronger interaction between different nanocrystals.  

![Graph](a) C1 and (b) C2. $T_B$ was determined as the peak temperature of ZFC curves. $T_F$ was determined as the temperature where FC and ZFC curves split.

![Graph](a) C1 and (b) C2. $T_B$ was determined as the peak temperature of ZFC curves. $T_F$ was determined as the temperature where FC and ZFC curves split.

**Figure 3-14** Magnetization vs. temperature measurements for both ZFC and FC processes. (a) C1 and (b) C2. $T_B$ was determined as the peak temperature of ZFC curves. $T_F$ was determined as the temperature where FC and ZFC curves split.

<table>
<thead>
<tr>
<th></th>
<th>$T_B$</th>
<th>D (diameter of a single nanocrystal)**</th>
<th>V</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>150 K</td>
<td>9.7 nm</td>
<td>$1.41 \times 10^{-27}$ m$^3$</td>
<td>$1.1 \times 10^6$ erg/cm$^3$</td>
</tr>
<tr>
<td>C2</td>
<td>125 K</td>
<td>8.1 nm</td>
<td>$6.64 \times 10^{-26}$ m$^3$</td>
<td>$1.5 \times 10^6$ erg/cm$^3$</td>
</tr>
</tbody>
</table>

* $k=1.38 \times 10^{-23}$ J/K

** The diameters were obtained from TEM images of Fig. 3-5(a) and Fig. 3-6(c) for C1 and C2, respectively.

The most obvious difference between C1 and C2 was the freezing temperature ($T_F$). $T_F$ is determined as the temperature at which the irreversible magnetization ($\Delta M=M_{FC}-M_{ZFC}$), becomes different from zero. In a spin-glass, magnetic spins experience random interactions with other magnetic spins, resulting in a state that is highly irreversible and metastable. This spin-glass state is realized below $T_F$. Recent study reported that lower $T_F$ in nanocrystalline nickel ferrite implied stronger magnetic interactions. In the present work, the observed $T_F$ of C2 (~125K) was much lower than...
that of C1 (~265K). It could suggest that stronger interactions were present in C2 which was consistent with the calculation of anisotropy constant. Another hint showing the same conclusion was that below $T_B$ the FC magnetization curve of C2 increased very slowly with decreasing temperature (Fig 3-14 (b)). The constant magnetic moment over a wide temperature range was also resulted from the strong interactions between crystallites\(^{30}\). Together with the earlier discussion in morphologies, it was summarized that crystallites in C2 were in close contact with each other and were characterized by strong interactions.

The driving force for C2 aggregation could be due to strong inter-crystallite interaction. The reason for such strong interaction might be the insufficient surfactant coating in C2. Because of the complex of oleic acid and Fe, less oleic acid was found on C2 which was not sufficient to provide complete coverage to every crystallite. Thus agglomeration of crystallites resulted in order to achieve the lowest surface energy. Moreover, the TGA results in Fig. 3-8 implied that during the process of agglomeration, some changes happened in the oleic acid molecules. The onset points of the second stage weight loss represented the thermal decomposition of oleic acid molecules on particle surface. The higher decomposition temperature in C2 (250°C) indicated that it required more energy to decompose oleic acid. This could be explained as oleic acid molecules on adjacent crystallites interacted with each other by overlapping and entangling of long carbon chains. As proposed by Patil et al.\(^ {31}\), interdigitation occurred in the self-assembled aggregates of silver nanoparticles coated with lauric acid. Similar structure might also develop in C2.

Figure 3-15 shows the hysteresis loops of C1 and C2 at 300K. Zero remanence and coercivity were found in both C1 and C2, suggesting their superparamagnetic
properties at room temperature. Neither C1 nor C2 magnetically saturated at 50 kOe. The magnetization at 50 kOe was 27 emu/g and 46 emu/g, respectively. Compared to the saturation magnetization of stoichiometric NiFe$_2$O$_4$ (55 emu/g), the lower magnetization was probably attributed to surface spin disorder and broken exchange bonds between surface spins. C1 and C2 had different $x$, $y$ values in Ni$_{x}$Fe$_{y}$O$_{4}$ ($x$=1.26, $y$=1.83 in C1 and $x$=0.79, $y$=2.21 in C2, as shown in Table 3-1). Compared with NiFe$_2$O$_4$, some Fe$^{3+}$ ions were missing and some extra Ni$^{2+}$ ions were present in C1. In C2, some Ni$^{2+}$ ions were replaced by Fe$^{2+}$. The number of un-paired electrons in Fe$^{3+}$, Fe$^{2+}$ and Ni$^{2+}$ was 5, 4 and 2 respectively. Therefore, the magnetic moments of the above three ions were in the order of Fe$^{3+}$> Fe$^{2+}$>Ni$^{2+}$. In C1 the substitutes (Ni$^{2+}$) had lower magnetic moments than the original Fe$^{3+}$. And in C2 the substitutes (Fe$^{2+}$) had higher magnetic moments than the original Ni$^{2+}$. It could be the reason why the magnetization of C2 was higher than that of C1.

Figure 3-15 Hysteresis loops of C1 and C2 at 300K.
3.2 Mechanochemical method

We aimed to synthesize bare nickel ferrite particles with similar particle sizes as what were obtained in the above nonhydrolytic sol-gel method. Strictly following the reported mechanochemical method\textsuperscript{1}, ~10 nm particles were obtained. Large particles were prepared by modifying the heat treatment process in the reported method.

3.2.1 Experimental procedures

The mechanochemical method involved two steps i.e. co-precipitation and mechanical milling of the co-precipitated precursor.

In the co-precipitation process, NiCl\textsubscript{2} powders and FeCl\textsubscript{3} powders were dissolved in deionized water to make a solution containing Ni\textsuperscript{2+} and Fe\textsuperscript{3+} ions. By adding sodium hydroxide (NaOH) solution into the above solution, nickel hydroxide Ni(OH)\textsubscript{2} and iron hydroxide Fe(OH)\textsubscript{3} formed and co-precipitated according to the following reaction.

\[
\text{NiCl}_2 + \text{FeCl}_3 + 5\text{NaOH} \rightarrow \text{Ni(OH)}_2 \downarrow + \text{Fe(OH)}_2 \downarrow + 5\text{NaCl}
\]

The product in the above reaction was a mixture of hydroxides and NaCl. Two hydroxides were expected to mix at molecular level. NaCl was dissolved in aqueous solution. The suspension of hydroxides in NaCl solution was freeze dried for 24 h to obtain mixed powders of precursors and NaCl.

In the mechanical milling process, the mixed powders were milled in a Fritsch planetary miller. The mechanical milling was carried out at 200 rpm for 30 h, and then the rotation speed was increased to 300 rpm for another 30 h. The co-precipitation precursors were dehydrated due to mechanical and thermal energy produced during milling process. The as-milled powders were heat treated at 650\textdegree C for 2 h in the presence
of NaCl and then washed with deionized water three times to remove NaCl after heat treatment. We denote the particles obtained through above procedures as B1. As reported, the particle size of B1 was around 10 nm\(^1\) which was very similar to the size of C1.

Large particles without surface coating were synthesized by modifying the above method. In this process, NaCl was removed before heat treatment because it was proven as a good dispersant to prevent crystallite growth and agglomeration\(^1\). As-milled particles were washed using deionized water three times to remove NaCl and then heat treated at different temperatures for 2 h. Detailed XRD and TEM analysis on obtained particles were reported in supporting information-2. It was found that 650\(^\circ\)C was the temperature to obtain particles with similar size as C2. Thus in the following discussion we used B2 to denote the particles after heat treatment at 650\(^\circ\)C for 2 h in the absence of NaCl.

The characterization of B1 and B2 were briefly discussed in the following.

### 3.2.2 Characterization

Figure 3-16 shows the XRD patterns of B1 and B2. The line broadening of B1 was much larger than that of B2, indicating crystallite growth in B2. Because the heat treatment conditions of B1 and B2 were identical (650\(^\circ\)C, 2 h), the above crystallite growth was attributed to NaCl. It confirmed the role of NaCl as a dispersant to prevent crystallite growth.
The crystallite sizes of B1 and B2 were estimated using Scherer’s formula and the results are shown in Table 3-5.

Table 3-5 Calculated crystallite sizes of B1 and B2 estimated using Scherer’s formula.

<table>
<thead>
<tr>
<th>Sample name</th>
<th>(h k l)</th>
<th>2 Theta (°)</th>
<th>Crystalline size (Å)</th>
<th>Average crystallite size (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>(220)</td>
<td>30.24</td>
<td>93</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>(311)</td>
<td>35.54</td>
<td>84</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(400)</td>
<td>43.39</td>
<td>79</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(511)</td>
<td>57.36</td>
<td>82</td>
<td></td>
</tr>
<tr>
<td>B2</td>
<td>(220)</td>
<td>30.29</td>
<td>141</td>
<td>147</td>
</tr>
<tr>
<td></td>
<td>(311)</td>
<td>35.74</td>
<td>147</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(400)</td>
<td>43.43</td>
<td>156</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(511)</td>
<td>57.43</td>
<td>137</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(440)</td>
<td>63.12</td>
<td>158</td>
<td></td>
</tr>
</tbody>
</table>

It is known that strain was always introduced in mechanically milled particles\textsuperscript{33}. However, the strain contribution was ignored in the above size estimation. The integral
breadth method was used to estimate the crystallite size with the consideration of strain.\textsuperscript{34} The integral breadth method can be described in the following equation.

$$\frac{(\delta 2\theta)^2}{\tan^2 \theta_0} = \frac{k\lambda}{L} \left( \frac{\delta 2\theta}{\tan \theta_0 \sin \theta_0} \right) + \eta^2$$

where $\delta 2\theta$ is the breadth at half-maximum intensity of the diffraction peak; $\theta_0$ is the position of the peak maximum; $k$ is particle shape factor, for spherical particles, $k=0.9$; $L$ is crystallite size and $\eta$ is defined as apparent strain. A linear plot of $\frac{(\delta 2\theta)^2}{\tan^2 \theta_0}$ against $\frac{\delta 2\theta}{\tan \theta_0 \sin \theta_0}$ is expected with slope of $\frac{k\lambda}{L}$ and intercept of $\eta^2$. The crystallite sizes and strains of B1 and B2 were calculated from the first and second orders of (220) diffraction. The results are listed in Table 3-6.

Table 3-6 Crystallite sizes and strains of B1 and B2 estimated using integral breadth method.

<table>
<thead>
<tr>
<th>Sample name</th>
<th>$L$, crystallite size (Å)</th>
<th>$\eta$, apparent strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>82</td>
<td>3.2x10^-3</td>
</tr>
<tr>
<td>B2</td>
<td>138</td>
<td>6.3x10^-3</td>
</tr>
</tbody>
</table>

It was found that the crystallite sizes estimated using integral breadth method and Scherer’s formula were similar. The contribution by strain broadening was therefore not significant. The strains were perhaps relieved by subsequent heat treatment after the milling process.

The bright field, dark field TEM and HRTEM images of B1 are shown in Fig. 3-17, respectively. The average particle size was 9.5 nm in the bright field image, which was consistent with the crystallite size estimated by X-ray line broadening. In the dark field image, each separate bright spot represented a crystallite. Its size and shape was
similar to the particle shown in bright field image. Combined with the HRTEM image in Fig. 3-17 (e), it suggested that each nickel ferrite particle was a single crystal.

The bright field, dark field TEM and HRTEM images of B2 are shown in Fig. 3-18, respectively. The average particle size was 146 nm in the bright field image. Each particle consisted of some small crystallites that were produced in the mechanical milling process. In the absence of NaCl, nickel ferrite particles tended to sinter and form aggregates during heat treatment. In the dark field image, the average crystallite size was 13.8 nm which was consistent with the crystallite size estimated by X-ray line broadening. In the HRTEM image Fig. 3-18 (e) which focused on the edge of a large particle, a crystallite of ~15 nm was observed. Compared with the crystallite size of B1, considerable crystallite growth was found in B2.
Figure 3-17 (a) Low magnification TEM image of B1, (b) corresponding histogram of particle size distribution (total counts: 50), (c) dark field image, (d) corresponding histogram of crystallite size distribution (total counts: 30), (e) HRTEM image of B1.
Figure 3-18 (a) Low magnification TEM image of B2, (b) corresponding histogram of particle size distribution (total counts: 30), (c) dark field image, (d) corresponding histogram of crystallite size distribution (total counts: 30), (e) HRTEM image of B2.
Figure 3-19 shows the relationship of zeta-potential vs. pH of B1 and B2. As mentioned earlier for bare nickel ferrite particles, the dependence of surface charge on pH was caused by surface absorbed hydroxyl groups (-OH). In an acidic environment, the surface shows positive charge due to the dissociation of -OH followed by the formation of -OH₂⁺. Meanwhile, the negative surface charge is expected due to the formation of -O⁻ in a basic environment.

The IEPs of B1 and B2 were within the range of 6-7 suggesting the surface charges varied from OH²⁺, OH to O⁻ with increasing pH. In this transition, the intrinsic acidity constant (pKₐ) is the dissociation point of water (pH ~7). It confirmed that there was no surface coating on B1 or B2.

![Figure 3-19 Relationship between zeta-potential and pH for B1 and B2.](image)

3.3 Summary

Nonhydrolytic sol-gel method and mechanochemical method were used to synthesize nickel ferrite particles. In the mechanochemical method, small (9.5±2.4 nm)
and large particles (146±27 nm) without surface coating were obtained. In the nonhydrolytic sol-gel method, by applying surfactants, as-prepared nickel ferrite particles were successfully coated with oleic acid. The zeta potential, FTIR, XPS measurements confirmed the presence of chemisorbed oleic acid and the formation of carboxylate on particle surface. By varying the composition of metal precursors, two microstructures could be achieved, i.e dispersed nanocrystals (9.7±1.8 nm) and aggregates (152±21 nm) consisted of many nanocrystals (8.1±1.3 nm). Instead of controlling morphology by directly changing the ratio of precursor to surfactant, variation of precursor compositions affected the concentration of surfactant. Oleic acid could complex with iron (III) ions, but not with nickel (II) ions. Increasing the concentration of iron precursor thus consumed more oleic acid and led to insufficient oleic acid coating on particle surface. Strong intercrystallite interaction was induced from less protected surface and was possibly the driving force for aggregation of nanocrystals.
Supporting information-1

To prove the effects of precursor composition on particle size, an intermediate ratio of nickel acetate tetrahydrate to iron acetate (1.0:2.7) was used in the synthesis. The same experimental procedures and conditions were followed. The obtained particles were denoted as C3. TEM image of C3 is shown in Fig. 3S-1. Different from C1 and C2, C3 composed of both small particles and large aggregates. The aggregates were irregular in shape. The morphology of C3 further confirmed that the composition in precursor was the key factor affecting the particle size.

![TEM image of C3](image)

Figure 3S-1 TEM image of C3. The molar ratio of nickel acetate tetrahydrate to iron acetate was 1.0:2.7.

Due to the formation of iron oleate complex, some oleic acid molecules could complex with iron acetate and the remaining could act as surfactant. Compared with C1 and C2, C3 possessed intermediate concentration of iron acetate in precursors. Therefore C3 was found as an intermediate situation of C1 and C2. Agglomeration occurred on some particles with less oleic acid coating, while some particles with sufficient coating were well separated. Thus both small particles and large aggregates were observed in Fig. 3S-1.
Supporting information-2

Aiming at synthesis of nickel ferrite particles with large sizes, as-milled particles were washed using deionized water for 3 times to remove NaCl. The obtained particles were heat treated at 400°C, 650°C and 900°C respectively for 2 h and cooled at the rate of 15°C/min. Figure 3S-2 shows the XRD patterns of obtained particles. It was found that crystallite size increased when the temperature increased from 400°C to 900°C.

![XRD patterns of particles after heat treatment in the absence of NaCl. Heat treatment temperature (a) 400°C, (b) 650°C, and (c) 900°C.](image)

The TEM images of above nickel ferrite particles are shown in Fig. 3S-3. The average particle size was approximately 39.5 nm, 146 nm and 415 nm when the heat treatment temperature was 400°C, 650°C, and 900°C, respectively. Without the separation of NaCl, sintering occurred with the increase of temperature. It was found that 650°C was the suitable temperature to obtain particles with similar size as C2.
Figure 3S-3 TEM images of nickel ferrite particles after heat treatment in the absence of NaCl at different temperatures and corresponding histograms of particle size distribution (total counts: 30). (a) and (b) 400°C, (c) and (d) 650°C, (e) and (f) 900°C.
Chapter 4 Composites of biodegradable polymer and nickel ferrite particles

To simplify the system, the biodegradable polymer was chosen as poly (D, L-lactide) PLA, an amorphous polymer widely used for medical and pharmaceutical applications\(^1\). PLA used in this study was purchased from Sigma-Aldrich and was completely amorphous with molar molecular weight of 75,000-120,000.

4.1 PLA microspheres

4.1.1 Experimental procedures

Double emulsion method is the most convenient and practical method to prepare colloidal spheres of PLA\(^2\). The technique includes formation of a water/oil (w/o) emulsion of an aqueous solution in an organic solution of the polymer. This emulsion is used to prepare a new emulsion in an aqueous surfactant solution so as to finally obtain a water/oil/water (w/o/w) double emulsion. In our study, a solution of PLA in dichloromethane (DCM) was first prepared. Water was then introduced to the organic solution and the mixture was homogenized with a Diax 900, Heidolph (Germany) homogenizer for 10 s. A whitish, slightly transparent emulsion was obtained. The emulsion was added to a poly(vinyl alcohol) (PVA) solution, and homogenized for another 10 s to obtain a second emulsion with white color. The next step was organic solvent evaporation, achieved by stirring the solution at room temperature with a mechanical stirrer. The polymer suspension was then subject to a cleaning process that included repeated cycles of centrifugation (10,000 rpm) and redispersion in deionized
water. The aqueous suspension was freeze dried for 24 h to obtain PLA spheres. The above experimental procedures are briefly illustrated in Fig. 4-1.

4.1.2 Effect of processing parameters on the size of PLA microspheres

There are several processing parameters which affect the size of PLA microspheres, such as PLA concentration, PVA concentration, DCM-to-water volume ratio, and homogenizer speed. Experiments were carried out by varying one but holding the other parameters constant to investigate the effects of individual parameter on the size of PLA microspheres. The results are listed in Tables 4-1 to 4-4. The particle size was averaged by counting 100 particles in the SEM picture.
Table 4-1 Mean diameters of PLA microspheres using different PLA concentrations. *

<table>
<thead>
<tr>
<th>PLA concentration (% w/v)</th>
<th>Mean diameter of microspheres (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.0</td>
</tr>
<tr>
<td>2</td>
<td>5.0</td>
</tr>
<tr>
<td>3</td>
<td>9.0</td>
</tr>
</tbody>
</table>

*PVA concentration (1.0 % w/v), DCM-to-water ratio (1:20) and homogenizer speed (3 x 3000 rpm).

Table 4-2 Mean diameters of PLA microspheres using different PVA concentrations. *

<table>
<thead>
<tr>
<th>PVA concentration (% w/v)</th>
<th>Mean diameter of microspheres (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>2</td>
<td>1.0</td>
</tr>
<tr>
<td>3</td>
<td>4.0</td>
</tr>
<tr>
<td>4</td>
<td>8.0</td>
</tr>
</tbody>
</table>

*PLA concentration (5.0 % w/v), DCM-to-water ratio (1:20) and homogenizer speed (3 x 3000 rpm).

Table 4-3 Mean diameters of PLA microspheres using different DCM-to-water ratios.*

<table>
<thead>
<tr>
<th>DCM-to-water ratio</th>
<th>Mean diameter of microspheres (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1:5</td>
</tr>
<tr>
<td>2</td>
<td>1:10</td>
</tr>
<tr>
<td>3</td>
<td>1:20</td>
</tr>
</tbody>
</table>

* PLA concentration (5.0 % w/v), PVA concentration (1.0 % w/v) and homogenizer speed (3 x 3000 rpm).

Table 4-4 Mean diameters of PLA microspheres using different homogenizer speeds. *

<table>
<thead>
<tr>
<th>Homogenizer speed (rpm)</th>
<th>Mean diameter of microspheres (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1x3000</td>
</tr>
<tr>
<td>2</td>
<td>2x3000</td>
</tr>
<tr>
<td>3</td>
<td>3x3000</td>
</tr>
<tr>
<td>4</td>
<td>4x3000</td>
</tr>
<tr>
<td>5</td>
<td>5x3000</td>
</tr>
</tbody>
</table>

*PLA concentration (5.0 % w/v), PVA concentration (1.0 % w/v) and DCM-to-water ratio (1:20).

From the above results, it was suggested that low PLA concentration, high PVA concentration, low volume ratio of DCM-to-water, and high homogenizer speed were suitable to achieve small PLA microspheres. On the other hand, for synthesizing large microspheres, the suitable conditions were high PLA concentration, low PVA concentration, high volume ratio of DCM-to-water, and low homogenizer speed.
Experimental parameters listed in Table 4-5 were used for the synthesis of PLA microspheres and their composites with nickel ferrite particles for further studies. The obtained particle size was $\sim 1.5 \mu m$.

Table 4-5 Experimental parameters used for the synthesis of PLA microspheres and their composites with nickel ferrite particles.

<table>
<thead>
<tr>
<th>PLA concentration</th>
<th>PVA concentration</th>
<th>Volume ratio of DCM-to-water</th>
<th>Homogenizer speed</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0 % w/v</td>
<td>1.0 % w/v</td>
<td>1:20</td>
<td>3x3000 rpm</td>
</tr>
</tbody>
</table>

4.2 Preparation of composites of nickel ferrite particles and PLA

Nickel ferrite particles with or without oleic acid coating was used to form composites with PLA. To separate the size effect, particles with similar sizes were discussed here.

4.2.1 Nickel ferrite particles without surface coating

As previously described in 3.2.1., B1 with particle size 9.5±2.4 nm and B2 with particle size 146±27 nm were used for the study in this chapter.

4.2.2 Introducing oleic acid coating using sonication

1 g B1 (or B2) was mixed with 15 ml 0.1 M oleic acid aqueous solution. The suspension was held in ultrasonic bath for 30 min. The obtained particles were centrifuged and washed three times using deionized water. In the following discussion, “B1+S” or “B2+S” was used to denote particles prepared through above procedures.
4.2.3 Nickel ferrite particles with oleic acid coating

C1 with size of 9.7±1.8 nm and C2 with size of 152±21 nm were used in this chapter.

4.2.4 Preparation of nickel ferrite/PLA composites

The procedures were similar to that described for the preparation of PLA microspheres except that the first water-in-oil emulsion was substituted by an (aqueous suspension of nickel ferrite)-in-oil emulsion. We first prepared a 5.0 % w/v solution of PLA in 2 ml DCM. To this solution, 2 ml suspension of nickel ferrite particles in water with the concentration of 3.0 % w/v (unless noted specifically) was added. The mixture was homogenized. Finally, 38 ml 1.0 % w/v PVA solution was added and again homogenized. The solvent evaporation was achieved using mechanical stirring. The cleaning routines described for the PLA microspheres were also used for the composites. B1/PLA, (B1+S)/PLA, C1/PLA, B2/PLA, (B2+S)/PLA and C2/PLA were used to denote the composites discussed in this chapter.

4.3 Morphologies of composites

Figure 4-2 shows the SEM images of pure PLA microspheres, nickel ferrite nanoparticles B1 and their composites. Pure PLA microspheres (a) showed very smooth surface. Sample B1 (b) showed agglomeration of small nanoparticles. After mixing with PLA (c), the exterior surface of PLA spheres changed from smooth to irregular and some small particles with similar morphology as B1 were found on PLA surface.
Figure 4-2 SEM images of (a) pure PLA microspheres, (b) nickel ferrite nanoparticles B1 and (c) their composites.

Figure 4-3(a) shows the SEM images of nickel ferrite particles with oleic acid coating (C1). After C1 formed composites with PLA, as shown in Fig. 4-3(b), some small particles with smooth surface were observed, and their sizes were significantly smaller than the sizes of PLA and were slightly larger than those of C1. It might indicate that C1 was encapsulated by PLA. To elucidate the structure of encapsulation, the microspheres were immersed in DCM to dissolve the PLA. After immersion for 30 min, the SEM image in Fig. 4-3(c) shows that the surface of the small particles became rough and morphologies similar to C1 emerged. At the same time, large PLA spheres were eroded to irregular morphology.
Figure 4-3 SEM images of (a) nickel ferrite nanoparticles C1, (b) the composite of C1 and PLA and (c) the composite after immersed in DCM for 30 min.

The SEM image of B1+S (Fig. 4-4(a)) does not show significant differences from the image of B1 in Fig. 4-2(b). After mixing with PLA (Fig. 4-4(b)), some B1+S particles were encapsulated by PLA spheres as its morphology was very similar to Fig. 4-3(b). Some B1+S particles were also found on PLA surface, as indicated with arrows at the top right corner of the picture.

In C1, the particle surface was hydrophobic because oleic acid aligned in the way that carboxylic groups attached to ferrite surface and alkyl groups exposed (as shown in Fig. 3-13(b)). During double emulsion process, nanoparticles with hydrophobic surface were readily dispersed in organic phase where PLA was fully dissolved. With evaporation of DCM solvent, PLA microspheres precipitated with encapsulation of nickel
ferrite particles inside the spheres. On the contrary, sample B1 with hydrophilic surface was predominantly dispersed in aqueous solution. It could only adsorb on the surface of PLA spheres after the spheres precipitated. In B1+S which were prepared from an oleic acid aqueous solution, both hydrophilic and hydrophobic surface existed. It resulted in mixed microstructures of encapsulation and adsorption.

![Figure 4-4 SEM images of (a) B1+S and (b) the composite of B1+S and PLA.](image)

SEM images of B2, B2+S and C2 in comparison with B2/PLA, (B2+S)/PLA and C2/PLA are shown in Fig. 4-5. It confirmed that particles without oleic acid coating (B2) were adsorbed on PLA surface. However, nickel ferrite particles with oleic acid coating (B2+S and C2) were also adsorbed on the surface of PLA spheres. Compared with the morphology of C1/PLA, no obvious encapsulation was found in C2/PLA. It was perhaps because the oleic acid coating on C2 was not as sufficient as C1 for encapsulation. In this sense, the series of large particle/PLA composites (B2/PLA, (B2+S)/PLA, and C2/PLA) were not as representative as small particles/PLA composites because the latter was composed of adsorption, encapsulation and a mixed situation. Moreover, some distinct defects of PLA microspheres were found in C2/PLA, such as wrinkles on the PLA surface and holes in the PLA spheres. These defects could deteriorate the
interconnectivity of PLA spheres which made the study of composite properties complicated. Consequently only the series of B1/PLA, (B1+S)/PLA and C1/PLA were used in the following discussion.

Figure 4-5 SEM images of (a) B2, (b) B2/PLA, (c) B2+S, (d) (B2+S)/PLA and (e) C2 and (f) C2/PLA. Some distinct defects in C2/PLA were indicated using arrows such as wrinkles on PLA surface, and holes in PLA spheres.
4.4 Surface charges of composites

As discussed in 3.1.4, zeta potentials of nickel ferrite particles depended on pH values. This dependence was not expected for PLA due to its non-polar nature. The obvious differences between nickel ferrite and PLA in zeta potentials allowed effective evaluation of surface properties of the composites.

The relationship between zeta-potential and pH of PLA, B1, B1+S, B1/PLA and (B1+S)/PLA is shown in Fig. 4-6. Because there were no potential-determining groups on PLA surface, zeta potentials of PLA were nearly zero at all measured pH values. For B1, the IEP was ~6.0 because their surface charges varied from OH\(^{2+}\), OH to O\(^{-}\) with increasing pH. The IEP of B1/PLA (5.7) did not change significantly from that of B1. The reason was probably because introduction of PLA did not affect the potential-determining groups in the system. Considering the dependence of zeta potential values on pH in the whole pH range, B1/PLA showed a distinctly different trend from that of PLA but similar to that of B1. This indicated that the surface of B1/PLA was mainly nickel ferrite particles and it was consistent with the SEM observation. After B1 particles were treated with oleic acid using sonication (B1+S), their zeta-potential results suggested an IEP shift from 6.0 to 4.6, which was close to the range of pK\(_{a}\) of carboxylic acid (pH=3-4). It implied that some oleic acid molecules were successfully coated on these inorganic particles. At a specific pH, the zeta potential value of (B1+S)/PLA generally lay in between the values of B1+S and PLA. It suggested that both nickel ferrite and PLA surface were present in the composite. It agreed well with the SEM observation in which both encapsulation and adsorption were found.
Figure 4-6 Relationship between zeta-potential and pH of pure PLA, B1, B1+S, B1/PLA and (B1+S)/PLA.

Figure 4-7 shows the relationship between zeta-potential and pH of C1 and its composite with PLA. The IEP of C1 was 3.5 suggesting that the surface charge was changed by oleic acid. This IEP was lower than the IEP of B1+S (IEP=4.6 in Fig. 4-6). The difference in the IEP values indicated that the coverage of oleic acid by physical method (sonication) (B1+S) was incomplete as compared to that obtained by chemical method (C1). As suggested in SEM image (Fig. 4-3(b)), encapsulation of C1 nanoparticles in PLA spheres was found in the composite of C1/PLA. However, in Fig. 4-7, the zeta potential of C1/PLA was not very similar to that of PLA at many measured pH values. It implied that the encapsulation was incomplete and nickel ferrite surface was also present in the composite.
Figure 4-7 Relationship between zeta-potential and pH of pure PLA, C1 and C1/PLA.

4.5 Surface energies of PLA and nickel ferrite particles

Surface energy is defined as the work required increasing the area of a substance by one unit area. Calculation of surface energy of a solid is based on measurement of contact angles of a series of liquids on solid surface. Geometric mean and harmonic mean are two important methods for such calculation. It is found that the total surface energies of the solid obtained by the two methods are generally close, and neither of the two conceptually different equations is clearly incompatible with the available experimental data. However, geometric mean approach is preferable because it consistently fits the data better. In our following study, only geometric mean was used for analysis.

Geometric mean equation can be described as

\[ (1 + \cos \theta) \gamma_L = 2 \left( \sqrt{\gamma_S^d \gamma_L^d} + \sqrt{\gamma_S^p \gamma_L^p} \right) \]

\[ \gamma_L = \gamma_L^d + \gamma_L^p \]
where $\gamma^d$ is the dispersive component and $\gamma^p$ is the polar component of the surface energy, $\gamma_L$ and $\gamma_S$ are the surface energies of liquid and solid, respectively and $\theta$ is the contact angle. Since this equation has two unknowns, i.e. $\gamma_S^d$ and $\gamma_S^p$, the contact angle data from two liquids are necessary. In general the liquids used are water (polar) and methyleneiodide (non-polar). For more precise calculation, another polar liquid formamide was also used. The surface energy was averaged using values derived from water/methyleneiodide pair and formamide/methyleneiodide pair.

The literature values for the surface energy components of tested liquids are given in Table 4-6.

<table>
<thead>
<tr>
<th>Liquid</th>
<th>Surface energy and its components (mJ/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\gamma_L$</td>
</tr>
<tr>
<td>Water</td>
<td>72.8</td>
</tr>
<tr>
<td>Formamide</td>
<td>58.2</td>
</tr>
<tr>
<td>Diiodomethane</td>
<td>50.8</td>
</tr>
</tbody>
</table>

The contact angles of different nickel ferrite particles and PLA are listed in Table 4-7.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Contact angle (deg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water</td>
</tr>
<tr>
<td>PLA</td>
<td>82.9±0.3</td>
</tr>
<tr>
<td>B1</td>
<td>14.5±1.3</td>
</tr>
<tr>
<td>B1+S</td>
<td>70.0±0.5</td>
</tr>
<tr>
<td>C1</td>
<td>50.1±0.4</td>
</tr>
</tbody>
</table>

Surface energies calculated using the software VCAOpitmaXE are listed in Table 4-8. The mean values of contact angle were used for the calculation.
Table 4-8 Surface energies and their components of nickel ferrite particles and PLA.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Surface energy and its components (mJ/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\gamma_s$</td>
</tr>
<tr>
<td>PLA</td>
<td>35.0</td>
</tr>
<tr>
<td>B1</td>
<td>66.5</td>
</tr>
<tr>
<td>B1+S</td>
<td>50.0</td>
</tr>
<tr>
<td>C1</td>
<td>37.3</td>
</tr>
</tbody>
</table>

The dispersive component $\gamma^d$ of surface energy arises from one intermolecular interaction called dispersion force. It exists in all types of matters and always provides an attractive force between adjacent atoms or molecules, no matter how dissimilar their nature may be. As shown in Table 4-8, the dispersive components of four tested materials were very similar.

The polar component of surface energy is not limited to dipole interactions but includes all of the non-dispersive forces such as hydrogen bonding, metallic bonding, electrostatic forces, etc. From Table 4-8, polar components increased with surface charge. PLA with non-polar surface and C1 with oleic acid protected surface had very low values of polar component. As revealed in zeta potential measurements, sample B1 was highly charged due to adsorption of -OH. This was also consistent with the high polar component in surface energy. In B1+S, as expected, an intermediate value was found.

Interfacial energy is one of the key parameters to determine the adhesion between two different components in a composite. It is also the most accessible parameter that describes the thermodynamic state and structure of interface. Interfacial energy between particles (S1) and polymer (S2) in the composite can be calculated by

$$\gamma_{S1/S2} = \gamma_{S1} + \gamma_{S2} - 2\sqrt{\gamma_{S1}^d \gamma_{S2}^d} - 2\sqrt{\gamma_{S1}^p \gamma_{S2}^p}$$
The calculated interfacial energies between nickel ferrite particles and PLA are shown in Table 4-9.

<table>
<thead>
<tr>
<th>Composite</th>
<th>Interfacial energy (mJ/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1/PLA</td>
<td>15.8</td>
</tr>
<tr>
<td>(B1+S)/PLA</td>
<td>3.7</td>
</tr>
<tr>
<td>C1/PLA</td>
<td>0.2</td>
</tr>
</tbody>
</table>

A stable interface possessed an interfacial energy such that work must be done to extend or enlarge the interface, i.e. a positive interfacial energy. In a two-component system, if the presence of the interface resulted in a higher interfacial energy, the interface was spontaneously reduced to a minimum - the two components tended to separate to the greatest possible extent within the constraints. Thus less adhesion between the two components was expected. Mittal also did a careful analysis of the existing literature and revealed that the lower the interfacial energy, the higher the adhesive strength7. Based on above reason, for achieving an optimum mixing, it is important to reduce the interfacial energy. As a result, C1/PLA was the best-mixed composite in our current study.

4.6 Interaction between PLA and nickel ferrite particles

It is known that mixing of nanoparticles with a polymer matrix can bring about changes in the mechanical and thermal characteristics of the resulting composite8. One of the unique features of nanoparticles is their extremely high specific surface area. For a well-dispersed system, even low filler volume fractions provide an enormous amount of interfacial area through which the bulk properties of the polymer can be altered9. A
benchmark used to compare interaction between different components in a composite is the glass transition temperature, $T_g$.

DSC was a conventional method to determine $T_g$ and a baseline shift is expected because heat capacity ($C_p$) changed during the transition from glassy state to rubbery state. The DSC result of pure PLA is shown in Fig. 4-8. Different from normal DSC curves, an endothermic peak was observed instead of a baseline shift. This peak was attributed to a relaxation endotherm which occurred accompanying the glass transition. The peak was so obvious that could superimpose on the baseline shift. Such relaxation endotherm phenomenon had been found in some highly amorphous substances\textsuperscript{10}. It was reported that the peak of the endotherm relaxation in non-reversing heat flow was well fitted with the glass transition in reversing heating flow (as shown in Fig. 4-9)\textsuperscript{11}. Compared to the method using the midpoint of two extrapolations of $C_p$ to determine $T_g$, the position of endotherm peak was more convenient to identify. In another work on PLA, the peak position was directly interpreted as $T_g$\textsuperscript{12}. In our following discussion on $T_g$, we also use the peak position to represent the $T_g$ of the composite. As seen, the endothermic peak was found to be 58.7\textdegree C in pure PLA, which is consistent with the other’s report on $T_g$\textsuperscript{13}. 


Figure 4-8 DSC curve of pure PLA. An endothermic peak was observed instead of a baseline shift.

Figure 4-9 Separation of glass transition in reversing heating flow and the endothermic relaxation in non-reversing heat flow.\textsuperscript{11}

DSC curves of composites B1/PLA, (B1+S)/PLA and C1/PLA are shown in Fig. 4-10 (a), (b) and (c), respectively. Two nickel ferrite concentrations (0.6 % w/v and 3.0 % w/v) were studied in each composite.
In all cases, it was noted that $T_g$ of composites shifted to lower temperatures. $T_g$ decreased further when the concentration of nickel ferrite particles was increased from 0.6 % w/v to 3.0 % w/v. Lower $T_g$ of PLA composites compared with pristine PLA had been reported before\textsuperscript{14}. In the reported system containing irinotecan hydrochloride particles (anti-tumor agent) and PLA, two glass transitions were found. The new peak appeared at lower temperature was due to the phase transition of particle-miscible PLA. The decrease of $T_g$ was attributed to the proportional change of these two peaks\textsuperscript{14}. In our composites, nickel ferrite nanoparticles and PLA were not miscible because the DSC
results only indicated one endothermic relaxation, which was the \( T_g \) of pristine PLA. In other word, nickel ferrite nanoparticles and PLA were mixed but could not form a homogeneous phase. The decreases in \( T_g \) may be attributed to the interaction between PLA and nanoparticles at the interfacial regions. As reported, the interaction of polymer chains with the surface of the particles can drastically alter the chain kinetics in the region immediately surrounding the nanoparticle\(^{15}\). Depending upon the strength of the interaction, the interface region could either have a lower or higher mobility than the bulk material. It resulted in an increase\(^{16}\) or decrease\(^{17}\) in \( T_g \), respectively.

The interaction between polymer and inorganic nanoparticles can be defined by (1) chemical interaction which introduce new chemical bond between two phases. For example, in alumina/PMMA composites, PMMA could react with alumina surfaces and form ionic bonds through hydrolization of the ester bond in the side chain of the polymer\(^{18}\) or (2) physical interactions which only involve physical changes before and after mixing, such as changes of interparticle distances and changes of interconnectivity of polymer regions\(^{19}\), etc. To show whether there is formation of new chemical bond, FTIR was used to characterize the composites in comparison with pure PLA and pure nickel ferrite particles.

FTIR spectra of PLA, B1, B1/PLA are shown in Fig. 4-11. The strongest peak in B1 was located at 586 cm\(^{-1}\), which was attributed to Fe-O stretch and was the characteristic peak of nickel ferrite. Comparing the FTIR results of PLA and B1/PLA, there was no apparent difference between the two curves, except for the distinct peak at 586 cm\(^{-1}\). It suggested that the nickel ferrite particles were successfully loaded in PLA. However, there was no detectable new chemical bond between B1 and PLA.
FTIR spectra of PLA, B1+S and (B1+S)/PLA are shown in Fig. 4-12. In Fig. 4-12 (a), the characteristic peak of nickel ferrite at 586 cm$^{-1}$ was found in B1+S. Alkyl stretches became visible in the range of 2400-3400 cm$^{-1}$, as shown in Fig. 4-12 (b). The peaks at 2963, 2922 and 2856 cm$^{-1}$ in the spectrum of B1+S were assigned to asymmetric methyl and methylene stretches $\nu_{as}(\text{CH}_3)$, $\nu_{as}(\text{CH}_2)$, and the symmetric methylene stretch $\nu_{s}(\text{CH}_2)$, of oleic acid molecule, respectively. This indicated that using ultrasonic treatment oleic acid could be successfully introduced to the surface of nickel ferrite particles. In the spectra of PLA in Fig 4-12 (b), peaks of 2998, 2948 and 2886 cm$^{-1}$ were attributed to asymmetric methyl and C-H stretches $\nu_{as}(\text{CH}_3)$, $\nu_{as}(\text{CH})$, and the symmetric C-H stretch $\nu_{s}(\text{CH})$, of PLA molecule, respectively. There was a shift in the position compared to the stretches in oleic acid because of the influence of -C-O-C- and C=O on the CH$_3$ and CH groups in PLA molecular chain. Comparing the spectra of B1+S and
(B1+S)/PLA in Fig.4-12 (a) and (b), no new chemical bond was found between PLA and B1+S.

Figure 4-12 FTIR spectra of PLA, B1+S, and (B1+S)/PLA. (a) in full scan range 400-4000 cm$^{-1}$ and (b) in alkyl vibration range 2400-3400 cm$^{-1}$. 
FTIR spectra of C1, PLA and C1/PLA are shown in Fig. 4-13. In the spectrum of C1, two adsorptions at 1565 and 1415 cm\(^{-1}\) showed that carboxylate group was connected to nickel ferrite surface through one oxygen atom. After C1 particles were mixed with PLA, again, the only detectable difference was the peak at 586 cm\(^{-1}\) that was attributed to nickel ferrite.

![Figure 4-13 FTIR spectra of C1, PLA and C1/PLA.](image)

From Fig. 4-11, 4-12 and 4-13, it could be concluded that no new chemical bond was detected between nanoparticles and PLA. The shift of \(T_g\) was not attributed to chemical interactions. The observed change in \(T_g\) was therefore caused by physical interaction at the interfacial regions of the composites. It was reported that \(T_g\) of ultrathin films of PS and PMMA was correlated with the interfacial energies between polymer film and the substrate. A linear relationship was proposed between interfacial energy and \(\Delta T_g = (T_g^{\text{film}} - T_g^{\text{bulk}})^2\). Figure 4-14 shows the relationship between interfacial energy and \(\Delta T_g = (T_g^{\text{composite}} - T_g^{\text{PLA}})\) for our nickel ferrite nanoparticles/PLA composites. There were
three kinds of composites, i.e. B1/PLA, (B1+S)/PLA and C1/PLA. Their interfacial energies were obtained from Table 4-9. Good linearity was found in two additive concentrations, i.e. 0.6 % w/v and 3.0 % w/v. The results suggested that the difference in interfacial energies could account for the decrease in $T_g$ in our work.

![Figure 4-14 Relationship between interfacial energy and $\Delta T_g$ in three kinds of composites with two different concentrations.](image)

4.7 Loading capacity of nickel ferrite particles

Spectrophotometric and chromatographic methods were used to determine the amount of loading and free additives in composite system as long as the additives were photo-sensitive\(^{21}\). However, in our work, nickel ferrite particles could not be detected by spectroscopic means. The magnetic properties of nickel ferrite particles were instead studied. It was reported that surface modification increased the saturation magnetization of manganese ferrite nanoparticles when there were surface coordination ligands. These ligands were easily detected by FTIR\(^{22}\). Based on FTIR spectra (Fig. 4-11, 4-12 and 4-13),
there was no new chemical bond between PLA and nickel ferrite particles. We therefore assumed that PLA matrix did not contribute to the magnetization. The magnetizations of the composites were proportional to the mass of nickel ferrite particles that were properly loaded. Magnetizations of as-synthesized nickel ferrite particles and their composites with PLA were measured at room temperature. All the magnetization curves did not saturate at 50 kOe. It was due to the superparamagnetic properties of B1, B1+S and C1. Table 4-10 lists the experimental magnetization values at 50 kOe and the ideal magnetizations if all nickel ferrite nanoparticles were properly loaded to PLA. The loading capacity was calculated by taking the ratio of the experimental to the ideal magnetization.

The loading capacity was as high as 100% when the nickel ferrite particles were not coated with oleic acid. It meant that nearly all magnetic particles were successfully adsorbed on the surface of PLA microspheres even if the nickel ferrite concentration was as high as 3.0 % w/v. However, the microstructures consisted of nanoparticles adsorbed on PLA were not suitable for application as drug carrier because PLA degraded via surface erosion. With the degradation of PLA, magnetic particles on PLA surface would be removed, significantly deteriorating the magnetic properties of the composites.
Table 4-10 Loading capacities of nickel ferrite particles in PLA calculated by dividing the experimental magnetization by the ideal magnetization.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Experimental magnetization at 50 kOe (emu/g)</th>
<th>Ideal magnetization (emu/g)</th>
<th>Loading capacity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure PLA</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B1</td>
<td>49.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B1 0.6 % w/v + PLA</td>
<td>5.3</td>
<td>5.2</td>
<td>101.9%*</td>
</tr>
<tr>
<td>B1 3.0 % w/v + PLA</td>
<td>19.5</td>
<td>18.4</td>
<td>106.0%*</td>
</tr>
<tr>
<td>B1+S</td>
<td>42.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(B1+S) 0.6 % w/v + PLA</td>
<td>3.3</td>
<td>4.1**</td>
<td>80.5%</td>
</tr>
<tr>
<td>(B1+S) 3.0 % w/v + PLA</td>
<td>12.4</td>
<td>14.2**</td>
<td>87.3%</td>
</tr>
<tr>
<td>C1</td>
<td>27.3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C1 0.6 % w/v + PLA</td>
<td>0.9</td>
<td>2.2***</td>
<td>41.0%</td>
</tr>
<tr>
<td>C1 3.0 % w/v + PLA</td>
<td>4.2</td>
<td>7.7***</td>
<td>54.2%</td>
</tr>
</tbody>
</table>

*The calculated loading capacity was slightly higher than 100% which was probably due to the experimental errors in the measurement of magnetization.

**Based on TGA result (not shown), 90 wt% of (B1+S) was nickel ferrite.

***Based on Fig. 3-8, 76 wt% of C1 was nickel ferrite.

With the introduction of oleic acid onto particle surface (either by physical or chemical method), the loading capacity reduced significantly. The capacities were also different between (B1+S)/PLA and C1/PLA as shown in Table 4-10. Higher capacity in (B1+S)/PLA could be explained by the adsorption of (B1+S) on PLA surface, as revealed in Fig. 4-4 (b). The adsorption was the same as in B1/PLA because the surfaces of some (B1+S) particles were not properly modified by oleic acid. The above discussion suggested that loading capacity was reduced if encapsulation instead of adsorption occurred.

Figure 4-15 presents the effects of C1 concentration on magnetization in C1/PLA. The dotted line indicated the ideal magnetization. Experimental magnetization of the composite increased with increasing C1 concentration and was significantly lower than the ideal value. The actual loading capacities did not increase significantly with the
increase of concentration. Further studies aiming to increase loading capacity are required to enhance the magnetic response of the composite.

Figure 4-15 The function of experimental magnetizations in C1/PLA and loading capacities with different C1 concentrations. The dotted line suggested the ideal magnetization.

4.8 Summary

Nickel ferrite nanoparticles with or without oleic acid surface coating were respectively used to form composites with PLA. The morphologies, surface charges, surface energies of the obtained composites and interface energies, interactions between nickel ferrite nanoparticles and PLA were studied. If the nanoparticles were prepared without oleic acid coating, they could attach to the PLA surface. For the nanoparticles coated with oleic acid, they were readily encapsulated within the PLA microspheres. Since no new chemical bond was found between the particles and PLA, the mixing was physical in nature. The observed decrease of $T_g$ could be related to the difference in interfacial energies between the two components. The best-mixed composite (C1/PLA)
was achieved by reducing the interfacial energy. However, its loading capacity was limited. Increasing the amount of nickel ferrite nanoparticles was not useful to increase the loading capacity.


Chapter 5 Cytotoxicity studies of nickel ferrite particles

5.1 MTT assay to assess cytotoxicity

Neuro-2A (mouse neuroblastoma, CCL-131, ATCC) cell line was cultured and maintained in Dulbecco's Modification of Eagle's Medium (DMEM, provided by National University Medical Institutes (NUMI)) supplemented with 10% Fetal Bovine Serum (FBS) and maintained at 37°C under a humidified atmosphere with 5% CO₂.

Cells were seeded into 96-well plates at the density of ~20,000 cells/well in DMEM supplemented with 10% FBS. Different concentrations of nickel ferrite particles were suspended in serum free medium using ultrasonic bath for 1 h to prevent agglomeration. After plating cells for 28 h, the medium with 10% FBS was removed and replaced by 200 μl of particle suspensions with different concentrations. Controls were carried out with cells treated with an equivalent volume of serum-free medium without any particles. Cells were incubated for another 40 h with test particles before the cell viability assay was performed.

Cytotoxicity was assessed using an MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) test to measure the succinate dehydrogenase mitochondrial activity. Plates were centrifuged at 2000 rpm for 10 min to harvest total cell contents. Hundred μl of fresh media with 20 μl 5 mg/ml MTT stain (Sigma-Aldrich in vitro toxicology assay kit) were added into each well and incubated for 4 h. During incubation, formazan crystal of purple color developed in living cells. Acidic isopropyl alcohol was used to dissolve formazan after incubation. The absorbance in each well was measured at 570 nm in the BIO-RAD 680 microplate reader. Background absorbance was measured
in medium containing net particles (without cells) and net cells (without particles). All experiments were repeated between 5~15 times to ensure reproducibility. Statistical analysis was carried out using two-tailed paired t-test with a level of 95% significance.

Figure 5-1 shows the typical observed morphologies of Neuro2A cells of control (without any particles), about 50% viabilities and about 10% viabilities. In control, Neuro2A cells were found to differentiate and acquire neuritis after 40 h. In cytotoxic conditions, the cell membranes were compromised and distinct cell shape were no longer visible (b and c).

Figure 5-1 Morphologies of Neuro2A cells. (a) control, (b) about 50% viabilities and (c) about 10% viabilities.
5.2 Cytotoxicity of nickel ferrite particles

The cytotoxicity of oleic acid coated nickel ferrite particles was studied and compared with that of non-coated nickel ferrite particle. The surface coating effect and size effect of nickel ferrite particles were investigated.

5.2.1 Experimental methods to prepare particles with different sizes and coatings

Nickel ferrite particles without oleic acid coating were prepared using mechanochemical method. Two particle sizes, B1 (9.5 ± 2.4 nm) and B2 (146 ± 27 nm) were used. The synthesis and properties of B1 and B2 were discussed in Chapter 3. Nickel ferrite particles with limited coating of oleic acid (hydrophobic surface) were prepared by nonhydrolytic sol-gel method (also described in Chapter 3). C1 and C2 with respective size of 9.7 ± 1.8 nm and 152 ± 21 nm were used in the study. The particles with 2 layers of oleic acid coverage (hydrophilic surface) were prepared by re-dispersing C1 or C2 in excess oleic acid solution and mixed in an ultrasonic bath. The coverage of the 2nd layer of oleic acid was confirmed by zeta-potential and TGA measurements. We used C1+S and C2+S to denote the small and large particles with 2 layers of oleic acid coating. In summary, Table 5-1 shows the denotations of nickel ferrite particles used in the cytotoxicity study.

<table>
<thead>
<tr>
<th>No. of oleic acid layers on particle surface</th>
<th>Small size</th>
<th>Large size</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>B1</td>
<td>B2</td>
</tr>
<tr>
<td>1</td>
<td>C1</td>
<td>C2</td>
</tr>
<tr>
<td>2</td>
<td>C1+S</td>
<td>C2+S</td>
</tr>
</tbody>
</table>
5.2.2 Characterization of prepared nickel ferrite particles

A. Characterization of size and surface areas

Figure 5-2 shows the TEM micrographs of the nickel ferrite particles used in the cytotoxicity study. Small and large particles without oleic acid coating are shown in Fig. 5-2 (a) and (b), whereas those coated with 1 layer of oleic acid are shown in Fig. 5-2 (c) and (d). The TEM micrographs of particles coated with 2 layers of oleic acid are shown in Fig. 5-2 (e) and (f), respectively.

Table 5-2 summarizes the properties of as-synthesized nickel ferrite particles. Surface atomic composition and bulk atomic composition were compared. The compositions appeared to depend on the synthetic methods and the particle sizes. The surface composition was very similar to the bulk composition, indicating there was no composition segregation at the surface. The surface areas of individual particle were calculated using the average particle size and the results are also listed in Table 5-2. In a system of particles with fixed mass i.e. 1 mg, the surface area of individual particle increased with increasing particle size. However, the total surface area of all particles increased with decreasing particles size because the actual number of particles increased dramatically.
Figure 5-2 TEM micrographs of nickel ferrite particles of different sizes with 0, 1 or 2 layers of oleic acid coating (a) uncoated small particles, (b) uncoated large particle, (c) small particles with 1 layer of oleic acid coating, (d) large particle with 1 layer of oleic acid coating, (e) small particles with 2 layers of oleic acid coating, and (f) large particle with 2 layers of oleic acid coating.
Table 5-2 Properties of nickel ferrite particles used in the cytotoxicity study.

<table>
<thead>
<tr>
<th>Sample name</th>
<th>B1</th>
<th>C1</th>
<th>C1+S</th>
<th>B2</th>
<th>C2</th>
<th>C2+S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Particle size (nm) *</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>150</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>Surface area per nickel ferrite particle (m²)</td>
<td>$3.1 \times 10^{-16}$</td>
<td>$7.1 \times 10^{-14}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The number of nickel ferrite particles **</td>
<td>$3.7 \times 10^{14}$</td>
<td>$1.1 \times 10^{11}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total surface area (m²)***</td>
<td>$1.1 \times 10^{-1}$</td>
<td>$7.8 \times 10^{-3}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* For consistency, for the smaller particles, their sizes were rounded to the nearest whole number. For the larger particles, sizes were rounded to the nearest ten.

** Use total mass 1 mg as an example. The mass of individual nickel ferrite particle was calculated by $d \times \frac{4}{3} \pi \left(\frac{D}{2}\right)^3$ where $d$ is the density and $D$ is the particle size. The density used here was the bulk density of nickel ferrite, i.e. 5.368 g/cm.⁴

*** Multiply surface area per particle and the number of particles.

B. Characterization of surface charges

Table 5-3 lists the IEPs of different nickel ferrite particles and the zeta potential values measured at two extreme pHs (pH = 2.3 and pH = 11.5). For particles without oleic acid (B1 & B2), their IEPs were within the range of 6-7 because their surface charges varied from OH²⁺, OH to O⁻ with increasing pH. If nickel ferrite particles were treated by excess oleic acid (C1+S & C2+S), their IEP shifted to lower pH which is in the range of pKₐ of carboxylic acid (pH=3-4)². In this case, there were two layers of oleic acid coated on the particle surface with the outer layer being hydrophilic. The first layer of oleic acid aligned in the way that the carboxylic group (hydrophilic) attached to the ferrite surface and alkyl groups (hydrophobic) pointed to organic solvent during the synthesis. The second layer of oleic acid developed when the particles were further treated in excess oleic acid and aligned in the way that was opposite to the first layer. Nickel ferrite particles prepared by the nonhydrolytic sol-gel process using limited
amount of oleic acid (C1 & C2) also showed a shift of IEP, indicating that free –COOH groups developed on the surface. However, comparing zeta potentials, the particles with limited oleic acid had significantly lower absolute values than the particles with excess oleic acid in both low (pH=2.3) and high pH (pH=11.5) values. It implied that the amount of free oleic acid was insignificant in particles C1 and C2. The entire surface could be treated as hydrophobic.

Table 5-3 Zeta-potential values and isoelectric points (IEP) of nickel ferrite particles.

<table>
<thead>
<tr>
<th>Sample name</th>
<th>B1</th>
<th>C1</th>
<th>C1+S</th>
<th>B2</th>
<th>C2</th>
<th>C2+S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zeta potential at pH=2.3</td>
<td>48.7</td>
<td>4.3</td>
<td>22.0</td>
<td>69.5</td>
<td>9.8</td>
<td>25.7</td>
</tr>
<tr>
<td>Zeta potential at pH=11.5</td>
<td>-16.3</td>
<td>-7.2</td>
<td>-47.2</td>
<td>-34.5</td>
<td>-13.9</td>
<td>-65.9</td>
</tr>
<tr>
<td>IEP</td>
<td>6.0</td>
<td>3.5</td>
<td>3.7</td>
<td>6.5</td>
<td>3.0</td>
<td>3.2</td>
</tr>
<tr>
<td>No. of oleic acid layer</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Hydrophilic/hydrophobic</td>
<td>Hydrophilic</td>
<td>Hydrophobic</td>
<td>Hydrophilic</td>
<td>Hydrophilic</td>
<td>Hydrophobic</td>
<td>Hydrophilic</td>
</tr>
</tbody>
</table>

C. Characterization and calculation of surfactant coverage

Based on the discussion on surface charges, the following configurations (Fig. 5-3) were proposed for monolayer or bilayer of oleic acid on particle surfaces.

![Figure 5-3 Configurations of monolayer or bilayer of oleic acid on particle surfaces.](image-url)
TGA was used to quantitatively characterize the 1\textsuperscript{st} and 2\textsuperscript{nd} layers of oleic acid coating. Nickel ferrite particles were heated to 700°C in air. The TGA results of C1, C1+S and C2, C2+S are shown in Fig. 5-4 (a) and (b), respectively.

![TGA results of nickel ferrite particles. (a) C1, C1+S and (b) C2, C2+S.](image)

The particles with 2 surfactant layers showed a larger weight loss. To evaluate the surfactant coverage of each layer, the weight losses were assigned to certain fractions of adsorbed species. As discussed in Chapter 3, the first 4-5% weight loss was attributed to desorption and evaporation of water. For the particles coated with monolayer, the weight loss from 95-96% to the plateau was due to desorption and evaporation of the layer of oleic acid. For the particles coated with bilayer, the extra weight loss excluding the loss of 1\textsuperscript{st} layer coating was attributed to the 2\textsuperscript{nd} layer of oleic acid coating.

The experimental data used for calculation of surfactant coverage and the final results are shown in Table 5-4. In the table, the mass of nickel ferrite was obtained from the final remaining weight after the TGA measurement. Diameters of C1 and C2 were obtained from their average crystallites sizes in TEM images. The diameters of C1+S and
C2+S were estimated as $D+2l$, where $D$ is the diameter of C1 particle or C2 aggregate, respectively and $l$ is the fully extended chain length of oleic acid. The number of particles was calculated by dividing the total mass of nickel ferrites by the mass of each ferrite particle. The latter was obtained by multiplying the volume of each particle and the particle density. The volume was calculated by the TEM average size. The density used here was 5.368 g/cm$^3$.

Table 5-4 The experimental data used for calculation of surfactant coverage and the calculated surfactant coverage (Å$^2$/molecule).

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Mass of nickel ferrite (%)</th>
<th>Diameter of particle (nm)</th>
<th>Number of particles</th>
<th>Mass of surfactant (%)</th>
<th>Surfactant coverage (Å$^2$/molecule)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>76</td>
<td>10</td>
<td>2.71x10$^{19}$</td>
<td>19</td>
<td>21</td>
</tr>
<tr>
<td>C2</td>
<td>88</td>
<td>10</td>
<td>3.13x10$^{19}$</td>
<td>8</td>
<td>58</td>
</tr>
<tr>
<td>C1+S</td>
<td>67</td>
<td>10.4</td>
<td>2.12x10$^{19}$</td>
<td>9</td>
<td>37</td>
</tr>
<tr>
<td>C2+S</td>
<td>85</td>
<td>150.4</td>
<td>8.89x10$^{15}$</td>
<td>3</td>
<td>10</td>
</tr>
</tbody>
</table>

It was reported that the area occupied by densely packed fatty acid in monolayer films on water are 20-22 Å$^2$/acid molecule$^3$. If the coverage area by each oleic acid molecule was larger than this value, it meant that oleic acid was insufficient. If the coverage area was smaller than this value, it meant that oleic acid was in excess. In C1, i.e. small particles with one layer of oleic acid coating, the calculated coverage area (21 Å$^2$/molecule) was equivalent to that of the densely packed case. It suggested that the surfactant layer had highly organized structure which prevented the particles from aggregation. It was consistent with the TEM image of C1. In C2, i.e. large particles with 1 layer of oleic acid coating, the coverage area (58 Å$^2$/molecule) was much larger than that of the densely packed case. It suggested that oleic acid was not sufficient to cover all crystallites, which could lead to aggregation. This also agreed well with the TEM results of C2. From Table 5-4, it was also found that in C1+S the packing of oleic acid in the 2nd
layer (37 Å²/molecule) was not as dense as that in the 1st layer (21 Å²/molecule). For C2+S, the very small coverage area, i.e. 10 Å²/molecule suggested that there were sufficient oleic acid molecules in the 2nd layer.

5.2.3 Cytotoxicity of particles without oleic acid coating

Cell viabilities of nickel ferrite particles without oleic acid coating are illustrated in Fig. 5-5. As shown in Fig. 5-5(a), there was no obvious decrease of cell viabilities with increasing particle mass concentration. The differences in cell viabilities between 150 nm and 10 nm particles were also not statistically significant. Figure 5-5(b) shows the relationship between cell viabilities and the total number of particles per well which was calculated using the average particle size given in Table 5-2. Consistently, the increase of particle number did not lead to the increase of cytotoxicity in both small and large particles. With the similar particle number (around 10⁹), the 10 nm particles showed a much higher cell viability than that of 150 nm particles. In this sense, the smaller particles were less toxic. Since cells were only accessible to the surface areas of the present particles, the total surface areas per well were calculated and represented in Fig. 5-5(c). The differences of viabilities between large and small particles were also not statistically significant in terms of total surface areas per well. Normalizing the x-axis using total surface area, the cell viabilities showed similar values when the total surface area was similar, regardless whether the viability values were obtained from large or small particles.
Figure 5-5 Cell viabilities of nickel ferrite particles without oleic acid coating. (a) dependence of cell viabilities on mass concentration, (b) dependence of cell viabilities on the number of particles per well, and (c) dependence of cell viabilities on the total surface area per well.

In summary, when the nickel ferrite particles were not treated with oleic acid, their size did not affect cell viabilities in terms of mass concentration and the total surface areas. However, smaller particles were less cytotoxic compared with large particles in terms of total number of particles per well. This was reasonable because small particles had a smaller mass concentration and smaller surface areas than larger particles if both of
them were normalized by the number of particles. In further discussion, the mass concentration and total surface area were used to discuss the cytotoxicity.

5.2.4 Cytotoxicity of particles coated with oleic acid

Figure 5-6 and Figure 5-7 show cell viabilities of nickel ferrite particles with 1 layer or 2 layers of oleic acid coating respectively. Compared to the uncoated particles (hydrophilic surface, -OH is the functional group exposed to cells), the cell viabilities statistically decreased, as seen in both Fig. 5-6 (a) and Fig. 5-7 (a). Such decrease depended on the concentrations. It implied that the oleic acid played an important role irrespective of the number of layers on the particle surface. Comparing Figs. 5-6 (a) and 5-7 (a), the particles with 1 layer of oleic acid (hydrophobic surface, -CH\textsubscript{3} is the functional group exposed to cells) were more cytotoxic than the particles with 2 layers of oleic acid (hydrophilic surface, -COOH is the functional group exposed to cells). It seemed that a hydrophilic surface was less cytotoxic than a hydrophobic surface. Similar results had been reported in bile acids and solid lipid nanoparticles because hydrophobic groups were more accessible to cells and may cause more interaction between cells and invasive particles\textsuperscript{4}. 
Figure 5-6 Cell viabilities of nickel ferrite particles with 1 layer of oleic acid coating. (a) dependence of cell viabilities on mass concentration, and (b) dependence of cell viabilities on the total surface area per well.

Figure 5-7 Cell viabilities of nickel ferrite particles with 2 layers of oleic acid. (a) dependence of cell viabilities on mass concentration, and (b) dependence of cell viabilities on the total surface area per well.
5.2.5 The effect of particle size

In both Figs. 5-6 (a) and 5-7 (a), the smaller particles were less toxic than the larger ones in medium concentrations (at 20, 50, 100 μg/ml) with either 1-layer or 2-layers surface coating. The size effect could not be explained by the different surface areas because cell viabilities of small particles were still much higher than large particles even if both of them were normalized by surface area, as indicated in Figs. 5-6 (b) and 5-7 (b).

Two possible reasons on the size effect are suggested. First, the surface energy of small particles was higher than that of large particles. The different surface energies could have different effects on the surfactant adsorption and conformation which had been revealed in Table 5-4. In this way, the same surfactant might behave differently when it interacted with cells. Another possible explanation involved consideration of different interaction areas of large and small particles, as shown in Fig. 5-8. The effective interaction area that a large particle could access the cell was greater than that for a small particle. Within this specific area, there were more functional groups on the surface of individual large particle. Thus each large particle exerted a stronger stimulus on the cells. However, if an equal mass of particles with different sizes was considered, the significantly larger number of small particles increased the number of interaction points. These points were randomly distributed around the cells. However, due to the likelihood of a smaller number of functional groups on individual small particles, each interaction point exerted a weak stimulus on the cells. The experimental results seemed to suggest that the sum of weak stimuli at different locations was not as toxic as one localized strong stimulus from a bigger particle.
Figure 5-8 A schematic picture of different interaction areas of individual particle with 1 layer of oleic acid coating, in which single large particle possessed larger interaction area with more function groups. The big number of small particles increased the number of interaction points that were randomly distributed on the cell, but there were less functional groups on each interaction point.

5.3 Cytotoxicity of oleic acid

As mentioned earlier, oleic acid as a surfactant of nickel ferrite particles showed significant effects on their cytotoxicity. In this part, details were discussed in the cytotoxicity of oleic acid molecules in different structures, i.e. monomers, micelles and coatings.

5.3.1 Pure oleic acid system

Oleic acid monomer was critical for the cellular structure and functions of bacteria, fungi, and humans. Because of the presence of the double bond in the center of the molecule, it helped govern the critical nature of fluidity of the membrane matrix. The external oleic acid monomer could pass through the bilipid membranes because it had very similar structure as lipids which are the main component in cell membranes. Such membrane-transport minimally involved adsorption, translocation and desorption.
(as shown in Fig. 5-9, adopted from Hamilton et al.’s work). None of these steps had a universal requirement of protein transporter. So the mechanism of cellular uptake of oleic acid was largely agreed by diffusion transport rather than by specific transporter\textsuperscript{5}.

![Diagram of oleic acid monomer passing through cell membranes](image)

**Figure 5-9** A schematic picture showing oleic acid passing through cell membranes as monomer.\textsuperscript{5}

To study the cytotoxicity of oleic acid, the cell viabilities of oleic acid alone were carried out and the results are shown in Fig. 5-10. At the concentrations of 20 and 200 μg/ml, the cell viabilities were above 80%. When oleic acid concentration increased to 500 μg/ml, cell viabilities decreased dramatically to 54.3%. When the concentration increased further to 1000 μg/ml, cell viabilities decreased to 10.1% and remained very low when the concentration further increased to 2000 μg/ml.
At low concentrations, oleic acids were present in the form of monomer. They could pass through the bilipid membranes as shown in Fig. 5-9. As the interface between air and solution became crowded with oleic acid, more molecules arranged into micelles. At a higher concentration, the interface became completely loaded with oleic acid and any further additions arranged as micelles. This concentration was called Critical Micelle Concentration (CMC). Above this characteristic concentration the appearance and development of micelles brought about sudden variation in some physico-chemical properties of the solution, such as surface tension, conductivity and light absorption. For example, the relationship between surfactant concentration and surface tension is illustrated in Fig. 5-11. The CMC of specific surfactant depended on ion strength, temperature and pH, etc.
Figure 5-11 Surface tension vs. concentration.

The CMC of oleic acid in aqueous solution was reported as 0.72-3.5 mM, (i.e. 200-1000 μg/ml)\(^6\). Due to the higher ion strength of cell culture medium, the CMC of oleic acid in DMEM might be different from the CMC in water. In our current work, the CMC of oleic acid in DMEM was measured using UV absorption and surface tension to assess the formation of micelles. The results are shown in Fig. 5-12. The CMC of oleic acid was measured as 1000 μg/ml using UV absorption and 1600 μg/ml using surface tension, respectively. Although there were some differences in the CMCs determined by different methods, it was noted that the cell viabilities dropped significantly near the range of CMC of oleic acid. Similar results had been reported that the action of lysophosphatidylcholine (LPC, a naturally occurring phospholipids metabolite) on rabbit perivascular cells was related to micelle formation such that the alternation of cellular
function occurred at concentrations lower than, and cytotoxicity occurred at concentration higher than CMC of LPC \(^7\). In micelles, oleic acids could form aggregates in which the hydrophobic portions were oriented within the cluster and the hydrophilic portions were exposed to the aqueous solution. The cytotoxicity above CMC could be attributed to the conformation of oleic acid molecules in which functional groups of oleic acids were aligned orderly.

![Graph showing CMC of oleic acid in DMEM](image)

Figure 5-12 CMC of oleic acid in DMEM (using UV absorption and surface tension respectively to assess the formation of micelles).

### 5.3.2 Comparison of oleic acid itself and its coating on particles

Compared with the pure oleic acid system, the oleic acid-coated particles showed lower cell viabilities at similar concentrations. This finding was in good agreement with the report by Olivier et al.\(^8\) showing an increased cytotoxicity of Tween 80 in combination with polybutylcyanoacrylate (PBCA) nanoparticles. Furthermore, Scholer et al.\(^9\) reported the increase in cytotoxicity of surfactant cetylpyridinium chloride (CPC) when it was introduced to solid lipid nanoparticles. They both attributed this increased
cytotoxicity to the conformation in which the surfactant was adsorbed on the nanoparticle surface.

In our case, the highest concentration of nickel ferrite particles, i.e. 200 μg/ml caused dramatic cytotoxicity in both Figs. 5-6 (a) and 5-7 (a). However, for this particle concentration, the required concentrations of oleic acid to cover 150 nm nickel ferrite particles were 24 μg/ml (1 layer coverage) and 48 μg/ml (2 layer coverage), respectively. Whereas for 10 nm particle they were 50 μg/ml (1 layer coverage) and 100 μg/ml (2 layer coverage), respectively. If the above concentrations were present as pure oleic acid, they were far below the toxicity tolerance level of oleic acid (about 500 μg/ml). The above comparison suggested that the aligned oleic acids on particle surface caused pronounced cytotoxicity.

5.4 Summary

It was demonstrated that cytotoxicity of nickel ferrite particles strongly depended on the surface coating, which in turn depended on the method of synthesis and processing used. When there was no oleic acid coating on surface, nickel ferrite particles were not cytotoxic for both large and small particles. If nickel ferrite particles were coated by 1 layer or 2 layers of oleic acid, cytotoxicity was observed and larger particles were more cytotoxic than smaller ones. The size effect could be related to the surface energies and surface interaction areas that were size-dependent. For randomly distributed oleic acid monomers, their organized assemblies (micelles) and coating of oleic acid on nickel ferrite particles, surfaces had significant effects on the cytotoxicity. If oleic acid molecules were present as monomers, they were not cytotoxic. However, if they
developed micelles or coated on the ferrite particles, i.e. with their functional groups spatially aligned, cytotoxicity was observed.

Chapter 6 Conclusions and future work recommendation

6.1 Conclusions

Nonhydrolytic sol-gel method and mechanochemical method were used to synthesize nickel ferrite particles with controllable particle size. Using reported mechanochemical method, particles without surface coating were obtained. In the nonhydrolytic sol-gel method, by applying surfactants, as-prepared nickel ferrite particles were successfully coated with chemisorbed oleic acid. By varying the composition of metal precursors, two microstructures were achieved; i.e individual nanocrystals and aggregates consisted of many nanocrystals. Different from common methods to control particle size by changing the ratio of precursor to surfactant, the variation of precursor composition contributed to the difference in microstructures. Oleic acid could form complexes with iron (III) ions, but not with nickel (II) ions. Increasing the concentration of iron precursor thus consumed more oleic acid and led to insufficient oleic acid coating on particle surface. Strong inter-crystallite interactions were induced from less protected surface and were possibly the driving force of aggregation.

Nickel ferrite nanoparticles with or without oleic acid coating were used to prepare composites with PLA. The microstructure of the composites was strongly dependant on the presence of oleic acid coating. If there was no oleic acid coating on the surface, nickel ferrite particles attached on the PLA surface. For the nanoparticles coated with oleic acid, they tended to be encapsulated by PLA microspheres. No new chemical bond was detected between the particles and PLA. A slight decrease of $T_g$ was found in the composites which could be related to the difference in interfacial energies between
the two components. The optimal mixed composite (C1/PLA) was achieved by reducing the interfacial energy. However, its loading capacity was limited. Increasing the amount of nickel ferrite nanoparticles was not useful to increase its loading capacity.

The cytotoxicity of nickel ferrite particles significantly depended on the surface coating and surface characteristics which in turn depended on the type of synthesis and processing used. When there was no oleic acid coating on particle surface, nickel ferrite was not cytotoxic for both large and small particle sizes. When 1 layer or 2 layers of oleic acid were coated on the surface, larger particles were more cytotoxic than smaller ones. The size effect could be related to the surface energies and surface interaction areas that were size-dependent. For oleic acid molecules in different conformations, the surface characteristics had significant effects on the cytotoxicity. If oleic acid molecules were present as monomer, they were not cytotoxic. However, if they developed micelles or coated on the ferrite particles, i.e. when their functional groups were spatially aligned, cytotoxicity was observed.

6.2 Recommended work in the future

The most interesting findings in this thesis were that the presence, conformation, and density of oleic acid on the surface could influence the microstructure of nickel ferrite particles, the properties of their composite with PLA and even their cytotoxicity. To investigate these effects, a detailed study on the interface between particles and surfactant coating is recommended for future work. Though, interdigitation structure or intermolecular interaction had been suggested by others, there was no direct proof to support any of them.
In this thesis, encapsulation of nickel ferrite nanoparticles in PLA microspheres was achieved. Such morphology was suitable for drug loading and controlled release. However, the drawback of such composite was its limited loading capacity. Further studies aiming to increase loading capacity are required to enhance the magnetic response of the composite.

By using non-hydrolytic sol-gel method, the synthesized nickel ferrite particles were not stoichiometric NiFe$_2$O$_4$. To maintain electron balance in a nickel ferrite cell unit, cation substitution was expected in both C1 and C2. To investigate the distribution of substituted cations on $A$ and $B$ sites, further Mössbauer and neutron diffraction studies are suggested.

Finally, although it was demonstrated that orderly aligned oleic acid molecules were cytotoxic, the mechanism of cell death is unclear. Further study such as tracing nanoparticles should be carried out via modifying particle surface with fluorescence dye. In addition, detailed TEM study on cells in cytotoxic conditions would be another good approach to investigate the interactions between cells and nanoparticles.