

From Micro to Nano Magnetic Spheres: Size-Controllable Synthesis, Multilayer Coatings, and Biomedical Applications

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ABSTRACT

Size-controllable monodispersed ferrite microspheres to nanoparticles have been synthesized with a range of particle sizes from 10 to 1500 nm. The particle's grain size can also be tuned from 5.9 to 21.5 nm. Some of these spheres have been coated efficiently with silica, poly(aniline), dendrimer, etc., to yield well-defined core/shell-like structures. These magnetic microspheres have been functionalized successfully with organic moieties such as crown ethers, acting as a tool for the magnetic separation of discrete functional gold nanoparticles. Moreover, ultrasmall magnetic nanoparticles have been employed as both *in vitro* and *in vivo* magnetic resonance imaging contrast agents.

Keywords: contrast agent, magnetic resonance imaging, magnetite, microsphere, nanoparticle

1 INTRODUCTION

Magnetic material has become a particularly important research area and is attracting a great deal of attentions because of their potential technological applications, such as in ferrofluids, colored pigments, high-density magnetic recording media, chemical sensors, electrophotographic developers, biological assays and magnetic resonance imaging [1-9]. High-quality nanocrystals with well-defined shapes and controllable sizes play an important role in nanotechnology because the chemical and physical properties of nanomaterials are strongly related to their mean size and size distribution. To date, therefore, a range of techniques have been developed for fabrication of magnetic nanostructures with sophisticated nanostructures, among which spherical nanoparticles are of particular interest owing to their shape and size-dependent properties [10-18].

Spinel ferrites (MFe_2O_4 ; $M=Fe, Mn, Zn, Co,$ and Ni) exhibit interesting magnetic, magnetoresistive, and magneto-optical properties that are useful for a broad range of applications [19-22]. In principle, the magnetic properties of the ferrite can be systematically varied by changing the identity of the divalent M^{2+} cation or by partial substitution while maintaining the basic crystal structure. Besides the composition, the size and crystallinity of the crystals also have great influence on the properties of

the ferrite nanomaterials. For this reason, various approaches, including co-precipitation, hydrothermal, sol-gel, reversed and normal micelles, sonochemical reactions, ball milling, etc., are being adopted to produce spinel ferrites to realize the desired properties [23-26]. In order to attain reliable and high performance for biomedical applications, ferrite particles should possess narrow size distribution, large surface area, high magnetic saturation, good dispersion in liquid media, well-defined shape and surface texture [27-29]. Therefore, the synthesis of monodispersed ferrite nanospheres with tunable sizes and magnetic properties has been intensively pursued for their technological and fundamental scientific importance.

2 RESULTS AND DISCUSSION

Up to now, the general method that was employed for the preparation of monodispersed ferrite nanospheres, was usually based on a wet chemistry-based synthetic route such as co-precipitation [30]. Another effective method for the size and shape-controllable synthesis of the ferrite nanoparticles is *via* a high-temperature thermal decomposition route, by using organometallic complex, such as metal acetylacetonates, carbonyls, etc., as the precursors along with some aliphatic acids, amines and diols [31-36]. In this work, a modified solvothermal method [37] was developed to construct a series of superparamagnetic ferrite microspheres/nanoparticles which comprised of tunable particle sizes, grain sizes, and magnetic properties. The as-prepared ferrite particles can be coated with different functional groups or layer of materials such as silica, poly(aniline), dendrimers, etc. The superparamagnetic properties equipped with functional coatings render the particles ideal candidates for practical applications such as magnetic separation, magnetic resonance imaging and drug delivery.

2.1 Synthesis

When $FeCl_3 \cdot xH_2O$ and $MCl_2 \cdot yH_2O$ ($M = Mn, Zn, Co,$ or Ni) were used as the metal source, the grain size and particle size of the secondary structures can be tuned rationally by using different amounts of ethylene glycol

(EG), diethylene glycol (DEG), sodium acrylate (Na acrylate), and sodium acetate (NaOAc) (Figure 1) [38,39].

By way of examples, monodispersed ferrite microspheres with different grain sizes that have an average particle size about 280 nm, can be synthesized by controlling the ratios of Na acrylate/NaOAc from 1.5/0 to 1.0/0.5, 0.5/1.0, 0.3/1.2, and 0.1/1.4 in EG at a temperature of 200 °C for 10 h. These processes afford the ferrite microspheres which possess a grain size from 5.9 to 6.9, 8.3, 10.1, and 13.5 nm, respectively [38]. When Na acrylate was introduced to the FeCl_3/EG solution, a significant amount of $\text{Fe}(\text{OOCCH}=\text{CH}_2)_3$ complex was formed. Then, in the presence of EG, the complex was transformed into Fe_3O_4 nucleates under hydrothermal treatment [37]. Meanwhile, the freshly formed nanocrystals are unstable because they contain high surface energy, and thus they have a great tendency to aggregate rapidly. Because of the strong coordinations between iron(III) ions and carboxylate, the as-formed Fe_3O_4 nanograins possessed a coating of polyacrylate. By increasing the amount of Na acrylate, more polyacrylate will bind to the primary Fe_3O_4 nanocrystals' surface. This results in a decrease of the size of Fe_3O_4 nanograins. Consequently, the main driving force for the aggregation of nanograins is generally attributed to the reduced high surface energy through both the attachment among the primary nanoparticles and the polyacrylate. Additionally, the grain size tunability may also be the result of slight differences in alkalinity caused by varying the amounts of NaOAc. The hydrolysis rate of FeCl_3 could be accelerated by increasing alkalinity so as to promote the formation of larger Fe_3O_4 nanocrystals.

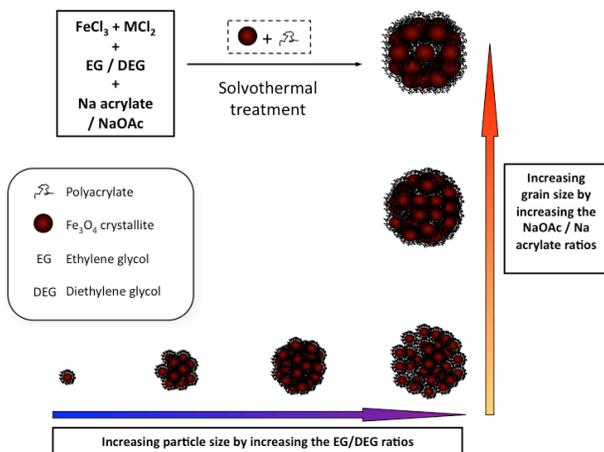


Figure 1: Schematic representation of the formation of the monodispersed ferrite particles with tunable grain size and particle size. The particle sizes are also tunable when Na acrylate is replaced by PVP.

On the other hand, by varying the EG/DEG ratios in the system instead of Na acrylate/NaOAc ratios, the particle sizes can be tuned. EG plays both roles as a reducing agent and a solvent during the formation of the particles. Since the reaction temperature in the sealed container is 200 °C,

which is slightly higher than the boiling point of EG (196 °C), therefore, EG may exist as a superfluid. At this temperature, Fe_3O_4 nanograins and polyacrylate assemble to form large secondary structural microspheres. When DEG (b.p. = 240 °C) was introduced together with EG, the size of the particles can be reduced. The diameter of the as-prepared Fe_3O_4 particles are ranging from 6 to 60, 120, and 170 nm when the EG/DEG ratio changes from 0/20 to 5/15, 10/10, and 20/0, respectively. These particles are superparamagnetic which were characterized by vibrating sample magnetometer (VSM).

From the above reaction conditions, if Na acrylate was replaced by poly(vinylpyrrolidone) (PVP), the sizes of ferrite nanoparticles/microspheres can be tuned with size from 20 to 300 nm, by simply varying the EG/DEG ratios. For example, the EG/DEG ratios of 1/19, 5/15, 10/10, and 20/0 lead to the formation of Fe_3O_4 nanoparticles/microspheres with average sizes of 20, 90, 165, and 300 nm, respectively, at a temperature of 200 °C for 10 h [39]. Additionally, if the reaction suspension was heated under a continuous flow of N_2 with mechanical stirring, the as-prepared particles are superparamagnetic.

2.2 Coating

Core/shell-structured composite materials which combine with the advantageous properties of both materials of the core and the shell, have attracted increasing interest due to their unique physicochemical properties. Various types of bifunctional or trifunctional core/shell materials, such as electronic/optical, electronic/catalytic, magnetic/electronic, etc., could be technically obtained. In our work, ferrite particles were coated with functional shells to give magnetic core/shell composite materials.

Among these shell materials, poly(aniline) (PANI) has received more attention for its unique electrical properties. In our group, $\text{Fe}_3\text{O}_4@$ PANI microspheres with blackberry-like morphology have been successfully obtained by using an *in situ* surface polymerization method in the presence of aniline and PVP [40]. Naked Fe_3O_4 particles (350 nm) were coated with a thin silica shell with amine functional group, prior to PANI coating. The thickness of the PANI shells (30-60 nm) depends on the polymerization time, the initial concentration of aniline, and the reaction temperature. Furthermore, a more uniform PANI coating (25 nm) apart from the rough blackberry-like morphology has been obtained for a carboxylic acid-modified Fe_3O_4 microspheres (160 nm) [41]. The uniform $\text{Fe}_3\text{O}_4@$ PANI microspheres are then used as templates for supporting gold nanoparticles (4 nm). For the synthesis of the $\text{Fe}_3\text{O}_4@$ PANI@Au composites, nitrogen atoms on the $\text{Fe}_3\text{O}_4@$ PANI microspheres' periphery were protonated such that the citrate-coated, negatively charged Au seeds could be immobilized by electrostatic interactions [41].

Silica has been frequently used to coat ferrite particles, unambiguously for biological applications [30]. This is because silica has good biocompatibility and hydrophilicity,

and can prevent aggregation of particles in liquids and improve their physicochemical stability. Silica can be effectively coated on hydroxyl functionalized ferrite particles by a sol-gel method using silica precursors such as tetraethyl orthosilicate (TEOS) and aminopropyl triethoxysilane (APTES).

Despite inorganic coating shells, organic shells are practical to coat ferrite particles to provide specific functions and properties. In our work, poly(arylether) dendrons have been successfully attached onto the surface of Fe_3O_4 microspheres [42]. The dendron attachment/detachment processes on ferrite particles are controlled by water concentration via dynamic covalent chemistry, wherein the hydrophilicity and hydrophobicity of the ferrite particles' surface can be reversibly tuned [42]. Organic, supramolecular molecules such as crown ethers were attached on $\text{Fe}_3\text{O}_4@SiO_2$ microspheres for magnetic separation purposes [43]. Crown ethers such as dibenzo[24]crown-8 that are attached on $\text{Fe}_3\text{O}_4@SiO_2$ microspheres, can be recognized by dibenzyl ammonium derivatives upon pH changes [44]. Discrete functional gold nanoparticles that are linked with dibenzyl ammonium groups, can be magnetically separated and purified by crown ether-coated ferrite microspheres. The pH-switchable property of the recognition unit allows the detachment of the final product from the ferrite microspheres [43].

2.3 Properties and Applications

Magnetic separation by using functional ferrite nanoparticles/microspheres is attractive. In our work, the $\text{Fe}_3\text{O}_4@PANI@Au$ composite materials are effective co-catalysis for reduction, that can be magnetically recyclable [41]. Furthermore, one of the important applications of ferrite is the use as contrast agents for magnetic resonance imaging (MRI). As MRI contrast agents, superparamagnetic ferrite should be employed wherein superparamagnetic materials behave as magnets only if external magnetic field is applied.

$\text{Fe}_3\text{O}_4@SiO_2$ nanoparticles (8 nm) appeared to be the preferred material for magnetic labeling of stem cells without the addition of any transfecting agent [30]. This is because (1) they provide a strong change in signal per unit of metal, in particular on $T2^*$ -weighted images; (2) they are composed of biodegradable iron, which is biocompatible and can thus be reused/recycled by cells by using normal biochemical pathways for iron metabolism; (3) they can be easily detected by light and electron microscopy. After biodegradation, iron oxide nanoparticles are turned into soluble iron ions. Our amine-functionalized $\text{Fe}_3\text{O}_4@SiO_2$ nanoparticles (8 nm) have been employed as contrast agents for *in vitro* and *in vivo* MRI. For *in vivo* MRI, nanoparticle-labeled stem cells were implanted to rabbit's brain (Figure 2) and spinae muscle, demonstrating a long-lasting, durable MRI labeling efficacy after 8-12 weeks [30].

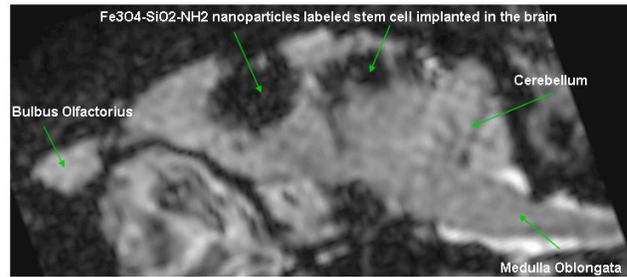


Figure 2: *In vivo* MRI. Amine-functionalized $\text{Fe}_3\text{O}_4@SiO_2$ nanoparticle-labeled stem cells were implanted into rabbit's brain.

The $T2$ relaxation rates of some of our as-synthesized superparamagnetic ferrite particles are summarized in Table 1. From the results, the ferrite microspheres/nanoparticles with a larger grain size yields a higher $T2$ relaxation rate. The $r2$ relaxivities of commercially available Fe_3O_4 -based MRI contrast agents range from 33.4 for VSOP-C184 and 38 for Supravist to 120 for Feridex and 189 for Resovist [45]. It is well-known that the effects of crystallization nature of the ferrite are related to the $T2$ relaxation rate [46]. Also, $r2$ relaxivity of ferrites is closely related to their particle size, and that larger particles tend to have higher $r2$ relaxivity. The grain sizes of some of our ferrite microspheres/nanoparticles are close to that of VSOP-C184 and Supravist. From this aspect, because of the difference in crystallization nature, our ferrite microspheres/nanoparticles demonstrate stronger relaxivities than the VSOP-C184 and Supravist.

Particles	Particle size (nm)	Grain size (nm)	$T2$ relaxation rate ($s^{-1}mmol^{-1}$)
Fe_3O_4	280	5.9	65.3
Fe_3O_4	280	10.1	80.4
Fe_3O_4	280	13.5	84.6
Fe_3O_4	90	10-20	93.7
ZnFe_2O_4	90	7-10	30.4
$\text{Fe}_3\text{O}_4@SiO_2$	10	3-4	18.9
$\text{Fe}_3\text{O}_4@SiO_2$	8	3-4	43.5

Table 1: $T2$ relaxation rates of some of our as-synthesized superparamagnetic ferrite particles.

3 CONCLUSIONS

Superparamagnetic ferrite nanoparticles/microspheres have been successfully prepared with tunable particle sizes and grain sizes. Coatings of these particles with selected inorganic and organic shells are feasible. It has been demonstrated that these particles are ideal contrast materials for *in vitro* and *in vivo* MRI.

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